VIRCON 2024

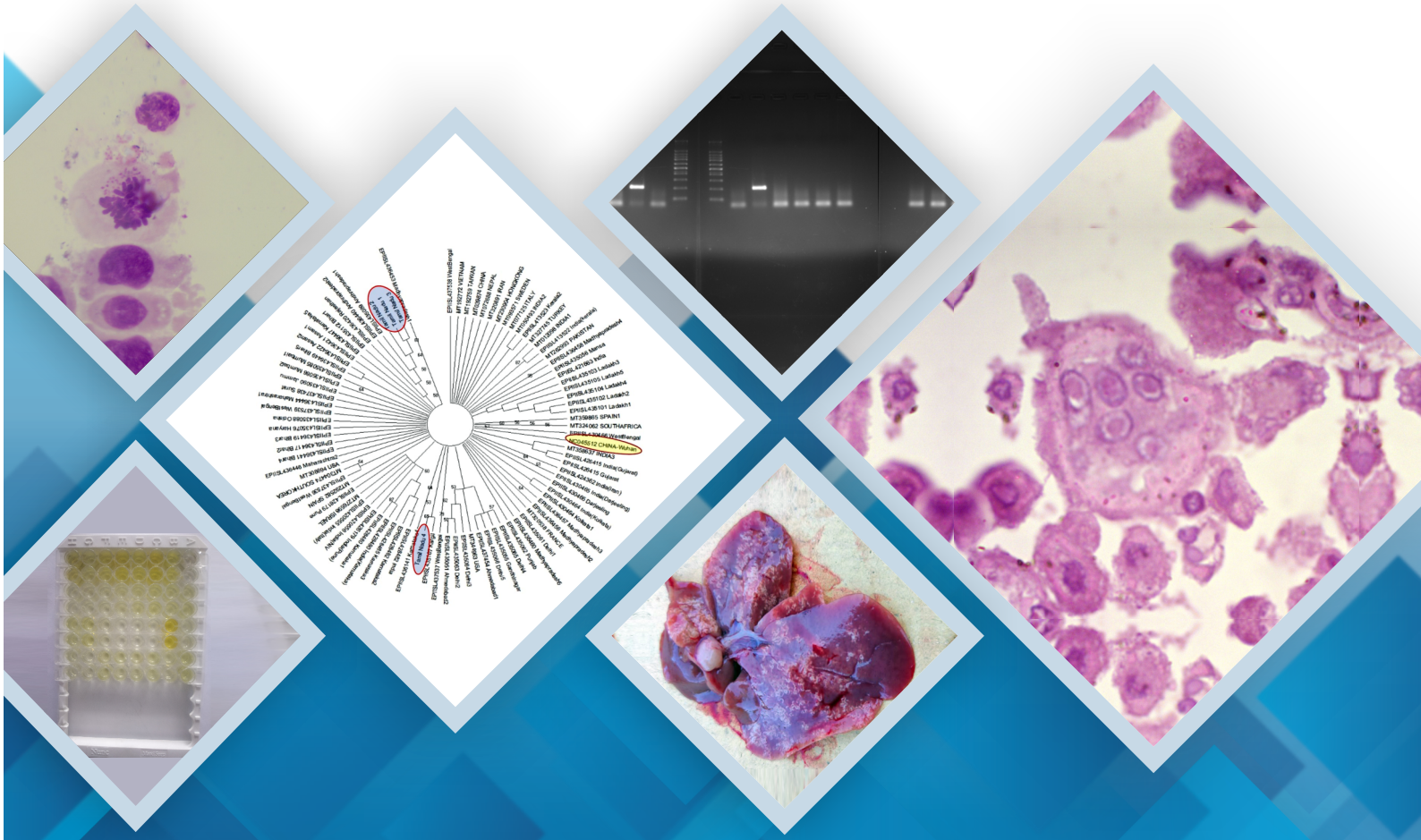
XXIX Annual Convention of ISVIB and National Conference

on

CHALLENGES IN ANIMAL HEALTH AND PRODUCTION AMIDST CLIMATE CHANGE: INNOVATIVE SUSTAINABLE SOLUTIONS AND THEIR TRANSLATION

26.09.2024 to 28.09.2024 | Madras Veterinary College, Chennai

COMPENDIUM



Jointly organized by

Tamil Nadu Veterinary and Animal Sciences University

&

Indian Society for Veterinary Immunology and Biotechnology

INTERNATIONAL INSTITUTE OF VETERINARY EDUCATION AND RESEARCH

BAHU AKBARPUR, NH-9,
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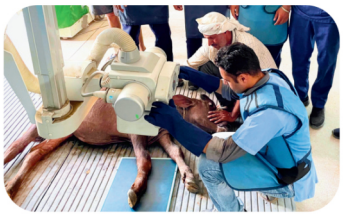
IIVER students consistently rank among the top 3 in the state, with several securing top positions in the industry.



Global Recognition

IIVER is listed among the world's recognized veterinary colleges by the American Veterinary Medical Association (AVMA).

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VIBCON 2024

XXIX Annual Convention of ISVIB and National Conference on

"Challenges in Animal Health and Production amidst Climate Change: Innovative Sustainable Solutions and their Translation"

COMPENDIUM

26.09.2024 to 28.09.2024 | Madras Veterinary College, Chennai

Jointly organized by

Tamil Nadu Veterinary and Animal Sciences University

&

Indian Society for Veterinary Immunology and Biotechnology

ACKNOWLEDGEMENT

The organizing committee of **VIBCON-2024** immensely thank the following funding agencies for their generous financial support in conducting this National conference

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DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science and Technology

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NABARD

FOREWORD VC

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

Dr. K.N. SELVAKUMAR, Ph.D.,
VICE-CHANCELLOR

FOREWORD

With immense pleasure, I welcome you all to the National Conference on **"Challenges in Animal Health and Production amidst Climate Change: Innovative, Sustainable Solutions and their Translation" – VIBCON 2024**. This esteemed gathering, hosted by the Tamil Nadu Veterinary and Animal Sciences University, Chennai (TANUVAS) promises to be a pivotal event in shaping the future of veterinary science.

The interconnectedness between human health, animal welfare, and environmental sustainability is now becoming increasingly evident. Rising temperatures and extreme weather events are disrupting delicate ecological balances, leading to habitat loss, food shortages, and the emergence of new diseases. Animals, particularly livestock, are vulnerable to these changes, as they are often directly exposed to the elements and rely on healthy ecosystems for their survival. The consequences of climate change on animal health are far-reaching, affecting not only individual animals but also entire populations and communities. The Indian Society for Veterinary Immunology & Biotechnology (ISVIB), in partnership with TANUVAS, has taken a significant step towards addressing this pressing issue by organizing VIBCON 2024.

The conference theme, "Innovative, Sustainable Solutions and Their Translation," highlights the importance of developing and implementing practical strategies that can mitigate the negative impacts of climate change on animal health while ensuring the sustainability of livestock production. From the intricacies of host-microbe interactions to the groundbreaking advancements in veterinary vaccines and disease diagnosis, the conference is expected to delve into a wide array of topics such as genomic research, disease surveillance and epidemiology, providing valuable insights into the prevention and control of animal diseases.

I am confident that VIBCON 2024 will be a catalyst for innovation and a source of inspiration for all participants. By fostering dialogue, sharing expertise, and exploring new avenues of research, we can collectively work towards a future where animal health and well-being are safeguarded in the face of emerging challenges.

I extend my warmest wishes to all participants and appreciate the organizing team for all the efforts in the happening of this event. I look forward to a fruitful and inspiring conference.

Date: 10.09.2024
Place: Chennai

(K. N. SELVAKUMAR)
VICE-CHANCELLOR

Telephone (O) : 0091-44-25551574 / 75 Email : vc@tanuvas.org.in; Website : www.tanuvas.ac.in

FOREWORD REGISTRAR

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

MADHAVARAM MILK COLONY
CHENNAI – 600 051, INDIA

Phone : 0091-44 2555 1584 (Dir.)
0091-44 2555 1586 / 87
E-mail : registrar@tanuvas.org.in

Dr.V.Appa Rao, Ph.D.
Registrar i/c

Foreword

It is with immense pride and pleasure that I extend my heartfelt greetings to all the esteemed participants of the XXIX Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Challenges in Animal Health and Production amidst Climate Change: Innovative, Sustainable Solutions and their Translation"—VIBCON 2024. This prestigious event, to be held at the historic Madras Veterinary College under the aegis of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), brings together a wealth of expertise from across veterinary science, biotechnology, and allied disciplines.

ISVIB, a vibrant and dynamic conglomerate of veterinary scientists, has been instrumental in fostering the growth of veterinary immunology and biotechnology. With its membership now exceeding 1000 scientists from both India and abroad, ISVIB continues to serve as a critical platform for the exchange of ideas, scientific advancements, and innovative technologies. The organization's focus on gene-based technologies in animal production and health is particularly relevant in today's era of rapid biological advancements. ISVIB has consistently motivated its members to engage in rigorous discussions, critical analyses, and the formulation of strategies aimed at safeguarding animal husbandry.

The theme of this year's conference is more timely. As climate change poses significant challenges to animal health and production, it is imperative that we, as scientists, researchers, and policy makers, seek innovative and sustainable solutions. VIBCON 2024 promises to be an important forum where such critical issues will be addressed, with the goal of translating cutting-edge research into practical applications for the benefit of both animals and society.

I congratulate the organizers for their dedication and hard work in bringing this compendium. I wish all the participants a successful and enriching experience, and I look forward to the valuable contributions that will emerge from this gathering.

Best wishes for a productive and insightful conference.

18.09.24

(Dr.V.Appa Rao)
Registrar

Place : Chennai
Date : 18.09.2024

FOREWORD DCAHS

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Dr. C. SOUNDARARAJAN, M.V.Sc., Ph.D.,
Director
Centre for Animal Health Studies
Madhavaram Milk Colony
Chennai - 600 051

Phone : 044 – 2555 5151
Mobile : +91 - 95005 63853
e-mail : dcahs@tanuvas.org.in
Website : www.tanuvas.ac.in

Message from Director's desk

It is with great anticipation and enthusiasm that I cordially welcome all the delegates participating in the **"XXIX Annual Convention of ISVIB and National conference on challenges in animal health and production amidst climate change: Innovative sustainable solutions and their translation"**. This national level conference stands as a testament to the collective commitment of veterinary scientists to advancing the fields of animal health and production through the integration of groundbreaking technologies and innovative practices to deal with the impact of global warming and climate change.

In the event of climate change, the stakes for ensuring animal health and optimizing milk and meat production are higher than ever before. To ensure our country's food and nutritional security, we need to explore sustainable and ecofriendly solutions. I am happy to know that the countries best scholars in the field of veterinary Immunology and biotechnology gathered here to discuss and interact in finding the tools and strategies to meet our countries food security.

Scientists from veterinary immunology and biotechnology fields have the potential for addressing the most pressing issues in animal health and production in a sustainable way.Recent advances in genomics, proteomics and nanomedicine are paving the way for novel approaches to diagnosis, treatment, and prevention animal diseases. I am sure that this conference would facilitate the interaction among the best minds across the country that would eventually lead to harnessing the power of genetic and biotechnological tools in developing novel vaccines and diagnostics for livestock diseases.

The sessions of this conference will cover a wide range of topics, including host-microbiome interactions, advancement in veterinary vaccines and diagnostics, genomics, disease surveillance and epidemiology, production and reproductive biotechnology, nano-biotechnology in animal health and production and one health problems and solutions. I expect numerous oral and poster presentations of international standard on these topics in this conference that will stimulate discussions and collaborative opportunities to drive the future of animal health and production.

As we embark on this journey together, I encourage all the participants to engage fully with the presentations, discussions, and networking opportunities at this national level scientific get together. Your participation is vital in translating innovative ideas into real-world solutions that can make a meaningful impact animal health and production in future. May this XXIX ISVIB National conference be characterized by productiveand thought-provoking discussions, which will lead to the betterment of farming community.

Date: 21.09.2024
Place: Chennai

Warm regards,

Dr. C. SOUNDARARAJAN, M.V.Sc., Ph.D.
Director
Centre for Animal Health Studies
TANUVAS, Madhavaram Milk Colony
Chennai-600 051.

Dr. R. K. Singh
President ISVIB and
Former Director & Vice Chancellor,
IVRI, Izat nagar

It is my pleasure to introduce the 29th Annual Convention of the Indian Society of Veterinary Immunology and Biotechnology (ISVIB), a prestigious event that brings together leading scientists, researchers, and practitioners in the field of veterinary immunology and biotechnology. With over 30 years of history, ISVIB has established itself as a prominent organization in the Indian scientific community, with a current membership of over 1000 scientists from India and abroad.

Since its inception in 1990, ISVIB has been committed to fostering the growth of veterinary immunology and biotechnology, and creating a sense of camaraderie among its members. To achieve this, the society has recognized and honored outstanding contributions in the fields of veterinary immunology and biotechnology through various awards, fellowships, and medals.

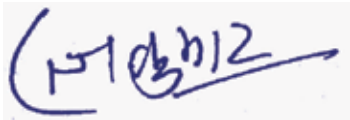
This year's convention, titled "Challenges in Animal Health and Production Amidst Climate Change: Innovative, Sustainable Solutions and Their Translation", is a testament to the society's commitment to staying at the forefront of scientific innovation and addressing some of the most pressing issues facing our planet. The conference is being jointly organized with Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), a premier veterinary institution in India that has been pioneering the field of veterinary teaching, research, and extension.

TANUVAS is a natural partner for this conference, given its long-standing reputation for excellence in animal health and production. The university's expertise in these areas will undoubtedly enrich the discussions and debates that will take place during the conference. I also wish to record that the national institutes like ICAR's IVRI at Bareilly, Mukteswar, Bengaluru, NIVEDI, NDRI and GADVASU always support the society's annual conventions wherever it is organized.

The conference will take place from 26th to 28th September 2024 at Madras Veterinary College, Chennai, providing a unique opportunity for scientists, researchers, and practitioners from around the country to come together and share their knowledge, expertise, and experiences. I am confident that this event will be a milestone in our collective efforts to address the challenges posed by climate change in animal health and production.

I extend my warm welcome to all delegates, speakers, and sponsors who will be joining us for this exciting event. Let us work together to create a platform that fosters collaboration, innovation, and sustainability in animal health and production.

Welcome to the 29th Annual Convention of ISVIB.



(R.K.Singh)

Dr.A.THANGAVELU,Ph.D.,
Secretary
Indian Society for Veterinary Immunology and Biotechnology

Message

In our country, contribution of livestock and poultry production to national Gross value added (GVA) is around five percent. This sector also provides employment to the rural people. Climate change affects growth, reproduction and disease transmission in animals. Higher environmental temperatures apart from causing heat stress to animals will also decrease water availability leading to shortage of feed and fodder. Global warming causes spread of vector borne diseases to new areas due to expansion of geographic range of vectors. Climate change can also lead to increased occurrence of emerging and re-emerging animal diseases. In this context it is timely and appropriate to hold a National conference on "Challenges in Animal Health and Production Amidst Climate change: Innovative, Sustainable solutions and their Translation"

I hope the deliberations in this national conference will help scientists to formulate research projects for developing vaccines to prevent animal diseases emerging due to climate change and also cost effective sustainable methods to reduce the effects of climate change in animal production.

I appreciate the efforts taken by the organizing committee for holding the XXIX Annual convention of ISVIB and National conference in Madras Veterinary College, TANUVAS, Chennai.

I offer warm felicitations to the organizers and participants of the National conference.

I wish the event a grand success.

Date: 19.09.2024
Place: Bhavani

19/9/2024
(A.THANGAVELU)

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Dr. S. MANOHARAN, M.V.Sc., Ph.D.,

Professor,
Vaccine Research Centre – Bacterial Vaccines
Centre for Animal Health Studies,
Madhavaram Milk Colony,
Chennai-600 051,

Message from the Organizing Secretary

I am very honored and privileged to organize this XXIX Annual Convention of ISVIB after a gap of 9 years in this campus. I have also the pride of donning dual role as Treasurer of ISVIB and as Organizing Secretary of ISVIB's convention. Now a day, it is challenging to organize a national conference amidst fast growing science, media and availability of information in the IOT world. We have selected the topic as "Challenges in animal health and production amidst climate change: Innovative, sustainable solutions and their translation", as the global warming effect on all living things on the earth including microbes leading to many pandemics, outbreaks of many exotic diseases, remerging of diseases in both human and livestock community. It will be more appropriate to discuss those challenges and suitable innovative, sustainable solutions to enhance the income of the farming community by the scientists of this country through this conference.

We have introduced separate best oral and poster awards for promoting student's participation who are the future of this country's growth. The deliberations from this conference will be communicated to the concerned authorities for required follow up action. We have received fullest funding support from all central govt. agencies whom we have applied and many private companies also for successful conduct of the conference. The organizing team is a great support for me and our Director, Centre for Animal Health Studies, TANUVAS also fully co-operative for this conference activities.

I hope this conference will bring a greater opportunity to the scientists, scholars and students to get more information, interactions and collaborations in their future career and Chennai is the most accommodative city in this country and all the delegates will have a fruitful, memorable and a joyful stay during this conference.

Chennai welcomes you all.

Date: 23.09.24
Chennai-51

(S. MANOHARAN)
Organizing Secretary
VIBCON 2024

ABOUT TANUVAS

The Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) was formed on 20.09.1989 as the first Veterinary University of the country, through the Tamil Nadu State Government Act 42 of 1989. The seed for the establishment and growth of TANUVAS was sown as early as 1876, when the Madras Veterinary College was started as an Agricultural School in Chennai to offer diploma and certificate course in the field of veterinary and animal sciences. The institute attained the status of a college on 01.10.1903, when it started functioning

at Dobbin Hall, Chennai and admitted 20 students for a three-year diploma course. Now TANUVAS has grown into a premier veterinary institution in the country with 9 constituent colleges, 12 research stations, 15 research laboratories and 30 outreach centers. TANUVAS offers 4 undergraduate programmes, 29 post graduate programmes, 23 PhD programmes and 25 PG Diploma programmes.

ABOUT ISVIB

The Indian society of veterinary immunology and biotechnology, popularly called as "ISVIB" is a conglomerate organization of primarily veterinary scientists from microbiology, biotechnology, preventive medicine, parasitology, animal reproduction, fisheries and related disciplines with the objective of fostering the growth of veterinary immunology and biotechnology in the era of biological technology. The organization was started in the year 1990 ISVIB motivates the members to discuss, analyze, formulate suitable strategies for safeguarding animal health and thus to ensure improved animal production. To achieve this, annual national conventions and symposiums are organized to critically analyze and update knowledge and understand issues concerning with gene based technologies in animal production and health. ISVIB has a current membership of over 1000 scientists from India and abroad.

ABOUT THE CONFERENCE

The Indian Society for Veterinary Immunology & Biotechnology (ISVIB) in collaboration with the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) is organizing the XXIX Annual Convention of ISVIB and National conference on "Challenges in Animal Health and Production amidst Climate Change: Innovative, Sustainable Solutions and their Translation" – VIBCON 2024" at Madras Veterinary College, Chennai from September 26 -28th, 2024. The conference will host an array of expert lectures in addition to presentations from researchers in the field of veterinary immunology and biotechnology. ISVIB welcomes the scientific community to participate in this conference.

SCIENTIFIC SESSIONS

The conference deliberations will focus on the following areas

1. Dr. Richard P. Masillamony Oration Award Lecture
2. Keynote address
3. Host - Microbes interactions
4. Advancements in veterinary vaccines and disease diagnosis
5. Genomics, Disease surveillance and epidemiology
6. Production and Reproductive Biotechnology
7. Nano-biotechnology in animal health and production
8. One Health - problems and solutions
9. Award session

CONFERENCE VENUE

The Conference will be organized in the Conference Hall at Madras Veterinary College, Vepery, Chennai. Vepery is a suburb in the north of Chennai which is around 2 kms from Chennai Central Railway Station and 20 kms from Madras International Meenambakkam Airport (MAA)

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XXIX Annual convention of ISVIB and National Conference on "Challenges in Animal Health and Production amidst Climate Change: Innovative, Sustainable Solutions and their Translations"

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	Dr. G. Elaiyaraja Assistant Professor, Education Cell, MVC, Chennai -7
	Dr. Sweetline Anne Assistant Professor, VRC-VV, DCHAS, Chennai -51

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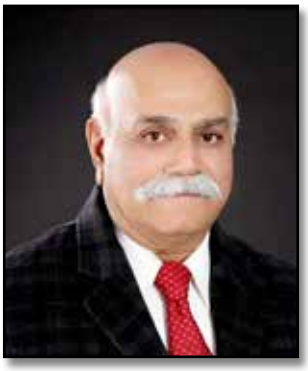
In loving memory of

Dr. MALLESHAPPA RAJASEKHAR



10.09.1942 – 08.09.2024

The Indian Society for Veterinary Immunology and Biotechnology on this occasion takes this opportunity to remember Dr. Malleshappa Rajasekar a remarkable individual whose contributions to veterinary science have made a lasting impact. Dr. Malleshappa Rajasekhar is a distinguished gold medallist in Veterinary Sciences and had his Ph.D. from the University of Edinburgh in 1981. Dr. M. Rajasekar was the Founder Director of the National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), where he was instrumental in advancing veterinary research, disease informatics and management in India. Further, he also held the position of Professor of Veterinary Microbiology at Bengaluru Veterinary College and served as Technical Director of the Royal Western India Turf Club for 14 years.

His dedication to veterinary medicine was instrumental in the successful eradication of rinderpest in India which fetched him the prestigious OIE award from the FAO in 2002. Dr. M. Rajasekar's legacy of excellence, innovation, and compassion with a pleasing personality will continue to inspire future generations.

AWARD SESSION

YOUNG SCIENTIST AWARD

YS_1: First report on Whole genome sequencing and comparative genomics of *Salmonella enterica* serovar *Abortusequi* isolated from Donkey in India

Rajendran Manikandan, Sithanandam Rajagunalan, Shanmugasamy Malmarugan and Chhavi Gupta
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Salmonella enterica subsp. *enterica* serovar Abortusequi (*S.* Abortusequi) is a leading cause of abortion in equines that hinders the rapid growth of equine industry. *S.* Abortusequi infection in equids has re-emerged over last ten years. In India, there are very few reports available on isolation of *S. Abortusequi* serovar from equines. The present study was undertaken to isolate and molecular characterize *S. Abortusequi* serovar from donkeys during an abortion storm in the southern peninsular region of India. *Salmonella* spp. was isolated and characterized by biochemical and molecular techniques. Further, the obtained isolate was subjected to whole genome sequencing using Illumina technology. Phylogenomic analysis revealed that the present isolate was clustered among *S. Abortusequi* clade. Further, core genome MLST (cgMLST) analysis based on hierarchical clustering and single nucleotide polymorphism (SNP) core-genome dendrogram of the present isolate against ten S. Abortusequi isolates revealed that our isolate established a distinct clade compared to all previously reported isolates. Comparison of cgMLST and SNP analyses revealed same clustering concordance between isolates. In addition, comparative genomics and phylogenetic analysis was carried out with six *S. Abortusequi* serovars for comprehensive genomic characterization of the outbreak-related isolates. In this study, isolation, whole genome sequencing and comparative genomics of *S. Abortusequi* from donkeys in India were carried out for the first time and this would be the first report on comparative genomics of *S. Abortusequi*, worldwide. This core genome information and analysis will aid in the development of diagnostics, target drug and vaccine prediction for the effective surveillance and disease control.

YS_2: First complete genome sequence of Duck Hepatitis A Virus genotype 2 from India

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Duck virus hepatitis (DAH) is economically important viral disease which caused by three viruses, DHAV- 1, DHAV-2 and DHAV-3. DHAV is a positive sense single stranded RNA virus which belongs to the *Picornaviridae* family. Here, we are reporting the whole genome sequence of DHAV-2 genome for the first time in India and interestingly the third genome sequence globally. The phylogenetics of the whole genome and viral capsid (VP1) gene sequences revealed that the sequence belonged to DHAV-2. This

sequence showed 79% identity with DHAV-1, 93% with DHAV-2 and 77% with DHAV-3. The DHAV-2 genome is 7769 bp long coding for nascent polyprotein which gets cleaved into a leader protein (L), and three structural (VP0-VP1-VP3) and nine non-structural proteins (2A1, 2A2, 2A3, 2B, 2C, 3A, 3B, 3C and 3D). A homology of 93.4% was identified with previously reported DHAV-2 VP1 sequences from Taiwan and significant mutations were noticed within the hypervariable regions of VP1 gene. We identified highly conserved central ATP binding domain in the 2C protein. The protein structural investigations were carried out for 2A2 and 3Dpol proteins by homology modelling which was further validated by molecular dynamics and simulations. The nuclear localization signal in 3Dpol protein and the GTPase binding sites in the 2A2 protein were found to be conserved as in DHAV-1.

YS_3: Harnessing canine stromal vascular fractions: Unraveling immunomodulation in coculture with PBMCs for enhanced tissue repair and regeneration

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Stromal vascular fractions (SVFs) have translational significance in regenerative medicine in humans and companion animals. SVFs are derived from adipose tissue, are a rich source of mesenchymal stem cells (MSCs), and are available for immediate administration, expansion of MSCs, multilineage differentiation, and other in vitro studies. This study provides information on the proliferation kinetics of canine SVFs and demonstrates the immunomodulatory potential of SVFs in lymphoma. Furthermore, this study demonstrates the immunomodulatory properties of SVFs in canine peripheral blood mononuclear cells (PBMCs) using a coculture system. SVFs were isolated from periovarian adipose tissue of healthy female dogs (n = 3) undergoing cesarean section with owner consent, while PBMCs were collected from healthy (tumor-free) and lymphoma affected dogs (n = 5 each). After flowcytometry, the presence of MSCs was evaluated by gene expression studies (OCT4, CD44, CD90, and CD105) and expansion in culture. Furthermore, SVFs were co-cultured with PBMCs and assessed for divisional kinetics, cell proliferation (Ki67 expression), cell viability (MTT assay), and immunomodulatory properties (TNFA and PTGS1 cytokine expression). Canine SVFs contain MSCs, evidenced by the expression of stem cell surface markers, namely, D105+/ CD90+/ CD44+ and pluripotency transcription factor, OCT4. Coculturing SVFs and PBMCs increased the population doubling time (PDT) of proliferating PBMCs in both the healthy and lymphoma groups and decreased (3.5-fold downregulation of Ki67) the proliferation of PBMCs in lymphoma in a ratio-dependent manner. Immunomodulatory properties of SVFs were evident by the down-regulation (44x; p-value = 0.0003) of the inflammatory cytokine tissue necrosis factor-alpha (TNFA) and up-regulation (88x; p-value = 0.008) of the anti-inflammatory cytokine cyclooxygenase 1 or prostaglandin endoperoxide synthase 1 (PTGS1). In conclusion, SVFs showed immunomodulatory effects on the immune cells of dogs with lymphoma, complementing their potential for therapeutic applications.

ISVIB mid-career Scientist Award

MCS-1: Molecular screening, isolation, and complete genome analysis of Porcine Parvovirus-1 in domestic pig, Tamil Nadu

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In the current era of global swine populations, porcine parvovirus (PPV) mediated reproductive failure poses a serious threat to swine communities all around the world, which calls for a comprehensive genomic study to understand its molecular epidemiology and identify potential vaccine candidates. In India, PPV infection was first reported in Uttar Pradesh in 2010, followed by Kerala, Punjab, North Eastern states and Tamil Nadu. As of now, there are no complete genome sequences available in GenBank for PPV1 strains from India. A total of 100 field samples including post-mortem tissues (n=80) and serum (n=20) were collected from both suspect and diseased pigs in Tamil Nadu for this study. PCR assays targeting NS1 and VP2 genes were used to screen samples for PPV. Molecular detection revealed 13% positivity (n=13) for PPV with 11 in tissues and 2 in serum. Two PPV positive samples were subjected to three blind passages in PK15 cells and the infected cells were monitored daily for any cytopathic change with non-infected monolayers as control. The PPV genome was confirmed by PCR assay on both the cell culture lysates. One PPV isolate (L17) from a stillborn fetus in Chennai, Tamil Nadu subjected for whole genome sequencing through I*llumina next-generation sequencing* platform by outsourcing. Raw sequence data were trimmed, assembled and annotated in Galaxy online tool along with reference PPV and PPV full genome contig sequence obtained. The whole genome of the PPV strain isolated in this study has 5,075 bp in length, with a G+C content of 37.72%, Coding region ORF1 is located at the 5′ end codes for NS1 protein (1,989 bp, 662 aa), NS2 (486 bp, 161 aa), and NS3 (324 bp, 107 aa). ORF2 is located at the 3′ end encodes the structural proteins VP1 (2,190 bp, 729 aa), VP2 (1,740 bp, 579 aa), and a late non-structural protein, SAT (207 bp, 68 aa). PPV full genome in this study showed 98.26% to 99.19% nucleotide sequence identity to published PPV strains, 99.09% with the reference PPV strain NADL-2 strain (NC_001718) and 99% with the virulent PPV strain Kresse strain. Phylogenetic and evolutionary analysis in MEGA-X revealed PPV isolate (L17) in this study clustering along with European PPV1 isolates by Maximum Likelihood method whereas, in Neighbor-Joining method it clustered along with Chinese PPV1 isolates evidencing its possible origin and its spread. The VP2 region is the main protective antigen, which contains primary neutralizing epitopes and can trigger neutralizing antibodies. Deduced AA analysis of VP2 protein core site 89 to 97 (ESGVAGQMV) involved in antibody recognition evidenced completely conserved. Further analysis of three core epitopes of PPV1 virion and VP2 monomer namely 228QQITDS233 exposed on the virion surface and other two epitopes 284RSLGLPPK291, and 344FEYSNGGPFLTPI356 located inside virion are highly conserved. Few AA variations of VP2 at 45th(T→ S), 59th(L→ M), 215(I→ T), 378(D→ G), 383(H→Q), 414(A→S), 436(S→ T) and 565(R→ K) positions needs to be analysed further for its impact on immune function. Deduced AA sequences of NS1 gene are highly conserved except in three locations 59(N→ S), 195(K→ R) and 562(K→ R) when compared to reference NADL-2 strain. The complete genome analysis of the PPV1 isolate (L17-PPV1-ABT2024) from domestic pig in Tamil Nadu provides crucial insights into the genetic characteristics and evolution of PPV1 in the region.

MCS-2: Development of classical swine fever bait vaccine and evaluation of its immunogenic potential in pigs

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Classical swine fever (CSF), also known as hog cholera is a highly contagious and economically devastating viral disease of domestic, pigmy hog, and wild swine. Systematic implementation of conventional parenteral immunization for CSF control in backyard settings is a challenge. Therefore, suitable bait for the oral delivery of the CSF virus (CSFV) vaccine considering the pig's bait flavor preferences (sweet corn/anise oil/dry shrimp fish) was designed as an alternative vaccine delivery method. Simultaneously, the stability of live attenuated cell culture adapted CSFV C-strain in liquid and freeze-dried forms was evaluated using different combinations of stabilizers at 4°C, 25°C, and 37°C for use as an oral CSFV vaccine. Dry shrimp fish (DSF) bait was found to be the most preferred and accepted bait by pigs of all age groups. Baits (hemisphere shape, 4.5 cm diameter and 1.5 cm thickness) were prepared using a cereal-based matrix composed of piglet feed, sacarose, paraffin, wheat flour, peanut butter, turmeric oil extract, sodium chloride and DSF attractant with gelatin capsules dipped into the matrix to introduce vaccine formulation. The physical stability test demonstrated that baits were stable for three days at 37^0 C. However, moist environments affected stability after 48 hrs. Hence, the designed baits are stable and suitable for the oral delivery of vaccine formulations in field conditions. A novel formulation containing 10% (w/v) trehalose, 2.5% (w/v) gelatine, and 10% (w/v) pullulan (TGP) was found to be the most efficient stabilizer formulation based on lyophilization loss and thermal stabilization of CSFV in both liquid and freeze-dried form. Notably, TGP freeze-dried CSFV C-strain maintained stability for up to 3 months at 4^0C, without any loss of virus titer with an estimated half-life of 18.54 months.The oral CSFV vaccine induced limited immune response at lower virus concentrations ($log_{10}3.0$); however, a double oral dose with booster vaccination triggered a protective antibody titer up to 180 days post-primary immunization. Vaccination of sows with CSFV C-strain oral vaccine during pregnancy elicited CSFV-specific neutralizing antibodies. The persistence of maternally derived antibody titer in piglets is directly proportional to the corresponding antibody titer of dams at farrowing. The results of this study will aid in establishing an effective oral immunization regimen as a substitute of parenteral immunization for CSF control in backyard settings.

MCS-3: Selection of candidate Orf Vaccine strains for Sheep and Goats

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Sheep and goats rearing is one of the paramount economic activities of low income people particularly women in Tamil Nadu. Orf is a viral disease that affects sheep and goats and considered as one of the top 20 most important viral diseases of sheep and goats in terms of impact on the poor. However, there is no commercial vaccine available in our country till date to control the disease. The objective of the current study was to characterize the Orf virus isolates from Tamil Nadu and develop a live attenuated and inactivated Orf vaccine. Scab samples were collected from clinically infected goats at different districts of Tamil Nadu. Orf was diagnosed by polymerase chain reaction using Orf specific GIF/IL-2 gene. The positive samples showed a specific amplicon of GIF/IL-2 gene (408bp). Further, partial major envelope protein gene (B2L), late transcription factor gene (VLTF1), viral interferon resistance protein gene (VIR) and GM-CSF and inhibitory factor gene (GIF/IL-2) was amplified, sequenced and phylogenetic tree constructed using the

neighbour-joining (NJ) tree. The results of the phylogenetic analysis revealed that Orf isolates from Tamil Nadu belonged to the same cluster. Further, Orf viruses were isolated and adopted using VERO (ORF/Thiruvallur/TN/2021) and MDBK (ORF/Madurai/TN/2020) cells, respectively. The experimental live attenuated vaccine was prepared by continuously passaging the ORF/Thiruvallur/TN/2021 vaccine strain in VERO cells. Similarly, the experimental inactivated orf vaccine was prepared using binary ethylenimine inactivated ORF/Madurai/TN/2020 vaccine strain and blended with water-in-oil adjuvant. Both the experimental live attenuated and inactivated vaccine safety and immunogenicity studies will be carried out in sheep and goats. The developed orf vaccines can help to control the disease in sheep and goats.

MCS-4: Non-Tuberculous Mycobacteria in Captive Elephants: Unveiling the Antibiotic Resistance Profile and Implications for Public Health

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The genus *Mycobacterium* includes more than 200 species including the *Mycobacterium tuberculosis* complex as well as non-tuberculous mycobacteria. Non-tuberculous mycobacteria (NTM) are environmental opportunistic organisms with about 190 species and subspecies and can cause disease in humans of all ages. Pulmonary disease (PD) is the most common clinical presentation of NTM infection accounting for 80 to 90% of all NTM-associated diseases in humans. In the present study, 96 captive elephant trunk wash samples were screened for Mycobacterium species using the gold standard 'isolation in culture' method. Fifteen of these samples were found to have acid fast bacilli. Molecular characterizations revealed them to be non-tuberculous mycobacteria and were not belonging to MTBC. The fifteen cultures were represented by five different species of non-tuberculous mycobacteria. These non-tuberculous mycobacteria showed varying patterns of antibiotic resistance especially to the first- and second-line antibiotics (Kanamycin, Amikacin, Rifampicin, Isoniazid and Levofloxacin) used for treatment of tuberculosis. Whole genome sequencing of non-tuberculous mycobacteria isolated from captive elephants revealed several antibiotic resistance genes. This study is significant considering the global emergence of broad-spectrum antibiotic-resistant NTM. The prevalence of such resistant organisms in captive elephant species poses a greater risk considering the unique nature of this human animal interface and therefore warrants further investigation

GADVASU WOMEN SCIENTIST AWARD

WS-1: Comparative pathology, Molecular pathogenesis and phylogenetic analysis of Pathotype of Infectious Bronchitis Virus (IBV) outbreaks among Poultry flocks in Tamil Nadu during 2019 to 2021

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Infectious Bronchitis (IB) is an acute, highly contagious and economically important viral disease of commercial meat and egg type birds caused by infectious bronchitis virus (IBV).Twenty four outbreaks of Infectious Bronchitis were investigated among the poultry flocks in Tamil Nadu during November, 2019 to December, 2021.The affected birds were presented with tracheal rales, nasal discharge, wet droppings, periorbital cellulitis, produced poor quality eggs like misshapen eggs, soft to rough shelled eggs, watery albumin with significant reduction in egg production in 31-40 weeks. The highest morbidity and mortality rate were 37.35%and 21.44%, respectively. Pathological changes revealed catarrhal laryngotracheitis, bronchopneumonia in lungs, urate nephropathy, haemorrhagic oophoritis and salphingitis. Immunohistochemical localization of IBV antigen was observed as cytoplasmic expression in the epithelial cells of nasal cavity, lungs, kidneys, oviduct and lymphoid organs. The ultrastructural changes in oviduct showed collapsed cells containing numerous small round virions in the cytosol, distortion and indentation of nuclear membrane, numerous distended mitochondria, dilated and hyperplastic RER. The degenerated tubular epithelial cells of kidneys showed distorted RER, abundant round virion particles and vesiculated mitochondria. Virus isolation and identification studies in SPF embryonated eggs showed reduced motility, death of all embryos, severe haemorrhage, dwarfing and curling of embryos, clubbing of down feathers, urate deposits in the mesonephrons in second and third passages. RT-PCR, Genome sequencing and phylogenetic analysis of S1gene-Spike protein (393bp) of VPP/MVC/IB/S1gene/001 isolate of IBV virulent strains revealed 99.00% genetic homology with the nephropathogenic IBV variants of UK 4/91 strain, UK B1648 and Holte variants from different countries. The other two study isolates VPP/MVC/IB/S1gene/002 and VPP/MVC/IB/S1gene/003 of IBV strains had 98.00% genetic homology with the pathogenic QX like IBV variants from India and other countries targeting reproductive system. Molecular characterization by evolutionary generation of IBV genetic lineages showed that the IBV variants circulating in the region in the present study belonged to generation GI-13 and GI-14 IBV lineages related to UK 4/91 nephropathogenic genotype. Diagnosis of IBV pathotypes in the present study by pathological, immunohistochemical, electron microscopic studies, virus isolation, RT-PCR sequencing and molecular characterization inferred that different forms of genotypes and pathotypes of IBV variants were under circulation among the chicken flocks in Tamil Nadu and these variants confirms their affinity and tropism more towards the reproductive and renal system of poultry.These findings indicate the need for continuous surveillance of IBV pathotypes and provide useful information for devising diagnostic and vaccination strategies for effective control of IB in poultry including native chickens.

WS-2: *XIST* - An alternative gene marker for sexing of bovine embryos

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Embryo sexing involves determining the gender of the growing embryo before the actual characteristics of the gender is expressed phenotypically. Although a variety of techniques are available, molecular methods utilizing the sex determining markers like *SRY* are commonly employed. The present study is undertaken to check the suitability of an alternative gene namely *XIST* for gender determination in pre implantation cattle embryos. Bovine embryos were produced *in vitro* from oocytes derived by ovum pick-up and fertilized by frozen thawed sexed and conventional semen to obtain embryos of both the sexes. Sex determination was carried out on whole embryos by amplification of *XIST* and *SRY* gene. It was found that the accuracy of sex determination was almost 95% in consensus between the *XIST* and *SRY* genes. Further, sex determination was attempted utilizing the embryo conditioned medium and it is suggested that *XIST* could be a potential alternative molecular marker in embryo sexing.

DR. LINGARD MEMORIAL AWARD FOR PRODUCT DEVELOPMENT

LMA 1: Smartphone Guided Portable Rabies Specimen Collection Kit

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Rabies is a fatal viral disease affecting the central nervous system of mammals, including humans and dogs. Accurate and timely diagnosis is crucial for controlling outbreaks. Traditionally, brain samples for rabies testing are collected from deceased dogs through opening the skull. These methods have several disadvantages: (i) Contamination Risk. (ii) Safety Concerns (iii) Procedure Time (iv) Need for Specialized Equipment. The rabies specimen collection kit is designed to enhance the process of collecting brain samples occipital foreman route from rabies infection susceptible dogs for rabies testing. It includes several key components: Medical-Grade Stainless Steel Rod with an internal diameter of 6mm and a length of 20cm, is the primary tool for boring into the skull to reach the brain tissue safely and precisely. A compatible drill attachment ensures controlled and accurate penetration through the skull, minimizing the risk of accidental damage. The internal camera Integrated within the rod, provides real-time visualization of the procedure. This ensures precise positioning and accurate sample collection, reducing the chances of error. The camera connects to a mobile device via an app, providing a live feed and guiding the user through the procedure. The app also records the procedure for documentation and further analysis. The rabies specimen collection kit addresses these challenges by offering a safer, more efficient, and user-friendly method for sample collection. The rabies specimen collection kit represents a significant advancement in veterinary diagnostic practices. By enhancing precision, reducing contamination risks, improving safety for veterinarians, and simplifying the procedure, this kit offers a reliable, efficient, and user-friendly method for rabies testing. It addresses the limitations of traditional methods and provides a comprehensive solution for accurate and timely rabies diagnosis.

LMA_2: SaloVac: A Recombinant Lactobacillus-Based Vaccine for Effective Salmonella Control in Poultry

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Salmonella infections in poultry represent a critical challenge to both public health and the poultry industry, contributing to significant economic losses and food safety concerns. This study introduces SaloVac, a novel recombinant Lactobacillus-based vaccine designed to combat Salmonella in poultry. SaloVac utilizes Lactobacillus plantarum NC8 engineered to express key Salmonella antigens—PagN, SopE2, and FliC—to enhance immune protection across multiple serovars. In a series of efficacy trials involving broiler and Keystone chicks, the vaccine demonstrated impressive protective efficacy. Vaccinated birds exhibited robust cellular and humoral immune responses, with significant increases in interferon-gamma (IFN-γ) and CD8+ T cell proliferation. Following challenge with *Salmonella gallinarum, Salmonella enteritidis*, and *Salmonella typhimurium*, SaloVac conferred up to 96% survival rates compared to lower survival rates in control groups. Notably, the vaccine led to the clearance of Salmonella from critical tissues, including the liver, and prevented contamination of eggs, underscoring its potential to enhance food safety. These results highlight SaloVac's capability to offer broad-spectrum protection against diverse Salmonella serovars, addressing the limitations of existing vaccines and providing a promising solution for effective Salmonella control in poultry. Future studies will focus on evaluating long-term efficacy, safety across different environments, and potential applications for other animal species and human vaccines.

Burnett Memorial award for Team Research

BMA -1: Thermo-adapted Newcastle disease virus isolate from Muscovy duck (AAUPSD44C) – a prospective thermostable vaccine

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Newcastle disease (ND) is one of the most significant viral diseases affecting poultry globally in terms of economic impact. The only affordable, efficient, and safe method of preventing ND epidemics is immunization and biosecurity. Conventional live vaccines, which have been used for decades, require a cold chain (2-8°C) from production to administration to remain effective. Once the cold chain breaks, the potency of the product is lost by more than 50% or even lost completely in a few hours at ambient temperature. Besides, up to 80% of the cost of vaccination programs is consumed by the cold chain infrastructure. Keeping the live vaccines refrigerated and/or maintaining the cold chain at remote locations is challenging. Therefore, there has been a need for a more stable local vaccine candidate in the country for a strategic ND control programme. We created an NDV library recovered from domestic, wild, and captive birds. Following biological and molecular characterization, the thermostability profiling of the NDV isolates was performed. Finally, based on immunogenicity, a lentogenic NDV isolate from Muscovy duck (AAU/2015/44/PSDC) was selected for thermo-adaptation. Further, the thermostability of the virus was enhanced using a novel stabilizer composition. Our results showed that the replication-competent thermo-adapted NDV was able to retain its hemagglutination activity and infectivity for up to 18 hours at 56°C. There was no significant difference ($P<0.01$) between the immune response of the thermo-adapted NDV and the conventional Lasota vaccine. In conclusion, the thermo-adapted NDV AAU/2015/44/PSDC is expected to aid in the ND control program especially in the remote areas where cold-chain maintenance is a major concern. This study led to the "Thermostable Newcastle Disease Virus Vaccine AAUPSD44C" developed by Assam Agricultural University, which has already been technology transferred to Ventri Biologicals, Venkateshwara Hatcheries Pvt. Ltd., Pune, Maharashtra.

KEY NOTE ADDRESS

Transforming Livestock Disease Epidemiology in India: Innovations and Strategic Initiatives

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Epidemiology of livestock diseases is important for management of the vast, diversified livestock population of India. This country has more than 536 million cattle, buffalo, sheep, goats, pigs, and poultry. Not only is it a vital sector of the agricultural GDP, but it also provides a source of income generation for sustaining economic security and food safety to the very large section of subsistence farmers. There are many diseases through which livestock have to suffer in the world, but the major diseases that pose threats are Peste des Petits Ruminants (PPR), Lumpy Skin Disease (LSD), African Swine Fever (ASF), Brucellosis, Foot and Mouth Disease (FMD), Avian Influenza, and Bluetongue, in addition to several others, which actually impact the productivity of the livestock and public health hazard.

Livestock disease epidemiology is critical for managing the health of India's vast and diverse livestock population, which exceeds 536 million animals including cattle, buffaloes, sheep, goats, pigs, and poultry. This sector is not only pivotal to the nation's agricultural GDP but also essential for ensuring economic stability and food security for subsistence farmers. Livestock are susceptible to numerous diseases, with key threats including Peste des Petits Ruminants (PPR), Lumpy Skin Disease (LSD), African Swine Fever (ASF), Brucellosis, Foot-and-Mouth Disease (FMD), Avian Influenza, and Blue Tongue, all of which significantly impact livestock productivity and public health.

Since 2019, LSD has spread across 29 states, resulting in over 5 million cases and more than 150,000 cattle deaths. The ASF outbreak beginning in 2020 has led to substantial losses in the pig sector, forcing the closure of numerous farms. Concurrently, FMD continues to impair livestock productivity and international trade. The expansion of Avian Influenza into new regions and during atypical seasons such as summer highlights the evolving dynamics of disease transmission, influenced by factors like climate change and migratory bird patterns.

In response to these challenges, extensive vaccination campaigns under the National Animal Disease Control Programme (NADCP) have been instrumental in reducing the incidence of diseases like FMD. The predominant FMD serotypes—O, A, and Asia 1—have experienced a dramatic decline in cases. Prior reports indicated approximately 25,000 annual cases, but post-vaccination data shows a reduction of over 60%, even with sporadic outbreaks due to incomplete vaccine coverage. Notably, the Asia 1 serotype, once widespread, is now confined to very few cases in 1-2 states. This progress highlights the need for ongoing vaccination efforts and vigilant monitoring to ensure vaccines match circulating serotypes, thus optimizing FMD control.

Further initiatives under the National Livestock Mission (NDLM), spearheaded by the Department of Animal Husbandry and Dairying (DAHD), aim to digitalize animal production, breeding, and health systems. Specifically, ICAR-NIVEDI is developing a national framework for real-time disease reporting and analysis. This includes the establishment of the National Animal Disease Information Control Centre (NADICC) at NIVEDI, which will utilize a cloud-computing based system to capture and analyze livestock disease information from across India, providing detailed analytics up to the block and epidemiological unit level.

Despite these advancements, the field faces significant challenges. The emergence and spread of transboundary animal diseases, inadequate infrastructure for border surveillance, and fragmented data management systems continue to impede progress. Early detection and reporting mechanisms, crucial for effective disease surveillance and control, are often compromised by outdated methods and insufficient training of veterinary personnel. Moreover, pathogens are constantly evolving, with strains developing resistance to vaccines. This necessitates ongoing research into host-pathogen interactions and the pathogenesis of diseases in both natural and unnatural hosts. Additionally, the rise of antimicrobial resistance (AMR) pathogens poses a real threat. These pathogens can be introduced through various ports, highlighting the urgent need to develop strategies to prevent their entry and spread within the country.

Looking ahead, the integration of advanced technologies such as artificial intelligence (AI), machine learning (ML), geographic information systems (GIS), remote sensing, and genomic surveillance is set to revolutionize disease management. These tools will greatly enhance the ability to predict outbreaks, understand pathogen evolution, and aid in the development of effective vaccines. Innovations in surveillance that incorporate data analytics and machine learning will refine the prediction of outbreak patterns and facilitate the identification of potential hotspots, while genomic surveillance will provide deeper insights into the genetic diversity of pathogens, supporting the creation of targeted vaccines and therapeutics.

Beyond the existing strategies of vaccination, surveillance, and monitoring, there is a pressing need to embrace advanced methods to address endemic, emerging, and transboundary diseases effectively. Novel statistical and mathematical models are essential for understanding the transmission dynamics of livestock diseases. The application of GIS and remote sensing can be particularly valuable in understanding and quantifying the risk associated with livestock diseases. Furthermore, the adoption of newer technologies such as thermal sensors, camera traps, and drones will prove instrumental in the enhanced monitoring and surveillance of transboundary diseases in identified hotspots. These combined efforts are crucial for managing disease threats more effectively and safeguarding the health of livestock nationally.

Continued investment in these technologies and their integration into national disease surveillance programs are crucial. Strengthening infrastructure, enhancing the skill sets of veterinary personnel, and improving regulatory frameworks are essential for advancing livestock disease epidemiology in India. This comprehensive approach promises not only to mitigate the impacts of current and future outbreaks but also to ensure the economic stability and health security of India's vital agricultural sector.

In conclusion, while significant challenges remain, the strategic use of modern technologies and robust management strategies are pivotal in safeguarding India's livestock resources. Proactive and integrated efforts at national and regional levels are essential for moving towards a more secure and prosperous agricultural future.

TECHNICAL SESSION - I

HOST - MICROBES INTERACTIONS

Lead paper -1

Immune Evasion by the Pathogens

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The immune system is a highly sophisticated network consisting of primary and secondary lymphoid organs, diverse immune cells (including innate immune cells like dendritic cells, monocytes, macrophages, granulocytes, and NK cells; and adaptive immune cells such as T and B lymphocytes) and a large range of molecules (such as complement proteins, antibodies cytokines, and chemokines) aimed at detecting and eliminating the pathogens such as bacteria, viruses, fungi, and parasites. Despite its robustness, plasticity and diversity, the immune system faces constant challenges from ever evolving pathogens that tend develop mechanisms to evade recognition and elimination. This dynamic interplay between immune system and pathogen evasion strategies has profound implications for health and disease.

Mechanisms of Immune Evasion

Pathogens have evolved a range of strategies to evade the protective immune responses, reflecting their diverse biological contexts and life cycles. These strategies can be categorized broadly into several categories: hiding from immune surveillance, molecular mimicry, antigenic variation, immune suppression, and concealment.

1. **Hiding from immune surveillance:** Pathogens can mask their identity so that they are not detected by the immune system. By using *Aspergillus fumigatus,* a ubiquitous airborne opportunistic fungal species as a model, we show that filamentous fungi use this strategy as one of the immune evasion mechanisms (1, 3). The air we breathe contains a large number of microorganisms, including a multitude of fungal spores or conidia, which can number in the several thousand per cubic meter in certain environments like compost piles. These conidia come from various fungal species, predominantly from the genera *Aspergillus, Cladosporium*, *Penicillium* and *Alternaria*. Despite containing numerous antigens and allergens, airborne fungal conidia do not continuously activate the host's innate immune cells nor induce chronic inflammatory responses upon inhalation. Our research indicates that the surface rodlet layer of dormant conidia made by hydrophobin plays a crucial role in masking their recognition by the immune system, thus preventing a harmful immune response. Removal of this rodlet layer by chemical, genetic or biological methods, rendered the resulting morphotypes immunostimulatory, thus confirming the essential role of the rodlet layer for the fungal survival in vivo.

2. **Molecular Mimicry:** Pathogens often produce molecules that closely resemble host cell components, a strategy known as molecular mimicry. By mimicking host antigens, these pathogens can avoid detection by immune cells. For instance, some bacteria and viruses display surface proteins that are structurally similar to host molecules, thus evading recognition by antibodies and T cells. This will also explain in part on the emergence of autoimmune diseases following a bout of infection.

3. **Antigenic Variation:** Another common evasion strategy is antigenic variation, where pathogens frequently change their surface antigens to avoid immune surveillance. This is particularly evident in viruses such as influenza, Corona and HIV. Influenza viruses undergo frequent genetic changes through antigenic drift and shift, resulting in new viral strains that are not recognized by pre-existing antibodies. Similar strategy was also deployed by SARS-CoV-2 (11-13). We and others have reported that the plasma (or immunoglobulins) from COVID-19 patients infected with the ancestral variant of

SARS-CoV-2 exhibit strong neutralizing activity against the ancestral virus and the Alpha, Beta, and Delta variants of SARS-CoV-2, whereas the Gamma and Omicron viruses were less sensitive to sero-neutralization. Infectious bronchitis virus (IBV) is another good example of virus that exhibits high antigenic variation (6).

4. **Immune Suppression:** Some pathogens actively suppress the protective immune response by stimulating the expansion of $CD4^+CD25^+$ regulatory T cells (2, 5, 22) and by exploiting the PD-L1-PD-1 pathway to facilitate their survival. We show that *Aspergillus fumigatus,* could exploit this pathway through its pathogen-associated molecular patterns like α-(1,3)-glucan to selectively mediate regulatory T cell responses (18, 21). Although regulatory T cells prevent infection-associated inflammation and tissue damage, they also suppress protective immune response to pathogens and enhance their persistence. Mechanistically, we found that *Aspergillus fumigatus* induces regulatory T cell expansion via the C-type lectin receptor-dependent, Wnt-β-Catenin signaling pathway-dependent induction of immune checkpoint molecule PD-L1 (CD274) on dendritic cells. Blockade of Wnt signaling in dendritic cells abolished the *Aspergillus fumigatus*-induced expression of PD-L1. Also, blockade of either PD-L1 or Wnt signaling in dendritic cells abrogated the *Aspergillus fumigatus* induced regulatory T cell responses and reciprocally enhanced protective Th1 responses.

   Further, we found that other pathogens like *Mycobacterium tuberculosis*, Japanese encephalomyelitis virus, filaria also exploit PD-L1 pathway on dendritic cells to induce expansion of regulatory T cells and to suppress protective immune responses (15, 16, 19, 23).

5. **Concealment and Intracellular Survival:** Many pathogens avoid immune detection by residing within host cells, where they are shielded from extracellular immune responses. Viruses like herpes simplex virus (HSV) can establish latent infections within host cells, evading immune surveillance and persisting in a dormant state. Furthermore, some pathogens have developed mechanisms to modulate host cell processes to their advantage. For instance, *Plasmodium falciparum*, the parasite responsible for malaria, alters the surface properties of infected red blood cells, preventing their recognition and destruction by the immune system.

   Other mechanisms of immune evasion by the pathogens include inhibition of the complement pathway, inhibition of cytokines/interferons/chemokines, and their signaling, blockade of acquired immunity, modulation of autophagy and apoptosis processes (17), interference with toll-like receptors and suppression of intrinsic cellular pathways, Inhibition of antigen processing & presentation by interfering with MHC expression and peptide transportation.

Implications of Immune Evasion for Disease Progression and Treatment

The ability of pathogens to evade immune responses has significant implications for disease progression and treatment strategies. Immune evasion can lead to chronic infections, increased virulence, and make therapeutic and vaccine development challenging.

Moreover, the suppression of immune responses by pathogens like HIV and *Mycobacterium tuberculosis* not only affects the host's ability to fight the primary infection but also increases susceptibility to secondary infections and complications. This underscores the importance of developing therapeutic strategies that not only target the pathogen directly but also support and enhance the host's immune responses.

Counteracting Immune Evasion Strategies

Understanding the mechanisms of immune evasion has led to the development of various countermeasures. Vaccines are designed to stimulate immune responses that can overcome antigenic variation and provide protection against multiple pathogen strains. For example, the development of

multi-valent vaccines and those targeting conserved viral regions aims to address the challenges posed by antigenic variation in influenza and other pathogens.

In addition, research into immune modulators and therapies that enhance immune function or target specific evasion strategies is ongoing. For instance, checkpoint inhibitors that block immune suppression pathways have shown promise in treating cancers and chronic infections. Furthermore, we have developed an innovative strategy of targeting regulatory T cells to boost protective immune responses to infection and vaccines by transient inhibition of regulatory T cell migration without depleting them and interfering with immunoregulatory functions (4, 7-9, 14, 20). Thus, by combining bioinformatics, functional assays, in vitro and in vivo models, we have identified small molecule antagonists to chemokine receptor CCR4, expressed by the regulatory T cells. By inhibiting the migration of regulatory T cells in response to the chemokines CCL17 and CCL22 produced by activated innate immune cells like dendritic cells, CCR4 antagonists could circumvent immunosuppressive effect of regulatory T cells on innate cells at the time of antigen or vaccine exposure and could enhance intensity and duration of protective immune responses. In fact, CCR4 antagonists functioned as 'molecular adjuvants' in vivo in experimental models and amplified cellular and humoral immune responses when injected in combination with antigens (without any other adjuvant molecules) including Mycobacterium, hepatitis B virus, Aspergillus or *Plasmodium yoelii* (4, 7-10, 14, 20). The significant adjuvant activity observed in diverse models without noticeable side effects provided strong evidence that CCR4 is a sustainable adjuvant target for prophylaxis and therapeutic vaccines.

Conclusion

Immune evasion by pathogens is a complex and multifaceted phenomenon that highlights the never-ending fight between pathogens and the immune system. By employing strategies such as molecular mimicry, antigenic variation, immune suppression, and intracellular survival, pathogens can evade immune detection and establish persistent infections. Understanding these mechanisms is crucial for developing effective treatments and vaccines to combat infectious diseases. As research progresses, novel approaches to counteract immune evasion and bolster host defenses hold promise for improving health and managing infectious diseases in the future.

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Lead paper -2

Computational strategies for understanding and combating African Swine Fever

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Introduction

African swine fever (ASF) is a deadly disease caused by African swine fever virus (ASFV) in pigs. After its first report from Kenya in year 1921, ASFV spread through-out Africa, Asia and Europe, and most recently it reached to America. For the effective diagnostic and vaccine assay is needed to curb the various divergent isolates of the ASFV. Due to lack of efficient treatment and highly infectious nature of the ASFV, it is the general policy of the most of the governments to culling the infected pigs and other pigs which are there in a certain vicinity of the confirmed case. This causes huge economic loss to farmers. Therefore it is important to develop effective and universal diagnostic assay for most of the isolates as the wrong diagnosis will still lead to huge economic loss to the whole area of the false positive case. Therefore, whole genome based computational study of the various isolates is also important, which will need the whole genome sequencing, assembly, and further whole genome-based analyses. Further, whole genome based or certain specific gene/protein based genetic variability, diversity, and characterization, can be done *in silico*, which would include phylogenetic, selection pressure analyses etc. Further, immunoinformatic analyses could be performed to detect highly immunogenic proteins to finally develop vaccine candidate, which could be based on multi-epitope to target the most of the divergent isolates of ASFV. This write-up will provide a brief of various computational strategies to understand and combat the African swine fever in pigs.

Whole genome sequencing, assembly and annotation

Small genome sequencing enables whole genome study of ASFV genome. NGS techniques for small whole genome sequencing are faster and cost effective, such as shotgun sequencing, Illumina sequencing, Ion Torrent sequencing, 10X Genomics, and pyrosequencing etc. First raw read libraries are obtained from the whole genome sequencing performed on extracted DNA of ASFV. First, raw reads are pass through quality check and adapter removal to remove the reads with length less than 20 and Phred score less than 20. Tools such as seqtk (https://github.com/lh3/seqtk), FastQC (Andrews, 2010), FaQCs (Lo *et al*., 2014), FASTX-toolkit (http://hannonlab.cshl.edu/fastx_toolkit), and FastProNGS (Liu *et al*., 2019) etc. are there for quality check. While, adapter removal can be done by tools such as FASTX (http://hannonlab.cshl.edu/fastx_toolkit), CutAdapt (Martin, 2011), Adapter-removal (Lindgreen, 2012), AlienTrimmer (Criscuolo and Brisse, 2013), Adapter-removal v2 (Schubert *et al*., 2016), and TrimGalore (Krueger *et al*., 2021) etc. Filtered reads are assembled using de novo assemblers such as ABySS (Simpson *et al*., 2009), SOAPdenovo (Li *et al*., 2009), SPAdes (Bankevich *et al*., 2012), and Clover (Hsieh *et al*., 2020) etc. After assessment of the assembly (using assembly-stats (https://github.com/rjchallis/assembly-stats), QUAST (Gurevich *et al*., 2013), SQUAT (Yang *et al*., 2019), and GenomeQC (Manchanda *et al*., 2020) etc.), whole genome based analyses could be performed for the investigation of genome wide/novel genes (using INFERNAL (Nawrocki and Eddy, 2013), tRNAscan-SE (Chan *et al*., 2019), Augustus (Stanke *et al*., 2005), GlimmerHMM (Majoros *et al*., 2004) etc.), variants, other genetic changes, and biomarkers (using Samtools (Li, 2011), GATK (McKenna *et al*., 2010), RepeatMasker (https://www.repeatmasker.org/cgi-bin/WEBRepeatMasker), and RepeatModeler2 (Flynn *et al*., 2020) etc.). Currently, 324 whole/nearly whole genomes of world-wide ASFV isolates are available in GenBank. These 324 genomes were having ≥92.64%

sequence similarity with ≥91% coverage among themselves isolated from various continents and countries, such as Africa (Tanzania, South Africa, Namibia, Malawi, Kenya, Congo, Burundi, Benin, Zimbabwe, Zambia, Uganda, Nigeria, Ghana, Mauritius, and Mozambique), Asia (Singapore, Russia, Mongolia, Thailand, Hong Kong, South Korea, Japan, India, Serbia, Philippines, China, Vietnam, Indonesia, Timor-Leste, and Ukraine), Europe (Hungary, Portugal, Poland, Italy, Germany, Georgia, Czech Republic, Spain, Cameroon, Belgium, Estonia, Latvia, Lithuania, and France), and South America (Dominican Republic only).

Genomic variability, diversity and characterization

Whole genome based variability, diversity and characterization could be studied from computational analysis of the whole genome assembly sequences or some specific gene/proteins of various isolates of the ASFV by performing multiple sequence alignment (using MUSCLE (Edgar *et al*., 2004), Clustal omega (Sievers and Higgins, 2018) etc.), phylogenetic tree (using PHYLIP (Felsenstein, 1981), MEGA11 (Tamura *et al*., 2021) etc.) and selection pressure analyses (using Datamonkey (Weaver *et al*., 2018)). Singh *et al*. (2024) reported higher sequence similarity among various Asian isolates of ASFV based on the multiple sequence alignment of p72 and CD2v proteins. Njau *et al*. (2021) were also reported high sequence similarity (99.6%) among most of genotypes II of ASFV. Most of the phylogenetic studies are based on divergence among few genes or proteins of ASFV, however, few studies are based on the whole genome sequences of ASFV. Michaud *et al*. (2013) provided phylogenetic analysis of B646L, CP204L and E183L genes from 356, 251 and 123 ASFV isolates, respectively, and reported that currently circulating ASFV strains evolved over 300 years by molecular clock analyses. Imdhiyas *et al*. (2023) performed phylogenetic analysis of ASFV isolates based on p30 and p54 proteins. Similarly, Qu *et al*. (2022) performed phylogenetic analysis based on p72 gene sequences to analyze genotypes and serotypes of ASFV and Kim *et al*. (2023) on p72 gene sequences of 30 isolates of ASFV. de Villiers *et al*. (2010) studied 11 genome sequences of ASFV isolates from Spain and reported high diversity within limited set of genome sequences. Similarly, Mazur-Panasiuk *et al*. (2019) and Hakizimana *et al*. (2023) analyzed diversity among whole genome sequences of ASFV isolates from Poland and Tanzania, respectively. Aslanyan *et al*. (2020) done phylogenetic analysis on 40 genome sequences of ASFV isolates, and reported that *Asfarviridae* evolved relatively recently with close relatedness. Similarly, Shi *et al*. (2024) performed phylogenetic analysis of ASFV isolates based on 40 genome sequences. Bao *et al*. (2022) performed whole genome sequence diversity analysis on 123 isolates of ASFV. Zhang *et al*. (2023) also performed phylogenetic analysis on 39 genotype II ASFV genomes.

Detection of Immunogenicity

Further, assessment of immunogenicity of certain proteins could be done using computational strategies such as assessment of variability using the PVS server (Garcia-Boronat *et al*., 2008) in terms of Shannan entropy, assessment for solvent accessibility using SABLE (Adamczak *et al*., 2004), assessment of immunogenicity by VaxiJen 2.0 (Doytchinova and Flower, 2007). Singh *et al*., 2024 reported that p72 and CD2v proteins are mostly conserved as the Shannon score was less than 1.5 throughout the sequence and higher scores for few amino acids. Relative solvent accessibility was found to be higher in C-terminal domains in p72, CD2v, p30 and p54 proteins (Singh *et al*., 2024; Petrovan *et al*., 2019; Imdhiya *et al*., 2023). Both p72 and CD2v proteins are highly immunogenic, which is important to develop a diagnostic assay or in epitope-based vaccine designing.

In silico vaccine designing

In silico vaccine designing involves prediction of highly immunogenic T-cell and B-cell epitopes from a particular protein sequence, assessment of predicted epitopes, joining of best epitopes using linkers and adjuvants to prepare vaccine construct, evaluation (assessing antigenicity by VaxiJen (Doytchinova *et al*., 2007), non-allergen by AllerTOP (Dimitrov *et al*., 2014), no homology by BlastP (Boratyn *et al*., 2013),

overexpression in Escherichia coli by SCRATCH SolPro (Magnan *et al*., 2009), and stability by Expasy Protparam (Gasteiger *et al*., 2005)) and secondary structure prediction of vaccine construct (Modeller (Fiser and Sali, 2003), GOR (Garnier *et al*., 1996), GlobPlot (Linding *et al*., 2003), I-TASSER (Yang *et al*., 2015), ColabFold (Mirdita *et al*. 2022), AlphaFold (Jumper *et al*., 2021) etc., simulation of immune response (prediction of conformational epitopes by Discotope server (Kringelum *et al*., 2012), immune response by C-Immsim (Rapin *et al*., 2010)) by vaccine construct, molecular docking and MD-simulation (docking of vaccine construct with receptors using AutoDock, GalaxyPepDock (Lee *et al*., 2015), ClusPro (Kozakov *et al*., 2017)), codon optimization (by JCat (Grote *et al*., 2005), Codon Usage Analyzer (http://www.kazusa.or.jp/codon/) etc.), and finally, in silico cloning (by SnapGene tool (http://www.snapgene.com)).

For epitope prediction, residue-wise epitope probability is assessed using Ellipro (Ponomarenko *et al*., 2008), linear B-cell epitopes are predicted using Bepipred Linear Epitope Prediction 2.0 (Jespersen *et al*., 2017) and discontinuous B-cell epitopes are predicted using ElliPro, T-cell epitopes (peptides binding with MHC class I alleles of porcine and MHC class II alleles of human as not available from porcine (Hou *et al*., 2023)) are predicted using TepiTool (Paul *et al*., 2016). Further, predicted epitopes pass through various assessments such as antigenicity using Vaxijen (Doytchinova *et al*., 2007), non-allergen using AllerTOP (Dimitrov *et al*., 2014), non-toxic using ToxinPred (Gupta *et al*., 2013), immunogenicity using IEDB immunogenicity prediction (Calis *et al*., 2013 for Cytotoxic T-cell; Dhanda *et al*., 2018 for helper T-cell), inducibility of helper T-cell epitopes using IFNepitope (Dhanda *et al*., 2013a) for interferons, using IL4pred (Dhanda *et al*., 2013b) for interleukin 4, using IL10pred (Nagpal *et al*., 2017) for interleukin 10. As ASFV is an antigenically complex virus and it could not rule out the possible role of many proteins in immunogenicity. Recently, Simbulan *et al*. (2024) considered pan proteome for the extraction of epitopes of genotype I and II of ASFV and utilized epitopes for an *in silico* vaccine designing from minor capsid protein p49, major capsid protein p72, envelope protein p22, transmembrane protein, polyprotein p220, transmembrane, cysteine protease, MGF 360-13L, MGF 360-18R, guanylyltransferase, termination factor, helicase/primase, EP424R and MGF 360-11L. Singh *et al*. (2024) highlighted the possibility of B cell and T cell epitopes of p72 and CD2v to render immunoprotective function within genogroup and between genogroups of ASFV isolates from India and Asia.

Conclusions

This write-up provides a brief of various computational strategies to understand and combat the African swine fever in pigs at various levels. Due to importance to develop effective and universal diagnostic assay and vaccine for most of the isolates, whole genome based computational study of the various isolates is important, which needs the whole genome sequencing, assembly and further whole genome-based analyses. Further, whole genome based or certain specific gene/protein based genetic variability, diversity, and characterization, can be done *in silico*, which would include phylogenetic, selection pressure analyses etc. Further, immunoinformatic analyses could be performed to detect highly immunogenic proteins to finally develop vaccine candidate, which could be based on multi-epitope to target the most of the divergent isolates of ASFV.

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Lead paper – 3

Toll-like receptor agonists as adjuvants and prophylactic agents in chickens

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Abstract

Adjuvants are added to the vaccines for enhancing the antigen-specific immune response. Pattern recognition receptors (PRRs) recognize the conserved molecular structures of pathogens known as pathogen-associated molecular patterns (PAMPs). Innate immune response is triggered by PRR activation, which further modulates adaptive immunity. Toll-like receptors (TLRs) are well-characterized PRRs. TLR agonists have been used as adjuvants and prophylactic agents in livestock, poultry, and humans. This topic focuses on TLR agonists as immunomodulators in chickens with a special emphasis on our lab reports. Resiquimod (R848), a TLR 7 agonist, induced the expression of IFNβ, IFNγ, IL1β, IL4, and iNOS genes in the peripheral blood mononuclear cells (PBMCs) of chicken both *in vitro* and *in vivo* conditions. Overall, R-848 was found to induce a mixed Th1 and Th2 response in chickens. R-848 has shown potential adjuvant activity with inactivated Newcastle disease vaccine in chicken, as evidenced by enhancement of antigen-specific humoral as well as cellular immune response. In addition, R-848 was found to enhance both systemic and mucosal immune response when used with infectious bronchitis vaccine. Further, prior administration of R-848 could induce partial protection in chickens against very virulent infectious bursal disease virus. The combination of resiquimod (R848) and lipopolysaccharide (LPS) induced a synergistic response in the chicken peripheral blood mononuclear cells (PBMCs) pertaining to both Th1 and Th2 responses. Further, we have evaluated the combination of LPS and R-848 as an adjuvant with inactivated NDV vaccine in chickens. The combination of Pam3CSK4 (TLR2 agonist) and poly I: C (TLR3 agonist) de-repressed the very virulent infectious bursal disease virus vaccine-induced immunosuppression in chickens. In conclusion, TLR agonists will be soon employed as immunomodulators in the poultry industry.

Keywords: TLR, adjuvant, prophylaxis, vaccine, immune response, chicken

Introduction

Vaccination serves as the main means of controlling infectious diseases. In search of safer vaccines, the developed subunit and new-generation vaccines have weak immunogenicity. Adjuvants are added to these weakly immunogenic vaccines for enhancing the immune response (Ravikumar *et al*., 2022). Adjuvants nonspecifically enhance antigen-specific immune response and reduce the required vaccine dose and boosters through an antigen-sparing strategy. (Facciola *et al.*, 2022). Pattern recognition receptors (PRRs) detect the conserved molecular structures of microorganisms called pathogen-associated molecular patterns (PAMPs). Along with this, it can also be activated by host danger-associated molecular patterns (DAMPs). On activation by PAMPs or DAMPs, the PRR pathway triggers innate immune responses such as the production of cytokines and chemokines, and co-stimulatory molecular expression. Immune potentiators can act as a ligand for PRRs and activate innate immune responses (Facciola *et al.*, 2022). One of the most commonly studied PRRs is Toll-like Receptors (TLR). To date, 10 TLRs have been discovered in poultry (Abdelaziz *et al*., 2024). TLR1LA, TLR1LB, TLR2A, TLR2B, TLR3, TLR4, TLR5, TLR7, TLR15, and TLR21 are the ten TLRs discovered in chickens (Nawab *et al*., 2019). Many TLR agonists have been tried and found as potential adjuvant candidates to stimulate the innate immune response which in turn enhance adaptive immune response.

Table. 1 Chicken Toll-Like Receptors and their microbial ligands and synthetic agonists

TLR	Microbial Ligands	Synthetic agonists
TLR1LA	Triacyl lipopeptides and bacterial lipoproteins	Pam3CSK4, LTA
TLR1LB	Triacyl lipopeptides and bacterial lipoproteins	Pam3CSK4, LTA
TLR2A	Lipoproteins, zymosan, Lipoarabinomannan, peptidoglycan, lipoteichoic acid	Pam3CSK4, Pam2CSK4, LTA
TLR2B	Lipoproteins, zymosan, Lipoarabinomannan, peptidoglycan, lipoteichoic acid	Pam3CSK4, Pam2CSK4, LTA
TLR3	Viral dsRNA	Poly (I:C), poly-ICLC, Poly (I:C12U) poly (A:U)
TLR4	LPS	MPLA, GLA, KLA
TLR5	Flagellin	Recombinant flagellin derivatives
TLR7	Viral and bacterial ssRNA	Thiazoquinoline and imidazoquinoline derivatives (e.g.,resiquimod, imiquimod)
TLR15	Yeast	Proteases from Candida guilliermondi
TLR21	Viral and bacterial CpG DNA, DNA: RNA hybrids	CpG ODN

CpG ODN: CpG Oligodeoxynucleotides; TLR: Toll-like Receptor; LPS: Lipopolysaccharide; Poly I:C: Polyinosinic: Polycytidylic acid; Pam3CSk4: Pam3CysSerLys4; LTA: Lipoteichoic acid; MPLA: Monophosphoryl lipid-A; Poly (A: U): Polyadenylic-polyuridylic acid, GLA: Glucopyranosyl lipid A, KLA: Kdo2-lipid A

Mechanism of action of TLR agonists

Cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) trigger an inflammatory response after activation by PAMPs. In contrast, intracellular TLRs (TLR3, TLR7, TLR8, and TLR9) recognize nucleic acids from the pathogens and induce type I IFN and inflammatory responses (Duan *et al*., 2022). TLR include three functional domains as follows: leucine-rich repeats (LRRs), which contain an amino (N)-terminal domain responsible for ligand binding and fold into a typical horseshoe-like structure, a transmembrane spanning region, and a carboxyl (C)-terminal cytoplasmic domain that resembles the cytoplasmic region of globular Toll/interleukin-1 (IL-1) receptor (TIR) (Chakraborty *et al*., 2023). The TIR domain initiates downstream signaling, and various cytosolic adaptor proteins containing the TIR domain, such as myeloid differentiation factor 88 (MyD88), MyD88 adaptor-like (MAL or TIRAP), TIR-domain-containing adaptor protein-inducing IFN-β (TRIF or TICAM1), TRIF-related adaptor molecule (TRAM or TICAM2), and sterile α- and armadillo-motif-containing protein (SARM) and are involved in TLR signaling pathways (Kayesh *et al*., 2024). Except for the TLR3 pathway, practically all TLR signaling pathways involve the MyD88 adaptor protein. IRF3 is ultimately activated by the TRIF route, which is activated by TLR4 and TLR3 responses. The TLR4 signaling pathway can activate both the MyD88 and TRIF signaling pathways (Kayesh *et al*., 2024). The cytosolic TIR domains of each TLR undergo coordinated dimerization upon binding by particular ligands (Duan *et al*., 2022). Upon dimerization these signaling pathways will drive the induction of Type I IFN in addition to pro-inflammatory cytokines, based on the specific supramolecular complexes that are formed (Duan *et al*., 2022). All TLRs will undergo homodimerization except TLR2 and 6 which form heterodimers (Diaz-Dinnamarca *et al*., 2022). Two receptor proximal membrane adaptor proteins—the TIRAP-inducing IFN-β (TRIF)-related adaptor molecule (TRAM) and the TIRAP-containing adapter protein (TIRAP; also known as MAL)—identify dimerized receptor TIR domains. Through the actions of an N-terminal phosphoinositide binding domain of TIRAP or a bipartite localization domain of TRAM made up of an N-terminal myristoylation motif and a phosphoinositide binding motif, these peripheral membrane proteins survey the inner leaflets of the plasma and endosomal membranes (Duan *et al*., 2022). Myeloid differentiation primary response protein 88 (MyD88) and TRIF can be further recruited by TIRAP and TRAM respectively, and they can also promote the formation of a vast oligomeric scaffold known as a Myddosome or Triffosome. These supramolecular complexes are made up of kinase enzymes

and downstream signaling elements. Elevated regional concentrations of signaling molecules stimulate innately feeble allosteric connections and start the transmission of cytosolic signals (Duan *et al*., 2022). The MyD88-dependent pathway mainly initiates the activation of signal transduction pathways that promote inflammation, like NF-κB and mitogen-activated protein kinases (MAPKs). On the other hand, the TLR3 and TLR4 signaling pathway that is TRIF-dependent stimulates IRF production of IFN-I, preparing the cells for anti-viral activity (Kircheis *et al*., 2023).

TLR agonists as adjuvants and prophylactics

TLR3 agonists

TLR3 is an intracellular TLR that primarily recognizes the dsRNA of viruses. This receptor is involved in the induction of antiviral response and contributes to the induction of adaptive immune responses through the stimulation of conventional dendritic cells (cDCs) for cross-priming (Ong *et al*., 2021). Due to its capacity to elicit a type 1 interferon response, TLR3 has garnered significant attention in studies as an adjuvant for cancer vaccines (Kaur *et al*., 2022). Microbial ligands for TLR3 include double-stranded RNA from viruses (Sakaniwa *et al*., 2023). Poly (I: C), poly-ICLC, Poly (I: C12U) poly (A: U) are the known synthetic agonists of TLR3 (Chakraborty *et al*., 2023). The MyD88-independent pathway, activated by poly I:C binding to TLR3, signals through TRIF to activate IRF-3 and NF-κB, leading to the production of type I IFN and pro-inflammatory cytokines (Bashir *et al*., 2019). The upregulation of CD8α molecules, and IFNγ expression on circulating and lung γδ T cells was significantly induced by poly I: C (Matsuyama-Kato *et al*., 2022). This study also found a higher percentage of γδ T cells in chicken liver and lungs, suggesting they may play a crucial role in pathogen elimination and tissue homeostasis (Matsuyama-Kato *et al*., 2022). Results seen in the chicken PBMCs, bursal cells, and RAW 264.7 cells that are administered with infectious bursal disease (IBD) vaccine along with poly I: C are consistent with the upregulation of IL-1β, IFN-β, IFN-γ, IL-12, IL-4, IL-13, IL-10, and iNOS transcripts as well as an increase in NO generation in chicken PBMCs upon stimulation with poly I: C (Bashir *et al*., 2019). Combining TLR agonists with hot IBD vaccinations in SPF chicken preserves immunocompetence and poly I: C administration maintains B cell responses (Bashir *et al*., 2019). To preserve chicken immunocompetence, poly I: C treatment either alone or in combination with Pam3CSK4 (TLR2 agonist) decreased the harmful effects of hot IBD vaccination by reducing bursal damage and B cell destruction, restoring T cell and macrophage activities, and enhancing weight gain (Bashir *et al*., 2019). Early plasma cells were encouraged to become late/long-living plasma cells by poly (I: C), which demonstrated the ability to stimulate the BM plasma cells. According to this observation, the longevity and effectiveness of plasma cells in combating infections are dependent on poly (I: C) (Tandel *et al*., 2024). Although poly I: C has been applied as an adjuvant its instability and toxicity were the major shortcomings noticed. Hence it led to the synthesis of its derivatives like poly I: C12U and poly A: U (Ko *et al*., 2023). Poly-ICLC considerably increases resistance to nuclear solubilization (adverse effect of poly I: C) while retaining the biological activity of the parent Poly (I: C) (Fan *et al*., 2022). A poly-L-lysine found in carboxymethyl cellulose called poly (ICLC) increases the synthesis of IFN. It has a great ability to activate the Th1 response and is employed in vaccine candidates against *Plasmodium falciparum* (Kastenmuller *et al*., 2013), HIV (Saxena *et al*., 2019), and cancer (Ohlfest *et al*., 2013). It also increases the expression of the inflammasome and the complement system. This adjuvant's strong immunostimulatory activity and remarkable resistance to serum nuclease led to the development of poly (IC12U). This novel compound exhibits a mismatch between the uracil and guanosine residues, which results in decreased toxicity (it does not bind to MDA5) and decreased IFN-I production (Martins *et al*., 2015).

TLR4 agonists

TLR4, a cell surface TLR, is activated by lipopolysaccharides of the Gram-negative bacteria. In addition to LPS, TLR4 can also be activated by DAMPs released from the damaged host cells. Extracellular matrix

molecules and intracellular components, such as DNA-binding proteins like high-mobility group box 1 (HMGB1) and cellular HSPs, are examples of DAMPs obtained from cells (Kim *et al*., 2023). Unlike other TLRs, two distinct pathways—MyD88-dependent and independent (TRIF mediated) signaling—compete with one another to deliver the intracellular signal of TLR4 (Guven-Maiorov *et al*., 2015). LPS connects to TLR4 through the adapter protein myeloid differentiation factor-2 (MD-2) because it is unable to interact with TLR4 directly (Shimazu *et al*., 1999). Through interactions with extracellular proteins, LPS receptors form complexes with TLR4, CD14, and MD2, or myeloid differentiation protein 2 (Nawab *et al*., 2019). When LPS binds to TLR4 through the adaptor protein complex, it triggers a signaling cascade that causes NF-κB to become active and release chemokines and pro-inflammatory cytokines (Nawab *et al*., 2019). Once the TLR4-ligand complex has been internalized, endosomes initiate MyD88-independent signaling. It is triggered by the TIR domain's coupling to TRIF and TRAM. Through TRAF3, this signaling pathway activates TBK1 and IKKε, facilitating the transcription factor IRF3's trafficking into the nucleus. IRF3 starts the synthesis of type I interferons (Zanoni *et al*., 2011). LPS induces of iNOS and IL-1 transcripts in the chicken PBMCs (Ramakrishnan *et al*., 2015). LPS also up-regulate the expression of IL-2 and IL-4 in the chicken PBMCs (Gupta *et al*., 2013). The addition of LPS to other oil-based adjuvants like montanide increased both humoral and cell-mediated immune response indicated by increased antigen-specific antibody production and a remarkable rise in IL-2 and IFNγ gene expression (Mahmoud *et al*., 2024). Expression levels of TNF-α and IL-1β were enormously increased following administration of LPS (Senevirathne *et al*., 2022). Unfortunately, LPS's endotoxic action, which results in intolerable reactogenicity, significantly restricts its usage as an adjuvant (Zariri *et al*., 2015). The development and assessment of modified products, such as monophosphoryl lipid A (MPLA) and glucopyranosyl lipid A (GLA), Kdo2-lipid A (KLA) (Kim *et al*., 2018) which are structurally related to LPS, have been the focus of recent work on TLR4 agonists. These products maintain strong immune potentiating properties while lacking high pyrogenicity, making them more viable for use in clinical settings (Kaur *et al*., 2022). Hence these modified TLR4 agonists can be implemented as a vaccine adjuvant either solitarily or in conjugation with other TLR agonists.

TLR5 agonists

TLR5 is a cell membrane receptor expressed on many immune cells, which engages and recognizes bacterial flagellin. The primary protein constituent of the flagellum, flagellin, is recognized via receptors like TLR5 (Murtaza *et al*., 2022). TLR5 expression in chicken was reported in the testes, colon, spleen, kidney, lung, and heart (Iqbal *et al*., 2005). Moreover, TL5 expression has been found in chicken immune cells, including monocytes, T and B cells of the adaptive immune system, Langerhans cells, heterophils, and NK cells (Gupta *et al*., 2014). With its four fundamental domains (D0, D1, D2, and D3), flagellin binds to TLR5. Among the four fundamental domains D0 and D1 are highly conserved and are indispensable for TLR5 binding and activation (Doan *et al*., 2020). Binding of flagellin to TLR5 causes its dimerization and triggers downstream signal transduction pathways (Bagheri *et al*., 2022). Further, it triggers downstream inflammatory pathways, releasing a variety of inflammatory mediators, including TNF-α, IL-1, IL-6, and nitric oxide (Ong *et al*., 2021). For potentially larger levels of TLR5 activation and immunological response, (1) FliC99, residues 1–99, and (2) FliC176, residues 1–176 can be used (Murtaza *et al*., 2022) and the vaccinated chickens with FliC 99 and FliC176 exhibited a considerable rise in Th1 (IFN-γ) and Th2 (IL-4) cytokines. For an IBDV VP2 subunit vaccine, the N-terminus of flagellin, FliC99, and FliC176, can function as an immune activator to boost vaccination efficacy (Murtaza *et al*., 2022). Genetically fused nFliC exaggerated antibody response, expansion of CD4+ and CD8+ T cells, pro-inflammatory, Th-1 and Th2 cytokine expression (Chuekwon *et al*., 2022). nFliC enhanced the expression levels of IL-1β, IL-6, and IL-8 in addition to an accelerated increase in antibody levels and CD4+ and CD8+ T cells when used with vaccines (Doan *et al*., 2020). VP-2 protein-specific T-cell proliferation, nitric oxide synthesis, antibody genesis, and expression of pro-inflammatory cytokines were evidenced when FliC protein was injected along with virulent IBDV challenge in chickens (Deb *et al*., 2015). Using Salmonella flagellin as an adjuvant in

conjunction with the H5N2 avian influenza virus vaccination has increased the mucosal immune response in chickens and generated humoral and cellular immunity (Chaung *et al*., 2012). Co-administration of flagellin with the ND vaccine resulted in an increase in TNF-α and other pro-inflammatory cytokines (Barkhordari *et al*., 2021). Salmonella enterica serovar Enteritidis infection of chicken TLR5-expressing HeLa cells induced a dose- and flagellin-dependent response involving high levels of NF-κB (Iqbal *et al*., 2005).

TLR7 agonists

TLR7 is an intracellular TLR that recognizes the ssRNA of viruses. TLR7 agonists are being investigated as stand-alone treatments for a range of infectious diseases. Imiquimod (R837), Resiquimod (R848), selgantolimod, vesatolimod, gradiquimod and loxoribine are the most widely studied synthetic TLR7 agonists (Bhagchandani *et al*., 2021). The most potent imidazoquinoline (IMDQ) acting as TLR7 agonist is1-benzyl-2-butyl- 1*H*-imidazo[4,5-*c*] quinolin-4-amine (BBIQ) (Kaushik *et al*., 2024). It activates both MyD88 and TRIF signaling pathways resulting in the production of pro-inflammatory cytokines and interferons respectively (Bhatnagar *et al*., 2022). Among these two pathways inducing pro-inflammatory cytokine expression is a crucial aspect of TLR7 activation (Bhatnagar *et al*., 2022). Both cDCs and plasmacytoid DCs are stimulated and expanded, and CD14+ CD16+ (inflammatory monocytes) and CD14dimCD16++ (patrolling) monocytes are mobilized by TLR7 agonists (Kwissa *et al*., 2012). R-848 induced the expression of IFN-β, IFN-γ, IL-1β, IL-4, iNOS, and TLR7 genes in the chicken indicating a mixed Th1 and Th2 response (Annamalai *et al*., 2015). Pre-administration of R-848 before very virulent IBDV challenge showed a delay in the development of clinical symptoms, a reduction in its severity, and a decreased death rate compared to the control, indicating the protective effects of the agonist. Bursal damage was significantly lower in the R-848 pre-treated group, demonstrating R-848's effectiveness in shielding the bursa from the vvIBD challenge. For the chickens challenged with vvIBD, R-848 pre-treatment offered full protection against immunosuppression and partial protection against mortality (Annamalai *et al*., 2016). Further, R-848 administration with infectious bronchitis (IB) vaccine enhanced both mucosal and systemic immune response in chickens (Matoo *et al*., 2018). R-848 also showed potential adjuvant activity when used with inactivated ND virus vaccine in chickens. It enhanced NDV specific humoral and cellular immune response and also afforded complete protection against virulent NDV challenge as wells as virus shedding in chickens (Sachan *et al*., 2015). Administration of imiquimod along with the NDV vaccine substantially reduced the shedding of ND virus from the cloaca, increased antibody titre, and expression of IFN genes (Lee *et al*., 2023). Upregulation of expression of MHC-I and MHC-II, enhanced synthesis of cytokines specific to Th1 and Th2 immune response, and enhanced synthesis of pro-inflammatory cytokines were noticed when imiquimod was used as an adjuvant in various combinations with influenza virus vaccine (Zhang *et al*., 2022).

TLR 21 agonists

TLR21 is one of the intracellular TLRs located in the endosome. TLR21 is activated upon binding of CpG motifs present in dsDNA (natural microbial ligand/ synthetic CpG ODNs) (Alves *et al*., 2024). The lack of TLR21 in mammals where TLR9 is the primary CpG sensor and the absence of TLR9 in birds where TLR21 is the only known CpG sensor are two notable differences between these TLRs in mammals and chickens (Guabiraba *et al*., 2023). TLR21 ligation triggers several signaling outputs in different cell types, such as the generation of nitric oxide in myeloid cells and transcription of antiviral genes, which includes the generation of type I interferon and inflammatory cytokines (Guabiraba *et al*., 2023). In chickens, the major interferon reaction to CpG DNA is IFN-β production. Chicken macrophages are stimulated by TLR21 to produce a strong type-I interferon response in addition to activating NF-κB (Guabiraba *et al*., 2023). CpG ODNs also exhibit anti-allergic, anti-viral, anti-infection, and anti-tumor properties (Li *et al*., 2022). Type-A CpG ODNs enhance IFN-γ and IFN-α secretion by NK cells and plasma cell-like DCs (pDCs), respectively (Krug *et al*., 2001). Type-B CpG ODNs stimulate IL-6 secretion by monocytes and DCs and

encourage B cell activation, proliferation, and secretion of IgM and IL-6 (Vollmer *et al*., 2004). Increased production of cytokines and specific antibodies, as well as accelerated proliferation of lymphocytes, were indicative of both the innate and adaptive immune response triggered by CpG ODNs administered with avian influenza virus vaccination (Li *et al*., 2022). Immunostimulatory CpG-ODN molecule (TLR21 agonist), in conjunction with the recombinant HVT-LT (rHVT-LT) vaccine *in-ovo* enhanced the immunological responses of chickens as early as on the day of hatch. Adjuvanting rHVT-LT vaccine with CpG-ODN showed an increase in the Th1 immune response, improvement in splenic macrophage and γδ T-cell responses and induction of spatially regulated (lung and spleen) inflammatory response in the chicken (Gaghan *et al*., 2023). CpG ODN stimulated IFNγ production from circulating γδ T cells in chickens (Matsuyama-Kato *et al*., 2022). *In-ovo* administration of CpG ODNs increased the hatchability and reduced caecal shedding of *Salmonella* Heidelberg in chickens (Alves *et al*., 2024). As a nanoparticle CpG-NP (Nanoparticle) increased the expression of 53/84 immune-related genes, including IL-1β, IL-6, IL-12, and IFN-γ when administered along with H6N1 avian influenza virus and infectious bronchitis virus. These increased genes indicate a bias in the immune system toward T h1 and showed signs of enhanced mucosal immunity following CpG-NP immunization. (Lin *et al*., 2020). Further, administration of CpG ODNs drastically reduced the shedding of H9N2 AIV through oral and cloacal routes (Raj *et al*., 2023). After administering CpG ODN the phagocytic activity of heterophils was increased against *Salmonella* Typhimurium. Further, it also enhanced immune protection against septicaemia induced by *Salmonella* Typhimurium in neonatal chickens (Subhasinghe *et al*., 2024). In addition to enhanced synthesis of pro-inflammatory cytokines, CpG ODNs also showed anti-inflammatory effects on HD11 cells induced with LPS. It was confirmed by the enhanced expression of IL-10 mRNA which is an anti-inflammatory cytokine (Ichikawa *et al*., 2023).

TLR-TLR cross-talk (TLR agonists in combination)

Simultaneous activation of different TLRs using their cognate agonists would simulate the natural infection. By including the microbial molecules in the experimental vaccines, the outcomes of these TLR interactions can be prudently utilized to induce the intended immune response in the host. The polarization of naive CD4+ Th cell responses to either Th1 or Th2 type, as well as other characteristics of the subsequent immune response, can be influenced by the stimulation of distinct TLRs. The combination of LPS and R-848 synergistically up-regulated the expression of IL-1β, IFN-β, IFN-γ, IL-4 transcripts indicating the synergy of these agonists on the pro-inflammatory, type I interferon, Th1 and Th2 reponses in the chicken (Ramakrishnan *et al*., 2015). However, the combination of LPS and R-848 showed suppressive effects on the humoral and cellular immune response when used with the inactivated NDV vaccination in chickens (Sharma *et al*., 2022). Further, the combination of Pam3CSK4 and poly I: C synergistically upregulated IFN-β, IFN-γ, IL-12, IL-4, and IL-13 transcripts and cross-inhibited IL-1β, IL-10, and iNOS transcripts in the chicken PBMCs (Bashir *et al*., 2019). Antigen-specific IgG and IgM responses were increased when R848 and poly I: C was co-administered with antigen (Tandel *et al*., 2024). The combination of CpG and poly I: C enhanced cell-mediated and humoral immune responses when used with influenza vaccine in chickens (Paul *et al*., 2014).

Conclusion

Toll-like receptor (TLR) agonists were explored as adjuvants and prophylactic agents in chicken against various infectious diseases. The combination of TLR agonists provides us a way to achieve the desirable immune response in the host. The main drawback of using TLR agonists in the field is its cost. Synthesizing our own TLR agonists and optimization of the scale up procedures can be tried to make the usage of TLR agonists as a viable option. By using some delivery systems which would release the TLR agonists slowly the toxicity issues could be avoided. In near future it is feasible to witness the utilization of various TLR agonists as immunomodulators in the poultry industry.

Lead paper - 4

Ovine Foot rot in Andhra Pradesh –Problems and Solutions

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Sheep contributes 678 million Kgs of mutton, 40.26 million kgs of wool,41.6million pieces of skin, 31 million tons of manure annually (BAHS-2019). India stands 2nd position in Asia with 74.26 million of sheep population and 148.88 million goat population. (20th Livestock census) In A.P. 210.15 lakhs sheep and 64,27 lakhs goat population (A.P.20th Livestock census). Sheep and goat reared by landless laborers, tribes and marginal farmers of the country. This highlights the economic importance of these species of animals for rural development in our country. Sheep and goat farming is an important livestock industry of most of the state and serves as source of income to the rural farming community especially the scheduled tribes. At present, apart from the other diseases, virulent foot rot caused by a anaerobic bacterium, *D.nodosus* in sheep and goat is becoming problematic day by day not only in the state but also in adjoining states of Karnataka, Tamil Nadu and Kerala and Telangana.

Foot rot is a disease of sheep and goats caused by the synergistic action of several bacterial species of which *Dichelobacter nodosus* is the essential causative agent (Stewart,1989). Muddy pastures, frequent rains warm temperatures and injury to the feet predisposes the animals to infection. Though it is reported in cattle, deer and moufflon (Ghimire et.al, 2002) the infection is specific to sheep and goat. Virulent foot rot is highly contagious and economically important infection of sheep and goat because of its impact on the livestock production and health. Due to lesions in the feet, sheep show lameness inability to feed which results in a loss of body condition, reduced wool growth and wool quality, reduced meat production and decreased fertility (Stewart,1989; LaFontaine et. al.,1993 and Hill *et al*.,2010) Due to disease 10% production loss in body weight and wool growth in affected animals in addition to increased costs of treatment and control (Marshall.et. al., 1991; Mournane 1933).

The occurrence of foot rot from Andhra Pradesh and Telangana in tropical climate was reported during the period from 2009 to 2023. A total of 2499 samples were collected from 94 outbreaks and tested from 16 districts of Andhra Pradesh and Telangana. Out of 2499 samples tested, 631 samples were found positive for *D.nodosus* with 25.25% prevalence . Highest prevalence in Nellore district 37.5% (237) followed by chittoor34.07% (215), Krishna 8.2% (52), Anantapur 4.1%(26), and Kadapa 3.9%(25).Serogroup analysis of 631 positive samples revealed higher prevalence of B serogroup 40.7%(257) followed by I serogroup 29.% (183), A Serogroup 11.7%(74) and B+I serogroup 7.2% (43) respectively. A,B &I were detected in most of the districts and with the exception of E in East Godavari and Krishna districts and F in Krishna and Guntur districts . Similarly mixed serogrouping were observed in Chittoor (A+B; A+I;&B+I), Nellore (A+B;B+C;&B+I), Srikakulam (A+B) and Anantapuram (B+I) districts respectively. Foot rot is a seasonal occurring disease occurred during rainy season from June to December (South West Monsoon) with peaks during late spring and early summer season from July to December (North East Monsoon).

The high prevalence of the virulent foot rot in the state and also adjoining states, the disease being reported regularly during rainy season treatment of affected animals appears to be costly and not economical. The major approach in preventing foot rot was the development of vaccines. Infact, vaccination is considered as the easiest and most effective way of controlling the spread of foot rot among sheep. Vaccination provides threptic and prophylactic benefits to reduce prevalence and transmission respectively (Egerton

and Morgan 1972., Gurang *et al*.,2006) Vaccination programmes went successfully in Australia, Bhutan and Nepal to control the foot rot in sheep. The vaccines developed in abroad cannot be used in our country due to existence of various sero groups of *D.nodosus* and lack cross protective immunity. Sero group specific vaccination fond to have excellent efficacy. For immediate remedy action plan was initiated in the state with the request of A.H. Officers and taken consideration into the benefit of poor farmers SVVU, Tirupati, Andhra Pradesh, developed Bivalent(B+I) and Trivalent (B+I+A) inactivated whole cell vaccines using local isolates of Andhra Pradesh. This vaccine was under vent lab and field trials in the state and giving good immune response up to 120 days of vaccination with protective titers giving protection to susceptible period of infection i.e rainy season .However, the cultivation of the organism is a laborious and tedious process due to fastidious nature of growth of the bacteria and requirement of complex nutrients in the media for the growth. Further, constraint is lack of sufficient growth in the liquid media.

Commercial vaccines for the control of ovine foot rot were first manufactured and distributed in Australia during 1971. These early monovalent and bivalent vaccines effectively reduced foot rot incidence and severity in experiments carried out in a controlled environment, but lack cross protective immunity under field conditions where several serotypes were present in the flock. (Lewis *et,al.,* 1989). Subsequently, a multivalent, piliated, alum-oil adjuvanted bacterin of *D. nodosus* was also produced in England in 1984. (Smith et, al., 1990). However, they have limitations of low and short terms of antibody responses due to antigenic competition in contrast to higher and longer responses provided by monovalent/ bivalent vaccines. However, monovalent/bivalent vaccine were successful in eradication programmes in foot rot endemic areas of Bhutan, Nepal &Australia (Dhungyel &Whittington 2010). In Nepal, a serogroup specific vaccine was found to be superior to a commercial vaccine in protecting animals during seasonal migration (Egerton *et.al.,* 2002). The success of this because of monovalent only sero group E strains were involved. Similarly, in Bhutan, monovalent autogenous vaccine using sero B isolated from field eliminated the clinical signs of the disease. (Gurang et.al.2006).

Monovalent and multivalent recombinant vaccines against the disease were developed (Schwartzkoff et. al.,1993; Moore et. al.,2001) from fimbrial or protease genes but had their own limitations as in case of traditional vaccines. This poor protection is due to antigenic competition associated with such vaccines (Hunt *et al*.,1995), In addition, *D. nodosus* can undergo sero group conversion, involving genetic exchange of the fimbrial sub unit gene (Kennan et, al.,2003) highlighting the need for heterologous protection with antigens other than fimbrial.

The development of an effective vaccine against all ser groups requires the identification of additional protective factors or antigens. Due to existence of multiple serogroups the current vaccine research has been focused on the development of new universal vaccine using reverse vaccinology approach, thereby genome sequencing is used to identify potential antigens from *D.nodosus* that are not hampered by antigenic competition and can prevent infection by multiple sero groups/serotypes of *D. nodosus*. This reverse vaccinology approach going to be good success in future to overcome the limitations of either monovalent/ Bivalent/Multivalent vaccines in control of foot rot disease.

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ORAL PRESENTATION

OP_1.1: The p30 protein of the African swine fever virus behaves as an RNase

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The African Swine Fever Virus (ASFV) is responsible for causing the African Swine Fever (ASF), a severe contagious disease characterized by haemorrhagic symptoms. The p30 protein of ASFV is the most abundantly expressed viral protein. It is reported to be antigenic and has recognized phosphorylation, glycosylation, and membrane attachment sites. It also shows that the C-terminal region of p30 is more active than the N-terminal region. p30 interacts with heterogeneous nuclear ribonucleoprotein K (hnRNP-K) during infection and helps in virus attachment. Our work aimed to understand the RNase activity of p30, elucidate the kinetics of p30 among temperature, pH, and ions, and establish a mutated p30 protein that might alter the RNase activity of p30 protein. In this study, the RNA isolated from the BHK21 and PK15 cell lines was incubated with the p30 protein, and agarose gel was used to show the RNase activity. In addition, fluorometric analysis was used to show RNA degradation by p30 protein. The RNase activity of p30 was stable at an optimum temperature of 37 ºC, and the maximum activity was recorded at pH 7–9 in the presence of monovalent salts. The mutant of p30 (p30m), where cysteine was mutated to alanine at position 109, showed a loss of RNase activity. The RNase activity of the p30 protein is equally active in heterologous and homologous systems. After all the experiments, we concluded that the p30 protein of ASFV acts as an RNase. The role of this protein in the viral replication process remains unexplored. We tried to show the RNase activity of ASFV, which will help us better understand the host-pathogen interaction of ASFV.

OP_1.2: Assessment of three different culture systems for field fowl adenovirus isolation

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The present study was aimed to assess the three different virus culture systems for isolation of fowl adenovirus (FAdV) from field outbreaks. Fourteen liver samples positive for 897bp hexon gene of FAdV by PCR were used in this study. The clear supernatants from liver tissue samples treated with antibiotics (100 units of penicillin G, 100 µg of streptomycin and 0.25 µg of amphotericin B) and filtered through 0.45 µm Millipore membrane filter was inoculated into 11 day old embryonated chicken eggs through chorioallantoic membrane (CAM) route, primary chicken embryo fibroblast (CEF) cells and chicken embryo liver cells (CELi). There is no specific lesions on CAM, death of embryo and PCR amplification in CAM as well as liver tissues of embryo up to five passages. In CEF, nine out of fourteen PCR positive liver tissues produced CPE consisting of cell rounding, clumping and detachment and floating of cells at 72 hr at 5th passage level. In CELi cells, the CPE consisting of presence of vacuole and honey comb appearance in third passage at 24 hr post infection followed by cell rounding, clumping, detachment and floating of cells. All the fourteen isolates were recovered from liver samples using CELi cells and showed positive for PCR. The FAdV field isolates were well adopted and isolated in CELi cells followed by primary chicken embryo fibroblast cells.

OP_1.3: Over-expression of recombinant nucleoprotein (NP) of Ganjam virus (GANV) using heterologous expression system

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Ganjam virus (GANV) belongs to the genus *Nairovirus*, family *Bunyavirdae*. GANV is predominantly tick-borne disease but was also isolated from mosquitoes, man and sheep. This organism has a considerable veterinary importance in India, as neutralizing and complement fixing antibodies have been detected in animal and human sera collected from different parts of the country. Molecular studies have demonstrated that GANV is an Asian variant of Nairobi sheep disease virus (NSDV) of Africa which is highly pathogenic for sheep and goats and both these viruses are related to Crimean Congo haemorrhagic fever (CCHF) group viruses. Implementation of strategic 'One Health' approach in diagnosis could aid in effective prevention and control of the disease in endemic areas. In the present study, *in silico* analysis of nucleoprotein (NP) of GANV revealed a highly conserved nature of protein and then the target gene (1467 bp) sequence was codon optimized and chemically synthesized which was cloned in to prokaryotic expression vector- pET28a. Upon transformation in to expression host-*Escherichia coli* BL21(DE3) codon plus cells and chemical induction, the recombinant NP protein (~57 kDa) was over-expressed in *Escherichia coli* along with N-terminus hexa-histidine tag. The SDS-PAGE analysis indicated presence of over-expressed recombinant NP protein. Further, the protein was purified using Ni-NTA column and inoculated into rabbit and guinea pig model for hyperimmune sera raising. The study warrants further evaluation and standardization of indirect-ELISA using rNP for detection of anti-GAN specific antibodies in livestock serum samples.

OP_1.4: Characterization of Toll-Like Receptor-2 gene of Punganur cattle in relation to Paratuberculosis susceptibility

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We sought to characterize the TLR2 gene of Punganur cattle for the presence of putative mutations that were known to be associated with susceptibility to paratuberculosis. The Punganur cattle herd of 38 animals at the Livestock Research station, Palamaner was screened for paratuberculosis using ELISA and fecal IS*900* PCR. Seropositivity of 28 percent (11/38) was recorded but, none of the animals were positive in PCR suggesting that there was no fecal shedding of MAP and hence the herd is confirmed as free from paratuberculosis. The TLR2 gene of Punganur cattle (n=5) was characterized. Two overlapping fragments (1219bp and 1394bp) of the exon2 of TLR2 gene were amplified in two PCR reactions and the sequenced fragments were assembled to obtain the entire coding sequence of 2355 bp that encodes TLR2 peptide of 784 amino acids. The Punganur TLR2 sequences revealed 99.71-99.87 per cent identity with *Bos indicus* sequences except one Punganur animal (P555) that showed 100 per cent identity with *Bos taurus* X *Bos indicus* hybrid but, only 99.25, 99.38 per cent identity with other Indian cattle (*Bos indicus*). There were 14 nonsynonymous (amino acid substitutions) and eight synonymous mutations in Punganur TLR2 gene, in comparison to *Bos taurus* sequence. Fourteen mutations were found in the LRR domain out

of which 11 were nonsynonymous and, 5 mutations (2 non synonymous) were detected in the TIR domain of the Punganur TLR2. Three mutations were found in the transmembrane domain, out of which one was nonsynonymous. The mutations at the aminoacid positions 119, 335, 417, 605 and 766 found in the TLR2 of Punganur cattle were novel from the SNPs reported in cattle from other studies. The TLR 2 gene of Punganur cattle in the present study did not possess the putative/well known mutations associated with Paratuberculosis susceptibility (Arg677Trp, Pro681His, Arg753Gln) that were reported in the previous studies. In conclusion, the Punganur cattle in the present study, though seropositive for MAP antibodies, were free from paratuberculosis infection and the same was supported by the absence of putative mutations that are associated with increased susceptibility to paratuberculosis.

OP_1.5: Generation of N-acetylglucosaminyltransferase (*nctA*) deficient *P. multocida* mutant

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Haemorrhagic septicaemia (HS) has significant impact on livestock sector due to its opportunistic nature targeting the immunosuppressed animals. In India, HS is caused by *P. multocida* B:2 serotype, a Gram-negative bacteria belong to *Pasteurellaceae* family. It comprises many virulence factors; among them LPS is the major virulence factor. Currently killed vaccines with oil or alum adjuvant are available to prevent the HS. But the killed vaccines have drawback of providing inadequate immune response of shorter duration. Live attenuated vaccines are expected to provide better and longer duration of immunity. LPS, being the major virulence factor, targeting the LPS biosynthesis genes appears to be the better strategy. In the current study, the objective was to develop a *P. multocida* mutant lacking *nctA* gene (involved in LPS biosynthesis). The *nctA* gene deletion mutant of *P. multocida* was developed by allele-exchange method. The nctA gene deletion was confirmed by PCR and sanger sequencing of nctA gene region. The mutant was characterized by analysing the growth kinetics and the stability of mutant was checked by culturing the bacteria for several passages. To conclude, the nctA mutant *P. multocida* was successfully constructed and confirmed. The mutant will be checked for its vaccine potential.

OP_1.6: Adaptation and analysis of porcine circovirus-2b in PK-15 cells grown in serum deprived conditions

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Porcine circovirus-2 (PCV-2) is the small non-enveloped, non-cytopathic globally reported emerging viral pathogen in swine responsible for the post weaning multi-systemic wasting syndrome (PMWS), porcine circovirus associated diseases (PCVAD) with multiple systemic infections resulting in huge economic loss. Porcine kidney (PK-15) cell line has been widely used in PCV2 virus isolation, diagnostics, vaccine development and antiviral studies. Noncytopathic nature of PCV2 necessitates usage of indirect immunofluorescence assay for confirmation of its replication and titration. This study aimed to grow PK-15 cells in serum deprived growth conditions and subsequently adapt field PCV2b genotype in PK15 cells adapted to grown in serum deprived conditions and study both changes in cell and virus. Three levels of

serum supplemented growth medium 0%, 2% and 10% were used for cultivation of PK-15 cell. The PK-15 cells adapted to grow in varying serum composition medium were subsequently infected with genotyped field PCV2b and propagated up to 3 blind passages. Haematoxylin and Eosin staining of PK-15 cell grown in 0% and 2% serum supplemented medium with and without PCV2b infection evidenced granularity in cytoplasm when compared to PK-15 cells grown in 10% serum supplementation. The cell viability through MTT assay of PK-15 cells grown in 0% and 2% serum supplemented medium with and without PCV2b infection evidenced cell viability of 88.25 % and 94.66% respectively when compared to 99.27% viability in PK-15 cells grown with 10% serum supplementation. Cell lysates in all three passages of 0%, 2% and 10% serum supplemented medium showed positivity to PCV2b genome by specific PCR assay indicating the adaptability of PCV2b in PK-15 cell grown in serum deprived condition. Molecular analysis of major epidemiological marker gene, ORF2 of PCV2 grown in both 0% and 10% serum conditions revealed similar nucleotide (702bp) and amino acid composition (234aa). This study explores possibility growing PK-15 in serum deprived conditions and adaptability of PCV2b in PK-15 cell with serum deprived condition. Subsequent passage of PCV2 in serum deprived condition and analysis may add robust knowledge.

OP_1.7: A Novel Host Immune Evasion Mechanism Mediated by V Protein of Newcastle Disease virus

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Newcastle disease virus (NDV), an avian paramyxovirus, is a significant pathogen in poultry, infecting over 250 bird species worldwide. NDV possesses a negative-sense RNA genome that encodes six structural proteins and two accessory proteins, with the V protein being a multifunctional interferon (IFN) antagonist. MDA5, a crucial component of the host's antiviral defense, is an interferon-inducible helicase with a caspase activation and recruitment domain (CARD) at its N-terminus, responsible for detecting viral double-stranded RNA (dsRNA). The V protein of avian *Paramyxoviridae*, including NDV, inhibits MDA5, thus preventing the recognition of viral dsRNA and impairing the host's antiviral response. However, the precise mechanism of MDA5 inhibition by V remains unclear.

Our research focuses on elucidating the structural changes in MDA5 upon V binding. Through docking studies and MD simulations, we observed that the C-terminal domain (CTD) of V binds with high affinity to the CTD of MDA5, disrupting the oligomerization of its N-terminal domains. This inhibition hampers the activation of mitochondrial antiviral signaling protein and subsequent IFN induction. Additionally, during NDV infection, overexpressed V protein enhances viral replication by downregulating the expression of MDA5. The V protein also aggregates in the perinuclear region, co-localizing with replicating dsRNA and competing with MDA5 for dsRNA binding, thereby facilitating immune evasion. These findings reveal novel insights into how NDV's V protein disrupts host antiviral signaling, advancing our understanding of viral immune evasion mechanisms.

OP_1.8: Antiviral potency of repurposed drugs against Newcastle disease virus

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Newcastle disease virus (NDV) is an enveloped RNA virus, responsible for neurological, respiratory, and enteric diseases in birds worldwide. In the present study, NDV was used as a prototype virus to study

the anti-viral properties of five repurposing drugs namely Ivermectin, Hydroxychloroquine, Levamisole, 2-Deoxy Glucose, and Doxycycline. Newcastle disease virus was isolated by inoculating necropsied spleen, liver, kidney, and proventriculus samples in embryonated chicken eggs, which showed typical occipital hemorrhages in embryos and confirmed by PCR using NDV fusion protein primers and also by neutralization of Hemagglutination activity by NDV specific antisera. Thus isolated NDV was adapted in Vero cells and typical syncytia was observed. *In vitro* and *in-ovo* antiviral activity of aforementioned drugs were tested against NDV at three different drug doses using time-of-addition procedures to ascertain the antiviral potency relating to the stage of virus replication and MTT assay was performed to assess the viability of cells. Additionally, relative quantification of chINF-γ gene expression was done using real-time PCR in NDV infected Chicken embryo fibroblast (CEF) cells in order to find out the chINF-γ mediated antiviral activity of these drugs. The findings of this study both *in vitro* and *in ovo* antiviral assay concluded that the Doxycycline and Hydroxychloroquine were viable antivirals against NDV. Among these five repurposed drugs, Levamisole alone had shown significant chINF-γ expression.

OP_1.9: Identification of suitable culture system for the propagation and development of vaccine of Duck hepatitis A virus Genotype-2

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Duck hepatitis A virus (DHAV) causes a highly contagious duck viral hepatitis in young ducklings of less than 6 weeks of age. It is often associated with liver necrosis and a high mortality rate. 3 genotypes of DHAV (DHAV-1, DHAV-2 and DHAV-3) are reported. Development of vaccine against DHAV is needed to protect the duck population from this deadly disease. Though, vaccines for DHAV-1 and DHAV-3 are available worldwide, vaccines against DHAV-2 is not available. Recently, we have reported genotype 2 of DHAV (DHAV-2) from India. Hence, it is important to develop a suitable platform for virus isolation, propagation and development of vaccines. Therefore, in this study the susceptibility of the embryonated chicken eggs (ECE), embryonated duck eggs (EDE) and primary cells such as Chicken embryo fibroblast (CEF) , Duck embryo fibroblast (DEF), chicken embryo liver (CEL) cells, chicken embryo kidney (CEK) cells and duck embryo liver (DEL) cells, duck embryo kidney (DEK) cells for the growth and adaptability of DHAV-2 was evaluated. Similarly, continuous cell lines such as Vero and BHK were also evaluated. Adaptability of DHAV-2 to these platforms and ability to produce cytopathic effect were analyzed by sequential passaging in the respective system. Conventional Reverse transcription PCR was used to confirm the DHAV infection at every passage level. Our results revealed that the growth of DHAV-2 in ECE was not consistent. Similarly, Virus could not adopt and grow in Vero and BHK cell lines. Virus growth and adaptation in EDE, DEK, DEL, CEK, CEL, CEF and DEF platforms were successful with an average titer of ~ 5. However, cytological changes' indicating the infection was seen only in CEF cells. Also, DHAV-2 could not produce any type of plaques in all the 6 different primary cells and 2 cell lines evaluated in this study. To conclude that the CEF cell is susceptible to DHAV- 2, produces evident cellular changes and propagates the virus with satisfactory titer. This shows that CEF cells could serve as a valuable platform for the isolation, propagation and development of vaccines against DHAV-2. This is a pioneering research work on genotype 2 of DHAV and funded by DST SERB-POWER grant.

OP_1.10: Insights into polymorphisms of viral and host genes in recent infections of the Marek's disease

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Marek's disease virus (MDV) causes malignant lymphomas, neurological disorders and immunosuppression in infected poultry. Although the disease is currently controlled by vaccination, the recent past has witnessed a continuous increase in virulent MDV strains. This study aims at comparative analyses of the virulence-associated genes of MDV along with the exploration into a cancer-driving gene of the host. Isolation of MDV was carried from suspected cases of both vaccinated and unvaccinated poultry flocks in South India. The virus-associated virulence genes namely- *Meq, pp38, and vIL8* presented point mutations by sequence analysis. The *Meq* gene, an oncogene of MDV, is essential for tumorigenesis and regulates the expression of both viral and host genes. Hence the study postulated that the host genome may contain cancer-driver genes, whose somatic mutations can promote tumors. Mutations in the *Meq* gene have resulted in polymorphisms and deletions encoding S-*Meq* (short isoform of *Meq*) in the Proline-rich region, which may possibly contribute to the varying virulent nature of the MDV's isolates. The Ikaros (*IKZF1*) gene was identified for the exploration of the MDV-induced lymphomas in this study, based on prior research of the same gene in humans and mice oncology. One or more nonsynonymous somatic mutations in the Ikaros gene - a master regulator of lymphocyte development, have been identified. Thus, the virus-encoded virulent genes and their polymorphisms may be working in conjunction with one or more of the host-driven potential cancer driver gene(s) in oncogenic diseases like MD. Phylogenetic analyses of the virulence associated genes of MDV revealed clustering of the field strains of this study with a few North Indian strains and virulent viruses of Nigeria and China. However, the transactivation activity of *Meq* and its effect on the pathogenicity of MDV needs to be explored further. These findings of the virus and host gene are suggestive of the evolutionary pattern of newer virulent MDVs.

OP_1.11: Isolation of lumpy skin disease virus from recent outbreaks from Andhra Pradesh

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Lumpy skin disease (LSD) outbreaks have been recorded in several states of India. Since 2019, Andhra Pradesh being one of the most seriously affected areas, resulting in significant economic losses to the farmers. Skin lesions observed throughout the body especially on face. The disease has economic significance with a decrease in milk production; cows remain in angstrom for months, abortion in pregnant animals and infertility. Keeping in view of devastating nature , severity of the disease and varied clinical lesions affecting different systems of the body. Hence, the work has been focused on isolation of LSDV from different parts of the state to know the circulating strains during the period. A total of 38 blood samples and 39 skin scabs were collected from affected adult cattle and younger calves suspected for LSD in different outbreaks of Andhra Pradesh during the period from August 2022 to September 2023. The LSDV isolation was carried out in embryonated chicken eggs initially and later in lamb testicular cell culture and confirmed the presence of LSDV from field clinical samples. The molecularly confirmed LSDV isolates showed characteristic pock lesions on 5th day at 6th passage. The LSDV isolates recovered from embryonated chicken eggs were further adopted to primary lamb testicular cell cultures and characteristic

CPE of shrinkage, cell rounding between 48 to 96 hours of post infection and complete CPE was observed after 120 hours of post infection. Similar pattern of CPE was also observed during 2nd blind passage with same interval of time. Further the LSDV isolates recovered from both passaging through embryonated chicken eggs and primary Lamb testicular cell cultures were further confirmed with PCR by amplifying P32 gene. The present work revealed the presence of LSDV from blood and skin scab from different outbreak areas of Andhra Pradesh showing varied clinical signs and lesions compared to previous outbreaks in the state (2019,2020 &2021). This will help in selection of vaccine candidates to take action plan in control of the disease.

OP_1.12: Induction of innate immune training by beta-glucans in chicken monocytes

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Modulation of immunity via induction of innate memory (trained immunity) offers an alternative approach in effective prevention and control of infectious diseases. Innate memory is characterized by increased (training) or decreased (tolerance) innate immune responses such as proinflammatory cytokine production in response to homologous or heterologous secondary challenge following a priming event. *In vitro* induction of a trained or tolerized state is a prerequisite to identify a potent agonist with the intent of enhancing disease resistance. The present study was undertaken to investigate the innate training or tolerogenic potential of different beta-glucans (sourced from yeast, algae and bacteria) in chicken blood monocytes. The chicken peripheral blood mononuclear cells were isolated and enriched for monocytes by adherence method. The chicken blood monocytes were primed with different β-glucans and following resting for 5 days, challenged with heterologous toll-like receptor (TLR) agonists (Pam3CSK4, Poly I:C and LPS) to determine the induction of innate memory or tolerance. Priming of chicken blood monocytes with beta-glucan from yeast (*Saccharomyces cerevisiae*) or algae (*Euglena gracilis*) or bacteria (*Alcaligenes faecalis*) and heterologous challenge with any of the TLR agonist significantly upregulated (innate training) the expression of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 ($P \leq 0.05$). These results constitute the first detailed demonstration of trained immunity potential of different beta-glucans in chickens.

OP_1.13: Screening of cultured *Penaeus vannamei* shrimp for *Enterocytozoon hepatopenaei* at Tiruvallur district of Tamil Nadu

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Shrimp farming is one of the economically important aquaculture systems worldwide, the export commodity yielding high income to our country. *Enterocytozoon hepatopenaei* (EHP) is a disease causing microsporidian pathogen in cultured shrimp. Loss of revenue due to EHP was higher due to wide spread distribution and difficulty in controlling the disease, as the spores can be viable in the aquatic environment for months. The EHP infected shrimps shows symptoms like lethargy, pale hepatopancreas, growth retardation leading to size variations in the pond with high FCR. In this study we collected 134 samples from sixty Penaeus vannamei farms cultured in low to high salinity water resources from bore well and creek at different villages of Tiruvallur district, Tamil Nadu, India during September 2022 to July 2024. The shrimp tissue samples were preserved in 90% alcohol and processed for testing EHP by PCR. 44.70 % were positive for EHP screening using spore wall protein (SWP) gene PCR, out of these n=55 positive cases, 29 cases showed size variation as clinical sign, n=20 were first step (514bp) positive, rest all were detected

nested positive (148bp) indicating the EHP load in hepatopancreas of infected samples. EHP is frequently reported with white feces syndrome (WFS) due to mixed infection with other pathogens, eight positive samples had WFS during the culture and while 12 samples had WFS history in their previous culture was observed. Shrimp farms without proper biosecurity were at the risk of EHP infection at any time of their culture period even when the seeds were screened and found negative for EHP before stocking, disease occurs mainly through contaminated farm environment and spread mainly through inanimate objects and water resources

OP_1.14: Immuno informatic analysis and cross reactivity of recombinant Beta Propeller Repeat protein against multiple serovars of *L. interrogans* sps. in guinea pig and canine models

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Leptospirosis is the wide spread neglected zoonotic disease among domestic and pet animals and poses major threat to human public health. Although it is treatable with antibiotics, vaccination is the prime strategy to control the disease. However, the current vaccines are ineffective due to lack of cross reactivity and require booster doses. Therefore, it is inevitable to identify conserved protein candidates that can provide immunity against the majority of serovars. In this context, this study was aimed to mine the proteome data base of *L.interrogans* sps through reverse vaccinological approach to select a promising vaccine candidate. Immuno informatic analysis revealed 18 promiscuous proteins candidates. Based on MHC class I, II, and B cell epitope predictions and functional annotations of the selected proteins, it was found that Beta Propeller Repeat (BPR) is responsible for the virulence and pathogenesis in the host system. Hence, cloning and expression of BPR was carried out in prokaryotic system. Immunoblot analysis of recombinant BPR protein (36 kDa) showed a strong reaction against the rabbit antiserum raised against inactivated *L. interrogans canicola* and confirmed the specificity of the beta propeller protein of *L.interrogans canicola.* Microscopic Agglutination Test (MAT) analysis of the sera collected from the guinea pigs immunized with the recombinant BPR (rBPR) vaccine revealed cross reactivity with five serovars namely; *L.autumnalis, L.canicola, L.grippotyphosa*, *L.hardjo, L.icterohaemorrhagiae* except *L.pomona.* The MAT test in canine model also resulted similar immune response. The antibody titre varied from 1:40 to 1:160 and the highest titre (1:160) was recorded against *L.hardjo.* Conclusively this study ascertained the potential cross reactivity of the recombinant BPR protein against the multiple serogroups *of Leptospira interrogans*.

OP_1.15: Computational analysis of Newcastle disease virus: Investigating molecular interactions of anti-viral phytochemicals and synthetic drug with RNA polymerase

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Newcastle disease virus (NDV) is a major concern for avian populations worldwide. This study aimed to identify plant-based compounds from *Azadirachta indica* as potential antiviral agents targeting the NDV RNA polymerase protein and also to compare the result with the selected drug in pipeline. Molecular docking

analysis was performed to assess the interactions of these compounds with the NDV polymerase. Promising compounds, including syringic acid, norvaline, gallic acid, (-)-epicatechin, and cianidanol, exhibited favourable interactions with the active site of the NDV polymerase more than that of the drug in pipeline. These compounds showed non-carcinogenic effects and moderate to high gastrointestinal absorption, suggesting their potential for systemic distribution. Molecular simulation study confirms the stability of all the docked complex. However, i*n vitro* and *in vivo* studies, including cytotoxicity assays, pharmacokinetic evaluations, and animal models, are necessary to determine their antiviral activity and therapeutic potential. This study provides valuable insights into the potential of *Azadirachta indica* compounds as an antiviral agent against NDV by targeting the RNA polymerase protein. Further investigation is required to validate their effectiveness which will pave the way for the development of novel antiviral drugs against NDV, addressing a significant concern in avian health.

OP_1.16: Transcriptional response in duck thymus to infection with Duck Plague Virus and *Pasteurella multocida*

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An attempt was made to understand the transcriptional regulation in thymus during duck plague virus (DPV) and *Pasteurella multocida* (PM) infections by separate experimental infection models. The Next Generation Sequencing of the transcriptome was performed with the Illumina platform. The generated paired end data was analyzed with the DNAStar Laser gene NGS software suite version 17. In the course of DPV infection, the thymus tissue showed an up-regulation of 4735 transcripts, down regulation of 20266 transcripts and 2881 transcripts remained at basal expression level in comparison with uninfected thymus tissue. Infection with DPV resulted in 8-fold or higher up-regulation of 219 genes. 106, 43 and 11 genes were up-regulated between 10 and 20-fold, more than 20 to 50-fold and 50 to 73-fold respectively. One gene each was up-regulated 100.98 fold (Paralogue of Parvalbumin) and 251.27 fold (GRAP2 - GRB2 related adaptor protein 2). These two genes play a key role in cell signaling of immune cells and seem to be pivotal in the initial immune signaling during DPV infection. On the other hand, 1592 genes were down-regulated 8-fold or more. The duck genes whose expression levels were significantly up-regulated between 50 to 73-fold included genes involved in immune response, such as, RAG1 and RAG2 which are involved in V(D)J recombination, TCF7, a transcription factor involved in the development of innate immune T cells such as Natural Killer T cells, C-C motif chemokine receptor 7 involved in immune cell migration, cytokine CD28 involved in T cell activation, Carbohydrate sulfotransferase 1 involved glycation of proteins and lipids in Golgi, Paralogue of Parvalbumin and Junctional Adhesion Molecules (JAM) of the immunoglobulin super family which are involved in immune cell signaling. In addition, the genes up-regulated 50 to 73-fold also included genes active in energy metabolism, such as, Gastrokine 1 involved gut metabolism, Dispatched RND transporter family member 3 (DISP3) and Fatty acid binding protein 1 (FABP1) genes involved in fatty acid metabolism. The transcriptional signature of the top up-regulated genes indicates significant cellular immune response and energy mobilization. A STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis of all the 160 genes which were up-regulated 10 fold (confidence interval of 99%) or more in DPV infected thymus compared with uninfected thymus indicated protein networks linked to T cell activation, proliferation, differentiation and signaling.

During PM infection, the thymus tissue elicited an up-regulation of 9569 transcripts, down-regulation of 16624 transcripts and 1689 transcripts remained at the same expression level when compared with uninfected thymus tissue. Infection with PM resulted in 8-fold or higher upregulation of 72 genes. 9 genes

were up-regulated between 15-fold and 20-fold. One gene each was up-regulated 21.29 (Lipocalin -ApoM), 22.65 (Transglutaminase 4 - TGM4) and 30.16 (Fatty acid binding protein 1 - FABP1) fold. All the changes in gene expression were detected with a confidence interval of 99%. The genes that were significantly up-regulated more than 10-fold included Family with sequence similarity 110 member (FAM110C) involved in immune cell migration and signaling, PID/PDZ domain containing nitric oxide synthase involved cell signaling, Calcium binding proteins (CaBP), Collagen type XIV alpha 1 chain (COL14A1) involved in cell adhesion, C-type lectin domain-containing protein, StAR related lipid transfer domain containing 13 (STARD13) involved in immune cell signaling and migration, and SERTA domain containing 4 (SERTAD4), a nuclear protein with unknown function. Interestingly, the genes serpin family C member 1 Antithrombin -III SERPINC1, an inhibitor of coagulation, and Gallinacin 10 like (GLL10 protein) which has an antimicrobial function was also up-regulated 15-fold. In addition, the genes involved in energy metabolism;Lipocalin (Apolipoprotein M), aldehyde dehydrogenase 1 family member A1 (ALDH1A1) and Adenylate kinase 4, were up-regulated around 15-fold, striking a similar picture to antiviral (DPV) transcriptional response.

The study has identified the key role of the GRAP2 (GRB2 related adaptor protein 2) in the signaling events in the thymus during the generation of immune response to DPV. On the same note, the involvement of antithrombin activity and antimicrobial Gallinacin 10 like protein (of β defensin family), in the development of immune response to PM infection in the thymus are noted.

POSTER PRESENTATION

PP_1.1: Comparative assessment of *in vitro* non-cytotoxic dose of *Andrographis panniculata* extracts

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The present study illustrates the difference in the *in vitro* cytotoxicity of aqueous and ethanolic extracts of *Andrographis panniculata* in Vero and BHK- 21 cells. Briefly, the cells (1×10^5 cells/ ml) were seeded in a 96 well micro titer plate (100 µl/ well) and incubated for 12 hours to form a monolayer. The cells were treated with two fold dilutions of the aqueous and ethanolic extracts ranging from 3200 to 3.12µg and 1600 to 1.56 µg with three replicates per treatment respectively for 48 hours. The effect of extracts on cell viability was measured by MTT assay and the IC_{50} was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. The viability percentage of the cell (IC_{50} =A570(Sample)/A570(Control)] ×100. Both aqueous and ethanolic extracts exhibited substantial cytotoxic activity against tested cell lines in a dose-dependent manner. The IC_{50} of aqueous extract was 400 µgml^{-1} and 3200 µg ml^{-1} in BHK and Vero cells respectively. The IC_{50} of ethanolic extract was 1.56 µg ml^{-1} and 1200 µg ml^{-1} in BHK and Vero cells respectively. Cytotoxicity of the Andrographolide (98%) was 5.125µg ml^{-1} and 400 µg ml^{-1}in BHK and Vero cells respectively. The findings of the study demonstrate Vero cells could tolerate higher concentration of both types of extracts in comparison with BHK cells implying the tolerance level being determined by the cell type. Interestingly, the aqueous extract of *A. paniculata* produce less cytotoxicity in higher concentrations as compared to the ethanolic counterpart. The cytotoxicity of the ethanolic extract producing 50% cell death with much lesser concentration in both the cell types could be attributed to the phytochemical components present in them.

PP _1.2: TCID50 Assay for Avian Reovirus Titration in Chicken Embryo Fibroblasts

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Avian reoviruses (ARVs), also known as avian Ortho reoviruses, are a common virus that infects a wide range of birds. Belonging to the family Reoviridae, these RNA viruses can cause several diseases in poultry, impacting their health. Diagnosing Avian Reovirus infections in poultry can be challenging due to its ability to cause a variety of symptoms. TCID50 Assay can be used for directly measuring the concentration of ARV.

In the significance of ARV, the present study was envisaged for screening of the poultry samples from suspected poultry birds, isolation of field strains in Vero cell culture and genetic characterization of the suitable live vaccine candidate for ARV disease. For this, about 110 samples were screened for presence of ARV by PCR and 6 of them were found positive. The PCR amplification using primers specific to the L1 segment and S2 segment produced positive results. These were confirmed by observing bands on the agarose gel on 421 base pair and 409 base pairs respectively. Sigma C gene sequences obtained from Genbank were aligned for molecular investigation. Initial tree(s) for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances using Maximum Composite Likelihood (MCL) approach. There were total 801 positions in final dataset. The gene that encodes Sigma C exhibited the highest amount of sequence divergence and rapid evolution, indicating that the gene may be utilized to quickly differentiate and classify ARV isolates. Further, the virus was adapted to CEF cells for 21 generation and the titre of the virus was found to be 106.0 by Reed–Muench method. This study indicates that genotype cluster I ARV are the main virus lineage found in India and DVB 04 may be a good option for an attenuated ARV vaccine if attenuated in suitable cell culture system.

PP_1.3: Exploring *Theileria annulata* schizont infected bovine PBMC culture secretome derived bovine MMP-9 as a Novel Alternative to Trypsin for Cell Detachment

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Theileria annulata, a protozoan parasite, significantly impacts bovine health by infecting and transforming bovine peripheral blood mononuclear cells. Recent studies have highlighted the secretome of infected PBMCs as a source of various proteolytic enzymes, including matrix metalloproteinase-9 (MMP-9). MMP-9 is a zinc-dependent gelatinase that degrades extracellular matrix components and may offer an innovative and affordable alternative to traditional proteolytic enzyme, trypsin which is being used to detach the adherent cells in cell culture. However, its use can be limited by its non-specific proteolytic activity, leading to cell damage, and altered cellular physiology. In search for an affordable alternative to trypsin, we investigated the potential of MMP-9 from a secretome of T. *annulata*-infected bovine PBMCs as a novel alternative to trypsin. Native form of bovine MMP-9 was purified from the spent media of *T. annulata* schizont infected bovine PBMC culture, using a Gelatin Sepharose affinity chromatography. Further, the purified 92 kDa bovine MMP-9 was dialyzed and concentrated using PEG 6000. The yield range of the purified MMP-9 was 50 to 150 µg/ ml from 25 ml of 3-4 days spent culture media. The purified bovine MMP-9 was characterized by gelatin zymography that exhibited a unique substrate specificity profile, making it an attractive candidate for selective cell dissociation. Our study demonstrated that MMP-9 can effectively detach the adherent cells viz. Vero cells and MDBK cells attached on the cell culture plates at lower concentrations (2.5µg-7.5µg /ml of cell culture) while preserving cell viability and functionality. These findings suggest that *T. annulata* secretome-derived bovine MMP-9 is a promising alternative to trypsin for cell dissociation.

PP_1.4: Phage therapy of necrotic enteritis in poultry

Anindita Sarma and Luitmoni Barkalita

The objectives of the study included isolation and identification of bacteriophages from caecal samples, having lytic activity against *Clostridium perfringens*. *In vitro* assessment was done to assess the inhibitory potential of the bacteriophages and the stability of bacteriophage isolates in different environmental conditions. Bacteriophages were isolated from caecal contents of poultry birds and tested by double layer agar (DLA) method in temperatures 37, 40, 60, 70, 80 and 90˚ C and in pH 4.0, 6.0, 7.4 and 9.0. The ammonium sulphate technique was used to isolate bacteriophage DNA, and whole genome sequencing was carried out to determine the molecular characteristics of the phage isolate. Mega11 software was used for the phylogenetic analysis, and Swiss online tool was used for the homology modelling of the endolysin protein. Based on the DLA technique preparation of the culture plates, the bacteriophage isolate produced spots or transparent plaques. The procedure for isolation and preservation of bacteriophages (host *C. perfringens*) for up to three months was optimized. The optimal storage conditions for the phage isolate were found to be 4˚C in SM buffer, and were stable at 37˚C to 60˚C and pH 4.0-9.0. Using whole genome sequencing, the phage isolate was identified as a *Caudovirus*. The phage isolate was found to be closely linked to the phages of *Clostridium* and *Yersinia* through phylogenetic analysis and according to homology modelling, the bacteriophage endolysin exhibited 46.85 percent similarity to the *E. coli* phage Enc34.

PP_1.5: Flow cytometry-based analysis of immune response in shrimp against WSSV infection

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White spot syndrome virus (WSSV), a double-stranded, circular DNA virus from the *Whispovirus* genus of the *Nimaviridae* family, causes White spot disease (WSD) in shrimp. It affects brackishwater crustaceans and is particularly problematic for the economically important shrimp and crabs, because of its high mortality rate. Indian white shrimp, *Penaeus indicus*, is considered as economically important species in Indian aquaculture sector. Although crustaceans lack adaptive immunity, their innate immune system, which relies on haemocytes and associated immune factors, plays a crucial role in defense against pathogens. Flow cytometry-based haemocyte analysis has been used in studying immune responses in crustaceans, including their reaction to WSSV. Apoptosis, serves as a defense mechanism to prevent pathogen proliferation, and its role in response to viral infections like WSSV has been well-documented. Cytoplasmic free Ca2+ (cf-Ca2+), a crucial secondary messenger, influences various cellular processes and its dysregulation can lead to cell death. In this study, the immune response of Indian white shrimp to WSSV at temperatures of 27°C, 30°C, and 33°C at 12, 24, and 48 hours post infection (hpi) was examined through apoptosis and cf- Ca2+ analysis using flow cytometry. FITC Annexin V Apoptosis Detection Kit I revealed highest apoptotic rate of 31.65±3.52% at 33°C after 48 hpi. Additionally, cf-Ca2+ concentration assay using Fluo-3/Acetoxymethyl ester (Fluo-3/AM) revealed highest cf-Ca2+ concentration of 57.91%, 65.55%, and 80.10% at 33°C across all time points. Higher temperatures (33°C) inhibited DNA replication, while increasing innate immune response. Viral copy number showed 2.05-fold increase at 12 hpi and later decreased at 24 and 48 hpi, indicating that elevated temperatures enhance the shrimp's innate immunity against WSSV.

PP_1.6: Comparative Evaluation of Mechanical and Enzymatic Disaggregation Methods for Culturing Primary Duck Embryo Liver and Kidney Cells: Implications for Duck Hepatitis A Virus Propagation and Study

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Primary duck embryo liver (DEL) and duck embryo kidney (DEK) cells serve as valuable host systems for studying the Duck Hepatitis A virus (DHAV). This pathogen causes Duck Viral Hepatitis (DVH), a highly contagious, lethal disease of young ducklings. This study investigates the effectiveness of mechanical and enzymatic cell disaggregation methods for culturing primary DEL and DEK cells for DHAV isolation and propagation. Mechanically sifting tissues, which involves physically rupturing tissue, is a quick method but comes with a higher risk of cellular injury and decreased cell viability. Enzymatic disaggregation of primary tissues using trypsin yields higher cell viability and better preservation of cellular integrity but possesses a contamination risk of porcine-origin viruses. Therefore, to create a single-cell suspension of primary DEL and DEK cells from the primary tissues, we evaluated a mechanical disaggregation method in this study. The mechanical disaggregation method was compared with trypsin-based classical enzymatic disaggregation in terms of cell morphology, viability, integrity, and yield. The virus-host interaction characteristics of primary DEL and DEK cells produced by both methods were further compared by infecting those cells with DHAV. The outcomes of this study showed that there was no discernible difference in the cell morphology, viability, and yield for the methods. Moreover, primary DEL and DEK cells produced using both techniques have comparable susceptibilities to DHAV. The obtained titre of DHAV using the primary DEL and DEK cells produced by mechanical and enzymatic disaggregation methods was 5 ED50 and 4.5 ED50, respectively. Therefore, the mechanical disaggregation protocol used in this study is a simple, rapid, and user-friendly way to cultivate primary DEL and DEK cells. The DST-SERB-POWER Grant, which advances avian virology research, provided funding for this work.

PP_1.7: Expression profile of inflammatory cytokines of critical care dogs affected by *Babesia gibsoni* and co infection with other hemoprotozoa

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The common signs of canine babesiosis caused by an infection with small piroplasm *Babesia gibsoni* are fever, anorexia, lethargy, pulse alterations, anemia, and occasionally mild icterus. Nowadays mixed infection of hemoprotozoan diseases are very common. Severe forms of this nature of infections lead to systemic inflammatory response syndrome (SIRS) and sometimes death also. The client owned dogs presented to Critical care unit, Madras veterinary college, Chennai – 600007 for treatment has been used for this study. The study group was divided into with SIRS (n=21), without SIRS (n=9) and healthy dogs (n=10). Haematological and serum biochemistry including C- reactive protein were analysed and the results clearly showed that thrombocytopenia and leukopenia, elevation of liver and renal parameters. The positivity of infections within groups (n=30) showed 43% of *Babesia gibsoni* alone and 40% co infection with other hemoprotozoas. Cytokine profile of with SIRS group indicated elevation of IL-1α, IL-1β, IL-8, TNF alpha, enzyme 5-LO and lower expression of IL-10, TGF beta and enzyme iNO. The timeline of cytokine expression of non survivors comparing with survivors showed unchanged pattern of cytokine expression and progressive increase in expression. Hence cytokine expression pattern can be used as molecular marker for early diagnosis of prognosis of animal in the course of treatment for canine babesiosis

PP_1.8: Proteomic analysis of canine platelet lysates and *in vitro* study for evaluation of their anti-inflammatory effect

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Nowadays longstanding cases of inflammatory trigger with infectious cause were very common in small animal practice. The higher inflammatory trigger of dog is usually treated by corticosteroid therapy. If the animal is affected with simultaneous damage of infectious and inflammatory causes of the disease, an alternative to corticosteroid as anti-inflammatory is the need of an hour in treatment strategy. Platelet lysate can be used as potential immunomodulator. The platelet lysate preparations of thrombocytopenic with SIRS blood samples were estimated for protein quantity by Bradford assay. The protein quantity after removal of major serum proteins i.e. albumin and globulin were also assessed for both type of samples. Two preparations of platelet lysate from thrombocytopenic SIRS were analysed by PAGE. The two preparations of thrombocytopenic SIRS platelet lysates were analysed by Time-of-flight LC-QTOF-HRMS/MS chromatography with normal samples as control. The variations in peptide fragment pattern between the platelet lysates of thrombocytopenic SIRS and normal clearly showed the changes in protein composition for therapeutic study. Proteins annotated from referral canine platelet proteome were grouped based on cellular compartment, molecular function and platelet function by gene ontology. Platelet lysate preparations of thrombocytopenic SIRS preparations successfully reduce the cytokine expression of *in vitro* produced sepsis in RAW 264.7 cell line by LPS for therapeutic study.

PP_1.9: Concurrent infection of Dermatophilosis, Candidiosis and aspergillosis in cattle at Tiruvanamalai district of Tamil Nadu - its antibiotic sensitivity patterns and therapeutic management

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Dermatophilosis is more prevalent in tropical countries and has higher incidence during rainy and high humidity seasons. The disease investigation was attended at Arani division of Tiruvannamalai and a total of 57 animals investigated with severe skin lesion at the dorsal and ventral part of body neck area with black colored scabs and crusts. The animal does not have ticks and fleas infestation. The scabs and crusts were collected for direct microscopy and culture isolation and identification which reveals the presence of filamentous and branching zoospores in the impression smears confirmed to be dermatophilosis. The culture isolation and identification revealed *Klebsiella, Candida sp and Aspergillus sp*. The samples were subjected into DNA extraction and PCR was performed for bovine papilloma virus and LSD. The feed and water sample s were subjected in to toxicological analysis. The antibiotic sensitivity test shows sensitivity with ciprofloxacin, chloramphenicol, metronidazole and lincomycin. The animals were treated with lincomycin @ 10 mg/kg body weight intramuscular along with intramuscular chlorpheniramine maleate @ 0.2 mg/kg for first five days and it was followed by antifungal ointment and dusting powder for another 9 days. The animal showed marked improvement after 14 days of therapy with complete clinical improvement after 20 days with the disappearance of scabs and crusts.

PP_1.10: Assessment of antibacterial efficacy of *Andrographis paniculata* against bacterial isolates from clinical samples

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Antimicrobial resistance (AMR) is an important global public health concern in the present scenario necessitating critical attention. The present study was done to evaluate the antibacterial potential of

aqueous and ethanolic extracts of *Andrographis paniculata* against pathogenic bacteria such as E.*coli, Staphylococcus, Klebsiella and Proteus spp.* The pure culture of the bacteria was isolated from clinical samples like foot abscess, Ear swab, heart blood swab and nasal swab of canine, caprine, cow and canine species respectively. The minimal inhibitory concentration (MIC) and the antibacterial activities of both the extracts were determined by resazurin dye reduction assay and agar diffusion method respectively. The efficacy of the extracts was compared with standard antibiotic discs like Streptomycin, Oxytetracycline, Gentamicin, Enrofloxacin, Amoxicillin-clavulanic acid and Co-trimoxazole commonly used at field conditions. Further, phytochemical analysis was performed to determine the components in the extract responsible for antibacterial effect. The aqueous extract of *A. paniculata* at a concentration of 500µg exhibited strong *in vitro* antibacterial activity against *E.coli, Staphylococcus, Klebsiella and Proteus* spp. Interestingly, the ethanolic extract of *A. paniculata* at a concentration of 300µg exhibited strong *in vitro* antibacterial activity against *E.coli, Staphylococcus, Klebsiella and Proteus* spp,. The phytochemical analysis revealed that the antibacterial effect of extracts could be attributed to the flavanoids, terpenoids, saponins, tannins in the aqueous extract and additionally phlobatannins and cardiac glycosides in the ethanolic extract of *Andrographis paniculata*. The presence of these extra phytochemical components in the ethanolic extract resulted in greater antibacterial activity at a lower concentration compared to the aqueous extract. It is conceivable from the findings that the extracts or the components of *Andrographis paniculata* could be exploited as a potential alternate for the treatment of bacterial infections in the era of antibiotic resistance.

PP_1.11: An emerging trend in collagen mining—production using a sustainable resource

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Collagen is a triple-helix structural protein found in abundance in the extracellular matrix of multicellular organisms. The demand for collagen among consumers is on constant rise because of its wide range of applications in the cosmetic, food, pharmaceutical, and biomedical industries. The conventional sources of collagen are not able to produce sufficient collagen due to the rise in demand for this vital ingredient. It is important to discover novel and sustainable sources for collagen production. Adipose tissue is one of the best alternative source of collagen, as its extracellular matrix contains many types of collagen. In adipocyte culture, collagen can be harvested from the condition media, as collagen can be found in the adipocyte secretome. Harvesting and processing of cell secretome is less time-consuming, laborious, and cost-efficient than maintaining adipocytes. The assay for hydroxyproline quantifies the amount and presence of collagen in a given source. This particular amino acid is non-proteinogenic and is found in the elastin and collagen of mammals. Its presence is primarily restricted to collagen's triple helix, where it increases triple helix stability. Prolylhydroxylase is an enzyme that forms hydroxyproline from specific proline residues post-translationally. Tissue hydrolysates' hydroxyproline can be used as a direct indicator of the quantity of collagen present. Using adipocytes from chicken as an animal model and alternative sustainable resource for collagen production could indeed help in reducing the waste generated from poultry slaughtering. The technique can be applied for non invasive collagen rich source for regenerative medical applications such as non healing ulcers, skin wounds and in food industry for edible food packaging and so on. It's important to explore and develop sustainable sources for collagen production to meet the increasing demand while minimizing environmental impact. This approach has the potential to be both environmentally friendly and economically viable. Keywords - Secretome, Adipocytes, Hydroxyproline, Collagen.

TECHNICAL SESSION - II

ADVANCEMENTS IN VETERINARY VACCINES AND DISEASE DIAGNOSIS

Lead Paper -1

Commercialization of Veterinary Diagnostics – Rhetoric And Reality

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Vaccines and diagnostics are two sides of the coin of future-prophylaxis. In recent times, commercialization of veterinary vaccines has been happening in India with respect to vaccines for Bluetongue, Peste des petits ruminants, Newcastle disease, classical swine fever, Brucellosis, lumpy skin disease etc. But the same cannot be said for veterinary diagnostics. There are hardly any indigenously developed veterinary commercial kits available for field use at affordable costs. Certain diagnostic reagents such as California mastitis test reagent or Brucella plate antigen (RBPT) are procured through tendering process and supplied to veterinary dispensaries across the State. A few diagnostic kits that are still available are largely restricted to the poultry sector. Imported kits are available but at high costs and poor accessibility. We are unable to harness the totally naïve diagnostic market in our country.

Several research projects on diagnostics have led to proof-of-concept research on the development of diagnostic kits for most major diseases with limited validation studies. However, their technology readiness levels (TRL) which may be TRL3 or 4, to mature to TRL 7 or 8 does not happen easily.

Some of the hard lessons learnt in this domain are presented here so that there are good learnings from our experiences and these identified gaps are bridged, to achieve the ultimate goal of commercialization success in this field of veterinary diagnostics.

Our initial efforts during early 2000s on development of diagnostic kits were for poultry diseases such as infectious bursal disease, infectious bronchitis, Newcastle disease etc. The aim was to assess post-vaccinal seroconversion especially in breeder flocks to ensure that they are protected against these diseases. The assays involve single serum dilution ELISA which is the only assay amenable for such assessment since at least 1% of the samples are to be tested and flock monitoring is the objective. Other assays such as lateral flow or flow through does not lend itself for such application. Although these kits could be developed and validated in comparison with the available imported kits, the gap in our research was the lack of computer interface and data processing. The available commercial kits after reading the ELISA plate had a computer interface to convert the optical density values in to titres and calculate their mean and co-efficient of variation and predict their immune status. Vaccination index (VI) was also calculated VI = (Mean Titre) / %CV. The VI would be high with good vaccination (high mean titre, low % CV) and low in case of poor vaccination (low mean titre, high % CV). Although we could calculate these values with the OD values obtained after ELISA read-outs, this was not user friendly and hence did not find ready acceptance amongst users, who were primarily, poultry vaccine companies offering this service to measure post-vaccinal responses and to recommend the choice of vaccines and vaccination regimens.

During the chikungunya outbreak in Tamil Nadu in 2005-2006, the Department of Animal Biotechnology, MVC had offered chikungunya diagnosis as a service using PCR and nested PCR. However, the usage of this service was only lukewarm and on analysis of the reason, it was felt that a clinical diagnosis by the clinician was good enough as the diagnosis did not drive the line of treatment. What this means is that, the clinician may not need a laboratory confirmation of the disease suspected, as despite this lack of confirmatory diagnosis, the treatment would revolve around treating clinical signs alone. But the same was not true for leptospirosis also called as rat fever, since once diagnosis is done the choice of antibiotics is changed to doxycycline and an immediate improvement of symptoms would be seen in most cases. The

same logic was also true for diagnosis of canine diseases such as canine distemper, canine parvo, etc., or also for sheep / goat diseases such as bluetongue, PPR etc., since the treatment regimens are largely the same for which clinical diagnosis appears sufficient.

When these diagnostic assays were developed for disease diagnosis, another potential application of these serological tests were as demonstration kits for students. If there was a non-pathogenic antigen-antibody reaction system, all assays such as agar gel precipitation, counter immune electrophoresis, rocket immune electrophoresis, western blots, different types of ELISA etc., could be demonstrated and this commercialization was potentially very successful and resulted in receiving royalty also which meant that sales of these demonstration kits were happening.

Meanwhile, the lateral flow assay methods were gaining popularity due to their user friendliness and pen-side applications. The prototype kit in this regard is the pregnancy diagnosis kit that detected human hCG. These kits could be used either to detect antigen or antibody. For antigen detection, the conventional format used is the antigen capture ELISA that requires two antibodies, one to capture the antigen and the other to detect the captured antigen for which the detection antibody is labelled. This requires a set of 2 monoclonal antibodies or one monoclonal and one polyclonal antibody. For antibody detection, a purified antigen, preferably a recombinant antigen, is required and a detection system for the bound antibody that could be a coupled anti-species antibody or protein A/G is needed. However, the antibody detection method is only qualitative and may not be applicable for diseases for which vaccines are used. Hence this may be applicable only for chronic diseases such as Brucellosis or tuberculosis / paratuberculosis. We developed lateral flow tests for detection of antibody to PPR, Brucellosis, Wild life TB kit, egg drop syndrome etc. In addition to the above constraints, the requirement of such kits was very inconsistent and volumes were alarmingly low.

Antigen detection methods use either flow through or lateral flow techniques that are highly useful but suffer from the lack of sensitivity of detection. A negative test result does not rule out lack of infection. This again compromises application in a clinical setting where in only a confirmatory diagnosis would drive the treatment and/or prognosis.

Molecular diagnosis involves the use of polymerase chain reaction (PCR) and its variants, real time PCR or several isothermal amplifications methods for detecting the pathogen genome. All of us are aware that real time PCR was used as gold standard test for COVID diagnosis. Although the diagnosis did not involve quantification of the pathogen genome, this was used to overcome aerosol contamination and for obtaining a gel-independent readout. In the context of veterinary diagnosis, the application of this technology is prohibited by its cost. Further, diagnosis involving large, small ruminants, poultry or pigs need herd diagnosis for which number of samples to be analysed would be high which would further escalate the costs involved. Usage of pooled samples for testing may not be an ideal alternative as this could dilute the pathogen genome resulting in negative diagnosis.

However, application of PCR was widely successful for diagnosis of canine haemoprotozoan diseases for differentiation of *Ehrlichia, Babesia, Anaplasma* etc. Coupled with this diagnosis was the requirement of detection of immune mediated haemolytic anaemia or immune mediated thrombocytopaenia. This diagnostic service given by TRPVB was largely successful as conventional diagnosis was not always correct and sensitive and the diagnosis determined the line of treatment.

Another domain in diagnostics is the detection of certain conditions such as mastitis, pregnancy, heat detection, ovulation time, cardiac conditions etc. based on detection or quantification of particular analytes. These have also been widely successful as compared to disease diagnosis. Our somatic cell quantification kit (ABT-SCC kit) based on detection of an enzyme was commercially successful due to its low cost and

provided a solution, 'Teat Protect' to reduce the somatic cell counts in animals where it was found to higher. The bottom-line of this success is to identify the problem and provide a solution. In certain cases, in disease diagnosis such as brucella or tuberculosis, we may be able to identify the disease but cannot provide a solution to the farmer which discounts its applicability and thereby its commercial viability.

Another major issue of using latest isothermal technologies such as loop mediated isothermal amplification or recombinase polymerase assays is its patent protection. As long as these techniques are used for research work or publications this may be accepted but for developing a kit and commercialization, these techniques may have to be licensed which is a deterrent for companies' uptake.

Thefuture of diagnostics would probably involve a genome detection isothermal amplification technique including CRISPR-Cas with lateral flow assay to detect the protein/ small molecule tag attached to the nucleic acid as the read-out method. This would provide the best of both worlds that is, the sensitivity and specificity of detection of nucleic acid assays and the ease of detection and field deployability of protein assays through colour reaction and line formation.

Lead Paper - 2

Newcastle disease virus vectored vaccines for animal health

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Abstract

NDV (family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Avulavirus*) is an avian pathogen which naturally occurs as three pathotypes: velogenic strains, which cause systemic infections with a high level of mortality; mesogenic strains, which cause systemic infections of intermediate severity and lentogenic strains, which cause mild infections that are largely limited to the respiratory tract and which are used as live attenuated vaccines against NDV for poultry. The development of a reverse genetics system for the Indian vaccine strain R_2B is being preceded by elucidating the complete genome sequence of this strain and was carried out in our laboratory. Green fluorescent protein gene was introduced into the R2B backbone between the P and M genes of the virus and was rescued successfully in Vero cells. Reverse genetics has greatly benefited our understanding of NDV pathogenicity by introducing specific mutations into the genome of the virus and then analysing the phenotype of the rescued virus. NDV is an ideal vaccine vector for poultry. Live attenuated NDV vaccines are widely used all over the world. Therefore, a live attenuated NDV carrying the protective antigen of another avian pathogen can be used as a bivalent vaccine. Such a vaccine will be economical for the poultry industry. Nonetheless, the ability of NDV to infect a wide variety of non-avian species makes it also a promising vaccine delivery vector for other animal species. NDV can be a vaccine delivery vector for foreign animal diseases or diseases for which currently vaccines are not available. The present paper explores the current research that is being undertaken in the field of NDV reverse genetics and the generation of vaccine candidates against poultry and animal viral diseases.

Introduction

Newcastle disease (ND) is caused by Newcastle disease virus (NDV), belonging to the family Paramyxoviridae and genus Orthoavulavirus. NDV has a single stranded negative sense non-segmented RNA genome. The virus encodes six main structural proteins namely nucleoprotein (NP), phosphoprotein (P), matrix protein (N), fusion protein (F), haemagglutinin neuraminidase (HN) and large (L) proteins (Dey *et al*., 2014). The genome of NDV is approximately 15 kb. Based on the pathogenicity, NDV can be classified into three pathotypes namely velogenic (highly virulent), mesogenic (intermediate virulent) and lentogenic (low virulent). Among the factors for determining the virulence of NDV, fusion protein cleavage site (FPCS) has been characterized as a major determinant of NDV virulence (Peeters *et al*., 1999). Both mesogenic and lentogenic strains can be used for vaccination but mesogenic strains are used only in older birds above 6 weeks of age. Comparatively, mesogenic strains provide long term protection and have high immunogenicity whereas lentogenic strains require booster vaccination regularly. The frequently used strains of NDV for vaccination are Lasota, F that are lentogenic and Komarov and R2B are mesogenic in nature. The major viral surface glycoproteins, HN is also immunogenic and elicits neutralizing antibodies in the host during infection.

The advent of recombinant DNA technology in vaccinology has opened up new vistas in generating new vaccines by reverse genetics. Rescuing of virus from cloned cDNA refers to reverse genetics wherein desired mutations can be introduced in virus backbone or introduce new heterologous immunogenic genes in generating designer vaccines. The concept of reverse genetics in NDV model was proposed by two independent groups in 1999 (Peeters *et al*., 1999 and Römer-Oberdörfer *et al*., 1999). The various

reverse genetics system that followed in succession include those of Beaudette C (Krishnamurthy *et al*., 2000); Hitchner B1 (Nakaya *et al*., 2001); AF2240-I (Murulitharan *et al*., 2013); F (Dey *et al*., 2017); R2B (Chellappa *et al*., 2017). The system also helps researchers to develop avirulent viruses from virulent pathotypes by altering the fusion protein cleavage site, the primary virulent determinant of NDV (Xiao *et al*., 2012; Kim *et al*., 2017; Yadav *et al*., 2018).

Strategies for expression of immunogenic genes from NDV backbone

Given the modular nature of NDV genome and its propensity to carry foreign genes with maximum amount of RNA transcripts being produced from the 3' end, it is quite natural to introduce the foreign genes between NP and P genes. But that is not the case so. For efficient replication of the virus an optimum ratio of NP:P protein is essential and introduction of foreign genes in this junction would disturb the equilibrium of this ratio with decreased viral replication (Nagai, 1999). It has been proven by various studies that the optimal region of foreign gene expression is in fact the P and M junction.

The foreign genes are inserted as independent transcriptional units made up of gene end, intergenic region, gene start and Kozak sequences followed by the open reading frame of the gene of insert. Another strategy of expressing two foreign genes from a single mRNA transcript involves introduction of an internal ribosome entry site between the NDV and the foreign genes. In this scenario, during transcription, the two genes separated by the IRES sequence are transcribed into a single mRNA such that the first gene is translated using the default cap-dependent translational machinery while the translation of the downstream gene is cap-independent (Kieft, 2008). Yet another strategy of expressing two foreign genes involve the introduction of a 2A peptide between the foreign genes so that on maturation the 2A peptide cleaves the two foreign proteins.

Development of NDV-vectored bivalent vaccines for poultry

For many decades, the global poultry industry has used live attenuated or killed vaccines to control and prevent Newcastle disease. NDV vaccines can be combined with other poultry vaccines to control economically important poultry diseases. The production of NDV vaccines is highly cost-effective since they grow to very high titers in embryonated chicken eggs and in cell culture. This makes NDV vaccine strains attractive as viral vectors for the development of polyvalent vaccines against pathogens that could devastate the poultry industry. A recombinant NDV expressing the VP2 protein of infectious bursal disease virus (IBDV) provided dual protection against NDV and IBDV in chickens (Huang *et al*., 2004; Dey *et al*., 2017). NDV-vectored vaccines expressing the HA protein of H5N1 HPAIV (Park *et al*., 2006; Ge *et al*., 2007; Römer-Oberdörfer *et al*., 2008) have been shown to provide dual protection against NDV and HPAIV in chickens. Similarly bivalent vaccines against NDV and infectious bronchitis virus (Toro *et al*., 2014; Shirvani *et al*., 2018); NDV and infectious laryngotracheitis virus (Basavarajappa *et al*., 2014; Zhao *et al*., 2014), NDV and avian metapneumovirus (Hu *et al*., 2011), NDV and avian reovirus (Saikia *et al*., 2019), NDV and chicken anaemia virus (Chellappa *et al*., 2021) have also been reported. Further, recombinant NDV R2B strain has also been shown to induce oncolytic property in cancer cell lines *in vitro* (Ramamurthy *et al*., 2021).

Development of NDV vectored vaccines for other animal species

NDV can be used as an effective vaccine vector in various animal species as given below:

Host	Disease	Immunogen	Backbone	Immunity
Monkeys	Severe acute respiratory syndrome	SARS-CoV S	Beaudette C	SARS-CoV S - specific
Camel	Middle east respiratory syndrome	MERS CoV S	LaSota	Neutralizing antibodies, CD8+ T cells
Cattle	Rift valley Fever	RVFV-Gn	LaSota	Neutralizing antibodies

Cattle	IBR	BHV-1gD	LaSota	Neutralizing antibodies IgG, IgA
Cattle	Bovine ephemeral fever	BEFV G	LaSota	Neutralizing antibodies CD4+, CD8+ cells
Dogs, Cats	Rabies	RVG	LaSota, R2B	Neutralizing antibodies
Horse	West Nile Virus	WNV PrM/E	LaSota	Neutralizing antibodies, CD4+, CD8+ cells
Mice	Acute pneumonia	RSV F	Hichner B1	Neutralizing IgG, CD8+ cells
Mice	Nipah encephalitis	NV G and F	LaSota	T and B cells, Neutralizing antibodies
Mice	Vesicular Stomatitis	VSV G	LaSota	Neutralizing antibodies
Mice	Viral gastroenteritis	NV VP1	LaSota/ Beaudette C	CD8+ cells, Neutralizing antibodies
Minks	Canine Distemper	CDV F and HN	LaSota	Neutralizing antibodies
Monkey	Ebola	EBOV GP	Beaudette C	CD8+ cells, IgA and IgG
Pigs	Nipah encephalitis	Nipah virus G and F	LaSota	Neutralizing antibodies, T and B Cells
Monkeys	Parainfluenza	HPIV3 HN	Beaudette C	Neutralizing antibodies
Guinea Pigs	AIDS	HIV gp160	LaSota	Neutralizing antibodies

Unique attributes of NDV as a viral vector

NDV has a number of characteristics that make it an attractive candidate as a vaccine vector for both human and animal uses. A wide range of NDV strains exist that can be used as vectors. NDV replicates efficiently in embryonated chicken eggs, cell culture and in the respiratory tract of avian and non-avian species and induces strong local and systemic immune responses. Therefore, it will also induce a strong immune response to the foreign antigen. NDV does not integrate into host cell genome. Recombination involving NDV is extremely rare. NDV has modular genome that facilitates genetic manipulation. A number of strategies exist for attenuating NDV by reverse genetics. The level of expression of a foreign gene can be modified by altering its position in the gene order. NDV infects via the intranasal route and therefore induces both local and systemic immune responses. Local immunity is particularly important since most pathogens enter through the respiratory tract.

Conclusion

For several decades, NDV was only known as a poultry pathogen with some potential to treat human cancer. However, with the discovery of reverse genetics, so many other prospects of the virus have been unearthed. Today, the virus is easily programmable into a protective bivalent vaccine against highly virulent NDV and other economically important poultry diseases. The virus can also be manipulated to generate rationally designed vaccines against several emerging infectious diseases of various domestic animals. Furthermore, the virus has demonstrated the potential to not only deliver vaccine antigens against fatal human diseases, but also serve as an improved oncolytic agent against a variety of human cancers. Thus, the impacts of engineered NDV in modern vaccinology are enormous. Given its simple genome, efficient replication, host restriction, and non-pathogenicity in most mammals, NDV is likely to be the vector of choice against many other emerging diseases of man and domestic animals.

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Lead paper - 3

New generation vaccines for economically important poultry viral diseases

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Abstract

In India, poultry production has been increasing leaps and bounds and its growth is curtailed due to the economically important poultry viral diseases, with significant economic and welfare consequences. Conventional vaccines have narrow protection range, virulence reversion (live attenuated), short-term immunity and presence of maternally derived antibodies (MDA) drive the need for alternatives. Moreover, these vaccines fail to protect against rapidly evolving variants of endemic poultry pathogens thereby posing a huge threat to Indian poultry sector. In this context, new generation vaccines hold immense potential, produced through genetic engineering and recombinant DNA technology. It includes recombinant viral vectored vaccines, sub-unit vaccines or virus like particles as vaccines, DNA vaccines, etc. These vaccines offer broader protection, could work in presence of MDA, have enhanced safety and efficacy, improved stability and ability to protect against emerging poultry pathogens. The new generation vaccines have the ability to differentiate between infected and vaccinated birds (DIVA strategy) and fusion of potential antigens could create chimeric or multivalent vaccines like HVT-ND, which offer protection against both Marek's disease and ND. Many recombinant vaccines like VAXXITEK, INNOVAX, VECTORMUNE are used against various economically important poultry viral diseases such as Marek's disease, ND, ILT, IB, IBD globally. India still lacks indigenous recombinant vaccines for commercial use.

Introduction

Poultry sector contributes 1% to National GDP in Livestock 14% GDP. At present India has the distinction of being the third largest egg producer in the world and secured eighth position in meat production. Poultry farming is a thriving industry in the present scenario. It has grown leaps and bounds from the time of being backyard farming to a burgeoning and lucrative business in many countries of the world. As a cheaper and nutritious source of protein, poultry eggs and meat are becoming affordable and easily available commodity for most of the global population. With an ever-growing demand from the industry, poultry production in many countries including India has become intensified. This has led to the occurrence of disease outbreaks and introduction of previously unknown pathogens or prevalence of disease syndromes with multi-factorial etiologies. The best example would be the emergence of very virulent strain of Infectious bursal disease virus (vvIBDV) in the late 1980s and the virus is continuously evolving in the field with changes in antigenicity and virulence. As we live in an era of technological advancement in science, the traditional methods of detection and treatment of diseases are undergoing phenomenal changes.

The ICAR-Indian Veterinary Research Institute (IVRI) is one of the premier national institutions in the field of veterinary and animal sciences in the world. The Institute, the largest of its kind of whole of South East Asia, is widely known for its impressive contributions to all aspects of livestock and poultry health. Our laboratory for the past 20 years is working with the aim towards generation of recombinant DNA based diagnostics and vaccines for the economically important poultry viral diseases which are prevalent in our country.

Recombinant vaccines offer an alternative approach to animal disease prevention, overcoming limitations of traditional vaccines. New-generation vaccines used in poultry are of different types like

attenuated recombinant vaccines where pathogenic organisms are genetically modified and made non-pathogenic but immunogenic to be used as vaccines. Vectored vaccines, using viruses or bacteria as carriers for target antigen, offer diverse delivery options and stimulate both humoral and cellular immunity. Subunit vaccines, utilizing purified antigen fragments usually with an adjuvant, further minimize the risks while maintaining strong immune responses. Further *in-silico* analysis even enables the creation of chimeric vaccines targeting multiple strains or pathogens.

The possible reasons for vaccination failures are:

1. Major challenge in induction of protective immune responses both humoral and cell mediated immune responses.
2. Administration of a sub-optimal dose of vaccine
3. Live attenuated vaccines are excellent inducers of T cell as well as antibody responses, but there is always a slight risk of reversion to a more aggressive phenotype. Moreover, these vaccines induce moderate to severe clinical signs and immunosuppression.
4. Non-infectious subunits of pathogens like the killed vaccines are poorly immunogenic and have to be formulated with immune-stimulating adjuvants. Many potent adjutants cause considerable side effects - toxicity of inflammation.

Sub-unit vaccines

Recombinant subunit vaccines are proven to be effective against pathogens. They provide protection without requiring the entire pathogen, minimizing vaccine side effects by presenting only immunogenic antigens to induce a sufficient immune response. Currently, the live attenuated or inactivated IBDV vaccines for example are used but live vaccines can revert back to virulence and may also not give full protection against the vvIBDV strain. Hence, "hot" vaccines with intermediate/intermediate plus strains are used for very virulent strain but could not provide complete protection and induce moderate to severe clinical signs and immunosuppression. The inactivated or killed viruses are usually given to birds in the pre-laying stage to induce higher levels of antibody production for at least 2 weeks. Humoral immunity plays an important role in the clearance of many viruses but rapid infiltration of T cells into the bursa and upregulation of various cell mediated immunity (CMI) related genes following infection would lead to the hypothesis that CMI responses are also crucial for protection as well as clearance of the virus.

We have achieved success in development of newer generation vaccines namely the Subviral particle based IBD vaccine showing complete protection and stimulating both the arms of the immune responses as well. Virus-like particles are one of the highly effective types of subunit vaccines that mimic the authentic structure of virus particles without containing the genetic material. The IBDV major capsid protein VP2 led to the formation of subviral particles (SVPs) in Saccharomyces cerevisiae that was structurally similar to IBDV (Dey *et al*., 2009). The SVPs, in addition to their ability to stimulate the B-cell mediated immune responses, were highly effective in stimulating CD4 proliferative response and cytotoxic T lymphocyte responses. Moreover, when compared to the commercial live attenuated and killed vaccines, the SVPs alone and in combination with adjuvant were much safer and did not show the presence of the virus in BF post challenge. The clinical trials for the SVP based IBD vaccine was carried out in the poultry vaccine manufacturing company Globion Pvt Ltd, Hyderabad. The recombinant vaccine first of its kind in the country has been transferred to the Indian industry and soon will be available in the market.

Besides, F and HN proteins of NDV (Lee et.al*.,* 2008) and S protein of IBV (Moore, 1997; Yuan *et al*, 2018) are some of the proteins used for the development of subunit vaccines against economical important viral pathogens. Various studies suggest that VLPs produced by incorporating VP2 proteins of IBDV, S, M, and E proteins of IBV, and F, HN, and M proteins of NDV trigger stronger immune responses, both

antibody and cell-mediated (Th1 and Th2), reduce viral shedding more effectively and could offer broader protection against different virus strains.

Genetically modified vaccines/ Recombinant attenuated vaccines/ Modified live vaccines

Through reverse genetics or *invitro* recombination it is possible to genetically modify the pathogenic organism into a non-pathogenic form without affecting its antigenicity. Targeting deletions, insertions, or site-specific mutations in those genes involved in virulence, replication, pathogenesis and/or host interactions will generate an attenuated microorganism that has less chance to revert back to virulence after bird passages. **Conventional vaccines can protect against mortality caused by field NDV strains, but to protect against egg drop resulting from contemporary field NDV strains, is the need of the hour.** Yadav *et al*. (2018) engineered a lentogenic strain from the mesogenic strain Mukteswar (R2B), enhancing its efficacy while maintaining its capacity to generate protective immunity akin to the original mesogenic strain. This modification enables the utilization of the engineered strain in primary vaccination programs. Hein *et al*. (2021) demonstrated that modified live vaccines can successfully boost immunity against infectious bursal disease after maternal antibody levels have declined.

Vectored vaccines

Vectored vaccines represent a promising approach that utilizes modified carriers, such as viruses or bacteria, to introduce specific genes or antigens from target pathogens into the host organism. Those viruses with well-established safety profile are considered as carrier, with large genome size so as to incorporate the foreign genes and should be stable, and do not show reversion to virulence. These types of vaccines stimulate both humoral and cellular immune responses, ensuring a robust defence against the targeted pathogen. Avian vectored vaccines, frequently bivalent, commonly employ large DNA viruses like attenuated Fowlpox virus (FPV) and herpes virus of turkey (HVT) due to the possibility of genetic manipulations, stability and capacity to integrate large foreign sequences. However, challenges such as interference by pre-existing immunity against the vector and maternal antibodies can limit their effectiveness, particularly in FPV-based vaccines. Recently, the focus has shifted towards HVT-based vectors for enhanced efficacy. Newcastle disease virus (NDV) has emerged as a valuable vector for developing vaccines against poultry viral diseases due to its ability to induce various immune responses and accommodate different vaccine candidates (Dey et.al. 2017, Saikia et.al. 2019, Chellappa et.al. 2021). They offer advantages such as diverse administration routes, inherent adjuvant properties, and potential safety benefits. However, challenges like interference by maternal antibodies and delayed onset of immunity remain areas of concern, driving ongoing research to improve vectored vaccines. Additionally, advancements in genome editing technologies have facilitated the development of multivalent viral vectored vaccines, opening new avenues for precise and tailored vaccine design. Several recombinant vaccines, including VAXXITEK, INNOVAX, VECTORMUNE, ULTIFEND, and TROVAC, are extensively employed worldwide to combat economically significant viral diseases in poultry, encompassing ailments like Marek's disease, Newcastle disease (ND), infectious laryngotracheitis (ILT), infectious bronchitis (IB), and infectious bursal disease (IBD).

Present status of recombinant vaccines in India

A recent trend in the country's poultry vaccine industry involves a shift towards multivalent vaccine formulations. These innovative vaccines offer a one-shot solution, protecting against multiple pathogens simultaneously. This streamlined approach not only simplifies immunization for farmers but also minimizes stress on birds. The first indigenous veterinary recombinant vaccine, SVP-Gumboro Vac against IBD is on the way to commercialization. VAXXITEK® HVT+IBD - recombinant vectored poultry vaccine introduced in 2021 by Boehringer Ingelheim Pvt. Ltd., offers lifelong protection against both IBD and MD with a single dose. The approved imported vectored vaccines include Innovax-ND, Vectormune ND, and Vaxxitek HVT+IBD+ND, each offering protection against specific diseases like ND and IBD through

innovative technology. Additionally, immune complex vaccines like Novamune and Nextmune have also received approval for active immunization against IBD in broiler chickens. Currently, India does not have any indigenous new-generation vaccines for poultry which needs to be addressed in the future.

Conclusion

The advancements in the poultry industry towards new-generation vaccines mark a transformative shift in disease prevention strategies. These vaccines, including modified live vaccines, subunit vaccines, and vectored vaccines, address critical limitations encountered with traditional vaccine methodologies. This shift indicates a promising future for new-generation vaccines, offering safer and more effective disease control strategies in poultry farming. Continued research and development efforts remain crucial to overcoming existing challenges and realizing the full potential of these innovative new-generation vaccine technologies.

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Lead Paper - 4

Insights Into Development of Inactivated Vaccine for Lumpy Skin Disease

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Lumpy skin disease (LSD), an infectious disease of cattle, is characterized by raised nodules on the skin. Lumpy skin disease virus (LSDV), the causative agent of LSD belongs to the genus *Capripoxvirus* (CaPV) in the family *Poxviridae* (Tuppurainen *et al.,* 2017). Although the morbidity rate of LSD is low, it has a considerable fatality rate. The nodular skin lesions are distributed on the skin of animals along with inflammation in internal organs,fever, poor growth, and lymphadenopathy. Mechanical transmission of LSDV between cattle occurs by hematophagous arthropod vectors such as mosquitoes and stable flies (Tuppurainen*et al.,* 2011). Direct contact between cattle or contacts through milking procedure were also reported as potential transmission modes.

LSD is listed as a notifiable disease by the World Organization for Animal Health (WOAH) (WOAH, 2018) that originated in Africa (Haig, 1957). LSD is an exotic disease to India, affecting cattle and buffalo in recent past. It was reported for the first time in India from five districts of Odisha state (Sudhakar *et al.,* 2020) during August 2019. Subsequently, LSD outbreaks were reported in more than fourteen states of India (Kumar *et al.,* 2021). The disease is diagnosed by conventional and real time Polymerase chain reaction (Ireland and Binepal, 1998; Lamian *et al.,* 2011). Timely diagnosis plays an important role in the control of the disease.

Processing of scab material

The scab samples from cattle were received from November 2020 to February 2021 from different parts of the Tamil Nadu state, India and stored in -20°C freezer until analysis. The samples were thawed at room temperature and ground into a suspension . The clarified homogenate was used for DNA extraction.

DNA Extraction

Total DNA was extracted from swab samples by EZ-10 Spin Column Genomic DNA Kit as per the instructions of the manufacturer .Briefly, 200ul of clear supernatant fluid was taken into a 1.5 ml of micro-centrifuge tube and then 0.6 ml of Lysis- Buffer-V Reagent was added into the tube. The mixture was vortexed vigorously for 30 seconds and incubated at 65°C for 10 min. The mixture was transferred into the EZ-10 spin column and Kept at room temperature (23-25°C) for 2 minutes. The EZ-10 spin column was centrifuged at 10,000 g for 1 minute after that the flow-through was discarded. Wash solution (0.5ml) was added to the spin column and spin column was centrifuged at 10,000 g for 1 minute after that the flow-through was discarded. Wash step was repeated one more time.The column was transferred to a new 1.5 ml micro-centrifuge tube, 30 µl of TE Buffer was added onto the centre of the column and kept at room temperature (23-25°C) for 2 minutes. Finally, the column was centrifuged at 10,000 g for 1 minute. The purified viral DNA was used for PCR and real time PCR. The purity and concentration of DNA in the extract was estimated using Nanodrop and the OD 260/280 was greater than 1.8 for all samples.

Polymerase chain reaction

The Forward primer, 5′-TTTCCTGATTTTTCTTACTAT-3′ and reverse primer, 5′- AAATTATATAC GTAAATAAC-3′ for the gene of viral attachment protein VP32 (OIE, 2018) was used. Reactions volumes

of 25 µl containing 100 pmols each of forward and reverse primers, 12.5 µl master mix, 3 µl of DNA template (contains 200 ng) and nuclease-free sterile double distilled water up to 25 µl. Negative and positive controls were included for each reaction. Amplification was done under the following conditions: Initial denaturation cycle at 95°C for 2 min, 35 cycles (denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min), followed by a final extension cycle at 72°C for 10 min. PCR products (5 µl) were separated on a 1% agarose gel at 90 V for 30 min and 100 bp DNA ladder was used as a size standard. The PCR products were visualized with a gel documentation system. Out of eight LSD suspected scab samples, seven scab samples (except sample6) showed the positive amplification of 192bp product size. The negative control sample (primers without DNA template) showed negative amplification specific for LSD

Real-time PCR

SYBR green based real-time PCR assay was performed. The primer sets developed from the gene of viral attachment protein gene, VP32 was used. Viral DNA (1µl) was used as template in a SYBR green based real time PCR assay. Briefly, each reaction was constituted with 12.5 uL of 2X SYBR green PCR master mix 1 µl each of forward and reverse primers, 1 µl of template and 9.5 µl of nuclease free water to a final volume of 25 µl. The reaction was run in a Biorad CFX 96 real time PCR detection system with the following cycle conditions; 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 50°C for 30 seconds and 60°C for 30 seconds, with a plate read at the end of each 60°C incubation, followed by a melt curve analysis from 50°C to 95°C in 0.5°C increments with continuous plate read. The threshold value (Ct) for each sample was automatically called by the CFX Maestro software version 1. The melt curve and melt peak for each sample was analysed to rule out erroneous amplifications. The threshold value (Ct) of ≤35 was considered as positive.

The real time amplification curve and melting peak data are presented in figure.1. The Ct value for the known positive sample was 19.87. The suspected samples Ct values vary from 19.14 to 23.01. The sample number six which was negative in conventional PCR, was positive in real time PCR

Sequencing

PCR amplicons were sequenced by Sanger dideoxy sequencing platform. The sequences were submitted tothe Genbank and accession numbers were allotted viz., MZ337603, MZ337604, MZ337605, MZ337606, MZ337607, MZ337608, MZ337609. Sequences of seven positive samples ((Accession numbers MZ337603, MZ337604, MZ337605, MZ337606, MZ337607, MZ337608, MZ337609) were analysed by BLASTN

revealing a highest percent identity of 99.13% (114/115 bases) for MZ337603 with that of MN418202.1, LSDV p32 gene partial sequence from Egypt. All the seven sequences were showing highest identity with LSDV sequence MN418202.1 from Egypt. Overall, the seven sequences had highest sequence identity with LSDV sequences than with sheep pox and goat pox p32 sequences. Notably all the seven LSDV sequences p32 sequences from this studyhad **G** in the "TTTTT**G**ATTTGA" motif amplified with the primers suggested by WOAH and designed by Ireland and Binepal (1998) (Fig - 2). Interestingly, upon closer analysis the "TTTTT**G**ATTTGA" was found in all LSDV sequences, however this differed in sheep pox and goat pox p32 sequences retrieved from Genbank, which had A in the same motif; "TTTTT**A**ATTTGA". Thus the presence of this single nucleotide polymorphism "G" in the seven sequences generated in this study confirms the identity as LSDV.

Phylogenetic analysis

Phylogenetic analysis was performed using partial p32 gene sequences obtained from this study and five other partial p32 gene sequences from the Genbank submitted from India. The sequences were aligned by Clustal W slow accurate method using MegAlign – DNASTAR software Version 10.1.0. Sequences producing significant alignments with the LSDV p32 sequences generated in this study (MZ337603 to MZ337609**)** were retrieved by performing BLASTN (optimized for highly similar sequences - megablast) against nr/nt collection. Multiple sequence alignment was visualized using the Multiple Sequence Alignment Viewer 1.20.0 tool at the NCBI website.

Phylogenetic analysis of seven partial p32 sequences generated in this study and five other partial p32 sequences (from Orissa state of India), together representing all the publicly available LSDV p32 sequences generated from India at the time when this manuscript was prepared, revealed distinct clustering of the sequences from the two states (Figure 4). In addition seven partial p32 sequences from Tamil Nadu, obtained from different locations with LSDV cases, showed a higher degree of variation compared to the five partial p32 sequences of LSDV from Orissa.

Research Analysis

LSD causes huge economic losses to the livestock by way of decreased milk production, damage of animal skin/hide, abortion and infertility in female cattle, temporary orpermanent sterility of bulls, death in rare cases and trade restrictions on animals and animal by-produces. Live attenuated vaccines are available for LSD in many countries, but not in India where the current outbreak is being recorded (WOAH, 2018). As LSD is genetically closely related to sheeppox and goatpox virus and share many epitopes, vaccines against sheeppox and goatpox with a higher dose level are warranted for emergency use in prophylaxis against LSD (Tuppurainen *et al.,* 2015). Where vaccines are not available and on the face of an outbreak, the disease is controlled by isolation and quarantine of the infected animals and symptomatic treatment. Early diagnosis of the disease is very important to control the disease in bovine population.

In the present study, tissue biopsy samples from cattle with clinical presentation suggestive of LSD were subjected to conventional gel-based PCR using the primers specific for the gene encoding viral attachment protein (VP32) of LSDV and positive samples were identified, Similar results were reported earlier in India (Sudhakar *et al.,* 2020; Kumar *et al.,* 2021).

In addition in the current study, SYBR green real- time PCR was developed using the primers specific for the gene encoding viral attachment protein (VP32) of LSDV which was used for the conventional PCR assay. This real time PCR assay can be used for a sensitive detection of LSD in tissue biopsy samples. The developed assay can be used as a rapid diagnostic tool. The assay has the advantage of lack of post–PCR work flow (AgarosePAGE) and has a higher sensitivity than conventional PCR. TaqMan probe based real- time PCR assay were developed earlier for detection of LSDV (Bowden *et al.,* 2008). However, in

the present study SYBR green real–time PCR assay was developed for detection of LSDV in the biopsy samples which is economical than TaqMan probe based real- time PCR assay.

The positive PCR samples in this study were sequenced and the molecular identity was confirmed as LSDV based on multiple sequence alignment with other highly similar sequences retrieved by BLASTN search. In this process a single nucleotide polymorphism that could differentiate LSDV genome from Sheep pox or Goat pox genomes (all three are members of the Genus Capripoxvirus) was identified. This SNP of **"G"** in the "TTTTT**G**ATTTGA" motif in the p32 gene of LSDV differs from **"A"** in the **G** in the

"TTTTT**A**ATTTGA" motif in the p32 gene of Sheep pox and Goatpox virus. Thus this SNP could be used for the specific diagnosis of LSDV in samples.

The developed SYBR green real-time PCR assay, based on OIE recommended primers specific for p32 gene, is a cost-effective, sensitive and rapid diagnosis assay. This assay can be routinely used for detection of LSDV in the tissue biopsy samples. Further the SNP reported in this study can be used to discriminate LSDV sequences from other members of the Genus Capripoxvirus.

Development of LSD inactivated vaccine

LSD live vaccines have been reported to cause a local inflammation, drop in milk production and sometimes a mild generalized disease with skin lesions called "Neethling disease" (Ben-Gera *et al.,* 2015; Abutarbush *et al.,* 2016). Live vaccines also present a potential risk of transmission of extraneous agents; however, they are the only ones which had been used so far to prevent LSD.

Advantage of inactivated vaccine

The use of inactivated sterile products has the advantage of safety, no replication, no spread in co-habitant unvaccinated animals and no reversion to virulence. In addition, no consequences for the country sanitary status, allowing in principle pursue of trade activities and animal movements.

Preparation of vaccine virus

Lumpy skin disease virus was used for vaccine preparation. Initially, LSD virus (LSDV) was propagated in MDBK cell line. The harvested virus was characterized by virus titration, PCR, qPCR and immunofluorescence assay.

Preparation of inactivated virus

For inactivation of virus, binary ethylenimine (BEI) was used as it is a standard procedure for inactivation of viruses for commercial inactivated vaccines, e.g., foot-and mouth disease virus vaccine. Successful inactivation was validated by three passages on Madin-Darby bovine kidney (MDBK) cells and pan-Capri pox real-time qPCR analyses.

Immunogenicity studies in laboratory animals

Inactivated LSD vaccine was prepared using inactivated LSDV antigen and Freund's adjuvant. Institutional Animal Ethics Committee approval has been obtained to carryout immunogenicity studies in laboratory animals like rabbit and guinea pigs. Rabbits and guinea pigs did not exhibit any allergic or untoward reactions after vaccination. The results suggested that the inactivated LSD vaccine is safe and immunogenic in laboratory animals.

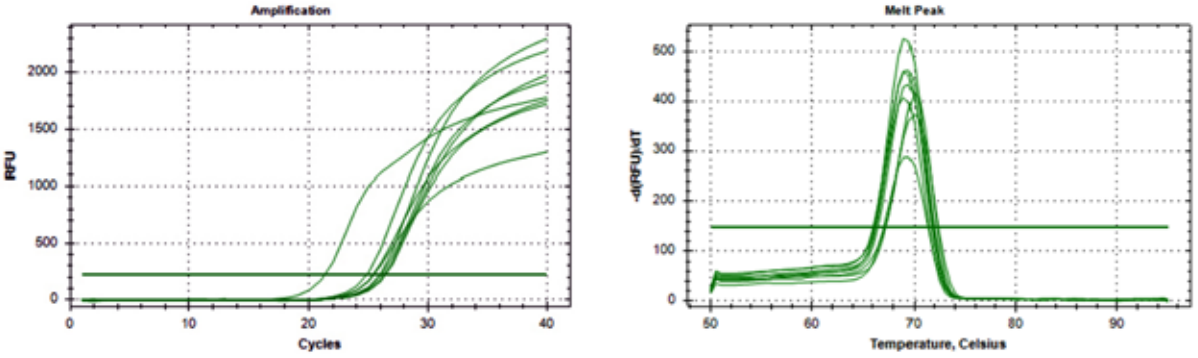
Inactivated LSD vaccine safety and immunogenicity studies in cattle

CPCSEA approval has been obtained to carry out Inactivated LSD vaccine safety and immunogenicity studies in cattle. Safety test was performed in cattle calves as per Indian Pharmacopeia and the vaccine is safe to use in cattle. Immunogenicity test was performed in cattle calves as per Indian Pharmacopeia and the vaccine is providing immunity up to 210 days post vaccination in cattle.

Conclusion

Lumpy skin disease (LSD) is an exotic disease to India, affecting cattle and buffalo. LSD originated in Africa, where many domesticated and wild ruminant species are affected. LSD was first recorded in India from five districts of Odisha state during August 2019. Subsequently, LSD outbreaks were reported in more than fourteen states of India. In view of the high risk of LSDV spreading to new territories, the European Food Safety Authority (EFSA) recommended the use of a safe and efficient inactivated or DIVA vaccine for prevention in disease-free countries (EFSA, 2015). LSD inactivated vaccine was successfully developed.

Fig 1: Real Time PCR detection of LSDV from tissue biopsies.
A. Amplification curve B.Melt peak profile



A **B**

Fig 2: Multiple sequence alignment view of the partial p32 LSDV sequence (MZ337603) with highly identical p32 sequences of LSDV, Sheep pox virus and Goat pox virus

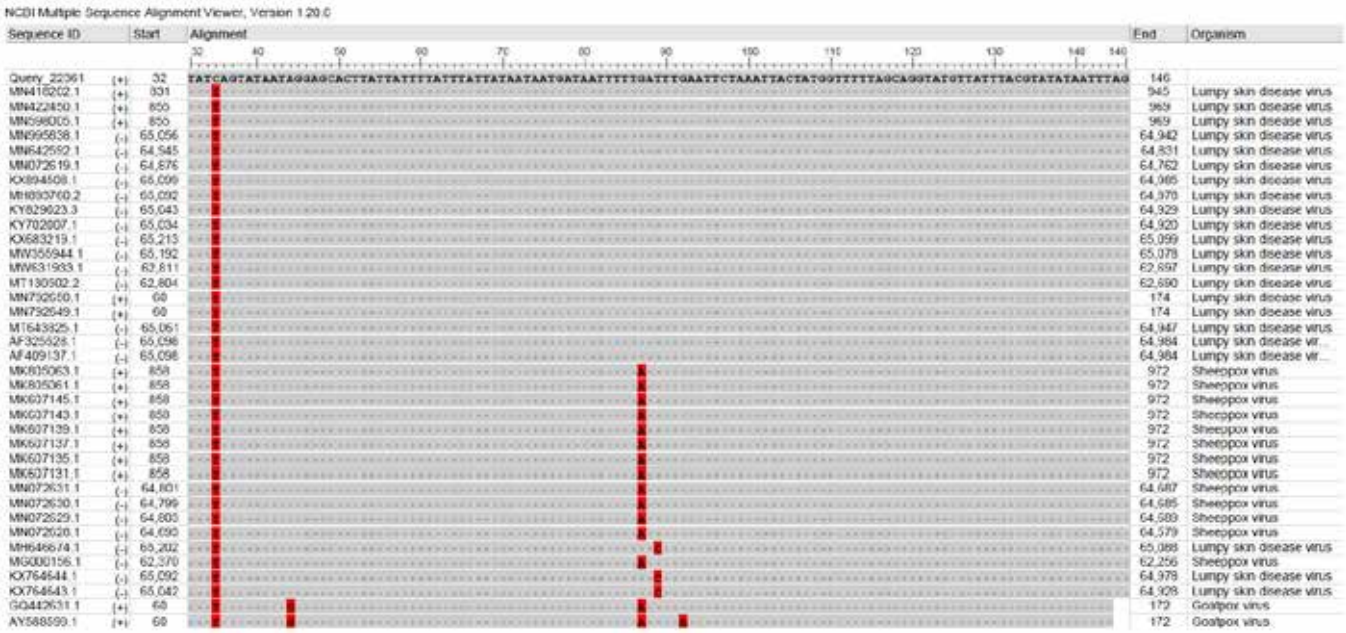
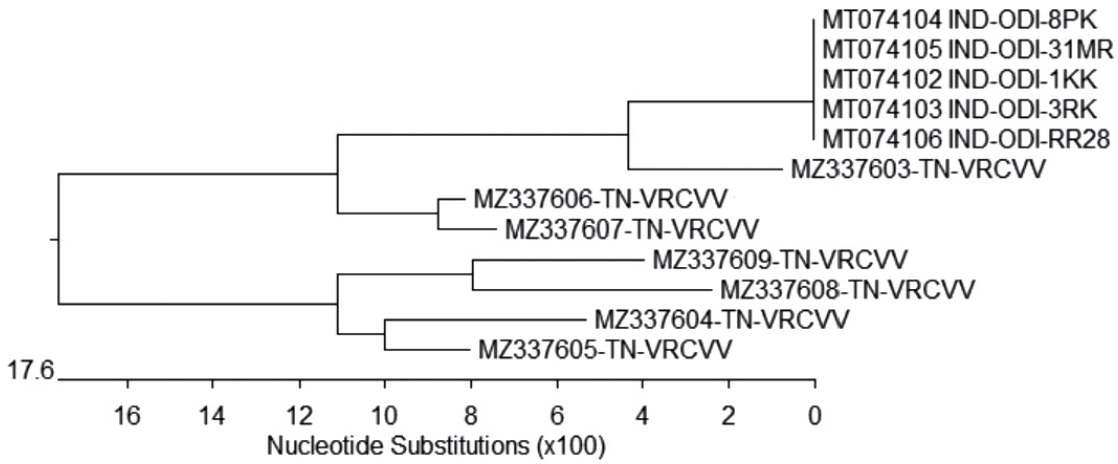


Figure 3: Phylogenetic analysis of partial p32 gene sequences from India. Nucleotide substitutions per 100 residues are indicated below the phylogenetic tree.



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ORAL PRESENTATION

OP_2.1: Cloning and Expression of Immunodomiant Lumpy Skin Disease Virus LSDV117 Recombinant Protein and its Diagnostic Evaluation

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Lumpy skin disease (LSD) is a transboundary pox viral disease primarily affecting cattle and Asian water buffalo. It is notifiable by World Organization for Animal Health (WOAH), resulting in both tangible and intangible losses to the farming community. Since its intrusion in India during 2019, outbreaks have been recorded throughout the country with limited research focus on diagnosis and control. Sero-surveillance based on recombinant proteins would be a better option for disease eradication programmes particularly in non-endemic countries. In this study, the immunogenic intracellular mature virion (IMV) protein (LSDV117) of LSDV was cloned and expressed in prokaryotic system using pRham™ N-His SUMO vector, purified under both native and denaturing conditions using Ni-NTA superflow cartridges and eluted in 300 mM imidazole. The predicted protein characteristics such as hydrophilicity plot, antigenic index, surface probability and predicted secondary structures revealed the immunogenic potential of this IMV protein. The expressed protein was analyzed in 10% SDS-PAGE and confirmed by western blotting. The diagnostic potential of the expressed LSDV117 recombinant protein (rA27L) was evaluated by optimizing an indirect ELISA and compared with the serum neutralization test (SNT) which is a gold standard test. The developed i-ELISA revealed a diagnostic specificity (DSp) of 95.83% (95% confidence interval (CI), 90.1 – 97.2%) and diagnostic sensitivity (DSn) of 92.77% at an optimum serum dilution of 1:160 and 31.25 ng of rA27L antigen per well, with a percent positivity (PP) cut-off value of 32. By this developed i-ELISA, 81.02% of tested samples (n=491) were found positive out of 606 random samples tested for LSDV antibodies and hence it can be employed over recombinant core protein-based ELISA in routine serodiagnosis.

OP_2.2: Efficacy studies of D58 strains of Newcastle disease virus against other circulating genotypes in chicken

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Newcastle disease caused by *Avian orthoavulavirus -1* (also referred as Newcastle disease virus, NDV) is one of the economically important diseases affecting more than 250 species of poultry worldwide. The currently used vaccine candidates belonging to genotype I or II are the lentogenic strains used as live or inactivated vaccines. The recent past has witnessed an emergence of new NDV genotypes and other NDV variants gaining virulence. This study was thus aimed at studying cross protection offered by two genotype II vaccine strains (F and D58 strains) in comparison, against the circulating genotype (NDV-XIII) and variants of NDV (Pigeon Paramyxovirus, PPMV-1, genotype VI). Specific-pathogen free birds were used for the vaccination and challenge experiment in a BSL-3 facility. Serology by ELISA /HI and virus shedding by PCR were used to assess the antibody titres and virus excretion respectively. Both the vaccine strains elicited a robust humoral immune response from a single vaccine dose. A progressive increase in serum antibody titres were observed in NDV/D58 and NDV/F vaccinated birds, with statistically significant

peak ELISA and HI titres on day 28 after vaccination. Upon challenge with virulent viruses (XIII and VI genotypes), a 100% cross-protection was observed in both the vaccinated groups against challenge with two virulent NDV-genotype XIII viruses and one PPMV- genotype VI virus.. A drop in the serum antibody titres 21 days after challenge, suggested that circulating antibodies effectively neutralized the challenge virus. The unvaccinated groups lacking antibodies to NDV, succumbed to challenge, resulting in neurological signs, virus shedding, diarrhoea and mortality. The absence of virus shedding of the virulent viruses in the vaccinated groups up to 21 days post challenge is clearly justified by the significant humoral immune response established by the lentogenic strains. However, differences in antibody titres elicited by the two vaccine strains before and after challenge were also observed. With the endemic scenario of NDV-XIII virus and the variant NDV reports in pigeons currently, these findings warrant the need for continuous seromonitoring and strict biosecurity measures in the poultry farms to curb the spread from pigeons to chicken.

OP_2.3: Generation of recombinant Virus-Like Particles of Porcine Circovirus 2 and its application in the development of indirect ELISA

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Porcine circoviruses (PCV) are small, non-enveloped viruses with single-stranded circular DNA genomes, belonging to the family *Circoviridae* and genus *Circovirus*. PCV includes PCV1, PCV2, PCV3 and PCV4, with PCV2 being a major pathogen causing postweaning multisystemic wasting syndrome (PMWS) and related diseases, significantly impacting swine production globally. PCV2 primarily spread through direct contact with infected pigs or contaminated environments, including semen and bodily secretions. The implementation of ELISA kits for PCV2 detection is crucial for effective disease management, control and prevention in the swine industry. The PCV capsid protein (ORF2) is capable of self-assembling into virus-like particles (VLPs). Recombinant PCV2 VLPs were generated using a bacterial expression clone carrying a codon-optimized ORF2. Following expression and purification, the yield of PCV2 rVLPs protein was found to be approximately 400 µg/L. The recombinant protein expression was confirmed by Western Blot (33 kDa) and the VLP formation was visualized by Transmission Electron Microscopy (TEM) which showed VLPs sized 17 nm to 25 nm. The ability of the rVLP to enter cells was validated by immunofluorescence assay in lab generated clonal PK15 cells devoid of PCV1. VLPs offer a versatile platform for various applications due to their ability to mimic the structure of native viruses while being non-infectious. They are pivotal in the development of vaccines, diagnostic tools and therapeutic agents. We optimized indirect ELISA (iELISA) using these rVLPs as antigens and tested 61 field sera samples. The cross-reactivity with other viral pathogens, such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Classical Swine Fever Virus (CSFV), was also evaluated. The in-house iELISA exhibited sensitivity and specificity of 77.5% and 80.9%, respectively, when compared to a commercial ELISA kit, and 72.7% and 78.9% when compared to Western Blot performed in house. Our work highlights the utility of recombinant VLPs generated using a bacterial expression system for developing iELISA suitable for large-scale epidemiological studies.

OP_2.4: Development of chemiluminescence ELISA for detecting nonstructural proteins during the foot and mouth disease virus vaccine manufacturing process

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The foot-and-mouth disease virus (FMDV), belonging to the genus *Aphthovirus* and family *Picornaviridae*, is the causative agent of foot-and-mouth disease, a highly contagious vesicular illness

that affects animals with cloven hooves. Vaccination followed by sero-surveillance are the major strategies followed to control FMD in enzootic areas. Vaccine quality depends upon the efficiency of method of purification to eliminate nonstructural proteins (NSPs). However, presence of NSPs in some vaccine preparations hinders DIVA strategy. Currently, the purity of the vaccine is evaluated through an *in vivo* approach. To minimize the use of animals and save time, *in vitro* purity testing is required. For this, an ELISA is developed using a monoclonal antibody raised against the 3B antigen (10H9D8). Checkerboard titration was employed to optimize various parameters. A stock of FMDV seed virus of serotypes A, O, and Asia 1 was produced separately, followed by large scale production using BHK 21 cell line. The produced virus was subjected to clarification, BEI inactivation, and purification via PEG precipitation and ultrafiltration methods individually. Subsequently, the purified virus harvest was examined for the presence of NSPs using the optimized kit and the commercially available kit. It was found that the PEG precipitation approach is more effective in elimination of NSPs from the virus harvest as compared to ultra-filtration. These findings were consistent with the earlier reports. Though in-house reagents performed well in the proof-of-concept study, the assay needs expensive plate modules and consumables to obtain consistent results.

OP_2.5: Biosecurity method of investigation of a suspected outbreak of anthrax in Salem, Tamil Nadu

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Anthrax is a bacterial zoonotic disease, caused by *Bacillus anthracis*, a rod-shaped, capsulated, spore-forming bacterium. Anthrax is endemic and several sporadic outbreaks have been reported in the state of Tamil Nadu. During, April 2022, a suspected outbreak of Anthrax was reported in Siruvachur village, Thalaivasal Talk, Salem Dt. Out of nine animals in a hamlet, four died within two days with signs of fever, bloat, and oozing of blood through the anal orifice. Blood smears were collected from dead animals and presumptively diagnosed by Polychrome methylene blue staining, revealing rod-shaped bacteria showing McFadyean reaction. The DNA was extracted from the other blood smears. The presence of virulence factors was detected by amplifying *lef* gene (385bp) and *capsular* gene (264bp) encoded in pXO1 and pXO2 plasmids respectively and chromosomal marker (Ba813) gene (152bp) by multiplex PCR, which is considered as a confirmative diagnosis. This biosecurity molecular method excludes the bio-risks on culture, isolation, identification of organisms and environmental contamination. A ring vaccination with the anthrax spore vaccine was carried out and the sporadic outbreak was contained.

OP_2.6: Generation of recombinant Newcastle disease virus strain R2B containing the S1 gene of avian infectious bronchitis virus by reverse genetics

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Respiratory viral infections cause serious problems in the poultry sector, leading to severe economic losses. Among these, Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) are the major respiratory poultry viral pathogens. Vaccines are crucial in combating poultry viral infections. Recombinant vaccines are an emerging technology that shows great promise in the poultry vaccine industry. A recombinant bivalent vectored vaccine was generated using the recombinant NDV strain R2B as a vector expressing the S1 immunogenic gene of IBV. The IBV-S1 sequence was constructed by generating a consensus sequence of the S1 gene from the prevalent circulating strains in North India. The constructed gene sequence was

synthesized in a pUC57 vector (pIBV-S1). pIBV-S1 was transformed, isolated, and screened for the presence of the IBV-S1 gene. The IBV-S1 gene fragment was cloned into the recombinant NDV strain R2B vector carrying the altered fusion protein cleavage site (FPCS). The cloned plasmid was isolated after transformation and screened for the presence of the insert by colony PCR and restriction digestion. The recombinant virus was generated in Vero cells by transfecting the cloned plasmid along with the support plasmids pNP, pP, and pL. RNA was isolated from the tissue culture of the 15th passage, and the virus was confirmed by RT-PCR and sequencing using NDV and IBV sequence-specific primers. Proteins of the viral genes were characterized from the infected tissue culture fluid by SDS-PAGE and western blotting. The generated recombinant virus will be tested as a bivalent vaccine candidate to study its immunogenicity and protective efficacy in specific pathogen-free (SPF) chickens.

OP_2.7: Isolation and Determination of Efficacy of Antimycoplasmal Agents against Avian Mycoplasma Species

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The production performance of the commercial broilers and layers are often challenged by numerous infectious hazards. Avian mycoplasmosis is caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important species which cause lifelong infection and are capable of horizontal as well as vertical transmission. The present investigation was undertaken to isolate MG and MS from desi, broiler breeders and commercial broilers and layer chickens and assessment of efficacy of antimycoplasmal agents against the isolates. Interestingly, *M. gallisepticum* (MG) could successfully be isolated only from the choanal cleft swabs of desi (1) and commercial broiler (4) birds against the fruitful isolation of *M. synoviae* (MS) from all the four types of birds under study showing respiratory signs, eggshell apex abnormalities and leg weakness in Namakkal, Krishnagiri and Tiruppur districts of Tamil Nadu, Karnataka and Andhra Pradesh and from the dead birds showing lesions suggestive of chronic respiratory disease. Upon successful isolation, the isolates were assessed for its sensitivity in a 96 well plate precoated with nine antimycoplasmal agents in a serial concentration viz., Tilmicosin, Tiamulin, Enrofloxacin, Doxycycline, Erythromycin, Lincomycin, Chlortetracycline, Tylvalosin tartrate, Tylosin tartrate. Tylvalosin tartrate (TVN) had the lowest MIC values followed by Tiamulin and Tylosin tartrate among the antimycoplasmal agent tested against all the *M. gallisepticum* field isolates. Similarly, Tylvalosin tartrate had the lowest MIC values followed by Tiamulin, Tylosin tartrate and Lincomycin among the antimycoplasmal agent tested against all the *M.synoviae* field isolates. The current study reports the successful isolation of *M. gallisepticum* (MG) and *M. synoviae* (MS) from chickens and first ever report on the assessment of efficacy of various antimycoplasmal agent in India.

OP_2.8: Comparison of Conventional PCR assay and SYBR Green Real-Time PCR assay for early detection of *Ornithobacterium rhinotracheale* from respiratory diseased poultry of Andhra Pradesh for sustainable production

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A total of 28 farms altogether, samples collected include 113 tracheal swabs, 79 nasal swabs, 104 oral swabs, 91 exudates of infraorbital sinuses, 52 heart blood swabs, 63 tracheal tissues, 63 liver tissues, 63 lung tissues, and 34 air sacs from 28 farms located in various districts of Andhra Pradesh . Molecular detection

of ORT was done by targeting the *16S r RNA* gene and *rpoB* gene and produced predicted amplicon sizes of 784bp and 538bp respectively. From 20 positive farms, 97, 49, 43, 28, 22,30, 37, 39, and 16 swabs of tracheal, oral, nasal, infraorbital sinus exudates, tissues of lung, liver, trachea, heart blood swabs and air sac tissues were positive with 85.84, 47.11, 54.43, 30.76, 34.92, 47.61, 58.73, 75 and 47.05% respectively. All the field samples were also screened by SYBR Green real-time PCR and the samples which were positive in conventional PCR were also positive in SYBR Green real-time PCR. The Ct values ranged between 12-24 for the *16S r RNA* gene and 15-30 for the *rpoB gene*. The Ct values inversely correlate with the conc. of DNA in the sample and vice versa. The Ct value 9.39 to 32.97 is found to be the optimum cut-off value for the *ORT* in real-time PCR. In the present study, the positive PCR *ORT* gene products (*16S r RNA and rpoB*) were sequenced (OR835801 and OR835802) and found 99.32-99.7% and 86.38- 98.05% homology with *O. rhinotracheale* strains of NCBI. The multiple sequence nucleotide analysis of the partial *16S rRNA* gene of the present *O.rhinotracheale* isolates revealed nucleotide substitutions. The isolate OR835801 showed 14 nucleotide substitution at T1A, C14A, T15C, 668C, A75G, G89A, T134C, A143G, A193T, T346C, G473A, C501T, A722T, A731G and OR835802 showed 13 substitutions T1A, C14A, T15C, 668C, A75G, G89A, T134C, A143G, A193T, G473A, C501T, A722C, A731G. The phylogenetic analysis revealed that our two strains were closely related to local Indian strains, Costa Rica and United Kingdom. The multiple sequence analysis of the published partial *rpoB* gene of the present *O. rhinotracheale* isolates nucleotide substitutions was revealed. The isolate seq R3 showed 6 substitutions at G190A, G232T, G268A, G271A, G410A, G454A and seq R6 showed 26 nucleotide substitutions at A5C, G21T, G28T, G34T, G55T, G64T, G66A, T68G, A189T, G190T, G223A, A231T, G232T, A243G, G244A, G268A, A311T, G328T, G370T, A407T, A406C, A420G, G427T, A432G, G454A, A475C. the phylogenetic analysis revealed that our two sequences were closely related to German strains. Concluded that early detection of ORT in common respiratory infections of poultry by molecular based methods helpful for implementation of control strategies to reduce the economic losses in poultry industry. This study concluded that the 71.42% incidence of the respiratory pathogen *O. rhinotracheale* was observed in all over Andhra Pradesh.

OP_2.09: Molecular Detection of Mycoplasma Respiratory Infections in Sheep and Goats

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The present research work was aimed to study the incidence of *Mycoplasma* in respiratory infections in sheep and goats. A total of 188 samples (44 nasal swabs ,49 lung tissues and 2 pleural fluids from sheep & 56 nasal swabs,33 lung tissues and 4 pleural fluids from goat) were collected from animals showing respiratory symptoms. DNA was extracted by using boiling method. All the samples were screened for the detection of genus *Mycoplasma* targeting

16S *r*RNA gene yielding 280 bp product size. The overall incidence of genus *Mycoplasma* was found to be 68.42% in sheep and 55.91% in goats. Genus *Mycoplasma* positive samples were screened for detection of *Mycoplasma mycoides* cluster targeting *glk* gene. Seven (6 lung tissues and one pleural fluid) samples collected from goats showed positive result and none of sheep samples turned out to be positive. On screening of seven *Mycoplasma mycoides* cluster positive samples 5(5.37%) for *Mccp*, 2 (2.15%) for *Mcc* and 2 (2.15%) for *Mmc* form goats were positive with the product sizes 316bp, 192bp and 194bp respectively. Other samples were screened for *Mycoplasma ovipneumoniae* and *Mycoplasma agalactiae* targeting 16S*r*RNA yielding 361 bp and 360 bp product and the incidence was 32.63% & 12.63% in sheep and17.2% &13.97% in goats respectively. The characteristic finding in present study was mixed infection of *Mccp* with both *Mcc* & *Mmc* noticed from different animals. Furthermore gene sequences was submitted for *Mccp*, *Mcc* and *M. ovipneumoniae* to the gene bank.

OP_2.10: Mycotic abortion in dogs- current perspective

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The infectious abortion is mainly caused by different types of microbes and causes great economic loss and public health significance. Mycotic abortion is an important reproductive problem of dogs all over the world. The 22 numbers of aborted dog's placenta, foetus and vaginal discharge form different breeds were collected from different parts of Tamil Nadu and subjected into culture isolation and identification by using specific media. 5 dog's aborted samples showed positive for klebsiella and staphylococcus and 7 dog's aborted samples showed *E.coli* and 12 dogs *Candida albicans* were isolated. The *Aspergillus fumigatus* were isolated from all the dog's aborted materials which are mostly distributed in the environment. Among the fungi, *Aspergillus fumigatus* is associated with most cases of abortions followed by zygomyectes. The blood samples and serum samples were collected from aborted dogs were subjected in to HPD and RBPT and DNA were extracted from aborted material subjected in to PCR. All the samples were negative for brucella in RBPT as well as by PCR. Fungal abortions in dogs have been recorded usually between 30-40 days of gestation particularly in summer month. The confinement of pregnant animals in humid, hot, old and unhygienic houses are recognized as predisposing factors for mycotic abortion. The transmission and epidemiology of disease are still inadequately studied. The direct microscopy and cultural isolation of the fungal agent in the clinical specimen still considered as the main stay of diagnosis. Currently, no treatment has been evolved for mycotic abortion. future studies on the pathogenesis and epidemiology of fungal abortion in dogs should be studied in future and application of molecular techniques for quick diagnosis of mycotic abortion in domestic animals should be attempted.

OP_2.11: Histopathological and Molecular characterization of avian reticuloendotheliosis virus in guinea fowl

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The present study was carried out to detect the causative agent of sudden death in guinea fowl flock maintained at organized poultry farm, Orathanadu. On post mortem examination, liver was enlarged and revealed multiple grayish areas. The spleen was enlarged and kidneys were pale and swollen. Breast muscle was very thin and the keel bone was prominent. The respective tissue samples from liver, kidney spleen were collected for histopathological examination and revealed lymphoid and reticuloendothelial cell in kidney, liver and spleen. PCR was used for detection as well as differentiation of avian neoplastic viruses such as Marek's disease virus, avian leukosis complex and reticuloendotheliosis virus using specific primer sets. The sample was found to be positive for avian reticuloendotheliosis virus and negative for Marek's disease virus and avian leukosis complex. The positive PCR amplicons were subjected to nucleotide sequencing for further confirmation of avian reticuloendotheliosis virus. The sequence analysis showed 99%homology with other published REV isolates available in the NCBI database. The present study reported the incidence of avian reticuloendotheliosis virus in guinea fowl flock based on gross as well as histopathological lesions and molecular methods.

OP_2.12: Expression of F1L, a vaccinia virus H3L transmembrane protein analogue of orf virus, and its successful purification as a diagnostic antigen

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Orf or contagious ecthyma is a highly contagious, zoonotic, and economically important global viral disease of small ruminants and is endemic in India. Vaccination of susceptible goats/sheep along with suitable recombinant protein-based serological assay will be useful in the control of the infection. In this study, the full-length and truncated versions of F1L encoding gene (ORF 059) of orf virus were cloned into pFasBac HT A vector, transformed in DH10Bac cells, and expressed in insect cells. The full-length and truncated recombinant F1L proteins were expressed as a 6xhistidine-tagged fusion protein for ease of purification by Ni-NTA affinity chromatography under denaturing conditions. A protein with ~ 40 kDa and ~35 kDa for full-length and truncated F1L protein, respectively were expressed and confirmed by SDS-PAGE and western blot. The protein reactivity evaluated by western blot analysis and indirect ELISA using ORFV hyperimmune serum was also found to be reactive. The results of the present study showed that the purified recombinant F1L protein can be used as a diagnostic antigen in sero-surveillance of ORFV infection in small ruminants. To the best of authors' knowledge, this is the first report on the expression of ORFV F1L in insect cells using a baculovirus vector and its successful purification to use as the potential diagnostic antigen in ELISA.

OP_2.13: Differentiating infected from vaccinated animals (DIVA) Nested polymerase chain reaction assay for detection of Marek's disease virus in chickens

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Poultry industry is one of the prime agricultural sectors that not only contributes to Global economy but also supports livelihood sustainability of the poultry farmers. However, this sector often faces severe economic losses due to various infectious diseases. Marek's disease (MD) is one of the common viral diseases of poultry, caused by Marek's Disease Virus (MDV). In spite of routine vaccination, events of vaccine failure as well as disease outbreaks are often seen in the field conditions. Several methods including polymerase chain reaction (PCR) based techniques are available for diagnosis of MD. Nested PCR (nPCR) is one of the highly sensitive and specific techniques used for disease diagnosis. Present study reports a highly sensitive *glycoprotein E* (*gE*) gene based nPCR for MDV detection. The outer set of primers amplified 567 base pair (bp) while inner set of primers amplified 230 bp of the of the MDV genome. The minimum detection limit was 17.6 picogram of genomic material in clinical sample. It was also shown as highly specific for the detection of field MDV and didn't amplify one of the commercial vaccine strains, mostly used for vaccination against MD in chickens *i.e.,* Turkey Herpes virus (HVT) FC 126 strain. It also didn't amplify the fowl pox virus (FPV) genome. The applicability of the technique was assessed with the field tissue (liver) samples (n=22), comprising of eleven (n=11) samples collected during post-mortem examination of birds suspected of MD and eleven (n=11) samples from apparently healthy birds collected

from commercial retail poultry outlets. It was found positive in eight out of eleven clinical samples in nPCR; while, all samples from healthy birds were tested negative. The developed assay was found highly sensitive and specific. The developed nPCR technique could be used for diagnosis of MD infection caused by the field strains in the vaccinated as well as non-vaccinated poultry flocks.

OP_2.14: Comparative evaluation of Infectious bursal disease immune-complex and live vaccine responses in SPF chicks

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Infectious bursal disease (IBD) significantly impacts the poultry industry due to its immunosuppressive effects and the resilience of the causative agent, Infectious bursal disease virus (IBDV), a double-stranded RNA virus from the *Birnaviridae* family. Conventional live attenuated and inactivated vaccines, novel formulation like immune-complex and next-generation vaccines like HVT vectored IBD vaccines are employed as preventive measures against IBD. Objective of this study is to evaluate the responses of SPF chicks to Infectious bursal disease immune-complex vaccines (IBD-Icxs) in comparison to a conventional live attenuated vaccine. Total sixty, day-old SPF chicks divided into four groups of 15 were used; first two groups receiving one of the two commercial IBD-Icx vaccines (Icx-1 and Icx-2), third group with live attenuated vaccine (Livevac), and fourth one with no vaccine (control). first three groups were vaccinated with respective vaccines on day 1, followed by evaluation of antibody titre in serum, viral load, apoptosis and pathological changes in tissues on 14, 28 and 42 days post vaccination (dpv). Serum antibody titers assessed by indirect ELISA kit at 14, 28, and 42 days post-vaccination (dpv) demonstrated significantly higher antibody titers in both Icx vaccinated groups compared to the Livevac. Viral load assessments by RT-qPCR revealed significantly higher copy number of IBD virus in the bursal tissue of Livevac group at 14 and 28 dpv, whereas viral load in spleen remained non-significant among the vaccinated groups. In the Icx groups, gene expression of apoptosis marker, caspase-3 in bursal tissue showed earlier elevation, indicating a more immediate response than that of Livevac. Results indicated the effect of Icx vaccines in eliciting stronger immune responses, however bursal atrophy was found to be more severe in Icx group. Our results indicate that IBD-Icx vaccines generated an earlier and stronger antibody response in chicks with a lower antigenic dose and viral load in the bursa compared to live vaccines, however the more pronounced inflammatory responses elicited in bursa should be taken care during vaccine development.

OP_2.15: Molecular investigation of Torque teno sus virus (TTSuV) in southern Indian domestic pigs

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Torque teno sus virus (TTSuV) is a ubiquitous single-stranded DNA virus identified in both animals and humans with unexplored clinical significance. As of now, TTSuV1 and TTSuV2 are two genetically distinct species documented with subtypes within each species. TTSuV may act as a primary pathogen and can be linked with respiratory, hepatic, and nephritic lesions in pigs and humans. The infection can also occur as a co-infection and potentiate the effects of other diseases. TTSuVs spread by both vertical as well as horizontal routes and pose a critical threat to swine husbandry. These viruses are now receiving more attention due to the latest results on their association with other viral diseases globally. TTSuVs have been circulating unnoticed in pigs for a long time and their prevalence is not explored in Tamil Nadu and nearby states. The primary objective of this study is to identify and characterize TTSuV incidence in commercial pigs from southern states of India. This study examined 100 field samples comprising of serum (n=50) and post-mortem tissues (n=50) collected from both suspected and diseased pigs from four southern states of India. All samples were screened for TTSuV1 and TTSuV2 by a specific PCR assay targeting conserved untranslated region (UTR) gene. PCR screening of all 100 samples revealed 15% (n=15) positivity for TTSuV1 genome, 34 % (n=34) positivity for TTSuV2 genome, and 11 % (n=11) positivity for both TTSuV1 and TTSuV2. Each of TTSuV1 and TTSuV2 specific amplicons was selected, sequenced, and annotated for its specificity and molecular characterization. The BLAST analysis of the contig sequence of TTSuV1 showed more than 99.34% homology with published TTSuV1a sequence and TTSuV2 revealed more than 94.05 % homology with published TTSuV2a sequences. Both the TTSuV1 and TTSuV2 partial UTR contig sequences obtained in this study were submitted to GenBank and accession numbers PP708691 and PP708692 respectively were obtained. The present study reports the first molecular evidence of TTSuV1a and TTSuVk2a in domestic swine of Tamil Nadu.

OP_2.16: Bulk production, concentration and purification of baculovirus expressed stabilized Virus Like Particles of Foot and Mouth Disease Virus for prophylactic and diagnostic use

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The Virus Like Particles (VLPs) have same epitopes as the native virus and offers a great alternative to conventional vaccines due to lack of genome along with eliciting both arms of immunity. The thermostabilized Foot and Mouth Disease VLP based antigen with inherent DIVA compatibility requires minimum biosafety and cold chain maintenance for production and transit process. It can potentially replace conventional virus handling during antigen production for prophylactic as well as diagnostic use. Preparation of Baculovirus expressed VLPs for vaccine formulation can be achieved by bulk production of VLPs in insect cell culture like Tn5 and Sf9 grown in shaker incubator at standard conditions followed by concentration of the harvested cell culture supernatant and purification by discontinuous sucrose density gradient with nine different gradients giving high yield. We have standardized the bulk production and purification of VLPs in refrigerated shaker incubator maintaining a temperature of 26°C and simplified sucrose density gradient purification, which could be used as immunogen or antigen for prophylactic and diagnostic use.

OP_2.17: Native antigen-based indirect-ELISA for detection of antibodies against *Clostridium chauvoei* in cattle

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Black Quarter (BQ) or Blackleg' caused by *Clostridium chauvoei* is a notable Clostridial infection. This disease is the second most reported bacterial bovine disease annually, following Haemorrhagic septicaemia (HS). *Clostridium chauvoei*, a Gram-positive, histotoxic, anaerobic organism forming spores, poses significant challenges for eradication due to its resilience in various environments and varying susceptibility to antibiotics and disinfectants. Vaccination remains a crucial preventive measure to control the spread of infectious organisms. Hence, the present study focused on developing a quantitative indirect enzyme-linked immunosorbent assay (ELISA) using the native whole cell/flagellar antigen of *C. chauvoei* to investigate the immunogenicity of blackleg vaccine. A checkerboard titration was done using BQ hyperimmune raised sera, convalescent sera, and negative sera. The concentrations of native flagellar and whole-cell antigen (160ng / well), sample serum (1:100) and goat anti-bovine immunoglobulin G labeled with horseradish peroxidase (1:10,000) were optimal for the assay. Screening of anti-*C. Chauvoei-specific* antibodies using field sera samples from bovines would aid in assessing the post-BQ vaccine titer among BQ vaccinated/susceptible bovines in endemic states.

OP_2.18: *In-vitro* Characterisation and Preliminary Stability Assessment of Novel mRNA Vaccine Constructs Expressing Major Antigenic Genes of Lumpy Skin Disease Virus

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The development of a mRNA vaccine requires precise processes to ensure a stable and effective delivery for the induction of the desired immune response. In this study, we concentrated on two critical requirements for the mRNA vaccine namely i) the stability of the synthetic mRNA upon delivery into cells and ii) the stability of the mRNA vaccine formulation under real-time and accelerated storage conditions. We designed a codon-optimized mRNA construct of the A33 (628 bp) and P32 (831 bp) genes of the Lumpy Skin Disease Virus (LSDV) to enable enhanced expression in murine and bovine cells, cloned into pUC19 and pEX plasmids respectively and confirmed by RE digestion and sequencing. *In-vitro* transcription using the Hyper Scribe™ All-in-One mRNA Synthesis System resulted in mRNA concentration of 530 ± 15ng/µL and 345 ± 20ng/µL of purified A33 and P32 mRNA respectively. The transcribed mRNA was reverse transcribed with mRNA-specific reverse primers and the amplicon size 187bp and 190bp confirmed the A33 and P32 mRNA respectively. LSDV A33 and P32 protein translation and cytoplasmic localization in MDBK cells transfected with mRNA constructs were confirmed by immunofluorescence using LSDV convalescent sera. The mRNA transcripts could be detected by gene-specific RT-PCR post-transfection into MDBK cells until 120 hrs (time point studied). The mRNA transcripts were encapsulated with cationic lipid nanoparticles (LNPs) to prepare the vaccine formulation, lyophilized, and stored at 4°C and 25°C. The A33 & P32 mRNA-LNP formulation stored at 4°C and 25°C has been ascertained for its stability up to 3 weeks by gene-specific RT-PCR post-transfection of MDBK cells. Studies are being undertaken to modify the formulation and the lyophilization protocol to ascertain the longer stability of the mRNA vaccine at 4°C.

OP_2.19: *Peste des petits ruminants* Marker Vaccine: From Design to Development

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Peste des petits ruminants (PPR) is lethal viral disease of sheep and goats causing estimated annual losses of about 4800 crores to India. In line with Rinderpest eradication, FAO and WOAH aim for global PPR eradication by 2030. Our lab has earlier developed indigenous PPRV/Sungri/96 vaccine and monoclonal antibody-based diagnostics with which the ongoing PPR control program of India was launched. To achieve disease-free status while continuing vaccination, it is essential to develop marker vaccines and assays for differentiating Infected from Vaccinated Animals (DIVA) and the same has been addressed here. We started with establishing a reverse genetics (RG) system for Sungri/96 and successfully rescued virus with identical growth and antigenic properties of the parent virus. For quick and sensitive tracking of genetic manipulations in PPRV, the cDNA clone was engineered as a vaccine vector and green fluorescence protein-based tracking system was embedded in the virus. Following this, multiple strategies were attempted for negative marker vaccine development, out of which targeted epitope knock out yielded success in recovery of PPR virus with the desired antigenic changes while still retaining the same growth characteristics and production scalability of the parent Sungri/96 virus. For companion DIVA assays, selected epitopes were tethered and expressed through baculovirus system and monospecific sera were raised against them. By optimising battery of ELISAs with the generated biologicals we successfully developed differentiation assays for both antigen and antibodies.

Lyophilized marker vaccine was prepared and its safety and efficacy was evaluated in sheep and goats as per Indian Pharmacopeia standards. All vaccinated animals effectively sero-converted and possessed PPRV neutralizing antibodies by two weeks while recording 100% protection upon challenge with virulent PPRV/Izatnagar/96 virus. On the other hand, all the control animals that were healthy post vaccination, succumbed to PPR infection upon challenge exhibiting characteristic signs, virus shedding and death with successful differentiation of infected from marker vaccinated animals using DIVA ELISA. Long term immunogenicity studies conducted in goats showed that a single administration of the marker vaccine provided sustained immunity for over three years.

The marker vaccine retains the all the safety, efficacy and long-term immunogenicity of the parent Sungri/96 strain while providing DIVA capabilities, significantly advancing efforts toward global PPR eradication by 2030.

OP_2.20: Development of cell culture-adapted live attenuated Goatpox virus vaccine against Lumpy Skin disease in cattle

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This study was designed to develop a cell culture adapted live attenuated goatpox vaccine using local isolate and to evaluate its safety profile. A total of six local goatpox virus isolates available in the virus

repository of the Department of Veterinary Microbiology, College of Veterinary Science, Khanapara, was revived in the Vero cell line and confirmed by PCR. The selected isolate yielding high titre was attenuated by continuous passaging in Vero cells up to passage 50 passage (P-50). The attenuated virus was subjected to both physicochemical and Molecular characterization. Further, the safety and potency of the prepared vaccine were tested in both homologous (goats) and heterologous (Calves) hosts inoculating different doses. The indigenous strain of goatpox (GTPV/AsKa/14) was successfully adapted and attenuated in Vero cells and the virus titre increased gradually as the passage levels were increased reaching a titre of 6.6 log10 TCID50/mL at P-50. The attenuated virus was found stable between pH 5.0 to 9.0 but was sensitive to pH 3.0 and 11.0 and 1% chloroform. Molecular characterization attenuated isolate at P-50 showed a 99.6% sequence similarity. Safety trial was conducted in two groups of homologous hosts (goats, n=6 in each group) inoculating with a recommended dose (3.0 $\log_{10}$ $TCID_{50}$) and 50 times the recommended dose (4.7 $\log_{10}$ $TCID_{50}$) respectively; similarly, two groups of heterologous hosts (Calves, n=6 in each group) inoculating with a recommended dose (4.0 $\log_{10}$ $TCID_{50}$) and 50 times of the recommended dose (5.7 $\log_{10}$ $TCID_{50}$) respectively. No significant signs of adverse reactions were recorded in the experimental goats and calves. Based on our findings we can conclude that the attenuated goatpox local isolate vaccine might be a potential and effective vaccine alternative to control LSD.

OP_2.21: A flow cytometry-based molecular detection of nucleic acid biomarkers for multiplexing of Zika and Rabies viral targets

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Design and development of ultrasensitive and highly specific bioassays form the core of precise and accurate molecular diagnostics of pathogens. Healthcare engineering has made rapid strides in the domain of nucleic acid-based detection, for both in situ tissue diagnostics and liquid biopsies. Towards the latter end, effective amplification of nucleic acid targets for detection of hypervariable viral strains is of great significance, given the challenges, diagnostic demands and prevalent gaps in their identification. An able readout platform to capture the amplicon signal and faithfully decode them to interpretable analytics is as important in diagnostics as the bioassay itself. In this context, we report a flow cytometry (FC)-based direct digital counting of DNA amplicons of Zika and Rabies gene targets, using padlock probes (PLP) and rolling circle amplification (RCA). PLP-RCA, an isothermal nucleic acid amplification test (NAAT), provides an unprecedented degree of multiplexing, specificity, versatility and amenability for compatible integration with diverse readout platforms (1). Protein-C synthetic oligonucleotide targets for Zika virus (2) and Nucleoprotein targets for Rabies virus were amplified using PLP-RCA, upon effective probe hybridization, ligation using T4 DNA Ligase and subsequent amplification using Phi-29 DNA Polymerase. The amplicons obtained were labelled using FITC-labelled detection oligonucleotide probes, that attaches to the repeated units of the concatemeric RCA products (RCPs) which measured close to a micrometer in size using dynamic light scattering. Various times of rolling and concentration of DNA targets were optimised on 3D microbeads for their capture. The RCPs were subjected to FC analysis (BD-FACS-Canto-Clinical-Flow-Cytometry-System), aptly exploiting the extended detection potential of FC from cellular to molecular levels. FC-coupled RCP analyses have already been reported in literature, including in situ and exosomal detection (3–5). However, the gap in multiplexing for parallel analyte detection with clinically relevant LoDs from biofluids would be the baseline for the current study. Distinguishable FC-profiles were obtained for the experimental parameters, demonstrating the platform's potential for NAAT-based diagnostics.

POSTER PRESENTATION

PP_2.1: Immunomodulatory effect of Phage Lysate vaccine against *P. multocida*

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Bacteriophage lysates of Gram-negative organisms consist of all the immunogenic components of the bacterial cells and have been shown to be of great value as vaccine candidates. The preparation was evaluated for its capability to induce cross-protective response. In the present study a standardized lysate of *P. multocida* was prepared using a lytic phage isolated locally against a pathogenic strain of *P. multocida*. The preparation was evaluated for its capability to induce cross-protective response against direct challenge in immunized mice and calf**.** Immune response trials in calf with *P. multocida* against plain lysate vaccinated group showed both homologous and heterologous protection. Assessment of antibody response by indirect haemagglutination test (IHA) towards *P. multocida* antigen reveals presence of protective antibody titer. Western blotting showed presence of immuno dominant epitope like 27 kDa, 35 kDa, 55 kDa and 70 kDa proteins detected in phage lysates. In calves, anti-P52 IHA titers were found significantly lower than that of anti-A:1 IHA titers throughout the period of investigations. The serum IHA antibody levels peaked on 35th DPI for both P52 and A:1 and slowly declined over the period up to180th day. However, the 180th DPI IHA titers were found equivalent to (for A:1) or more than the 21st DPI titers (for P52). In conclusion, the phage lysate preparation induced a cross protective response against H.S causing organisms of *P. multocida* and has shown great potential to be developed as commercial vaccine against Pasteurellosis.

PP_2.2: Detection of *Aliarcobacter* spp. in ruminants – An emerging threat

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Aliarcobacter is an emerging food borne pathogen and is a *Campylobacter* like organism causing bacteraemia, enteritis and diarrhoea in humans and animals. Occasionally, it causes reproductive diseases and mastitis in ruminants. In this study, a total of 262 rectal and ileal samples from cattle, sheep and goats were collected from slaughter house in Chennai. Isolation of bacteria was attempted from mucosal scrapping using *Campylobacter* selective medium with supplements, and incubated under microaerophilic conditions at 42°C. Suspected colonies which are transparent to pinkish coloured, round, convex and smooth were stained with dilute Carbol Fuchsin, revealed S, V, comma or spiral shaped organisms. PCR was performed using primers for 23S rRNA gene which is present in *Arcobacter/Aliarcobacter*, *Campylobacter* and *Helicobacter pylori*. 57 samples tested positive, of which six randomly selected DNA samples were subjected to 16S rRNA sequencing. Blast analysis indicated that the isolates belonged to the genus *Aliarcobacter,* predominantly *A. skirowii* and *A. cryaerophilus.* ABST for the isolates obtained showed resistance of the bacteria to Ampicillin (100%) and Nalidixic Acid (100%), but remained susceptible to other aminoglycosides and macrolides. This indicates that *Aliarcobacter* is prevalent in ruminants in Chennai, and abattoirs act as the potential source of spread to humans.

PP_2.3: Occurrence of *Thelazia rhodesii* infection in a cross- bred jersey cattle in Tamil Nadu, India

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Ocular thelaziasis is a vector-borne parasitic disease affecting the eyes of domestic/wild animals and humans caused by the spirurid nematode belonging to the genus *Thelazia*. A 3-year-old cross- bred jersey cattle was reported to Animal Disease Intelligence Unit, Tiruvannamalai with the history of Epiphora. The Clinical observations were half closed eyes, scratching of eyes on trees, wooden poles and on its own and being restless. Gross examination revealed six white thread like structures wriggling in the medial inner canthus of left eye. Two worms were wriggled out from the medial inner canthus of the left eye which was taken for identification. The collected worms were morphologically identified as *Thelazia rhodesii.* Ivermectin injection was given subcutaneously @ 200 µg/kg weight as single dose and complete cure was occurred 5 days after injection. Gentamycin eye drops was given for 5 days to prevent secondary infections and for quick recovery. From this study, it was concluded that single dose of ivermectin @ 200 µg/kg weight was effective to control ocular thelaziasis in cattle.

PP_2.4: Revolutionizing Lumpy Skin Disease diagnosis in cattle: A single-step SYBR green-based Real-Time PCR assay for absolute quantification and detection of virus

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Lumpy skin disease (LSD), a viral disease transmitted by vectors, poses substantial economic threats to bovine populations across the Indian subcontinent, urging the implementation of effective diagnostic and control strategies. This study streamlined SYBR green-based real-time PCR (qPCR) assay for the precise detection and quantification of Lumpy Skin Disease virus (LSDV) in clinical samples. Leveraging both conventional PCR and advanced qPCR techniques, the assay includes a precisely designed standard curve using specific GPCR amplicons (610 bp and 786 bp) to determine absolute quantification. This innovative approach promises enhanced accuracy in assessing LSDV prevalence and supporting targeted disease management efforts. In this study, we quantified the LSDV viral load in infected nodular tissues using two qPCR assays (Assay-I and II), revealing a virus copy number of 7.36±0.18 and 7.27±0.17 (log mean ± SE) respectively (n=37). The qPCR assays demonstrated high analytical sensitivity and precise quantification capabilities. These assays achieved impressive lower detection limits of 284 and 153 copies per microliter (µl), with corresponding threshold cycle (Ct) values of 25.75±0.27 and 32.10±0.64 (Mean Ct ±SE). The negative control samples had Ct values of greater than 35.00 (n=6). LSDV was successfully isolated in MDBK cell lines by the seventh passage, underscoring the potential for using MDBK cells instead of primary cells for viral isolation. In this context and against the backdrop of significant economic losses caused by LSDV in bovines, the SYBR green-based qPCR assay described in this study represents a substantial advancement in detecting and quantifying LSDV. It offers advantages over a traditional $TCID_{50}$/ml method by accurately determining viral titers in clinical samples. The qPCR assay is less expensive and provides rapid results, high sensitivity, and precise quantification, making it a superior alternative for assessing virus titers in vaccine production and quality control for mitigating significant economic threats to bovine populations.

PP_2.5: Molecular identification of *Rhipicephalus haemaphysaloides* from Sloth bear (*Melursus ursinus*) collected at The Nilgiris hills, Tamil Nadu

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Rhipicephalus haemaphysaloides is a widespread tick species in China and other South East Asian countries and transmits emerging zoonotic infections due to Rickettsial pathogens. Ticks were collected from a 7 years old male Sloth bear (*Melursus ursinus*) during post mortem at Katabetu forest range, The Nilgiris, Tamil Nadu. A total of 9 ticks were collected during post mortem in the month of July 2022. The ticks were morphologically identified under stereo zoom microscope and Scanning Electron Microscope as *Dermacentor auratus, Amblyomma integrum, Rhipicephalus haemaphysaloides* and *Rhipicephalus turanicus.* The PCR was employed for *Rhipicephalus haemaphysaloides* from Sloth bear (*Melursus ursinus*) using 16S primers (forward and reverse) rRNA subunit. The amplication of band was observed at 395 bp and molecularly confirmed as *Rhipicephalus haemaphysaloides* based on sequence analysis. The collected tick, *Rhipicephalus haemaphysaloides* from Sloth bear (*Melursus ursinus*) from the province of Tamil Nadu was assigned accession numbers as OP762024.

PP-2.6: Recombinant protective antigen (PA) based indirect-ELISA for the detection of antibodies against Anthrax in sheep serum samples

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Anthrax, a zoonotic bacterial disease of herbivores animals caused by the Gram-positive, spore-forming bacterium- *Bacillus anthracis.* The disease causes sudden death in affected animals including small ruminants. The bacterium-*B. anthracis* comprised of two virulence plasmids pXO1 and pXO2 that are responsible for the production of three exotoxins. Among exotoxins, Protective antigen (PA) plays a major role in pathogenesis along with lethal factor (LF) and edema factor (EF). Anthrax live spore vaccine is being effectively used immunize susceptible population in endemic areas. Post-vaccination monitoring the antibody titers in the immunized animals will provide insights into the effectiveness of the Anthrax control program. In the study, we used the purified recombinant protective antigen (rPA) produced using prokaryotic expression system for optimization of indirect-ELISA. The assay was optimized by checkerboard titration using rPA raised polyclonal sera, convalescent sera, and negative control sera. The concentrations of antigen, serum and conjugates were optimized. Further, assay was employed on randomly collected field sera samples from sheep to assess the immunoreactivity. The study warrants, large scale sero-surveillance of field sera samples in order to evaluate the antibody titers among vaccinated /non-vaccinated animals in anthrax endemic areas.

PP_2.7: Immuno-reactivity of recombinant flagellin protein of *Clostridium chauvoei,* a causative agent of Black quarter in bovines

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Black quarter (BQ)/black leg is highly fatal and the second most important reported bacterial disease causing death among ruminants in India. BQ is endemic in most of the geographical areas of India and is caused by *Clostridium chauvoei,* a Gram-positive and spore forming anaerobic bacterium. The disease is primarily diagnosed based on clinical signs, bacterial isolation and confirmation by molecular assays such as PCR and ELISA. The development of a highly sensitive and specific serological test is of paramount significance for the detection of antibodies against BQ. Flagellin, a subunit protein of flagellum, known to play a key role in providing motility to several bacteria including *Clostridium chauvoei* and also contribute for virulence and protective immunity. In this study, we describe the production of rFliA(C) protein of *Clostridium chauvoei* using prokaryotic expression system. Upon bioinformatics analysis based on sequence and structural features, a partial *fliA(C)* gene from *Clostridium chauvoei* was cloned in to pET28a vector and the recombinant mature protein with N- and C-terminal truncation, was over-expressed as a His-tagged fusion protein (~25 kDa) in *Escherichia coli*. The rFliA(C) protein was purified under non-denaturing condition by affinity chromatography using automated chromatography apparatus-AktaStart. The rFliA(C) specifically detected anti-flagellin antibodies in sera of rabbit and bovines in Western blot and iELISA format. Further, no cross reactivity was noted with antibodies against major bacterial and viral diseases endemic to bovines. The study warrants further evaluation and standardization of rFliA(C) based indirect-ELISA for sero-monitoring of bovine sera samples from endemic areas of India.

PP_2.8: Studies on the efficacy of Newcastle disease virus strain R2B expressing cap gene of Porcine Circovirus 2 (PCV2) vaccine candidates in mice

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The study evaluated the effectiveness of a recombinant Newcastle disease virus (NDV-R2B) vectored vaccine expressing the capsid (Cap) gene of porcine circovirus type 2 (PCV2) and the TMCT domain of the NDV F gene in BALB/c mice. Two vaccine candidates, rNDV-R2B-PCVcap-TMCT and rNDV-R2B-PCVcap, were compared with a commercial vaccine. The recombinant virus rNDV-R2B-PCVcapTMCT demonstrated genetic stability, and its growth kinetics showed that the insertion of the Cap gene did not significantly impact virus replication. Immunization studies indicated that both the recombinant viruses, and in particular rNDV-R2B-PCVcap-TMCT, elicited strong humoral and cell-mediated immune responses, surpassing those induced by the rNDV-R2B-PCVcap candidate. It was reflected with high PCV2-specific and neutralizing antibody levels, enhanced T cell proliferation, and balanced CD4+/CD8+ T cell responses. Cytokine analysis revealed that the recombinant vaccines led to elevated levels of pro-inflammatory

cytokines (IFN-γ, TNF-α, GM-CSF) and reduced levels of the regulatory cytokine IL-10, indicating a robust immune response. Upon PCV2d challenge, vaccinated groups, especially those receiving rNDV-R2B-PCVcap-TMCT, showed significantly lower viral loads in the lungs and lymph nodes, with no significant histopathological lesions compared to the empty vector (rNDV-R2B) and unvaccinated control groups. In conclusion, rNDV-R2B-PCVcap-TMCT emerged as the most effective vaccine candidate, inducing superior protective immunity against PCV2 infection in mice, outperforming the commercial vaccine, and showing promise as a potential candidate in controlling PCV2 infection.

PP_2.9: Investigation of Leptospirosis Outbreak in Swine: A Case Study from Mandya District, Karnataka

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In this study, a reported series of pig abortions in organized pig farms in Mandya district, Karnataka, suspected to be caused by *Leptospira,* was investigated to identify the cause and potential sources of infection for the outbreak that occurred between April 22 and May 2, 2024. Farm owners and workers were interviewed, and samples like serum, urine, vaginal swabs, tissue samples, water, soil and rodents were collected. The sera were tested using the Microscopic Agglutination Test (MAT) with 20 reference serovars representing 17 serogroups of *Leptospira*. A total of 60 DNA extracted from aborted pig tissues, rodent tissues, urine, soil, and water samples was subjected to pathogenic *LipL32*-gene-based PCR, with subsequent sequencing of selected ten samples targeting the *rpoB*, *LipL32*, and *LipL41* genes for species identification. Histopathological examination was conducted on the aborted tissues (kidney and liver=7) to identify lesions specific to leptospirosis. A total of 60 suitable samples were processed for *Leptospira* isolation using EMJH media. MAT results showed nine positive reactors, including two aborted, one pregnant and one lactating sow, and five young finisher pigs, with reactive titres ranging from 1:100 to 1:1600 with major reactive serogroups namely *Icterohaemorrhagiae* and *Canicola*. PCR results indicated that each of the seven aborted fetuses tested positive for pathogenic *Leptospira* in at least one of the samples, besides nine water samples. Further, sequencing identified *Leptospira interrogans* in the two aborted samples and *L. interrogans, L. meyeri*, and *L. biflexa* in the five water samples. Furthermore, histopathological examination of aborted tissues revealed diffuse cortical haemorrhages, tubular degeneration, inflammatory cell infiltration in the kidneys, and multifocal lymphocytic infiltration and hepatic cell degeneration in the liver. Five *Leptospira* isolates were successfully recovered from two placental (*L.interrogans*) and three water (*L.interrogans; L. meyeri, and L. biflexa)*. All rodent samples tested negative for both PCR and culture. The investigation confirmed the presence of pathogenic *Leptospira* infections in the affected pig farm with contributing factors like introduction of externally sourced mated sows, a new lot of purchased pigs, and contaminated water in the feeding area. These findings underscore the importance of regular screening for surveillance and diagnosis, as well as the implementation of stringent biosecurity measures to prevent further spread.

PP_2.10: Molecular and Morphological identification of *Cysticercus tenuicollis*, larval stage of *Taenia hydatigena* collected from goat

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Taenia hydatigena is a common worldwide parasite in wild carnivores, whereas its larval stage, *Cysticercus tenuicollis* in ruminants. This parasite occurs in the liver and cause hepatitis cysticercosis. The metacestode in liver from goat were collected from Perambur slaughterhouse, Chennai, Tamil Nadu during December 2023. The outer layer of the cyst was removed. Then the cystic fluid was collected from the cyst. Morphometric analysis of the hooks of *Cysticercus* was carried out to find the species of the metacestode. The metacestode was morphologically identified as *Cysticercus tenuicollis* under microscope. The DNA was isolated and PCR was employed for *C. tenuicollis* and observed 264 bp and 494 bp by using genus specific primers (CYS-F/ CYS-R) and species-specific primers (CYSTEN-F/CYSTEN-R) respectively. The phylogenetic analysis was employed using MEGA 11.0 software. The collected metacestode sample from a goat from Perambur slaughter house, the province of Chennai, Tamil Nadu was assigned accession numbers as PP510214 (genus-specific). The collected cysts sample from the liver of a goat, *(C. tenuicollis*) was assigned accession numbers as PP502426 (species specific). The Histopathological findings of the liver tissue showed multifocal mild infiltration of inflammatory cells, predominantly mature and degenerate neutrophils, with hepatocyte necrosis. Additionally, mild sinusoidal congestion and venule congestion were observed. These findings are suggestive of Hepatitis with the presence of a cyst.

PP_2.11: Molecular detection of *Clostridium piliforme* in rabbits

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Clostridium piliforme is an anaerobic, spore forming, obligate intracellular bacterial pathogen which causes Tyzzer disease in laboratory, wild, and domestic animals. *C. piliforme* has significant economic implications for laboratory and commercial animal facilities due to its impact on research integrity, increased veterinary and management costs, and the need for enhanced biosecurity measures. Addressing these challenges is crucial for maintaining the health of laboratory animal colonies and ensuring the success of scientific research. In the present study, 100 rabbit faecal samples were collected and nested PCR was performed to detect the presence of *C. piliforme*. The nested-PCR was carried out using *C. piliforme* specific primers and positive fecal samples yielded specific product size of 850 bp amplicon. *C. piliforme* was detected in all 5 rabbit farms and the overall prevalence of 40% was noticed in the present study. There was a statistically significant difference in the prevalence rate of *C. piliforme* in the farms studied in the present research investigation. Though more female animals (45.33%) were found to be infected with *C. piliforme,* it was not statistically significant. Similarly, more than 5 months old rabbits (42.05%) were commonly infected with *C. piliforme* than less than 5 months old (25%). However, the prevalence rate was not statistically significant. Rabbits' breed, weight, type of rearing and feeding did not influence the prevalence of *C. piliforme*. In conclusion, the presence *Clostridium piliforme* in rabbits could impact the health and research outcomes of these animals. Therefore, proper monitoring, preventive measures, and potential treatments should be considered to maintain the well-being of the animals and the integrity of research conducted with them.

PP_2.12: Detection of canine distemper virus by nucleic acid lateral flow assay (NALFA)

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Canine distemper virus (CDV) is responsible for a severe and often deadly disease affecting dogs and various other carnivores. Diagnosing canine distemper clinically is challenging due to its wide range of symptoms, which can be confused with other respiratory and gastrointestinal diseases in dogs. Thus, laboratory confirmation is necessary for suspected cases. Early detection can be achieved through PCR. This study was aimed to identify CDV in corneal swab samples from suspected dogs by Nucleic Acid Lateral Flow Assay (NALFA). A total of 65 swab samples were collected, and RNA was extracted using the Trizol method. The extracted RNA was then converted to cDNA using the iScript™ cDNA Synthesis Kit. Primers labeled to target the N gene of CDV were utilized in NALFA. A nitrocellulose membrane coated with avidin on the test line and an anti-mouse IgG antibody on the control line was employed for detection. Gold nanoparticles conjugated with anti-FITC monoclonal antibodies served as detector. The detection limit for the CDV vaccine was 1 ng using PCR and 10 ng using NALFA. The study indicates that PCR is more sensitive than NALFA. However, considering factors such as time, cost, equipment, manpower, and reduced chemical use, NALFA is a viable method for point-of-care testing.

PP_2.13: Development and Characterization of a Recombinant Adeno Associated Viral Vector (rAAV-A33)for Targeted Delivery and Expression of the A33 Gene of Lumpy Skin Disease Virus

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Lumpy skin disease virus (LSDV) a member of the *Capripoxvirus* genus responsible for severe economic losses in cattle due to decreased productivity and trade restrictions has resulted in significant outbreaks affecting the cattle population in various states of India in the last few years. Given the limited options available for effective prevention and control of the disease, innovative approaches are urgently needed to combat LSDV. In this study, we developed a recombinant Adeno-Associated Virus (rAAV) vector system to deliver and express the LSDV A33 gene, a key gene involved in the virus's pathogenesis and immune evasion. We employed the AAV pro-Helper Free System, to facilitate the production of AAV2 particles without needing a helper virus thereby enabling a more efficient and streamlined generation of viral particles. The full-length A33 gene of LSDV was cloned under the control of the strong, constitutive CMV promoter, flanked by inverted terminal repeats (ITRs), and the same was verified by sequencing. This recombinant AAV-A33 (rAAV-A33) plasmid was co-transfected into HEK293T cells with the pRC2-mi342 and pHelper vectors using the PEI linear transfection reagent. Following transfection, the cell lysates were treated with benzonase to ensure the presence of only encapsulated viral genomes, and the same was confirmed by PCR targeting the ITR (82bp) and A33 (591bp) to confirm the AAV particles and rAAV respectively. The rAAV-A33 was purified employing a chloroform extraction protocol and upon Transmission Electron Microscopy (TEM) analysis we observed small, spherical structures (of 20-25nm) with icosahedral symmetry and seen as uniform, discrete dots against a dark, negatively stained background. The presence of a biologically active rAAV-A33 virus to deliver the A33 transgene was confirmed by PCR

(targeting the ITR and A33) following infection of HeLa cells. These observations confirm the r-AAV-mediated delivery of the A33 gene of LSDV and currently in-vitro studies are being performed to assess the immunogenicity of this AAV-A33 virus. Further studies are being planned to confirm the utility of this AAV platform to develop a novel vaccine to control LSDV in livestock.

PP_2.14: Over-expression of recombinant protective Antigen (PA) domain fragments using prokaryotic expression system

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Anthrax, a zoonotic bacterial disease that primarily affects herbivore animals leading to sudden death, is caused by Gram-positive, spore-forming bacterium- *Bacillus anthracis.* Anthrax is endemic in several states of India especially southern and eastern states affecting herbivores domestic animals like cattle, buffalo, sheep and goat. The pathogenesis of anthrax is mainly driven by three exotoxins namely; protective antigen (PA), edema factor (EF) and lethal factor (LF), which are encoded by virulence plasmids pXO1 and pXO2. PA antigen is well defined by four domains and known to generate neutralizing antibodies in immunized animals. Currently, acapsular live spore vaccine is being used to immunize susceptible animals. In the study, we targeted to produce recombinant fragments PA antigens. The fragment genes encoding for N-terminal domain-1(PA20) and C-terminal domain-IV (PA4) were amplified and cloned in to prokaryotic expression vector (pET28a). Upon transformation in to *Escherichai coli* BL21(DE3) codon plus cells and chemical induction, the recombinant PA20 (~22 kDa) and PA4 (~19 kDa) domains along with N-terminal hexa histidine tags were over-expressed and same were confirmed by SDS-PAGE and Western blot. Further, bulk purification by affinity chromatography and evaluation of the antigenicity /immunogenicity of the recombinant PA fragment/domains in laboratory animal models would be advantageous to develop either subunit vaccine or diagnostic assay for anthrax in animals.

PP_2.15: Rapid and specific molecular diagnostic approach for detection of Porcine Parvovirus directly from field samples

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Porcine Parvovirus (PPV) infection is reported in many swine-raising countries worldwide and is associated with reproductive failure, including stillbirth, mummification, embryonic death, infertility (SMEDI), abortion, and neonatal death, particularly in gilts. In adult pigs, PPV is also associated with subclinical to mild infections. Molecular detection of PPV from field samples has significant applications in diagnosis, monitoring, and control measures. In this study, a total of 50 suspected porcine samples,

comprising post-mortem tissues (n=40) and serum (n=10) from different regions of Tamil Nadu and Karnataka, were analyzed for molecular detection of PPV through VP2 gene-based PCR directly from field samples without nucleic acid processing or extraction. Invitrogen platinum direct PCR universal master mix was used for direct screening of samples. Simultaneously, nucleic acid was extracted from all 50 samples using a commercial kit and subjected to PPV-specific VP2 gene-based conventional PCR. Results from both direct PCR and conventional PCR were compared to evaluate the specificity of PPV detection in field samples. Six field samples tested positive for the PPV genome by specific amplification of the 879 bp VP2 region using both direct and conventional PCR assays. This study demonstrates that molecular detection of PPV directly from field samples using a universal PCR master mix exhibited the same level of specificity as conventional PCR for detecting the PPV genome.

PP_2.16: A pilot study on the antiviral activity of ivermectin and nilavembu against Newcastle Disease Virus in Embryonated Chicken Egg

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Newcastle disease (ND) continues to pose significant challenges to the poultry industry around the world. In order to minimize the economic impact of ND, effective control strategies have been implemented, which includes improved vaccination programs, novel antiviral, biosecurity measures, and surveillance systems. However, threat associated with NDV remains constant in poultry sector due to vast genetic diversity with multiple genotypes and subtypes affecting a wide variety of poultry species. Research involving repurposing of existing drugs for its antiviral efficacy and development of cost effective novel antiviral with high safety are prime requirement to mitigate persistent challenges associated with NDV in the poultry sector. This pilot study aimed to explore the antiviral activity of ivermectin and nilavembu against NDV as an individual and combined candidate in chicken embryonated eggs through allantoic cavity route. This study evidenced that ivermectin completely inhibited haemagglutination (HA) activity whereas nilavembu treatment evidenced two-fold reductions in HA titre of NDV-Lasota vaccine strain when used as individual candidates. Evaluation of ivermectin and nilavembu in combined form evidenced only two-fold reduction in HA titre of NDV-Lasota vaccine strain as like that of individual nilavembu treatment. Activity of ivermectin against NDV has been affected when administered along with nilavembu but not vice versa. The viability and growth of embryo unaffected by both ivermectin and nilavembu treatments when used as individual and combined forms. This study shall be extrapolated against NDV virulent field strains both in-vitro and *in-vivo* studies for evaluating therapeutic antiviral efficacy of ivermectin and nilavembu as individual candidates.

PP_2.17: CRISPR/Cas12a based diagnosis of *Babesia gibsoni* and *Ehrlichia canis* infection in dogs using Lateral Flow Assay platforms

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Canine babesiosis and canine monocytic ehrlichiosis are two important tick-borne diseases in dogs caused by *Babesia gibsoni* and *Ehrlichia canis*, respectively. Recent advancements in the Clustered

Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas) systems have led to the development of highly specific, sensitive, and rapid nucleic acid detection tools, ideal for point-of-care diagnostics. This study aimed to evaluate the potential of CRISPR/Cas12a-based detection of *B. gibsoni* and *E. canis* DNA using a lateral flow assay (LFA) platform. Guide RNAs (gRNAs) targeting the *B. gibsoni 18S rRNA* gene and the *E. canis p43* antigen gene were designed and *in vitro* transcribed. The spacer regions of gRNAs were designed downstream to 5′-TTTV-3′ protospacer adjacent motif (PAM) sites within the target genes. PCR primers were designed to amplify short fragments of the target genes encompassing the PAM site and spacer sequences. Activity of the gRNAs was confirmed by agarose gel trans-cleavage assay using LbaCas12a-gRNA complex. The collateral cleavage activity of LbaCas12a was confirmed, and specificity was verified by the absence of cleavage in non-target amplicons. Using 5'Biotin---DIG 3' labelled ssDNA probes and gold nanoparticle-conjugated Goat anti-DIG IgG, the CRISPR/Cas12a-based LFA was optimized for specific detection of *B. gibsoni* and *E. canis* genome in blood samples by visual reading. Positive samples were identified by the absence of Cherry red colour formation on the test line of LFA strip. The limit of detection of the CRSIPR/LbCas12a-LFA assay was determined to be 100 nM for both gene fragments. Compared to nested PCR assay, the LFA showed a positive percent agreement of kappa value 47% (Moderate agreement) for *B. gibsoni* and kappa value 89% (Almost perfect agreement) for *E. canis*, for both pathogens. No cross-reactivity with related genomic samples were observed. These results suggest that the CRISPR/Cas12a-based LFA platform is a promising, rapid, and cost-effective point-of-care diagnostic tool for detecting *B. gibsoni* and *E. canis* in canine blood.

PP_2.18: Application of multiplex PCR for the detection of canine viral diarrhoea

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This study aimed to investigate the clinical epidemiology of canine viral diarrhoea and identify the viral pathogens associated with it using molecular techniques. The primary focus was on Canine parvovirus (CPV-2), Canine coronavirus (CCoV), and Canine adenovirus (CAV). A total of 50 rectal swabs collected from the clinical cases of dogs exhibiting foul-smelling diarrhoea with bloody diarrheal symptoms were screened using Multiplex PCR. Of these, 48% (24/50) tested positive for parvovirus infection, while neither CCoV nor CAV was detected in any samples. Out of 24 parvovirus-positive cases, 9 (37.5%) from vaccinated dogs and 15 (62.5%) from unvaccinated dogs. These findings suggest the alarming nature of the parvoviral infection in canines irrespective of the vaccination status. This also indicates the possible pathogenesis of multiple variants that are not being neutralized by the antibodies raised against the vaccine strain.

PP_2.19: Development of Monoclonal Antibody based antigen detection assay for *Anatid alphaherpesvirus 1* infection in ducks

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Duck Viral Enteritis (DVE)is contagious infection of the family anatidae that includes duck, swan and geese. The infection is caused by *Anatid alphaherpesvirus* 1(also known as Duck Viral Enteritis Virus (DVEV)that belongs to Alphaherpesvirinae subfamily of the Herpesviridae Genus *Mardivirus*. The disease is worldwide in distribution and live attenuated vaccines are available for prophylaxis. Efficient management

of DVE relies on the early detection of the virus and implementing control measures. Serological assays for DVE although used widely for prevalence studies, have limited potential to identify infection in vaccinated population. Considering this, we attempted to develop a monoclonal antibody based antigen detection assay for early detection of DVEV. A panel of monoclonal antibodies were developed in mice using purified DVEV antigen. The specific reactivity of these monoclonal antibodies was screened using recombinant immune-dominant glycoprotein G (gG) of DVEV. The recombinant glycoprotein G was expressed in bacterial expression system as a truncated protein. The recombinant protein expression was confirmed by western blotting followed by immune-labelling using anti DVE serum obtained from vaccinated ducks. Monoclonal antibodies reacting with recombinant glycoprotein G were selected for development of antigen detection ELISA. ELISA assay using glycoprotein G specific monoclonal antibody is being developed.

PP_2.20: Development of a recombinant Porcine circovirus (type 2b)virus-like particle and evaluation of its immunogenicity in mice model

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Porcine circovirus type 2 (PCV2) is a non-enveloped, circular single-stranded DNA virus that belongs to the *circovirus* genus of the family *Circoviridae.*PCV2 is the etiological agent of several diseases in pigs, collectively known as porcine circovirus associated diseases, which include post weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome,PCV2-reproductive disease,PCV2-enteric disease and PCV2-sub-clinical infection resulting in considerable economic losses within the swine industry. Commercially available virus-like particle (VLP)vaccines for PCV provide strong cell-mediated and humoral immunity against the disease however these are produced in insect cell expression systems which makes them expensive. This study aims to develop and evaluate a virus-like particle (VLP) vaccine against the prevalent PCV2b strain in a prokaryotic expression system. TheORF2region encoding the capsid protein was cloned into pET28a vector and transformed *Escherichia coli* strain BL21(DE3) pLysS. The transformed *E coli* could express the polyhistidine-tagged recombinant porcine circovirus capsid protein of 25 KDa. The expressed capsid protein was purified by affinity chromatography using Ni-NTA agarose beads. The identity of the recombinant protein was confirmed using a commercially available antiPCV2 serum. The expression was robust yielding 7mg of porcine circovirus capsid protein per 200 ml of *Escherichia coli* culture. The self-assembly of the recombinant porcine circovirus capsid protein into virus-like particles was analysed by transmission electron microscopy. The*in vivo* immunogenicity of this recombinant capsid protein is being tested in mice model. Future research will focus on immunogenicity evaluation in target species and scalability studies to enhance its potential for field use.

PP_2.21: Isolation, molecular detection and antibiogram of *Pseudomonas aeruginosa* from otitis externa of Canines

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Otitis externa is a complex multifactorial disease frequently affecting canine worldwide. It is mainly caused by bacterial and fungal pathogens such as *Staphylococcus sp., Pseudomonas sp., Proteus sp., Escherichia coli, Malassezia sp., Candida sp.* etc. *Pseudomonas aeruginosa* is considered as one of the

predominant organisms, often resist treatment and makes the condition complicated. Therefore, we aimed for the isolation and molecular detection of *Pseudomonas aeruginosa* from otitis externa cases of canines at Puducherry and its antimicrobial resistance pattern was studied. A total of 40 ear swabs were collected aseptically from otitis externa of dogs and subjected to pre-enrichment in Luria broth followed by streaking onto Muller-Hinton agar and cetrimide agar, for selective isolation of *P. aeruginosa*. The pure cultures were subjected to Gram's staining and biochemical tests for species level identification. PCR assay was performed for further confirmation of *P. aeruginosa,* using species-specific primers (16s rRNA gene) with the product size of 632 bp. Of the samples screened, 37.5% (n=15) were positive for *P. aeruginosa*. Antimicrobial susceptibility test was carried out for all the positive isolates and majority of the strains were susceptible to Ciprofloxacin (93%) followed by Enrofloxacin (85%) and Gentamicin (81%). The highest level of resistance was observed against Ampicillin (96%), Penicillin (93%), Cephalexin (85%) and intermediate susceptibility to Cefotaxime (56%). This study highlights the significant role of *P. aeruginosa* in otitis externa cases in dogs. The resistance of the organism to multiple antibiotic classes emphasize the need for further microbiological investigation on its resistance determinants to mitigate potential public health threats and to consider alternative treatment strategies.

PP_2.22: A simple rapid colorimetric PCR assay for Lumpy Skin Disease virus in bovines

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Lumpy Skin Disease (LSD) is a significant bovine viral disease caused by *Capripoxvirus,* which also affects sheep and goats. Recent past, it is highly prevalent globally among most of the countries including India. Most of the molecular techniques which are used to diagnose lumpy skin disease virus require advanced instruments and skilled personnel. This study focuses on developing a simple colorimetric assay for visual detection, combining the specificity of NATs (Nucleic acid amplification tests) with the selectivity of enzymatic assays using HRPzyme. The study utilized 10 samples, including scab and tissue samples from clinically suspected cows, with 2 testing positive for LSD. The assay showed high specificity for LSDV, with no cross-reactivity with other pox viruses or foot and mouth disease vaccine DNA. The sensitivity of this assay was found to be higher than that of conventional PCR as the former as it could detect up to (2 x10 ^{7}) copies of DNA while the latter could detect up to (2 x 10 ^{8}) copies of viral DNA. Hence colorimetric assay of this study holds great potential in mass screenings of LSDV with minimal resources and less trained personnel with further standardization.

PP_2.23: Production of recombinant capsid protein of Red-spotted grouper nervous necrosis virus: Challenges faced

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Nervous necrosis virus (NNV) a betanodavirus under the family Nodaviridae causes viral nervous necrosis also called as viral encephalopathy and retinopathy in large number of marine, brackishwater and freshwater species. Among the four genotypes reported red-spotted grouper nervous necrosis virus (RGNNV) is the most prevalent in tropical countries and is the only genotype reported in India. The virus causes acute mortality in larval and early juvenile stages whereas adult fish become carriers of the virus and transmit the virus vertically to the offsprings through eggs and milt. The only structural protein of the virus is the capsid protein weighing about 40 kDa coded by the RNA2 segment of the genome. The protein

expressed in prokaryotes or yeast folds into trimeric form and forms virus-like particles (VLPs). Hence the recombinant capsid protein is an excellent candidate for vaccine preparation.

The RGNNV isolated from infected Asian seabass, Lates calcarifer, in SSN-1 cells was plaque purified and characterized by whole genome sequencing. The capsid protein was cloned and expressed in Escherichia coli BL21 cells. For bulk production, the his-tagged protein expression was induced using 1 mM IPTG and the bacterial cells were harvested by centrifugation. The protein was found to express as inclusion bodies. Hence the protein was solubilized in solubilization buffer containing 8 M urea, 50 mM tris-HCl, 30 mM imidazole. The his-tagged protein was purified using Ni-NTA column using low pressure chromatography. The protein was eluted using buffer containing 8 M urea, 50 mM tris-HCl and 400 mM imidazole. The eluted protein was dialyzed against PBS overnight and concentrated by PEG sprinkling on the dialysis membrane. The purified protein upon dialysis resulted in precipitation of the protein. Hence, to solubilize the protein, buffer containing 10% glycerol was used. Use of glycerol resulted in homogenous mixture of the recombinant protein. This preparation can be used to emulsify in an adjuvant and used as a vaccine.

TECHNICAL SESSION - III

GENOMICS, DISEASE SURVEILLANCE AND EPIDEMIOLOGY

Lead paper -1

Experiences in accreditation and designation of WOAH Reference Laboratory for Leptospirosis

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Abstract

Obtaining WOAH reference laboratory status and ISO/IEC 17025:2017 accreditation has been transformative for our laboratory in ICAR-NIVEDI. These achievements have significantly enhanced our capabilities and credibility in veterinary diagnostics and research, appreciations to sustained efforts over time. We began with a thorough internal assessment to align our processes with ISO/IEC 17025:2017 requirements. A dedicated team of quality manager, technical manager, laboratory head, laboratory technicians, and administrative staff meticulously reviewed and audited our practices, documentation, and equipment calibration protocols, identifying gaps and creating a comprehensive action plan. Central to our success was implementing a robust Quality Management System (QMS) based on ISO 9001:2015. This included drafting new SOPs, revising documentation, participating in proficiency testing, and establishing rigorous record-keeping practices. Focused training ensured that all team members understood their roles within the QMS and the importance of ISO standards and WOAH guidelines. We invested in state-of-the-art instrumentation and third-party calibration services to validate equipment performance. Method validation followed international standards to ensure testing accuracy and reliability. Rigorous internal audits verified compliance with ISO/IEC 17025:2017, and feedback from auditors helped us refine our processes before achieving full accreditation. This preparation informed our online National Accreditation Board for Testing and Calibration Laboratories (NABL) application for ISO 17025 standards, highlighting both QMS compliance and laboratory competence in disease diagnostics.

We compiled a comprehensive dossier showcasing our expertise, research capabilities, and contributions to animal health. This formed the basis of our application for WOAH reference laboratory status, with experts noting our dedication to quality and alignment with WOAH's mission of impactful research and capacity-building. Achieving ISO/IEC 17025:2017 accreditation and WOAH reference laboratory status has elevated our laboratory's reputation, fostered a culture of excellence, and increased collaboration opportunities. We are now positioned as a leader in animal health diagnostics and research, committed to advancing global efforts in disease prevention and control through quality, innovation, and scientific rigor. ICAR-NIVEDI's designation as one of five WOAH Reference Laboratories for Leptospirosis places it at the forefront of international efforts to combat the disease. This achievement underscores ICAR-NIVEDI's commitment to improving global animal health and supporting agricultural economies worldwide, setting the stage for future successes in veterinary epidemiology and disease control.

Keywords: ISO 17025:2017 accreditation, WOAH, reference laboratory, animal disease, Leptospirosis, India.

Introduction

ISO/IEC 17025:2017 Accreditation: Achieving ISO/IEC 17025:2017 accreditation from the National

Accreditation Board for Testing and Calibration Laboratories (NABL) is a pivotal milestone for laboratories, signifying their commitment to high standards of technical competence and quality. This detailed overview explores what this accreditation entails, its benefits, the process, technical requirements, implications for laboratories, and the role of NABL.

Scope of Accreditation

ISO/IEC 17025:2017 specifies the general requirements for the competence of testing and calibration laboratories. This standard is applicable to all organizations performing laboratory activities, regardless of their size or number of personnel. It sets forth guidelines to ensure that laboratories operate competently and generate valid results.

Benefits of ISO/IEC 17025:2017 Accreditation

Global Recognition: NABL accreditation is recognized internationally, underscoring compliance with stringent standards and practices.

Enhanced Credibility: Accreditation demonstrates a laboratory's technical competence and reliability in producing test and calibration results.

Improved Quality: Ensures the consistent delivery of accurate, reliable, and traceable results through rigorous quality control measures.

Competitive Edge: Provides a distinct advantage in the marketplace by differentiating accredited laboratories from their non-accredited counterparts.

Customer Confidence: Builds trust among customers and stakeholders by adhering to high-quality standards and practices.

Accreditation Process

Application: Laboratories initiate the process by applying to NABL, submitting documented procedures, and providing evidence of compliance with ISO/IEC 17025:2017.

Assessment: NABL conducts a thorough assessment involving document reviews, on-site inspections, and proficiency testing to evaluate the laboratory's adherence to the standard.

Decision: Based on the assessment results, NABL grants accreditation if all requirements are met. If not, corrective actions must be taken to address any deficiencies.

Surveillance: Regular audits and reviews ensure ongoing compliance and continuous improvement.

Technical Requirements

Management Requirements: Include the laboratory's organizational structure, responsibilities, document control, and review of contracts to ensure effective management.

Technical Requirements: Cover the competence of personnel, calibration of equipment, testing methods, and handling of test items to maintain technical accuracy and reliability.

Quality Assurance: Addresses quality control measures, method validation, result reporting, and the handling of non-conforming work to ensure consistent and reliable outcomes.

Implications for Laboratories

Operational Excellence: Encourages continuous improvement through rigorous quality management practices, fostering operational excellence.

Training and Development: Requires ongoing training for personnel to maintain and enhance technical competence, ensuring high standards of performance.

Cost and Time Commitment: Involves significant financial investment and resource allocation for both initial accreditation and ongoing maintenance.

Market Access: Facilitates acceptance of test and calibration results across borders due to mutual recognition agreements (MRAs) between accreditation bodies.

Role of NABL for Accreditation

Accreditation Authority: NABL is the national body responsible for accrediting testing and calibration laboratories, ensuring adherence to international standards.

Technical Expertise: Provides guidance, proficiency testing, and training to help laboratories meet the requirements of ISO/IEC 17025:2017.

Promotion of Quality: Promotes the development of quality infrastructure and ensures that conformity assessment practices meet global standards.

Preparation of Application for WOAH Reference Laboratory Designation

To apply for accreditation or designation as a World Organisation for Animal Health (WOAH) Reference Laboratory for Leptospirosis, meticulous preparation and submission of comprehensive documentation are essential. This application aims to establish the laboratory's capability and commitment to international standards in disease diagnostics and control. Below is a detailed overview of the required essential documents and information requirements for submission.

Curriculum Vitae of the Designated Expert:

Content: Include detailed information on international recognition, national consultations, awards, professional memberships, and other relevant credentials.

Purpose: Demonstrates the expertise and global standing of the key individual representing the laboratory.

Laboratory Details

Content: Provide the laboratory's name, address, the head of the laboratory (Responsible Official), and the head of the institute.

Purpose: Establishes the identity and leadership structure of the laboratory.

Legal and Budgetary Provisions

Content: Document the sustainability and operational functionality of the laboratory, including budgetary allocations and legal frameworks.

Purpose: Ensures that the laboratory is capable of maintaining operations and meeting WOAH standards over the long term.

Accreditation to ISO/IEC 17025 or Equivalent

Content: Submit documented proof of adherence to quality management systems and an accredited scope of tests.

Purpose: Validates the laboratory's commitment to quality and reliability in testing and calibration.

Technical Expertise and Experience

Content: Provide an overview of the multidisciplinary team's capabilities, expertise levels, and experience in disease diagnostics as per WOAH standards.

Purpose: Demonstrates the technical proficiency and experience necessary for high-quality diagnostic work.

Diagnostic Techniques and Capabilities

Content: Detail the standardization, validation, and reagent production processes, including cultures, reference strains, and diagnostic kit capabilities.

Purpose: Shows the laboratory's capability in developing and using diagnostic techniques effectively.

International Shipment Capability

Content: Describe the laboratory's competence in packing, labeling, IATA Category A compliance, and sample handling expertise.

Purpose: Ensures the laboratory can manage international shipments of diagnostic materials safely and in compliance with regulations.

Research and Development Projects

Content: List completed projects related to leptospirosis, detailing roles, durations, and outcomes.

Purpose: Highlights the laboratory's research contributions and expertise in the field.

Inter-Laboratory Proficiency Tests (PT) / Inter-Laboratory Comparison (ILC)

Content: Provide a history of participation in proficiency tests, including organizing details and comparative analyses.

Purpose: Demonstrates the laboratory's engagement in quality assurance and its performance relative to other laboratories.

Training and Consultation Experience

Content: Detailed conducted training programs, workshops, and consultations, both nationally and internationally.

Purpose: Showcases the laboratory's role in knowledge dissemination and capacity building.

Scientific Meetings Participation

Content: List participation in scientific meetings and contributions to the field.

Purpose: Demonstrates active engagement and contribution to the scientific community.

Collaboration Agreements

Content: Provide a list of national and international collaboration agreements and memoranda of understanding.

Purpose: Indicates the laboratory's network and collaborative efforts in advancing research and diagnostics.

Guarantee Documents

Content: Include terms of appointment, confidentiality agreements, intellectual property rights policies, data security measures, and compliance monitoring protocols.

Purpose: Ensures adherence to legal and ethical standards, safeguarding intellectual property and data.

Confidentiality Undertaking and Declaration of Interests

Content: Submit a signed declaration ensuring confidentiality and a declaration of interests in accordance with WOAH Reference Laboratory rules.

Purpose: Affirms commitment to confidentiality and transparency, aligning with WOAH's ethical guidelines.

Annexure

Content: Provide a list of references and a declaration of interest for OIE Reference Centres.

Purpose: Supports the application with additional documentation and references.

Conclusions

ISO/IEC 17025:2017 accreditation by NABL is a mark of excellence and competence for laboratories, enhancing their global recognition and credibility. It signifies adherence to rigorous technical and management requirements, offering a competitive edge and fostering continuous improvement. By obtaining

this accreditation, laboratories demonstrate their commitment to high-quality standards, thereby increasing customer confidence and positioning themselves as leaders in the industry. Each document and detail contributes to portraying the laboratory's capability to uphold WOAH standards for disease diagnostics and control effectively. The thoroughness and accuracy of the application are crucial in demonstrating readiness and commitment to international excellence in animal health diagnostics and research.

ICAR-NIVEDI's Laboratory for Leptospirosis Designated as WOAH Reference Laboratory

In a landmark development for veterinary science in India, as a testament to its exemplary standards, the ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) was recently designated as a WOAH Reference Laboratory for PPR and Leptospirosis.

This prestigious recognition and designation was officially endorsed by the WOAH Council on March 26, 2024, under Resolution No. 33, "Designation of WOAH Collaborating Centers," during the 91st World Assembly of WOAH Delegates at the General Session held on May 30, 2024. This achievement underscores ICAR-NIVEDI's commitment to excellence in animal health diagnostics and its alignment with WOAH's global mission. Dr V. Balamurugan, Principal Scientist at ICAR-NIVEDI, has been appointed as the designated expert for the laboratory. This designation highlights ICAR-NIVEDI's pivotal role in monitoring, surveillance, diagnostic support, and capacity building for Leptospirosis across India and other neighbouring countries. The laboratory is ISO 17025:2017 accredited and their proficiency testing is regularly endorsed by international agencies. The ISO/IEC17025:2017 accreditation for the Livestock Disease Diagnosis Laboratory of ICAR-NIVEDI was obtained on December 4, 2023, from the National Accreditation Board for Testing and Calibration Laboratories (NABL), India. The accreditation was achieved with a dedicated team of scientists under the guidance of competent authority. Additionally, the support and guidance provided by the scientists, technical, and administrative staff of ICAR-NIVEDI were instrumental in achieving this significant milestone. Their collective efforts have not only marked an important achievement in the history of ICAR-NIVEDI but have also contributed to the broader success of ICAR and DAHD, as a whole. The significance of this achievement. With ICAR-NIVEDI's designation, India now hosts four WOAH Reference Laboratories, including those for Rabies at the Veterinary College, Bengaluru, and Avian Influenza at the ICAR-National Institute of High-Security Animal Diseases in Bhopal.

ICAR-NIVEDI's designation as one of five WOAH Reference Laboratories for Leptospirosis places it at the forefront of international efforts to combat the disease. This achievement underscores ICAR-NIVEDI's commitment to improving global animal health and supporting agricultural economies worldwide, setting the stage for future successes in veterinary epidemiology and disease control.

About the Laboratory

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics is a premier national institute in India dedicated to the surveillance and monitoring of livestock diseases and animal health information. Located in Bengaluru (India), ICAR-NIVEDI hosts the newly nominated WOAH Reference Laboratory for Leptospirosis. The institute specializes in epidemiology, forecasting, and economic analysis of livestock diseases, including zoonoses. Earlier, the Leptospirosis Research Laboratory of ICAR-NIVEDI is also the National Reference Laboratory for Animal Leptospirosis. The Leptospirosis Research Laboratory at ICAR-NIVEDI in Bengaluru is equipped with cutting-edge facilities for genomic characterization, molecular diagnostics, and large-scale surveillance. The laboratory supports the Government of India's goal of eliminating livestock diseases from India. Additionally, they serve as a regional coordinator under the National One Health programme for the prevention and control of zoonoses, managed by the One Health Centre of the NCDC, GoI. This role involves comprehensive surveillance, conducting capacity building training programme and monitoring and management of leptospirosis nationwide.

Aims and Objectives of the Leptospirosis Laboratory

- Enhancing Diagnostic Capabilities: Develop and implement advanced diagnostic technologies to improve the speed and accuracy of Leptospirosis diagnosis. Expand diagnostic services to support India and other neighbouring countries in disease control efforts. The laboratory routinely employs diagnostic assays/tests such as Microscopic Agglutination Test (MAT), PCR, and RT-PCR for Leptospira detection including molecular characterization and isolation.
- Capacity Building and Training: Organize regular training programs and workshops for veterinarians/ medicals/microbiologists and researchers to enhance diagnostic and epidemiological skills. Develop educational materials and technical guidelines for effective disease control.
- Advancing Research and Development: Conduct pioneering research on transmission dynamics, vaccine efficacy, and molecular epidemiology. Collaborate with international reference laboratories to share expertise and best practices. The laboratory is also involved in the development of rapid diagnostics and diagnostic ELISAs. This laboratory routinely conducts serological/molecular epidemiology studies of Leptospira in India.
- Global Health Initiatives Support: Contribute to WOAH's mission by providing high-quality diagnostics, engaging in impactful research, and building veterinary community capacity. Promote collaborative approaches to ensure livestock and public health well-being.

ICAR-NIVEDI's laboratories are committed to improving animal health through advanced diagnostics, innovative research, and capacity building. By supporting WOAH's goals, the institute contributes to global efforts in managing Leptospirosis, ultimately enhancing animal and public health outcomes worldwide.

Lead Paper 2

Recombinant Proteins: Applications in Animal Health

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Recombinant DNA/protein technologies have revolutionized almost all fields of biomedical research in recent times with enormous capability to generate a broad catalogue of proteins of interest which possess potential applications and solutions for each and every lingering problem that continue to pester the livestock production systems in India. Basically, 'Recombinant proteins' are proteins generated in an appropriate expression host system (homologues or heterologues) using DNA/gene of interest. The DNA, also called the gene of interest (GOI), is introduced into the host cells through a suitable expression vector and then expressed to proteins using the host genetic machinery. At present, recombinant protein production is one of the most powerful techniques used in life sciences including veterinary medicine. The produced recombinant proteins through a proven technologies have several advantages such as high purity, specificity, efficiency, safety, customization, scalability, and consistency. These recombinant proteins have been widely applied in various fields, including veterinary medicine/ health, research, biotechnology, and livestock production system etc. The most commonly generated proteins for the animal sector are those with an important role in reproduction, feed efficiency, and health. Although, there are good number platforms being used, nowadays, prokaryotic and mammalian cells are the significantly employed for recombinant production especially for bulk production of hormones and enzymes for reproductive purposes as well as to enhance animal performance through maintaining a good health.

Nevertheless, for livestock sector, the development of cost-effective products is always an utmost priority, the application of cell factories such as bacteria and yeast has notably increased over the decades to make the new developed recombinant proteins as a real alternative to the marketed ones. In addition, several notable efforts have also been made to develop new recombinant strategies for securing animal health especially in the areas of prevention and therapy, including passive immunization and modulation of the immune system. Such alternate strategies offer the possibility to reduce the burden of antibiotics usage thereby improving the efficacy of preventing infections. Thus, nowadays diverse recombinant antigens, enzymes, hormones, and therapeutic molecules with optimized functional properties have been successfully produced through relatively cost effective processes using microbial cell factories.

The present lecture highlights the introduction to the realm of recombinant proteins and their potential applications in animal health research and developing novel diagnostics and new generation vaccines. Although, recombinant proteins generated by various expression systems (bacteria, yeast, mammalian/ insect cells, cell free system and transgenic animals/plants), the most commonly employed method is application of basic molecular biology protocols along with cost-effective, easy upscale method and down-stream processing using prokaryotic expression system which targets either full/partial virulent gene from any pathogen (bacteria, virus, parasite, etc.) to recombinant proteins and subsequently, their utility in animal health diagnostics and vaccines. It basically involves a fundamental step involved in target gene analysis, amplification, cloning, over expression, purification, qualitative and quantitative analysis followed by functional analysis. Based on the fact that recombinant protein technology has made notable breakthroughs especially in the realm of discovery and development of various diagnostic antigens and/ or vaccines, it is presumed that future veterinary biological products based on recombinant proteins would

be of immense value for efficient sero-surveillance, rapid diagnostics, disease monitoring and protection of nation's treasured livestock. In view of growing demand for animal health experts armed with novel proteomic tools, the presentation highlights recent advances/ developments on production and utility of recombinant proteins. It is anticipated that a better understanding and production of quality recombinant proteins would likely to further strengthen the animal health research and development of new generation diagnostics/vaccines for infectious diseases (Endemic, exotic, and emerging/re-emerging) of livestock in India.

Biopharmaceuticals based on recombinant proteins form the largest category, encompassing enzymes, hormones, cytokines, growth factors, blood clotting factors, monoclonal antibodies (mAbs), subunit vaccines, vaccine delivery platforms, microbial adjuvants, and antibody-related products (e.g., Fc-fusion proteins and antibody fragments). They are used for therapeutics, diagnostics, and drug discovery and development, as well as vaccine development and production.

A) Therapeutic Recombinant Proteins

Most of therapeutic proteins that were earlier extracted from natural sources originally can be recombinantly produced in different expression systems including bacterial, yeast, mammalian, and plant hosts. Therapeutic recombinant proteins can be produced by modifying or altering the DNA/gene via genetic engineering techniques, providing important therapies for a variety of infectious diseases, autoimmune diseases, and genetic disorders involving deficiencies in cytokines (molecules such as interleukins, interferons, and colony-stimulating factors), which are signaling proteins regulating immune responses and cell growth. Recombinant cytokines can be administered to compensate for these deficiencies. Some recombinant cytokines such as immune checkpoint proteins also play a role in immunotherapy by amplifying immune response against certain types of cancer. They function to stimulate the immune system, evoke immune cells, and enhance anti-tumor reactions. Further Recombinant antibodies, especially monoclonal antibodies (mAbs), have revolutionized the treatment of various infectious diseases, cancers, and autoimmune diseases. Further, recombinant proteins enable the development of targeted therapies tailored to specific molecular targets or pathways, improving treatment outcomes while minimizing side effects. Recombinant technology allows for the customization of therapeutic proteins to match the specific genetic variations present in individual animal host species. Moreover, a major concern for all recombinant therapeutics is immunogenicity. All biotechnologically produced therapeutics may exhibit some form of immunogenicity. It is difficult to predict the safety of novel therapeutic proteins.

Additionally, recombinant proteins are also used in high-throughput screening assays to identify potential drug candidates. Researchers can efficiently test large compound libraries against specific targets, expediting the drug discovery process. Recombinant proteins are also used to design biological assays for studying the effects of potential drug candidates. In preclinical studies, these recombinant proteins help researchers understand the new drug's mechanism of action and assess its efficacy.

B) Diagnostic Recombinant Proteins

The complexities involved in use of either whole cell or partial/specific native antigens of bacteria, viral and parasite in development of immune-diagnostics led to employment of highly purified recombinant antigen/s as diagnostic reagent. They offered high specificity and sensitivity of immuno-assays in diverse platforms such as ELISA, dot-blot, and lateral flow assay. In recent times, the disease specific recombinant proteins are being used as antigens to identify specific antibodies in animal serum samples, facilitating the diagnosis of infectious diseases, autoimmune disorders, and allergies. Additionally, these proteins also serve as calibrators and controls in diagnostic assays to ensure consistency and accuracy in measuring analyte concentrations. This is critical for the reliability of diagnostic results and uniformly employability

across the country. Several recombinant antigens-based ELISA for diseases like bluetongue (BT), PPR, Classical swine fever (CSFV), buffalopox, lumpy skin disease (LSD), sheeppox, foot-and-mouth disease (FMD), goatpox, buffalopox, and orf have been developed. Further, recombinant exotoxins-based assays for anthrax, black quarter (BQ), and enterotoxaemia (ET) etc., have also been attempted and being employed for routine sero-surveillance and post vaccination monitoring of disease specific antibodies in livestock.

C) Subunit Vaccine Recombinant Proteins

As limitation of bulk production of virulent bacteria and virus either for killed /inactivated or attenuated conventional vaccines for majority of livestock vaccines, led to a newer approach using recombinant subunit or multi-component, chimeric /hybrid antigens-based vaccines for infectious agents. Pathogen specific 'recombinant proteins' engineered to express specific targeted antigens derived from pathogens such as viruses or bacteria, which are highly immunogenic and capable of inducing a protective immune response. These recombinant antigens such as surface proteins, subunits, or epitopes, are key components in various vaccine platforms, including protein subunit vaccines, virus-like particle (VLP) vaccines, and conjugate vaccines. Recombinant protein vaccines not only show a high safety profile but also are more stable in comparison to other genetic vaccine platforms like mRNA vaccines. In the recent past several antigens such as recombinant protective antigen (PA) for anthrax, recombinant epsilon for enterotoxaemia, recombinant flagella for foot rot, recombinant haemagglutinin for influenza virus etc., are being used as an alternate vaccine for livestock diseases. Additionally, recombinant B2L of Orf is also used as microbial adjuvant along with subunit vaccine. Chimeric/fusion recombinant antigens are also used to target multiple infectious agents. A novel invitro Phage T4 display platform is used to deliver multi-component vaccine against anthrax, tularemia, plague and others.

Although, recombinant proteins exhibit enormous potential utilities, they also have limitations. To note, in some cases, the production of recombinant proteins is complex, expensive, and time-consuming. Further, the recombinant proteins produced in expression systems may not be the same as the natural conformation forms. This difference may reduce the effectiveness of therapeutic, diagnostic and subunit vaccines - recombinant proteins and even cause unexpected effects. Additionally, these differences may affect the results of experiments and envisaged impact in addressing the challenges of animal health.

In conclusion, 'Recombinant protein technology' allows significant flexibility in modulating target gene/protein sequence, which subsequently makes it possible to obtain recombinant products/antigens with improved functional properties compared with native proteins extracted from respective hosts or infectious agents. This has contributed significantly in the development of recombinant products for a wide array of applications including animal health as presented in the lecture. A broad catalogue of microbial cell factories is being explored for the successful development of novel recombinant fusion proteins-based vaccine delivery platforms, subunit vaccines, diagnostics and therapeutic molecules. Nevertheless, to continue advancing in this approach, it is necessary to fortify novel strategies to overcome existing challenges in product refinement to make a giant leap towards the use of recombinant protein-based products with greater applicability in animal health. Additionally, merger of newer realms such as nanotechnology, especially Nano-structuration, could play a vital role in the development of a new generation of recombinant biomolecules with higher affordability for livestock farmers to safe guard animal health.

ORAL PRESENTATION

OP_3.1: Molecular Identification and risk factor analysis of the African Swine Fever Virus Outbreak in the Northeastern region of India

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This study has been carried out to detect the prevalence of the African Swine fever virus in pigs of the Northeastern region of India and associated potential risk factors. ASFV-suspected samples (2576 nos) were collected from different parts of NER states between 2020 and 2024, and samples were screened with OIE-recommended PCR protocol followed by sequencing and phylogenetic analysis. GPS-based mobile applications were used to record geographic coordinates of pig farms, major landmarks, and potential spatial risk factors. The outbreak location map with Infected Zone in affected villages was prepared using QGIS. Out of 2576 samples, 503 were found to be positive for ASFV genome in PCR. The incidence of ASFV was recorded in different parts of north-eastern states from 2020-2024 and Mizoram found with the highest incidence of ASF. Phylogenetic analysis of *B646L* gene showed the circulating ASF belongs to genotype II. Tempero-spatial distribution showed the highest incidence in the Aizawl district and moderately high in the East Khasi Hills district. Year wise the highest incidence of disease was observed in last year 2021. Non-meteorological risk factors like swill feeding (78%)*(P≤0.05), entry of visitors (81%)**(P≤0.01), non-usage of disinfectants (57%)*(P≤0.05) had a significant association with the outbreak. Again, the presence of a pig slaughter point less than 1 km vicinity of the farm (42.5%)**(P≤0.01), and the presence of a river within a 1 km range of the farm (41%)*(P≤0.05) had a significant association with the ASF outbreak. The spread of ASF into wild boar was recorded in Assam, Mizoram, Manipur and Arunachal Pradesh in India. In Assam, the spread of ASFV to the wild pigs of Assam might be due to spill-over from the domestic pigs. Probable spillover of ASFV to the wild boar of the Manas National Park might be through the Manas River, which is one of the major tributaries of the mighty Brahmaputra passing through the heart of the national park and infected carcasses disposed of in rivers. The circulating ASF in NER belongs to genotype II. To reduce the risk of ASF transmission farmers should receive proper awareness and education to reduce virus-infected animal movement.

OP_3.2: Molecular characterization of Sheep Pox Virus from field outbreaks using the major envelope protein P32 of Capripox virus

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The present study aimed at molecular characterization of Sheep pox virus from field outbreaks in Tamil Nadu by analysing the P32 gene sequences and establishing the evolutionary relationship of virus by phylogenetic analysis. A total of 16 samples collected from outbreaks in various southern districts of Tamil Nadu were used in this study. The DNA extracted from clinical samples were initially subjected to diagnostic PCR by amplification of partial p32 gene and then the positive samples were targeted for amplification of full length p32 gene. The sequences obtained in this study were subjected to phylogenetic analysis. The homologous sequences retrieved from GenBank were used for sequence comparisons by ClustalW. A phylogenetic tree was constructed by maximum likelihood method using 1000 bootstrap

replications in MEGA XI software. Among 16 clinical samples tested, 9 samples (scab 6/8; tissue 3/6, nasal swab 0/2) were found positive (56%) for partial amplification of P32 gene. All the 9 positive samples were successfully amplified for full length P32 gene. The sequence results of five samples were analysed by BLAST-n and submitted in GenBank with Accession numbers PP179173.1, PP179174.1, PP179175.1, PP179176.1 and PP179177.1. The phylogenetic analysis showed that these isolates were clustered into sheep pox virus clade with most of the other Indian sheep pox viruses available in the GenBank. They showed 98.6 to 99.2% nuclear identity based on pair distance analysis by ClustalW without codons. The present study elucidated the successful identification and molecular characterization of Sheep pox virus based on highly conserved P32 gene from the clinical outbreaks. The sequencing and phylogenetic analysis revealed the present sheep pox virus samples were closely related to most of the other sheep pox viruses circulating in India.

OP_3.3: Semi-nested polymerase chain reaction assay for detection of emerging Porcine Parvovirus 2 (PPV2) in pigs

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Porcine parvoviruses (PPVs) produce stillbirth, mummification, embryonic death, and infertility (SMEDI) in porcine populations. In PPVs, the ORF1 encodes large non-structural protein which is involved in genome replication, transcription regulation and cytotoxicity. The porcine parvovirus 2 (PPV2) genome has two major open reading frames (ORFs). The ORF1 gene of PPV2 encodes for large non-structural protein. Similarly, the ORF2 encodes the structural protein of the virus. PPV2 is generally associated with reproductive health problems in pigs. Diagnosis of the ailment is highly sought for detection of PPV2. In the present study, a semi-nested PCR was developed for detection of PPV2 targeting the nonstructural protein (NSP) encoding gene of PPV2. The analytical sensitivity of semi-nested PCR was found 10 fold higher compared to conventional PCR assay using same outer set of primers. The developed semi-nested PCR was found specific to amplify the genome of PPV2 and it did not amplify other homologous host DNA viruses like Porcine parvovirus 1 (PPV1), Porcine circovirus 2 (PCV2) and African swine fever virus (ASFV) as well as heterologous host DNA viruses. Further, the applicability of the semi-nested PCR was assessed using field clinical samples. In conclusion, the developed semi-nested PCR was found sensitive and specific. It could be used for detection of PPV2 infection in porcine herd.

OP_3.4: Proteomic Analysis of ATP*ase* Protein of Orf Virus Isolates from North Western Zone of Tamil Nadu

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The present study describes the prevalence of different strains of Contagious ecthyma virus at different localities of Northwestern zone of Tamil Nadu by nucleotide sequencing and phylogenetic analysis of full length A32L gene encoding ATPase protein involved in viral DNA packaging. Molecular characterization of A32L gene of the four CEV isolates showed that the nucleotide sequence was homologous with different

Indian and foreign sequences. The deduced amino acid sequences of Namakkal, Salem, Dharmapuri and Krishnagiri CEV isolates showed 96-99 per cent similarity with other compared isolates. Multiple nucleotide alignment of A32L gene revealed that nucleotide mutations/deletions at 12, 7, 5 and 17 positions in Namakkal, Salem, Dharmapuri and Krishnagiri CEV isolates respectively. Moreover, the phylogenetic analysis revealed Namakkal, Salem and Krishnagiri CEV isolates were closely related to Bangalore and Mukteswar 59/05 isolates whereas Dharmapuri CEV isolate showed close relationship with Assam, Fujian GO strain and Fujian SJ1 strain. The deduced amino acid sequences of CEV ATPase were analysed for five functional motifs in the N-terminal and KGD and RGD motifs in the C-terminal. The conserved motifs (I, II, III, IV and V) of N terminal was present in Salem and Dharmapuri CEV isolates whereas single amino acid substitution in motif V (**A**YDG→**S**YDG) of Namakkal CEV isolate and three amino acid substitutions in motif III (R**HIN**VSLVLLC**Q**→R**RIK**VSLVLLC**R**) of Krishnagiri CEV isolate was observed. The secondary structure of ATPase predicted using I-TASSER web portal revealed the presence of conserved arginine finger residue at position 125 in motif III. The deduced amino acid sequences in C- terminal region revealed the presence of two KGD motifs at position 247-252 and two RGD motifs in all the four CEV isolates. The occurrence of non-identical strains of CEV virus in the study area discloses the varying source of infection and molecular analysis based on A32L gene could be relied on for the characterization of virus isolates.

OP_3.5: Genomic surveillance of *Gallid herpesvirus* 1 based on *Infected Cell Protein-4* gene: Insights into the ultrastructural pathology of reproductive and lymphoid organs in Natural Outbreaks in chicken

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Infectious laryngotracheitis (ILT) is an emerging and highly contagious upper respiratory tract disease of chicken causing considerable economic loss to poultry industry. A total of 225 necropsies were carried out in sick and dead birds. The morbidity rate in ILT ranged from 14.53 % to 64.67 % and mortality rate from 11.39 % to 25.45 %. The tracheal and oviduct samples from suspected birds were subjected to virus isolation by egg inoculation of SPF embryonated chicken eggs (ECE) through chorio-allantoic membrane (CAM) route. All the positive samples produced severe congestion, thickening of CAM and characteristic pock lesions on 7th day post infection (dpi). These isolates were subjected to PCR amplification of 635 bp of GaHV-1 *ICP4* gene. Gross pathological changes of reproductive system showed salphingitis with inspissated albumin and yolk material, haemorrhagic oophoritis and egg peritonitis. Histopathologically, haemorrhagic oophoritis, degeneration and shortening of epithelial cells of oviduct, lymphoid depletion in spleen, bursa of Fabricius, caecal tonsil and plasma cell depletion in Harderian gland were recorded. Immuno-histochemical localization of ILTV was present as intracytoplasmic signals in the epithelial cells of oviduct and lymphoid organs. Genome sequencing and phylogenetic analysis of ICP4 gene revealed that five isolates of this study were grouped together in a separate branch (Cluster I), and were closely related to the Indian strains (MH128121) and isolates from Turkey (MN073049), Germany (JX273230), China (KC248168, KM214404) and Brazil (GQ499345) with 98% homology. Ultra structural pathology in ILTV infection revealed presence of numerous round enveloped intracytoplasmic and intranuclear virion particles in epithelial cells of oviduct and lymphoid cells of caecal tonsils. These findings establishes the localization of ICP4 gene responsible for regulating the expression of early and late genes of herpesviruses infection

and elucidates the affinity and pathogenesis of ILTV variant circulating in the poultry population targeting the reproductive system and immune system along with the respiratory system.

OP_3.6: Molecular characterization and phylogenetic analysis of lumpy skin disease virus from Tamil Nadu

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Lumpy skin disease (LSD) is an economically important transboundary viral disease caused by the Lumpy skin disease virus (LSDV) belongs to the genus *Capripoxvirus* in the family *poxviridae.* The LSD outbreak was first recorded in Tamil Nadu in February 2020. Between 2020 and 2023, LSD outbreaks in Tamil Nadu was occurred in two waves. In the first wave, LSD outbreaks were recorded in 19 districts, and in the second wave 24 districts were affected. A total of 577 samples (320 skin scab/ nodules and 257 blood samples) were screened for LSD using conventional PCR targeting the *p32* gene of *Capripoxvirus.* Out of 577 samples, 266 skin scab samples and 9 blood samples were found positive for LSD. Representative samples were isolated in embryonated chicken eggs and Vero cells. The full-length *RPO30* gene was amplified from selected samples and the amplified PCR products were sequenced by Sanger sequencing. Sequence analysis revealed differences in the *RPO30* gene sequences between first and second waves, both at nucleotide (nucleotide position 41 and 292) and amino acid (aa position 14 [T to N] and 98 [P to S] level. Phylogenetic analysis clustered the study sequences with strains from other parts of India, Kenya, and Bangladesh. However, the sequences from second wave samples formed a separate clade indicating variability. Therefore, it is necessary to constantly monitor the circulating strains for future vaccine and diagnostic development.

OP_3.7: Molecular epidemiology of bovine papilloma virus in Tamil Nadu

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Bovine papillomatosis is a contagious disease caused by bovine papilloma virus (BPV) belong to *Papillomaviridae* family. Even though papilloma virus is species-specific, BPV infections are reported in buffaloes, yak, equine, hamsters etc. BPVs are diverse, until now 44 types were reported throughout the world. In India, especially in the northern parts of the country BPV types 1, 2 & 5 are reported in cattle, buffaloes and yak. Cross protection across BPV types is reported to be minimal therefore, molecular epidemiology of BPVs is indispensable for the development of vaccines for prophylactic and therapeutic purposes. In the present study, wart tissues (n=10) were collected from lesions suggestive bovine papillomatosis in different parts of Tamil Nadu. Genomic DNA was extracted from wart tissues and amplified with universal primers targeting a 449 bp region of major capsid protein encoded by L1 gene of BPV. PCR amplification revealed presence of BPV infection in six out of 10 samples screened. Further, histopathological examination of the wart tissue revealed typical fibropapilloma indicative of papillomatosis. Sanger sequencing of 449 bp region of L1 gene and BLAST analysis revealed presence of BPV types 1 and 2. Even though type specific primers are available in the literature to identify specific BPV types, results were ambiguous. Phylogenetic analysis of sequences retrieved from public databases such as NCBI, The Papillomavirus Episteme (PaVE) and sequences obtained in the present study was performed. Maximum Likelihood trees were created for sequences from different regions across India and world, revealed that BPV types circulating in Tamil Nadu

were more closely related to BPV types present in Brazil. To the best of our knowledge this is the first study to report the presence of BPV 2 in Tamil Nadu. In conclusion, BPV types 1 and 2 were more commonly found in the skin lesions of bovine papillomatosis in Tamil Nadu.

OP_3.8: Sequence Analysis and Molecular Characterization of Lumpy Skin Disease Virus based on LSDV095 Virion Core Protein

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Lumpy skin disease (LSD) is a transboundary, high impact pox viral disease, notifiable by World Organization for Animal Health. It affects mainly cattle and Asian water buffalo and is caused by LSD virus, which genetically is closely related to sheeppox (SPPV) and goatpox virus (GTPV) and all together placed under genus *Capripoxvirus* (CaPV) of the subfamily *Chordopoxvirinae* under the family *Poxviridae*. The present report deals with isolation of the virus, sequence analysis and molecular characterization of circulating LSDV based on the immune-dominant LSDV095 core protein (A4L). As this protein is more immunogenic, the diagnostic and prophylactic potential of this A4L recombinant protein can be exploited as vaccine candidate in the non-endemic countries. Here, the samples collected from Tamil Nadu state were subjected to isolation in embryonated chicken eggs (ECE) and BHK 21 cells. The representative samples were subjected to sequence analysis based on A4L gene, during which it was revealed that GTPV, SPPV and LSDV clustered into three major separate clusters. The comparative sequence analyses of the A4L gene of Indian isolates of LSDV revealed G48 and C98 species-specific signature residues. Whereas GTPV revealed A93, A216, A315, G136 and G146 and incase of SPPV G47, A63, A168 and A276 were revealed. The Tamil Nadu isolates from the present study are closely related to other Indian strains recovered from both northern and southern parts of the country and to historically related Kenyan strains. Whereas the LSDV vaccine strains and recombinant vaccine like strains from Russia, China, Hong Kong, Thailand clustered separately. The dissimilarity of the current isolates from vaccine like strains confirms the circulation of virulent field strains responsible for the outbreaks in India.

OP_3.9: CONCURRENT INFECTION OF *AVIBACTERIUM PARAGALLINARUM* AND *MYCOPLASMA SP.* FOLLOWED BY PHYLOGENETIC CHARACTERIZATION OF *AVIBACTERIUM PARAGALLINARUM* IN DESI BIRDS

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In the present report, evidence of concurrent infection of this two major pathogen were identified in a farm located at Nagercoil maintain about 100 desi birds, in that nearly about 30 birds of three months old were showed the clinical signs of nasal discharge and swelling of infraorbital sinus. Randomly 15 samples were collected from nasal discharges and thick cheesy exudate from infraorbital sinuses. The initial inoculation was performed on to Chocolate agar plate (CAP) and simultaneously inoculated in the PPLO agar media. Samples showed characteristic growth of *A. paragallinarum* and *Mycoplasma sp.* on PPLO agar produced "fried egg" colonies. Direct HPG-2 PCR was also performed from the 15 clinical samples, of which 10 samples were identified as *A. paragallinarum* by producing a specific amplicon of 500bp. HPG-2 PCR positive samples were further subjected for serotype identification by serotype-specific PCR targeting the HMTp210 region and all the samples were identified as serovar A by producing an amplicon of 0.8 kbp. A sample was selected randomly and sequenced. BLAST analysis revealed 99% sequence identity with Indian (Pune) sequences followed by Iran and China. Simultaneously, 16S rRNA gene

amplification was performed among the 15 samples 05 samples showed positive for *Mycoplasma* sp. which are found already positive for *A. gallinarum*. This study emphasize the importance of ruling out the occurrence of concurrent infections during poultry disease investigation to make effective control measures to prevent the disease.

OP_3.10: Genotypic Characterization of field isolates of Newcastle disease virus (*Avian Orthoavulavirus-1)*

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Newcastle disease caused by *Avian orthoavulavirus -1 (AOAV-1)* affects more than 250 species of birds worldwide. The disease is endemic in many parts of the world especially Asian countries like India with outbreaks in vaccinated and unvaccinated flocks leading to great economic impact. The strains of Newcastle disease virus are divided into lentogenic, mesogenic and velogenic based on the virulence in chickens. Further based on phylogenetic analysis of F gene of NDV and recent unified classification, the strains are categorized in to 21 genotypes (I to XXI) organized in to class I and class II. The commonly used vaccine strains belonged to genotype II and in India the genotypes of ND viruses in ND outbreaks were genotype XIII, which is different from the vaccine strains. However, the genotypic characterization of AOAV-1 in apparently healthy backyard and vaccinated chicken is limited. Hence the present study was undertaken to genotypically characterize field isolates of AOAV-1 from both clinical ND outbreaks and apparently healthy vaccinated poultry. A total of 17 isolates (3 from vaccinated chicken from private farms, 10 from ND outbreaks and 4 from apparently healthy backyard chicken were included in the study. The MGB TaqMan Real time PCR assay identified fifteen isolates as virulent viruses with mean death time of 36-48 hours out of the total seventeen. Further genotypic characterization based on phylogenetic analysis of F gene carried out for seven isolates from different categories like vaccinated birds from private farms (D213, D217,D209), field outbreaks (D203, D230, D236) and apparently healthy chicken (D222) representing different geographical area over a period of 2017-2020 revealed two patterns of FPCS amino acid sequence characteristic of virulent AOAV-1 ^{112}RRQKRF^{117} and ^{112}RRRKRF^{117} and all the seven isolates were genotypes as genotype XIII.2.2. The study highlights the presence of genotype XIII ND virus in apparently healthy and vaccinated chicken and the need to continuously monitor and update the currently circulating genotypes of AOAV-1 to trace the evolutionary origin of AOAV-1 genotypes.

OP_3.11: Sero-positivity for haemorrhagic septicaemia (HS) in buffalo population of Andhra Pradesh

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Hemorrhagic septicemia (HS), an acute, fatal and septicaemic disease of cattle and buffaloes with high morbidity and mortality, is caused by Gram's negative bacterium- *Pasteurella multocida*. Although, HS vaccination is being carried out using killed whole cell antigen either with alum or oil adjuvanted, several cases of HS are being reported from endemic areas. The absence of routine assessment of HS vaccine effectiveness/ sero-conversion among susceptible population in a herd is greatly hampering effective immunization and infection control strategies in HS endemic states of India. Therefore, it's important to

assess the sero-positivity/ sero-prevalence against HS causing *Pasteurella multocida* among susceptible livestock in order to implement effective HS vaccination strategy in endemic areas. This would aid in assessing HS vaccine coverage as well as understanding the disease /immune status in population. In this study, we employed in-house standardized indirect-ELISA based on heat extract whole cell antigens of *Pasteurella multocida* B:2 strain P52 for detecting HS specific antibodies in buffaloes. To assess sero-positivity, a total of 1880 buffalo serum samples were screened from 20 different districts of Andhra Pradesh (AP). Out of which, 51.4% sera were positives and 48.5% were negative. The overall vaccine coverage was 56% in Andhra Pradesh. The maximum percentage of animals that showed sero-positivity were from Guntur (80%) district and minimum was observed in East Godavari (25%) district. The factors responsible for spatial variation in vaccine coverage was analyzed. The individual animal level factors showed that age and Murrah breed were positively and significantly associated with the seroconversion against HS vaccination. The factors influencing the vaccination response and future strategies are discussed.

OP_3.12: Morphometric and molecular characterization of wing louse (*Lipeurus caponis*) from backyard chickens of west-coast India

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Lice infestation in poultry presents a significant challenge to poultry farmers, with implications for bird health, welfare, and productivity. In this study, incidence of wing lice infestation was recorded in Polish Cap, Srinidhi, and Gramapriya chickens of an organized poultry farm located at North Goa during the winter season. The wing louse was found to be attached to underside of the wings between the feather barbs on the primary or secondary wing feathers. White clumps of louse's eggs (nits) were found attached at the base of feathers in breast and thigh region in addition to active louse populations. The affected birds showed signs of feather loss, irritation, annoyance, itching, restlessness, scratching and feathers plucking. Morphologically lice were dark brown coloured, elongated, had dorsoventrally flattened body, wingless, about 2mm in length and body structure resembled to *Lipeurus caponis* when observed under stereo microscope. After the morphological identification, the total genomic DNA was extracted from whole lice and universal primers L6625, H7005 were used to amplify the DNA of mitochondrial gene COI (Cytochrome Oxidase Subunit I). The PCR product was sequenced using Sanger sequencing and the gene sequence was submitted to the GenBank with accession number PP709001. Based on phylogenetic analysis of the COI gene, the sequence of the present study clustered with those of *L. caponis* hosted by Indian peacock of China and Jungle fowl of UK, whereas distinct clads were formed by other lice species sequences from different hosts and geographical locations. This is the first study recording the COI sequences of *Lipeurus caponis* from India.

OP_3.13: Proportional rate of health disorders and their seasonal influence in crossbred cattle maintained under small holder production conditions

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Surveillance and monitoring of disease occurrence pattern are basis for risk analysis and subsequent disease control programmes in animal health sectors. Data generated from disease reporting systems alone (e.g., National Animal Disease Referral Expert System, India), or in combination with other sources are normally used for understanding animal health status. The aim of this study was to estimate the proportional rate of various health disorders in dairy animals maintained under small holder production system in Bengaluru rural district of Karnataka. In this study, monthly summary records (two years) of disease

reporting system maintained by local veterinary authority at Bengaluru Milk Union Limited, Devanahalli taluk of Bengaluru rural district, Karnataka (India) was collected and the association between different disease categories and seasons were analysed using log-linear model. The proportional rate of endemic and infectious diseases was found to be significantly (P=0.001) higher followed by digestive tract and udder health disorders, than minor diseases as reference category. About 62% cases were treated once i.e., fresh cases and 38% were follow-up cases. Significant (P=0.01) influence of season was observed with overall more health disorders during winter than summer seasons. Endemic diseases were significantly (P<0.001) higher during winter and udder health disorders were significantly (P<0.001) higher during rainy seasons. About 61-65% of subclinical mastitis prevalence was observed in study area based on California Mastitis Test results. It is concluded that, endemic and infectious diseases followed by digestive tract and udder health disorders were more common health disorders, which requires prioritized control strategies in the study area.

OP_3.14: Molecular characterization of Foot and Mouth Disease virus Serotype-O from field outbreaks during 2024

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Foot-and-mouth disease (FMD) is a major disease of livestock in India and has immense economical importance. During 2024, 40 outbreaks have been reported from different parts of the country. A total of 317 clinical samples suspected for FMD were analyzed for serotype identification using sandwich ELISA/multiplex PCR and about 188 were found specific for serotype O. The genetic characterization of 38 isolates was done by VP1 coding region sequencing and phylogenetic analysis which showed 13 were clustered within O/ME-SA/SA-2018, and 25 isolates within lineage O/ME-SA/Ind2001e. The analysis showed that lineages O/ME-SA/Ind2001d could not be detected anywhere in the country. The lineages O/ME-SA/Cluster-2018 and O/ME-SA/Ind2001e were responsible for 35 % and 65 % of the FMD outbreaks recorded during 2024. Thus, further confirming the dominance of lineage O/ME-SA/Ind2001e and strong establishment of O/ME-SA/SA-2018 lineage in India.

OP_3.15: Molecular detection and prevalence of *Anaplasma* spp. in cattle, sheep and goats in Tamil Nadu, India

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Ticks and tick-borne diseases cause 61076.46 million economic losses in India. Anaplasmosis, babesiosis and theileriosis were listed among the top ten livestock diseases in India. Anaplasmosis is a tick-borne rickettsial disease of ruminants widely spread in tropical and subtropical areas. The present study was aimed in detecting different *Anaplasma* spp. in cattle, sheep and goats by using species specific PCR in Tamil Nadu, India. Blood samples of total 279 were collected from cattle (n=163), buffaloes (n=4), sheep (n=56), and goats (n=55), from nine districts of Tamil Nadu, India covering five agroclimatic zones. DNA was extracted from whole blood samples by NaOH lysis method and were screened with species specific primers for *Anaplasma marginale, A. bovis* and *A. ovis*. For each species, one sample was randomly taken for sequencing and phylogenetic analysis was carried out. The overall prevalence of *A. marginale, A.*

bovis and *A. ovis* was 33.33%, 9.32% and 12.54% respectively, in Tamil Nadu. Cattle were predominantly infected with *A. marginale* (50.31%) and *A. ovis* infection was most commonly seen in sheep (28.57%) and goats (34.55%) than *A. bovis*. In general, goats were infected with *Anaplasma* spp. than sheep. Phylogenetic and BLAST analysis of *A. marginale msp5, A. bovis groEL* and *A. ovis groEL* gene sequences revealed that they were 98.55% to 100%, 88.14% to 97.65% and 97.78% to 100% respectively, matched with nucleotide sequences from different geographical location, host and vectors deposited in NCBI GenBank database. This study imparted salient information on the epidemiology of *Anaplasma* spp. in Tamil Nadu, India. Since this study also included apparently healthy animals, it is suggested that healthy animals might act as the potential reservoir for uninfected animals.

OP_3.16: Foot and Mouth Disease epidemics in India during last two decades

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Foot and mouth disease (FMD) has been endemic in India for ages, with four serotypes (O, A, C, and Asia1) identified to date. Serotype O is the primary cause of FMD outbreaks in the country, followed by serotypes A and Asia1. The last recorded outbreak of serotype C occurred in 1995. Due to its endemic nature, FMD occurs sporadically throughout the year, with periodic epidemics occurring every 2-4 years. A vaccination-based FMD control program was launched in 54 districts in 2004, gradually expanding to cover the entire country by 2019. With large-scale vaccine application in the field, the severity of FMD outbreaks has generally decreased. Over the last two decades, India has faced three major epidemics—in 2013, 2018, and 2021—with decreasing severity. The 2021 epidemic was less severe and widespread compared to those in 2013 and 2018. All these epidemics were caused by emerging lineages of serotype O: O/ME-SA/Ind2001d in 2013, O/ME-SA/Ind2001e in 2018, and in 2021, a combination of the emerging O/ME-SA/SA-2018 and the existing O/ME-SA/Ind2001e lineages, with a strain ratio of 40:60. The cyclical nature of FMD outbreaks is primarily due to the waning of infection-induced immunity in the population and the introduction of new naïve animals. The current Indian serotype O vaccine strain INDR2/1975 is able to cover antigenically, the strains responsible for FMD outbreaks in the country. By improving herd immunity through regular vaccination, the country aims to break the virus transmission cycle and prevent future epidemics

OP_3.17: Molecular identification and Phylogenetic analysis of forensically important *Chrysomya* fly species based on Cytochrome Oxidase I and II genes

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Chrysomya flies are forensically important blow flies in vetro-legal cases because of its larvae found on dead wild animals. A total of 850 maggots were collected from 20 wild animals namely Elephant (*Elephas maximus indicus*), Leopard (*Panthera pardus*), Sloth Bear (*Melursus ursinus*), Indian gaur (*Bos gaurus*) and Wild boar (*Sus scrofa*) from 2019 to 2022 at Coimbatore, Kanniyakumari and the Nilgiris districts of Tamil Nadu and Nandyal district of Andhra Pradesh, India. The collected maggots were morphologically identified under the stereo zoom microscope and Scanning Electron Microscope as *Chrysomya megacephala*, *C. albiceps, C. rufifacies* and *C. villeneuvi.* Among the 4 species of maggots, *Chrysomya megacephala* (49.76%) was found with high prevalence followed by *C. albiceps* (40.70%), *C. rufifacies* (8.70%) and

C.villeneuvi (0.82%). Among the 20 wild animals, highest prevalence of larval stages of *Chrysomya* was observed on Elephant (75.00%), followed by Wild boar (10.00%), Sloth bear (5.00%), Leopard (5.00%) and Indian gaur (5.00%). The PCR was employed for *Chrysomya megacephala* and *Chrysomya villeneuvi* which was collected from Wild boar and Elephant respectively by using both cox I and cox II primers and molecularly identified as *Chrysomya megacephala* and *C.villeneuvi* based on the base pairs of 631bpand 624bp for cox I respectively. Similarly, *Chrysomya megacephala* and *C.villeneuvi* were binded with 559bp and 605bp respectively by using cox II primers. The Collected maggot, *Chrysomya megacephala* from Wild boar from the province of Tamil Nadu was assigned accession numbers as OQ621340 and OP697871 using CoxI and CoxII primers respectively. Similarly, *C.villeneuvi* from Elephant from the province of Tamil Nadu was assigned accession numbers as OQ826494 and OQ876862 respectively.

OP_3.18: Isolation, whole genome sequencing and comparative genomics of *Corynebacterium pseudotuberculosis*

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Corynebacterium pseudotuberculosis causes caseous lymphadenitis in small ruminants and ulcerative lymphangitis in equine. *C. pseudotuberculosis* TN_CUL_1 strain was isolated from Sheep affected with caseous lymphadenitis. Whole genome sequencing of the isolated showed that the genome was 2.32Mbp and an average G+C content was 52.17%. Protein subsystem analysis showed that maximum number of genes were involved in metabolism (n= 446) while 80 genes were involved in defence and virulence mechanism. Whole genome phylogeny of TN_CUL_1 along with 158 whole genomes available in public domain showed two major clusters namely biovar Equi and biovar Ovis. TN_CUL_1 corresponds to biovar Ovis and is closely related to strain 206 isolated from Brazil. TN_CUL_1 is the first report of whole genome of *C. pseudotuberculosis* from India. Comparative genomic study of all the 159 strains evinced the presence of 42 virulence genes predicted by VFDB tool. Phospholipase D coding gene *plD* is predicted among all the strains, indicating that all the strains are virulent. Total of 40 strains lacked virulence genes like *ureG*, *ureB, sapD* and *sigA*. Pangenome analysis showed that there were 2388 total genes of which 1909 are core genes and 479 accessory genes. This indicates that *C. pseudotuberculosis* has a closed pangenome structure. Multidimensional scaling showed separate cluster of some of the biovar Equi strains. Antimicrobial resistance gene prediction showed that 7 strains had *aph*(3')-IIa_2 and *blaTEM*-116_1 corresponding to resistance against aminoglycoside and β-lactam antibiotics. The study also identified specific genes for biovar Ovis and biovar Equi. This study documents the first report of whole genome of *C. pseudotuberculosis* from India and its comparative genomics.

POSTER PRESENTATION

PP_3.1: Seroprevalence and trend in co-occurrence of brucellosis among household domestic animals in South India

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A cross-sectional study was conducted in Tamil Nadu, Kerala, Andhra Pradesh, and Karnataka states of India from July 2022 to July 2023 to determine the seroprevalence of brucellosis in cattle, buffalo, sheep, and goat, and to determine the trend in co-occurrence of brucellosis among different livestock species. A total of 840 random serum samples were collected from household animals maintained in 18 village complexes of the four states. The sera collected from 406 cattle, 150 buffalo, 156 sheep and 128 goats were screened using blocking ELISA (BruAlert kit). Overall seroprevalence of brucellosis in all livestock species was 18.57%. The seroprevalence of brucellosis was 7.14%, 10%, 27.37% and 49.36% in cattle, buffalo, goat, and sheep, respectively. Principal component analysis of village wise and host wise percentage prevalence of brucellosis in 13 village complexes containing all the livestock species showed high level of co-occurrence of brucellosis between sheep and goat (Cov= 0.66) and moderate level of co-occurrence between sheep and buffalo (Cov= 0.31) & cattle and buffalo (Cov= 0.31). No co-occurrence was found between cattle and goat (Cov= -0.12), and cattle and sheep (Cov= 0.09). These results suggest the major role of buffalo as common reservoir host for brucellosis among livestock species. Hence, more emphasis should be given for prevention and control of brucellosis in buffalo, which in turns prevents brucellosis among other livestock species.

PP_3.2: Prevalence of enteric colibacillosis among post weaned piglets

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The enteric colibacillosis is a major reason for death of post weaned piglets worldwide. Porcine diarrhea is commonly caused by different types of E. coli particularly caused by the colonization of the small intestine by enterotoxigenic strains of *Escherichia coli.* The shiga toxin producing E. coli, necrotoxigenic *E. coli*, enterohaemorrhagic *E. coli*, entero aggregative *E. coli* and entero invasive *E. coli. A total of 52 fecal swab and 35 post mortem samples collected from* 3 piggery farms in Tamil Nadu having history of post weaning diarrhea. The post mortem lesions shows severe dehydration and distention of the small intestine with yellowish, watery mucoid fluid and the colon dilated with mucoid fluid. The stomach is dilated with partially digested food. The samples were subjected in to culture isolation and identification with Mac Conkey, XLD, MSA and BHI agar. Among the 87 samples 52 samples shows *E.coli* and 2 samples shows salmonella isolates by colony morphology and biochemical test. The intestinal content and rectal swab were subjected into parasitic examination which shows negative for Ascariasis and other parasitic eggs. The samples were subjected into DNA extraction and PCR with specific primers. The antibiotic sensitivity pattern shows sensitivity towards enrofloxacin, gentamicin, metronidazole and chloramphenicol. The increased prevalence of multi-resistant *E. coli* strains from diarrhoeic pigs is more attention on the alternatives to antibiotics such as vaccines, probiotics, prebiotics, additives and management practices.

PP_3.3: PORCINE CIRCOVIRUS-2 FROM HEALTHY PIGLETS FROM SOUTHERN TAMILNADU

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A total of 30 rectal swabs was collected from healthy piglets of about 6 weeks old belonging to three different piggery farms from Kanyakumari district. All the samples were processed and subjected to the screening and detection of the PCV2 genome by partial amplification of the *ORF2* gene which codes for capsid protein (Cp) and serves as an epidemiological marker. In that, about 15 samples were positive for PCV-2. Randomly, 04 partially amplified *ORF2* genes were sequenced and the aligned sequences were subjected to BLAST analysis and phylogeny in MEGA 11. BLAST analysis showed that they are having homology with PCV 2b genotypes sequences. The PCV2b genotype has a 100% sequence homology with Chennai, Kerala and Punjab isolates of India followed by 99.3% homology with China and Vietnam isolates. Amino acid analysis of all the ORF2 gene sequences showed there is single base change at the 115 and 116 position. This study emphasize the importance on increase in subclinical porcine circovirus -2 infection to make effective measures to control over the disease.

PP_3.4: Molecular Characterization of Fowl Adenovirus associated with Inclusion Body Hepatitis in broiler Chickens

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Inclusion Body Hepatitis (IBH) associated with fowl adenovirus (FAdV) infections are reported globally and responsible for tremendous economic losses in poultry production. The poultry industry in India has recently experienced disease outbreaks involving FAdV infections. The present study was undertaken to characterize the FAdV associated with IBH in broiler chickens in Tamil Nadu. Liver samples were collected from suspected IBH outbreaks in chicken from the Udumalpet and Tiruppur regions in Tamil Nadu. Necropsy showed enlarged pale yellow friable liver, haemorrhagic kidneys and congested spleen. Histopathological examination revealed eosinophilic intranuclear inclusions, mononuclear cell infiltration and vacuolar degeneration of hepatocytes. All the samples were subjected to FAdV hexon gene-specific PCR with an amplicon of 897-bp for confirmation. The PCR results were positive in eight number of samples collected from Udumalpet out of twenty number of samples examined. Sequencing of three selected FAdV positive PCR products and phylogenetic analysis revealed that all the samples showed close evolutionary relationship with FAdV serotype 11 of species D. This study demonstrated the involvement of FAdV11 in ongoing IBH outbreaks in Tamil Nadu. Given the genetic and serological diversity of the FAdV, it is essential to monitor the circulating serotypes and understand the molecular evolution of the FAdV to ensure that appropriate vaccines are being used for prophylaxis.

PP_3.5: Repetitive genes-based typing differentiates phenotypically identical *Pasteurella multocida* isolates from Sheep

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Pasteurellosis, caused by *Pasteurella multocida* strains, is a highly significant and economically important disease that affects small ruminants especially sheep and goats. The disease manifests in severe

respiratory symptoms leading to high morbidity and mortality rates. For rapid diagnosis and differentiation of strains, conventional methodologies are less sensitive and time-consuming. However, molecular techniques focused on genetic profiling based on repetitive genes like REP and ERIC markers are more sensitive, and rapid alternatives to diagnose and differentiate *P. multocida* strains. The present study aims to isolate and analyze the genetic diversity of *P. multocida* using REP and ERIC-PCR assays. A total of 352 clinical samples were collected and processed from sheep and goat farms across various regions of Karnataka. The initial identification was performed using conventional and specific PCR assays. Out of the 352 clinical samples, 9% were found positive, of which 17 *P. multocida* were isolated. These isolates were then processed for phenotypic and genotypic characterization using REP and ERIC-PCR assays. Both repetitive gene profiles generated multiple amplicons ranging from ~100 to ~2500 bp. Cluster analysis with ERIC profiles revealed three clusters, whereas REP formed two clusters based on banding patterns. Overall, the findings showed genetic diversity among circulating strains of *P. multocida* from sheep origin belonging to the same geographical state.

PP_3.6: Occurrence and Epidemiological Study of babesiosis in dogs

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The aim of this study was to determine occurrence and epidemiological investigation of canine babesiosis in and around Jaipur. This study was conducted on the dogs which were presented in medicine section of Veterinary Clinical Complex of PGIVER, Jaipur from April 2024 to July 2024. In this period total 642 dogs were reported in medicine section of VCC affected with various affections. Out of these 63 samples were received in veterinary Clinical diagnostic lab, suspected for protozoal infection (babesiosis) in dogs. Data regarding sex, age, breed and presence or absence of tick was recorded. Blood samples were prepared, stained with Giemsa stain and examine under light microscope for babesiosis. The results showed an overall occurrence of 25.39% (16/63) babesia spp. infection in dogs. Based on breed analysis, the infection was found in different breeds in following proportions: Golden Retriever 3/3, Pomeranian 3/3, Bull dog 1/1, non-descript 6/27 and Jerman Shepherd 2/18. Other breeds which were suspected and examined, but not found positive are as follows: Labrador (0/10), Begal (0/7), Pug (0/1). Of the infected dog, 8 (50%) were male and 8 were (50%) female. Dogs between 3month<36month old had higher prevalence of infection (14/16) while those of >48 month of age, (2/16). This study indicates alarming presence of babesia spp. infection in dogs in this region which requires preventive measures to curb the infection, Epidemiological outcomes require more no of samples to be study for more confirmatory findings.

PP_3.7: Occurrence of fowl adenovirus serotype 11 in commercial broiler chickens

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An investigation was carried out in 20 commercial broiler flocks with a history of retarded growth, lameness, 2 to 5% mortality and increased feed conversion ratio. The post mortem examination of the dead birds revealed hydropericardium, mottling of liver and undigested feed in the intestine. Samples such as heart, liver, spleen and intestine were collected for bacterial and molecular detection of viral etiologies. On cultural examination of the samples, no pathogenic bacteria could be isolated except in four lameness cases where only *Staphylococcus aureus* was isolated. The molecular screening of the above samples for

Newcastle disease virus, fowl adenovirus, avian reovirus, chicken anemia virus and reticuloendothelial virus revealed the presence of IBH (FAdV). Out of above-mentioned viruses, only FAdV was detected in 8 (40%) flocks of 2- to 4-week-old. The PCR amplicons of hexon gene of FAdV from two flocks were sequenced to identify the serotype involved in the outbreak and the analysis revealed serotype 11 FAdV. The findings of the investigation clearly demonstrate the prevalence of serotype 11 FAdV in broilers as the etiology for IBH emphasizing the need for vaccinating the parent birds with serotype 11 of FAdV to prevent the economic loss brought by FAdv in commercial broiler flocks.

PP_3.8: Potential diagnostic markers of antimicrobial resistant, biofilm forming pathogenic *salmonella* isolated from ducks and associated environments

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To explore suitable diagnostic markers for *Salmonella* spp. that exhibits distinct virulence, antibiotic resistance, and biofilm formation characteristics, 195 tracheal swabs, 192 cloacal swabs, and 75 environmental samples (soil, water, feed), were collected from Indigenous, Khaki Campbell, and Pekin ducks in West Bengal, India. All positive *Salmonella* isolates were screened by PCR for detecting antibiotic-resistant genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, and bla_{AmpC}), biofilm genes (*csgA*, *sdiA*, *rpoS*, *rcsA*), and the virulent gene *invA*. Outer membrane protein preparation (assessed by SDS-PAGE) was considered the selection criteria of a prominent candidate (C-10a). Out of the 436 *Salmonella* spp. isolates tested, 184 (42.20%) were identified as ESBL producers. PCR delineated the notable occurrences of bla_{TEM} (36.47%), bla_{CTX-M} (20.64%), bla_{SHV} (17.66%), and bla_{AmpC} (32.57%) genes. Concurrently, a diverse prevalence of biofilm-associated genes, including *csgA* (54.59%), *sdiA* (52.52%), *rpoS* (80.28%), and *rcsA* (63.76%), was observed. Remarkably, the virulence gene *invA* was detected in 32.34% of the isolates. SDS-PAGE analysis of outer membrane proteins (OMPs) from six *Salmonella* spp. isolates revealed varied bands ranging from 5 kDa to 109 kDa. Western blot analysis revealed the immunodominant polypeptides of 69 and 35 kDa in all those isolates (n=8). In short, the study revealed apparently healthy duck might carry *Salmonella* spp. having a distinctive triad of antibiotic resistance, biofilm formation, and virulence genes. The data suggested two cross-reactive polypeptides of 69 and 35 kDa that might serve as diagnostic markers for identifying *Salmonella* spp. with specific traits like antibiotic resistance, biofilm formation, and virulence gene expression.

PP_3.9: Maximizing the yield of therapeutic plasmids in maxiprep preparations through a modified alkaline lysis approach

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Plasmids play a crucial role in molecular biology and plasmid DNA vaccines are gaining importance nowadays. Commercial kits for plasmid purification are costlier and the traditional methods are suitable for lesser culture volume. Hence, ways to increase the yield of plasmid were studied. To optimize the yield of DH5α cells carrying the recombinant plasmid, two broth compositions viz. Luria Bertani (LB) broth

and Luria Bertani broth supplemented with 1.9 % added yeast extract (LBY) were used. The dry pellet yield (g/ 250 ml) of DH5α cells containing recombinant plasmid (pVAX1 -ILTV gB) in LB broth and LBY broth after 18 hours of overnight culture were 1.142 ± 0.016 and 1.535 ± 0.016. Additionally, the traditional alkaline lysis method was modified by using calcium chloride (5M), sodium chloride (2.5M), and 40% PEG6000, which significantly increased the yield of supercoiled plasmid DNA. The mean yield of plasmid purified from 250 ml of culture volume using the commercial kit and the modified method was 566 µg and 3530 µg, respectively. The modified alkaline lysis method yielded five times higher yield compared to purification by kit. The purity of plasmid in commercial kit and modified alkaline lysis ranged from 1.84 and 1.99, respectively. The plasmid extracted using modified method was further checked for its suitability in downstream applications by digesting with restriction enzymes and transfection studies in cell culture. Plasmids purified using modified method are cost effective, suitable for clinical use, cloning and transfection studies.

PP_3. 10: Studies on antimicrobial resistance, molecular detection and evaluation for co-infection of various respiratory pathogens of poultry in Punjab

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The present study was conducted to investigate the prevalence of bacterial and viral pathogens associated with respiratory tract of poultry in Punjab. The bacterial pathogens were evaluated for antimicrobial resistance both phenotypically and genotypically. A total of 115 samples including tracheal swabs, tracheal and lung tissues were collected from post-mortem of birds showing clinical signs of respiratory distress. A total of 52 isolates (45.2%) of different bacteria related to respiratory infections of poultry were obtained out of which 40 isolates (76.9%) of *Escherichia coli*, 6 isolates (11.5%) of *Klebsiella pneumoniae*, 4 isolates (7.69%) of *Proteus mirabilis*, 1 isolate (1.92%) of *Pseudomonas aeruginosa* and 1 isolate (1.92%) of *Staphylococcus aureus* were identified by conventional and molecular technique(s)-MALDI-TOF. On culture sensitivity test, all the isolates (52) tested showed multi-drug resistance (MDR). The *E. coli* isolates were 100% resistant to tetracycline, chlortetracycline, enrofloxacin, amikacin, tylosin, ofloxacin, ciprofloxacin and erythromycin. The isolates were evaluated for the antimicrobial resistance genes and 60% of the isolates were positive for the antimicrobial resistance gene *strA*, followed by *ere* (50%), *tetA* (47.5%), *aac-(3)-(IV)* (37.5%) and *blaTEM* (32.5%). On molecular detection for fastidious bacteria like *Mycoplasma* spp. (*M. gallisepticum* and *M. synoviae*) and *Avibacterium paragallinarum*, out of 115 samples, 25 samples (21.7%) were positive for *M. gallisepticum*, 22 samples (19.1%) were positive for *M. synoviae* and none of the samples were positive for *A. paragallinarum*. The samples were screened for the prevalence of viral pathogens like Infectious Laryngotracheitis Virus (ILTV), Infectious Bronchitis Virus (IBV), and Newcastle Disease Virus (NDV) which was found to be 13.04%, 56.5% and 20.8%, respectively. Multiplex Reverse-Transcription PCR was standardized using published primers for the known positive samples. This study inferred the presence of co-infection among the bacterial and viral pathogens with the highest percent between NDV and IBV. The increase of AMR among the bacterial isolates was indicated both phenotypically and genotypically. The presence of co-infection warrant a rapid diagnostic technique that can concurrently detect various infections.

PP_3.11: Molecular detection and characterization of infectious coryza from commercial laying hens of Namakkal district, Tamil Nadu

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Infectious coryza (IC) is an upper respiratory tract infection in chickens caused by *Avibacterium paragallinarum*. The present study aimed to detect and characterize *Avibacterium paragallinarum* serovars from outbreaks of infectious coryza in poultry by PCR and sequencing. A total of five IC outbreak flocks that occurred between 2019 and 2020 were investigated. The IC serovars were identified at molecular level by PCR and sequencing a portion of *hmtp210* gene which encodes outer membrane hemagglutinin protein. DNA sequencing of *hmtp210* gene indicated that three isolates were clustered with serovar C and one isolate was clustered with serovar B based on the nucleotide similarities percentage and phylogenetic analysis. Out of four flocks, two flock isolates showed 99.91% homology with serovars C and C4. One isolate showed 99.69% sequence homology to serovars C, C1, C2 and C3 and another isolate showed 99 to 100% sequence homology to serovars B and B1**.** It was concluded that the present study may provide preliminary data on molecular typing of IC serovars and formulating control measures since there was limited data on molecular typing of IC serovars based on sequencing of hypervariable region of *hmtp210* gene.

PP_3. 12: *Brucella* sero-positive indigenous cattle breeds were less likely to have LSDV infection

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A disease risk analysis among three different diseases such as brucellosis, Q-fever, LSD was performed in 176 random cattle samples collected in Tamil Nadu, Kerala, Karnataka and Andhra Pradesh states from July 2022 to July 2023. The samples were screened for presence of antibodies against brucellosis, Q-fever and LSD using BruAlert blocking ELISA kit, ID Screen Q Fever Indirect Multi Species ELISA kit and ID Screen Capripox Double Antigen Multi Species ELISA kit respectively. Seroprevalence data of each disease was used for the odds ratio and principal component analysis. The 176 cattle were categorized based on breeds such as indigenous breeds (N=41) and cross breeds (N=135) as well as based on age – Heifer (N=35) and Adult (N=141). Overall seroprevalence of brucellosis, Q-fever and LSD in 176 cattle was 13.63%, 1.70% and 43.18% respectively. Seroprevalence of brucellosis, Q-fever and LSD in adult cattle and heifer cattle were 10.63% and 25.71%, 1.41% and 2.85%, and 45.39% and 34.28% respectively. Seroprevalence of brucellosis, Q-fever and LSD in indigenous breed cattle and cross breed cattle were 31.70% and 8.14%, 2.43% and 1.48%, and 39.20% and 44.44% respectively. Principal component analysis was done for all 176 samples and negative correlation was found between brucellosis and LSD (COV= -0.11), and brucellosis and Q-fever (COV= -0.05). Odds ratio was calculated for all the 176 samples as well as breed specific sub-groups of cattle. Odds ratio of 176 samples suggest that *Brucella* sero-positive cattle were 50% less likely to have LSD (P=0.14). Odds ratio among different breeds also suggest that *Brucella* sero-positive indigenous cattle breeds were 93% less likely to have LSD (P=0.01). However, no significant risk was found when odds ratio between Q-fever and LSD, brucellosis and Q-fever was analyzed. The principal component analysis and odds ratio suggest that *Brucella* sero-positive cattle especially indigenous cattle breeds were immune to LSD. Exposure to *Brucella* might provide non-specific immunity against LSD in cattle.

PP_3. 13: Molecular evidence of Porcine parvovirus 6 (PPV6) infection in domestic pigs; first report in southern India

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Porcine parvovirus is a major causative agent of swine reproductive failure. During the last decade, several novel parvovirus species have been discovered in pigs namely PPV1 to 8. Porcine parvovirus 6 (PPV6) first identified in China during 2014 and later in other swine producing countries such as USA, Poland, Russia, China and found to cause porcine reproductive failure. PPV6 status in the Indian pig population remains undetermined. This study examined 100 field samples comprising of serum (n=50) and post-mortem tissues (n=50) collected from both suspected of reproductive failure and diseased pigs with systemic infections from four southern states of India. All samples were screened for PPV6 by specific PCR assays. Overall molecular positivity rate of PPV6 was found to be 5% (n=5) in southern India with. All the five PCR positive PPP6 samples are detected from systemic infected and reproductive failure pigs. Sequencing of three PCR positive amplicons revealed 394 nucleotides and BLAST analysis confirmed specificity of PPV6 genome. PPV6 sequence in this study revealed 99.5% homology with PPPV6 isolates from China. In the present study, PPV6 genome was identified in post-mortem tissue and serum samples collected from domestic pigs in southern India. This study evidences PPV6 associated with systemic infections and reproductive failure in domestic pigs for the first time in India.

PP_3.14: Molecular Characterization and phylogenetic analysis of Sheeppox Virus based on *RPO30* gene sequencing

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Sheep pox is an economically important disease caused by Sheeppox virus in the genus *Capripoxvirus*. Sheep pox causes high morbidity and mortality among sheep. The members of the genus *Capripoxvirus* have high genetic similarity and they are highly host specific. This study aims to characterize the sheeppox virus circulating in different districts of Tamil Nadu based on *RPO30* gene sequencing, A total of 50 sheeppox suspected samples from seven districts (Erode, Hosur, Pudukkottai, Karur, Tirunelveli, Tirupur, and Tiruvallur) were included in this study. Initial screening was carried out targeting p32 gene of *Capripoxvirus* . Out of 50 samples, 15 samples were found positive for *Capripoxvirus* with a specific amplicon size of 192 bp. Further seven representative samples from different regions of Tamil Nadu were chosen and the RPO30 gene was amplified in two fragments which yielded PCR amplicons of 520 bp and 550 bp size respectively.

The amplicons were sequenced by Sanger sequencing. The sequences were aligned using BioEdit software and phylogenetic tree was constructed using MEGAX software. Sequence analysis revealed five nucleotide changes at the positions 272 bp, 281 bp, 401 bp, 447 bp, 527 bp. Amino acid analysis revealed changes at 91 bp, 96 bp, and 175 bp. Phylogenetic analysis clustered the study sequences with Kazakhstan, Turkey, Russia, Saudi Arabia, and other Indian isolates. However, Sheeppox sequences from Hosur, Pudukkottai, and Tiruvallur form a different clade. This study indicates RPO30 gene is a valuable marker than p32 gene in differentiating the strain of the Sheeppox virus.

PP_3.15: Whole Genome Analysis of Porcine Circovirus1 from field swine samples - Foremost study in Southern India

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Porcine circovirus 1 (PCV1), first identified as a contaminant in a porcine kidney (PK-15) cell line in Germany in 1974, was long considered nonpathogenic. However, recent findings suggest that PCV1 can induce lesions in experimentally infected pigs. Although PCV1 has been detected in domestic pigs through serological and molecular studies, novel porcine circoviruses, such as PCV2, PCV3, and PCV4, which are linked to pathogenic conditions, have evolved from PCV1. Therefore, studying the molecular epidemiology and genetic variation of PCV1 remains crucial. In this study, five post-mortem porcine tissue samples suspected of systemic infections, including Classical Swine Fever Virus (CSFV), PCVs, and Porcine Parvovirus (PPV), were collected from Tamil Nadu and Karnataka. Viral nucleic acids were extracted and subjected to whole-genome sequencing using the Illumina NovaSeq 6000 platform. The raw sequence data were processed and annotated using the Galaxy online tool with reference PCV1 sequences. This study identified the complete genomes of two PCV1 sequences, K44ABT-2024 and L11ABT-2024, each measuring 1,759 base pairs with a G+C content of 48%. These sequences were submitted to GenBank under the accession numbers PQ303661 and PQ303662. The two major genes, ORF1 and ORF2, encode the replicase (rep) and capsid proteins, consisting of 938 base pairs (312 amino acids) and 701 base pairs (233 amino acids), respectively. BLAST analysis showed a 99.7% nucleotide identity with published PCV1 sequences. Phylogenetic and evolutionary analyses, conducted using MEGA-X through Maximum Likelihood and Neighbor-Joining methods, revealed that these PCV1 sequences clustered with sequences from India, the USA, and China. While the amino acid sequence of the rep protein was highly conserved, a notable substitution (A102V, where alanine is replaced by valine) was detected in the receptor binding domain (RBD) of the capsid protein in both PCV1 sequences. This mutated PCV1 RBD is similar to that of PCV2 and PCV4, suggesting a potential for binding to multiple cell types, akin to pathogenic PCVs. Furthermore, the analysis of three major B-cell epitopes 85GGTNPLP91, 162FTPKPELDKTIDWFHPNNK180, and 219YVQFREFILKDPLNK233 showed that the amino acid sequences were unaltered. This research represents the first complete genome analysis of PCV1 from domestic pigs in Tamil Nadu, providing valuable insights into its genetic features and potential implications for the future in this region.

PP_3.16: A molecular approach to understand the involvement of *Mycoplasma* in Suppurative Otitis cases of Cattle in Chittoor district of Andhra Pradesh

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In bovines the economic losses mainly due to the outbreak of diseases, leads to mortality, morbidity, treatment cost and reduced production. Recently in August month of 2024, received 13 bovine suppurative otitis samples from outbreak in Kuppam Mandal of Chittoor district. The history having the purulent discharges with foul smell from ear infections of cattle that to crossbreed cattle of all age groups are affected. After 5 days of appearing aural discharges the animals were also shown the nervous signs, head tilting and other clinical findings includes unilateral or bilateral ear droop, epiphora, ptosis, abnormal nystagmus, strabismus, regurgitation, stiff neck, opisthotonus, facial hyperesthesia, purulent aural discharge, nasopharyngeal collapse, recumbency and finally death were noticed in severely affected animals. All the samples were processed for cultural tests by inoculating in PPLO selective broth and selective media PPLO agar then incubated anaerobically at 37^0C and observed the color development in broth pink to yellow color after 3- 5 days of incubation indicative of positive growth and fried egg micro colonies on selective PPLO agar media after 8-14 days of incubation observed under low power and 40X. The DNA was isolated from all the samples and screened for presence of *Mycoplasma* by targeting *16s rRNA* gene and found that out of 13 samples 10 were positive for genus *Mycoplasma* and produced the predicted 280bp size product in all positive samples. The suppurative otitis in cattle usually is caused by *Actinomyces spp*., *Corynebacterium pseudotuberculosis*, *E. coli*, *Heamophilus*, *P.multocida*, *Pseudomonas spp*, *Streptococcus spp*., and *Mycoplasma bovis*. This study was targeted only the emerging pathogen i.e the cell wall deficient bacteria *Mycoplasma* because of infections is hampered by a lack of effective vaccines and specific treatments, leads to increasing trends in antimicrobial resistance. Concluded that isolation and identification of the pathogenic organisms is helpful for specific treatment to control the infection. The cultural methods are gold standard but time consuming; to overcome the difficulties more recently the molecular approach is an alternative for early detection followed by implementation of control strategies. In keeping in view, the objective of this study was a molecular approach to understand the involvement of *Mycoplasma* in suppurative Otitis cases of cattle in Chittoor district of Andhra Pradesh.

PP_3.17: First report of Lung worm infection due to *Metastrongylus elongatus* in a Non-Descriptive Pig in Tamil Nadu, India

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Lungworms are found to be a serious problem in Pigs by causing severe infections. The worms were collected during post mortem from a 6 years old male, non – descriptive pig at Periyeri, Hamlet of Thurinjikuppam, Panchayat polur taluk, Thiruvannamalai district, Tamil Nadu which was raised and hand fed as a single animal and had access to the agricultural field. On post mortem examination, the trachea was severely congested and occluded with pus and the lungs were congested in which the lung worms wriggled out with pus while sectioning the lungs. The worms were morphologically identified as *Metastrongylus*

*elongatus***.** This was the first report of lungworm infection due to *M. elongatus* in a Non- descriptive pig in Tamil Nadu, India.

PP_3.18: Occurrence of helminths and ectoparasites on sambar deer (*Rusa unicolor*), spotted deer (*Axis axis*) and barking deer (*Muntiacus muntjac*) in Tamil Nadu

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The study was under taken to know the occurrence of helminth and ectoparasites on wild and captive cervids such as Sambar deer (*Rusa unicolor*), Spotted deer (*Axis axis*) and Barking deer (*Muntiacu vaginalis*) from January 2018 to December 2023 in Tamil Nadu, India. A total of 13 dead deer viz., 9 sambar deer, 3 spotted deer and one barking deer were examined for the presence of helminth parasites and ectoparasites during post-mortem at The Nilgiris district (Nilakottai, Thalakunda, Bitharkadu, Panthalur, Ovalley forest ranges), Coimbatore district (Anamalai forest range) and Chennai district (Guindy deer park) of Tamil Nadu, India. Internal parasites of wild and captive cervids were identified as flukes like *Cotylophoron cotylophorum* and *Fischoederius elongatus* and round worms viz, *Setaria digitata.* The deer ked and the lice were identified as *Lipoptena cervi* and *Haematopinus tuberculatus,* respectively. The ticks were identified as *Amblyomma testudinarium*, *A. integrum*, *Haemaphysalis bispinosa H. birmaniae, H. aculeata, H. sambar, H. kutchensis, H. shimoga, H. spinigera, H. cornigera, Haemaphysalis* sp*, Rhipicephalus haemaphysaloides* and *Rhipicephalus* spp. The ticks were located mostly on the scrotum, ear, face, and body surface of the deer. Lice were observed on genital skin fold, anus, and tail. This is the first report of the occurrence of *Haematopinus tuberculatus* lice and *Amblyomma testudinarium* ticks on Sambar deer (*Rusa unicolor*) in India and elsewhere in the world.

PP_3.19: Ixodid tick species infesting wild Indian golden jackal (*Canis aureus indicus*) in Tamil Nadu

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The wild Indian golden jackal (*Canis aureus indicus*) is a medium-sized predator, omnivore found in Himalayan foothills and Western Ghats. A sub- adult, 3-4 years old male wild Indian golden jackal was rescued for treatment at Sivagangai range, Sivagangai district, Tamil Nadu, India. The wild Indian golden jackal was thoroughly examined for the tick infestation and the ixodid tick species viz. *Haemaphysalis paraturturis, H. intermedia*, *Haemaphysalis* spp, *Hyalomma isaaci*, *Rhipicephalus sanguineus R. turanicus* and *Rhipicephalus spp* were found feeding on the animal body. Of which, *Haemaphysalis paraturturis* and *Hyalomma isaaci* were observed on wild Indian golden jackal for the first time in world and *R. turanicus* was recorded on wild Indian golden jackal for the first time in India. This is the first study of Ixodid tick infestation on wild Indian golden jackal.

PP_3.20: Surveillance of parasitic infections in wild carnivores in the Nilgiris hills of Tamil Nadu

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The leopard cat (*Prionailurus bengalensis*) is the most widespread feline in Asia. The study was conducted to know the prevalence of parasitic infections in wild carnivores in the Nilgiris hills of Tamil Nadu from January 2018 to December 2022. During this period, 19 animals including 11 Leopard cats, 6 leopards, a tigress and a male Asian palm civet were found to be dead. The number of male and female in Leopard cats and Leopards are 9 and 2, 4 and 2 respectively with the age group ranging from 8 months to 6 years. The endo and ectoparasites were collected from the dead wild carnivores were identified morphologically by Stereo zoom and Scanning Electron Microscope (SEM). Out of 11 Leopard cats, 5 animals were infected with tape worms (45.45%) followed by each one with fluke (9.09%), with round worms (9.09%) and ticks (9.09%). Three animals showed the mixed infection namely tape worm with round worm, tapeworm with tick and tapeworm, round worm, ked with ticks. The flukes and round worms were identified as *Paragonimus westermani* and *Ascaris sp* respectively. The tape worms were identified as *Taenia taeniaeformis and Diphyllobothrium latum.* The ked were identified as *Lipoptena cervi.* The ticks were identified as *Rhipicephalus turanicus*, *Rhipicephalus haemaphysaloides*, *Ixodes scapularis* and *Haemaphysalis* sp. Among the tape worms, *Taenia taeniaeformis* (87.5%) was found to be more than *Diphyllobothrium latum* (12.5%). Out of 6 Leopards, 3 animals were infested with maggots (50%), 2 animals were infected with round worms (33.3%) followed by one animal with ticks (16.6%). No mixed infection was noticed in leopards. The round worms were identified as *Toxascaris leonina*. The maggots were identified as *Chrysomya rufifacies* and *Chrysomya megacephala*. The ticks were identified as *Haemaphysalis indica* and *Haemaphysalis turturis*. The tigress (*Panthera tigris*) had mixed infection with flukes (*Paragonimus westermani* and *Pseudodiscus collinsi)*, tapeworms (*Spirometra sp*), round worms (*Toxascaris leonine, Ancylostoma sp, Grammocephalus sp)* and ticks (*Amblyomma integrum)*. The Asian palm civet (*Paradoxurus hermaphroditus*) had mixed infection of round worms with ticks (*Ixodes ceylonensis*).

PP_3.21: Isolation and Identification of Bacteria from *Amblyomma integrum* tick

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Ticks represent a large group of pathogen vectors that blood feed on a diversity of hosts and are considered the second vector of human and animal diseases after mosquitoes. Tick-borne diseases and conditions are transmitted through the bite of an infected tick. These include Alpha-gal syndrome (AGS), Lyme disease, Anaplasmosis, Ehrlichiosis, Babesiosis, Powassan (POW), Rocky Mountain Spotted Fever, and Tularemia. Therefore, identification of ticks and associated pathogens is an important step in the management of these vectors. In Sri Lanka, *Amblyomma integrum,* a hard-bodied tick is an agent responsible for human otoacariasis disease. The present study focuses on the isolation and identification of a group of culturable bacteria associated with *Amblyomma integrum* females sampled in distinct geographical sites in Bargur village, Krishnagiri district, Tamil Nadu. Molecular identification of isolated bacteria was performed by PCR and BLAST analysis. BLAST and Phylogenetic tree analysis confirmed the presence of *Klebsiella pneumoniae.* This was the first report on the presence of *Klebsiella pneumonia*e on *Amblyomma integrum* tick.

PP_3.22: Molecular Characterization of Porcine Parvovirus (PPV) and Porcine Circovirus (PCV2) associated with reproductive failure in swine

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Reproductive failure in sows is a critical factor impacting pig breeding. Viral infections are the primary factors behind these reproductive issues. Given that reproductive failure in pig herds substantially reduces the profitability of pig farming, mitigating its effects is crucial for the industry. The study was carried out to evaluate the prevalence status and molecular characterisation of Porcine parvovirus (PPV) and Porcine circovirus 2 (PCV2) associated with reproductive failure in swine from Tamil Nadu, Karnataka, Andhra Pradesh and Kerala states. These two viruses are among the economically significant virus causing production loss to farmers. A total of 125 samples comprising both tissue (52) and serum samples (73) were collected from both organised and unorganised swine herds from Tamil Nadu, Karnataka, Andhra Pradesh and Kerala states. The samples were screened for PPV and PCV2 by PCR amplification of VP2 gene and ORF2 gene respectively. Out of 125 samples screened, 5 samples were positive for PPV and 17 samples were positive for PCV2 by PCR amplification of VP2 gene and ORF2 gene respectively. The phylogenetic tree revealed that the PPV isolates of present study are found to be grouping the other PPV1 isolates reported from China, USA, Colombia and other Indian PPV 1 strain which confirms that these isolates belong to PPV1.The PCV isolates of present study found to be belonging to PCV2h and PCV2d sub-genotypes and this study denotes diverse nature of circulating PPV-1 and PCV2 in India.

TECHNICAL SESSION - IV

PRODUCTION AND REPRODUCTIVE BIOTECHNOLOGY

Lead Paper -1

Sperm Phenome and Molecular Health: Implication for Bull Fertility Prediction

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INTRODUCTION

Reproductive biotechnologies are good model of transfer of technology between the laboratory and the field since the adaption of these technologies have been exemplary. Artificial insemination (AI) with cryopreserved semen, the first generation reproduction biotechnology, in one such technology that played an unequivocal role in genetic improvement and production enhancement, at least in large animals. For AI, it is imperative to use superior quality spermatozoa in terms of both genetic merit and fertilizing potential so that the desired results could be obtained in stipulated time frame. To achieve high conception rates with cryopreserved semen, at least two important criteria should be ensured. Firstly, the frozen semen dose should contain enough number of motile spermatozoa so that required number of spermatozoa reaches the site of fertilization. Secondly, the spermatozoa that reach the site of fertilization should have the desirable phenotypic and molecular characteristics so that they acquire the fertilizing potential during their stay at the female reproductive tract. Over a period of time the cryopreservation protocols had been optimized to recover higher number of motile spermatozoa after freezing and thawing, which is reflected in improved conception rates with frozen thawed semen; however, in spite of the advancements in the semen cryopreservation, approximately 50% of sperm are rendered immotile by cryopreservation and fertilizing capacity of spermatozoa is significantly decreased. Thus, precise determination of the fertility of the cryopreserved spermatozoa is utmost importance because semen from a single bull is used to inseminate thousands of females and using semen with poor fertility results in conception failure and colossal loss to the farmers. However, accurate prediction of sperm fertilizing potential still remains elusive. Even among the bulls that qualified all the breeding soundness evaluation assessments, the conception rates differ by 20–25% (Aslam *et al*., 2018; Kumaresan *et al*., 2021). With the developments in sperm analytical techniques like CASA, fluorescent microscopy, flow cytometry and a in molecular biology, now it has become possible to evaluate the spermatozoa in terms of specific functions that are well related to fertility. it is in this context, the recent developments in sperm phenotypic characterization, functional assessment, molecular health and their relationship with bull fertility are discussed here.

SPERM PHENOME AND FERTILITY

Traditionally semen quality control involves subjective assessment of motility, sperm morphology assessment and an estimate of the concentration of spermatozoa. However, they have limited value for predicting the fertility. Even today, post thaw sperm motility is considered as an indicator of fertilizing ability of the spermatozoa and it is mostly used for quality control in bovine breeding industry. Though good post-thaw sperm motility is prerequisite for the spermatozoa to reach the site of fertilization, it is not the sole attribute responsible for fertility. Attaining conception depends on structural and functional integrity of the spermatozoa. To achieve high fertility, sperm must be motile, retain their ability to produce energy via metabolism in mitochondria, maintain normal plasma membrane configuration and integrity, intact acrosome membrane, intact receptors that permit the spermatozoa to bind to the zona pellucida, maintain enzymes within the acrosome to allow penetration of ova and a nucleus capable of decondensation. Disruption of any of these functions or abilities will significantly affect the sperm's ability to achieve fertilization. Some of the important sperm phenome and their relationship with fertility are discussed below.

Sperm kinematics: In recent years, much progress has been achieved in automated sperm motility analysis, also known as computer-assisted semen analysis (CASA), the main goal of CASA is to eliminate the bias associated with subjective judgment (Amann & Waberski, 2014, Meza *et al*., 2018). Sperm kinematics comprises measuring the distance between each head point for a specific sperm at each stage of acquisition. According to Bompart *et al*. (2018) there are eight standard metrics for sperm motility; curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF). Various investigations have revealed associations of certain sperm kinematic parameters with fertility. Currently, CASA systems transmit successive images of a sperm suspension onto a detector array, detect objects based on the intensity of pixels in a frame or light scatter, and generate required output and information using specialized software (Amann and Katz 2004). Since sperm motility is thought to be strongly correlated with fertility, these electronic systems measure each sperm's motion or morphology and enable precise fertility prediction (Puglisi *et al*., 2012).

Plasma membrane integrity: Bull fertility and sperm plasma membrane integrity are linked, as evidenced by the various published studies (Kumaresan *et al*. 2017 ; Saraf *et al.* 2018), which supports the self-explanatory predictive value. While many fluorochromes have been employed for the purpose of assessment, the most commonly utilized ones include CFDA, Yo-Pro-1, propidium iodide, calcein violet, 7-amino-actinomycin D, and SYBR14 (Petrunkina & Harrison, 2011). Additionally, the hypoosmotic swelling test has been connected to bull fertility in the field and allows for the evaluation of plasma membrane functionality without requiring the fluorescent labelling of sperm (Rego *et al*., 2016). Therefore, evaluating the many structural and functional elements of the sperm plasma membrane, yields insights on more than just sperm survival and fertility

Acrosomal integrity: The fusion of the sperm plasma membrane with the outer acrosomal membrane, the creation of pores, and the release of acrosomal proteolytic enzymes into the extracellular environment are the hallmarks of the irreversible acrosome reaction (AR). The purpose of AR is to allow sperm to pass through the ZP and then unite with the oocyte. Fertility may be impacted by premature acrosome loss or inadequate acrosome development (Gerton, 2002). The most popular methods for determining acrosomal status involve the use of fluorescent-labelled lectins; in bull sperm analysis, the most commonly employed lectins are peanut and *Pisum sativum* agglutinin. Various studies, r = 0.59 (Singh *et al*., 2016) ; r = 0.88 Ahmed *et al*., 2016b) have demonstrated the substantial association between the percentage of sperm that preserve an intact acrosome or their capacity to undergo AR and bull fertility. The ability of sperm populations to conduct the AR in vitro corresponds with their fertilization success in vivo, suggesting that assessing the AR may serve as a predictive metric of fertility in bulls (Birck *et al*., 2010).

DNA integrity: Irregularities of nuclear integrity are regarded non-compensable components of sperm dysfunction; thus, evaluation of sperm chromatin integrity maintains an essential position next to traditionally performed spermatological examination. The following tests have been used to assess sperm chromatin structure: SCSA comet assay, terminal deoxynucleotidyl transferase deoxynucleotide triphosphate nick-end labelling assay, sperm chromatin dispersion test and light microscopy acridine orange test (Bollwein & Malama, 2023). Among these DFI is reported to be extremely sensitive and specific for detecting infertility in human sperm, and it has higher accuracy than the other methods (Santi *et al*., 2018). The greater the DFI %, the lower the fertility. Sperm chromatin integrity is critical not only for fertility, but also for the preservation of gestation and offspring health (Virro *et al*., 2004). Higher DFI has been connected with reduced fertility as shown in many studies (Kumaresan *et al*., 2017; Doğan *et al*., 2015), demonstrating the importance of chromosomal integrity. DNA damage is thought to have a negative impact on embryonic quality rather than affecting sperm's ability to fertilize the oocyte. For detailed information on the impact of sperm DNA damage on farm animal fertility, please refer to the recent review by Kumaresan *et al.* (2020).

Mitochondrial Integrity: Sperm mitochondria regulate the sperm motility and fertility. Low membrane potential of mitochondria significantly influence sperm function by impacting motility (Amaral *et al*., 2013). Mitochondrial activity can be assessed by Rhodamine 123 fluorescence, which is produced when dye diffuses into the cell and builds up in mitochondria (M al-Rubeai *et al*., 1993), depending on the amount of fluorescence, this fluorescence can be used to determine the amount of functioning mitochondria and enable the identification of mitochondrial damage. Along with R123, JC-1 or Mitotracker dyes can also be utilized. Spermatozoa having high MMP are positively associated with fertility, r = 0.81(Ahmed *et al*., 2016a) ; r = 0.88 (Selvaraju *et al*., 2009).

Capacitation status: Sperm capacitation is a sequence of events including the release of phosphatidylserine residues, alterations to the plasma membrane's lipid structure, cholesterol efflux, raised intracellular pH, and protein tyrosine phosphorylation, which occurs in the female reproductive tract and enables the sperm to achieve fertilizing ability. However, capacitation like changes (not physiological ones) do occur during the process of cryopreservation (Bailey *et al*., 2000) and are negatively associated with fertility, r = - 0.61 (Saraf *et al*., 2018) ; r = -0.51 (Singh *et al*., 2016). One of the most used techniques for assessing these variations is the chlortetracycline assay.

Oxidative stress: An appropriate amount of reactive oxygen species (ROS) is required for normal sperm activity and fertilization (Wagner *et al*., 2018). Oxidative stress (OS) is defined as an imbalance between the amount of ROS produced and antioxidant's ability to detoxify them. Higher ROS levels in semen can lead to OS, which plays a significant role in male infertility, impaired sperm motility, DNA damage, and hereditary disorders (Alahmar, 2019). Research indicates that ROS can damage plasma membranes and nuclear DNA, and negatively associated with fertility, r = -0.92 (Del Olmo *et al*., 2015). Dihydroethidium and 2',7'-dichlorodihydrofluorescein diacetate can be employed as a particular probe to identify superoxide anion, and MitoSOX can identify mitochondrial superoxide.

Apoptosis: Apoptosis is a type of cell death that occurs naturally and only affects individual cells, leaving the surrounding tissues uninflamed. During this process, the outer leaflet of the plasma membrane of healthy cells exposes phosphatidylserine (PS), which is typically found on the inner cytoplasmic leaflet (Martin *et al*., 1995). Annexin V is a phospholipid-binding protein that attaches to cells that have exposed PS and has a high affinity for PS which is reliant on Ca^{2+} (Vermes *et al*., 1995). Apoptotic spermatozoa had negative correlation with fertility when assessed in post thaw samples in buffaloes, r = -0.39 (Singh *et al*., 2016).

SPERM MOLECULAR HEALTH AND FERTILITY

The methodologies used to examine the morphological and functional properties of sperm have only moderate connections with fertility, making them ineffective for predicting the fertility of bulls (Rodríguez-Martínez, 2018). Furthermore, it has been demonstrated that the fertility of a considerable proportion of bulls with normal spermiograms, kinematics, and morphology remained suboptimal (Menezes, *et al*., 2019) indicating that there is something beyond the sperm phenome. Earlier, it was thought that the role of sperm was restricted to only transferring the paternal DNA to the oocyte. But, in recent decades, enough evidences accumulated for the other roles of spermatozoa during and after fertilisation. With the developments in high-throughput sequencing technologies, now it is possible for evaluation of thousands of molecules in spermatozoa in a given time. Recent developments in high-throughput sequencing technologies that allow the assessment of thousands of molecules in a given time has revolutionized the research on bull fertility. Using such techniques, several studies identified that bull fertility was influenced by several factors including sperm transcripts (Paul *et al*., 2021; Prakash *et al*., 2020; 2021), sperm and seminal plasma proteins (Aslam *et al*., 2014; 2015; Kumaresan *et al*., 2023) and sperm metabolites (Saraf *et al*., 2020;

DasGupta *et al*., 2021). Employing these techniques several studies identified the molecular differences between the spermatozoa of high- and low fertility bulls (Talluri *et al*., 2022).

Sperm transcripts: Beyond DNA, spermatozoa contains a variety of RNAs including Messenger RNAs (mRNAs), microRNAs (miRNAs), and various forms of long noncoding RNAs that are engaged in pre- and post-fertilization processes indicating the molecular well-being of spermatozoa in successful reproduction (Krawetz, 2005). Morphological changes that occur during spermiogenesis render the spermatozoa less capable of translating and transcribed information (Lalancette *et al*., 2008). According to Gilbert *et al*. (2007), spermatid and matured spermatozoa shared a fraction of transcripts, indicating that these transcripts might be used for sperm function and fertilization in the future. A number of transcripts (mRNA) and short non-coding RNAs, including piwi-interacting RNA (piRNA) and microRNA (miRNA), were shown to be connected to early embryonic development and were recognized as trustworthy biomarkers for male fertility based on the transcriptome data. Sperm RNA content research is important because it is a steady goal for the development of a fertility assay because sperm mRNA content varies across people and is constant between ejaculates (Das *et al*., 2010). Analysing sperm RNA can reveal details about previous spermatogenesis-related events (Yadav & Kotaja, 2014), sperm function (Savadi-Shiraz *et al*., 2015), fertilization (Lalancette *et al*., 2008), successful pregnancies and even the health of an offspring (Jodar *et al*. 2016). In a study published by Saraf *et al*. (2021) examined the sperm mRNA expression profiles of crossbred bulls with normal semen characteristics that were high- and low-fertile. In crossbred bull spermatozoa, a total of 6,238 transcripts were found; 559 transcripts (> 1.5-fold) showed differential regulation between bulls that were high-fertile and low-fertile. It was found that in low-fertile spermatozoa, transcripts linked to the oxidation-reduction process (p =.003) and mitochondrial membrane potential (p =.03) were considerably down-regulated, whereas transcripts linked to apoptosis (p =.04) were up-regulated. The dysregulated genes were linked to several significant cellular signaling networks, such as the MAPK, oestrogen, Wnt, cGMP-PKG, and oxidative phosphorylation pathways.

Sperm proteins: Proteomics helps us comprehend the molecular processes of mature spermatozoa, which are transcriptionally and translationally silent. Proteomics has become a reliable method in andrological research and is becoming increasingly important for understanding the molecular processes behind different sperm functions and fertility (Wright *et al*., 2012). Proteomics-based mass spectrometry, such as two-dimensional gel electrophoresis–mass spectrometry (2-DE/MS), LC-MS/MS, SELDI-TOF/MS, and MALDI-TOF/MS, is now utilized to identify the appropriate biomarker in biological samples. To identify the functionally significant proteins and understand their function in controlling the many processes leading to fertilization, sperm proteome analysis is crucial. Sperm proteins have functions related to spermatozoa's morphology, physiology, energy metabolism, and defence mechanisms. Semen quality and bulls' in vivo fertility are linked to seminal plasma and sperm-associated proteins. According to several studies, changes in the expression levels of particular proteins are always associated with changes in the ability of spermatozoa to fertilize (D'Amours *et al*., 2010; Aslam *et al*., 2014, 2018). Many proteins found in spermatozoa and seminal plasma are necessary for sperm to carry out processes including motility, sperm reservoir creation, capacitation, zona pellucida binding, acrosome reaction, and embryonic development (Wright *et al*., 2012).

Sperm metabolites: Metabolites are small molecules that reflect gene expression processes downstream, and they are thought to be more closely related to the real phenotype. Compared to genomic and proteomic investigations, whose findings are further confounded by transcriptional and post-translational processes, metabolites—the products of all metabolic pathways—are more superior and trustworthy indicators of phenotypic features. The by-products of cellular metabolism play a crucial role in growth, development, and reproduction, among other vital physiological processes. These metabolites have been referred to by Pearson (2007) as canaries, or indicators, of issues with the transcriptome, proteome, and genome. Testicular

tissue and seminal plasma were the most often used biological sources for metabolite detection, with blood plasma coming in second. Profiling seminal metabolite fingerprints in domestic animals is becoming more important in view of identification of fertility associated metabolites. In a study by Saraf *et al* (2020), a total of 3704 metabolites belonging to various chemical classes were identified in bull spermatozoa. After sorting the exogenous metabolites, 56 metabolites were observed to be common to both the groups while 44 and 35 metabolites were found unique to high- and low-fertile spermatozoa, respectively. Among the common metabolites, concentration of 19 metabolites was higher in high- compared to low-fertile spermatozoa (Fold change > 1.00). Spermatozoa metabolites with variable importance in projections of more than 1.5 included hypotaurine, D-cysteine, selenocystine. In addition, metabolites such as spermine, L-cysteine were identified exclusively in high-fertile spermatozoa. In conclusion, the present study established the metabolic profile of bovine spermatozoa and identified the metabolomic differences between spermatozoa from high- and low-fertile bulls. Among the sperm metabolites, Hypotaurine, selenocysteine, L-malic acid, D-cysteine and chondroitin 4-sulfate holds potential to be recognized as fertility associated metabolites.

EPILOGUE

The success of current method of breeding soundness evaluation and traditional cryopreserved semen quality control assays is limited for assessment of fertilising potential; therefore, there is an ardent need to develop new fertility prediction tools. Traditional semen quality control assays have limited value in estimating the bull fertility. Now-a-days fluorescent staining based tests to evaluate sperm functions that are directly related to fertility are available and can be performed easily at semen stations. Thus instead of continuing the traditional routine semen analysis tests, incorporation of advanced tests, using specific probes to assess specific functions related to fertilizing potential of spermatozoa, into the routines of semen stations is expected to yield good results in terms of identifying fertility potential of the bulls. To further simplify the process, it is essential to identify a battery of sperm functional tests with minimum number of sperm phenome but holding higher accuracy for fertility prediction. Once identified and incorporated into the semen stations, it is also possible to grade and sell the superior quality semen in a premium price, which would not only benefit the semen stations but also the end users in terms of improved fertility. The variations in fertility that are not explained by the sperm phenome could be due to the altered molecular health od spermatozoa. Advancements in high-throughput technologies, such as next-generation sequencing and mass spectrometry, will continue to expand our understanding of sperm molecular health. Although several studies identified sperm molecules that holds potential to use in fertility prediction, their practical use for the purpose still remains in its infancy. Nevertheless, continuing studies on sperm molecular health and fertility adds to the current knowledge and the time is not far to integrating molecular assessments into bull selection and fertility prediction, ultimately benefiting the end users.

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Lead Paper - 2

Adipose Tissue-Derived Mesenchymal Stem Cells in Therapy of Livestock Species

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Abstract

Adipose-derived stem cells (ADSCs) have emerged as a promising source of multipotent stem cells in regenerative medicine, particularly in veterinary applications. Unlike bone marrow-derived MSCs, ADSCs are more accessible to isolate, yield higher cell numbers, and involve less invasive procedures. Their immunomodulatory and anti-inflammatory properties minimise the risk of immune reactions in both autologous and allogeneic transplants, making them an attractive option for therapeutic use. Over the past few years, research in veterinary regenerative medicine has advanced significantly, with ADSCs being successfully applied to treat a range of conditions, including inflammatory bowel disease (IBD), feline chronic gingivostomatitis (FCGS), canine atopic dermatitis, and musculoskeletal disorders in dogs and horses. ADSCs have shown particular promise in addressing spinal cord injuries (SCI) and intervertebral disc disease (IVDD) in dogs, where they promote nerve cell survival and improve locomotor recovery post-injury. Additionally, experimental studies have explored the potential of ADSCs in treating acute liver failure, bone abnormalities, cartilage damage, and other conditions in various animal models. The future of ADSC therapy in veterinary medicine holds exciting prospects, including exploring hydrogel and scaffold-based delivery methods and cell-free therapeutics like exosomes and secretomes. However, challenges such as standardisation, large-scale production, and regulatory oversight remain. Despite the progress, a unified regulatory framework is lacking, especially within the European Union, where each country independently governs the use of stem cell therapies in animals. Addressing issues like donor age, genomic stability, and the risk of tumorigenicity will be critical to ensuring the safety and efficacy of ADSC-based treatments in veterinary practice.

Key words: Adipose-derived stem cells, Veterinary regenerative medicine, Immunomodulatory therapy, Musculoskeletal disorders, Cell-free therapeutics

Introduction

Since their discovery, stem cells have been recognized as a cornerstone of regenerative medicine and tissue engineering, particularly in veterinary medicine. Stem cells are undifferentiated cells with the unique ability to self-renew and differentiate into various cell types. Based on their origin, stem cells are classified into embryonic stem (ES) cells and adult stem cells, and their potency is categorized as totipotent, pluripotent, multipotent, or unipotent [1]. Adult stem cells, including mesenchymal stem cells (MSCs) and spermatogonial stem cells (SSCs), are shown in Figure 1. Additionally, induced pluripotent stem cells (iPSCs) are reprogrammed from adult or fetal somatic cells by expressing pluripotency-associated genes and are classified as stem cells[2]. In 1981, Martin Evans and Matt Kauffman, who later won the Nobel Prize in 2007, were the first to identify, isolate, and successfully culture ES cells from mouse blastocysts. These cells were initially valued for their high potential in genome editing to create genetically modified organisms. However, the application of ES cells has declined significantly with the advent of advanced gene-editing tools like CRISPR/Cas9 and somatic cell nuclear transfer (SCNT). The iPSCs were developed as an alternative to ES cells by reprogramming mouse embryonic fibroblasts. iPSCs hold promise in overcoming the ethical and immunogenic challenges associated with ES cells, yet their use in stem cell therapies has been limited due to their tumorigenic potential.

On the other hand, mesenchymal stem cells (MSCs) have emerged as a more attractive candidate for regenerative medicine due to their significant advantages over ES cells and iPSCs [3]. MSCs, multipotent

undifferentiated stem cells, can be harvested and cultured in vitro and found in various tissues throughout the animal body. In veterinary medicine, MSCs—particularly those derived from bone marrow and adipose tissue—have increased use due to their regenerative potential and the absence of ethical concerns. MSCs have been employed in treating various pathological and clinical conditions in livestock, domestic, and companion animals, including wound healing [4], canine spinal cord injuries [5], and equine tendon injuries [6].

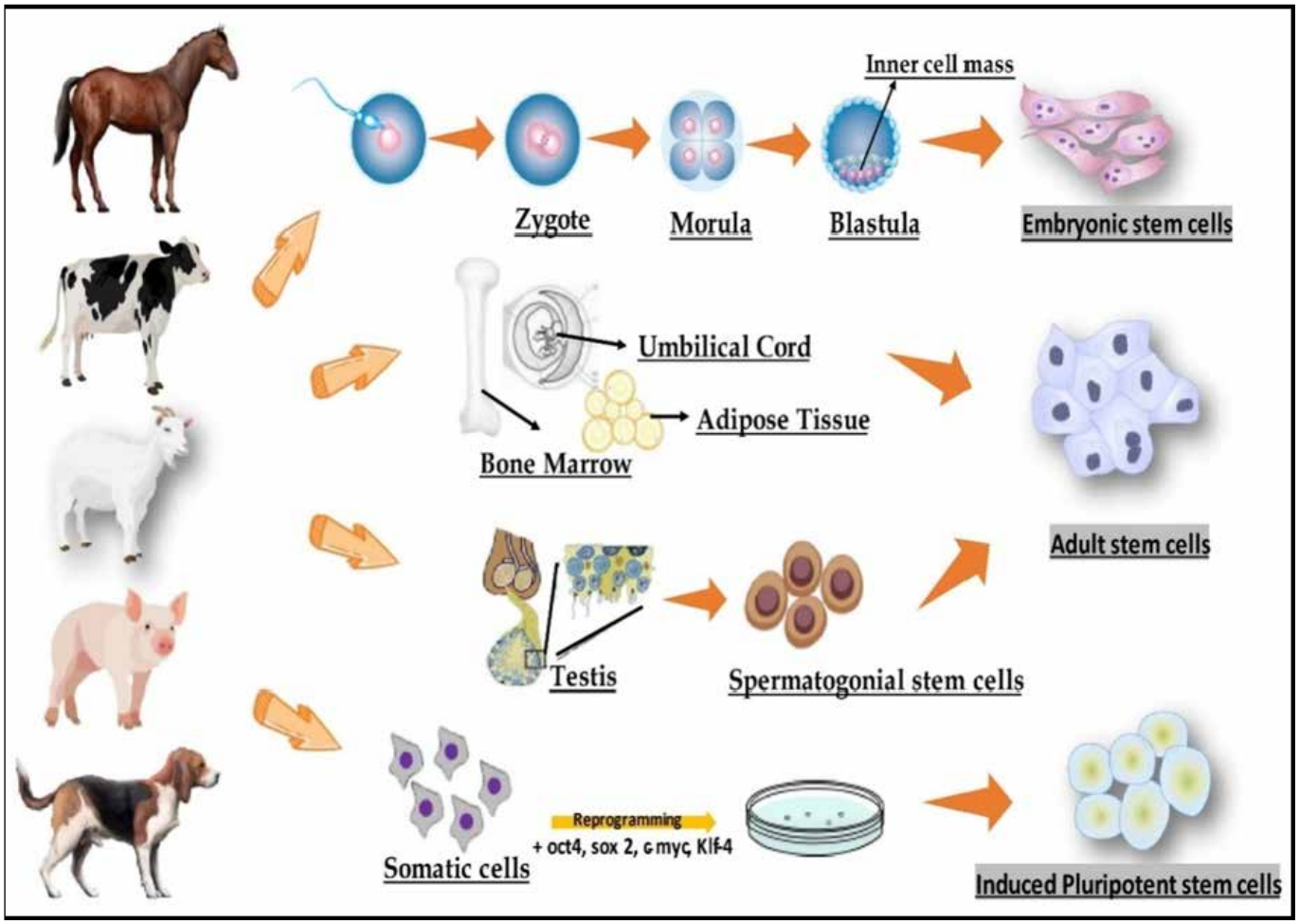

Figure 1. Different types of stem cells used in veterinary medicine for therapy. Image source: Goel, S. *et al*. (2023). Stem Cell Therapy: Promises and Challenges in Treating Animal Diseases. In: Mukhopadhyay, C.S., Choudhary, R.K., Panwar, H., Malik, Y.S. (eds) Biotechnological Interventions Augmenting Livestock Health and Production. Livestock Diseases and Management. Springer, Singapore. https://doi.org/10.1007/978-981-99-2209-3_2.

Mesenchymal stem cells (MSCs)

MSCs were discovered initially by Friedenstein in the 1960s as bone-forming cells in the bone marrow of guinea pigs [7], and Owen expanded such work to rats [8]. Since their discovery, stem cell therapy has gained significant attention and has been used in preclinical and clinical research for many years. MSCs are undifferentiated adult multipotent cells that can self-renew and differentiate into cells of other lineages[9]. In the past few decades, researchers have explored and discovered several properties of MSCs; perhaps some are yet to be discovered. Unlike hematopoietic stem cells (HSCs) originating from bone marrow, MSCs can be isolated from other sources, including placenta, adipose tissue, teeth, menstrual fluid, dental pulp, and umbilical cord [10]. MSCs are easy to isolate, grow readily in the culture dish, have intrinsic differentiation potentials, produce an abundance of helpful growth factors cytokines,

are genetically modified, and are immune-evasive, which permits use in allogenic conditions[11]. The International Society for Cellular Therapy (ISCT) defined MSCs functionally based upon negative (CD45, CD34, CD14/CD11b, CD19/CD20/CD79α, and HLA-DR) and positive (CD73, CD90, and CD105) cell surface markers, plastic adherence, and trilineage differentiation [12, 13]. However, these criteria were defined for human MSCs; hence, expression profiles may vary in animal models, posing confusion among the researchers. Studies suggest that activated MSCs secrete exosomes in response to stimulatory signals due to various pathological conditions, which further can be used for cell therapies using MSCs conditioned media. Exosomes are extracellular vesicles (ECV) discovered in sheep reticulocytes in 1983 [14]; due to the phospholipid bilayer on their surface, exosomes have good stability and permeability and can be secreted by almost all cell types [15]. The magnificent therapeutic potential of MSCs, primarily associated with their capabilities of initiating tissue regeneration, and inhibiting inflammation makes them suitable for stem cell therapy. Owing to their high regenerative potential, MSCs are widely used in tissue engineering and regenerative medicine in veterinary sciences. Several diseases in livestock remain difficult to treat due to the limitations associated with existing treatments and management of the disease, therefore, MSCs based therapies offer a great alternative to conventional therapies in livestock and companion animals. This shows the great promise of MSCs in regenerative medicine; however, isolation, in-vitro culture and storage remain an expensive task to make them available for their clinical applications.

Adipose-derived mesenchymal stem cells (ADSCs)

Over the past few years, it has been observed that fat is the energy reservoir and a good source of multipotent stem cells known as ADSCs. Isolation of MSCs from adipose tissues is much easier, less invasive, and yields higher than bone marrow. Also, these cells hold no ethical concern and can be isolated appropriately for *in vitro* culture and cell therapy. The ADSCs are immunomodulatory and anti-inflammatory, which excludes the chances of any immune reaction during autologous or allogeneic transplantation in the host [16]. Different methods for isolation and culture of ADSCs have also been established across different countries and laboratories.

Research on regenerative medicine in veterinary medicine is ongoing. Recently, significant advancements have been made in developing reliable stem cell therapeutics [17]. Significant progress in treating disorders like inflammatory bowel disease (IBD), feline chronic gingivostomatitis (FCGS), feline asthma, canine atopic dermatitis and wound healing has also been accomplished using ADSC therapy [18, 19]. ADSC treatment has documented remarkable results, particularly for musculoskeletal conditions in dogs and horses [17]. For clinical purposes, veterinarians mainly use MSCs from adipose tissue and bone marrow. In companion animals, they are used therapeutically on oro-dental disease, musculoskeletal conditions, osteoarthritis, and equine metabolic syndrome that affect the tendons, ligaments, and joints [20]. ADSCs have shown promise in preventing spinal cord injuries (SCI) and intervertebral disc disease (IVDD) in dogs, according to several studies. A single injection of 1×10^7 allogeneic ADSCs was given after hemilaminectomy into the injury site in dogs with acute SCI, leading to elevated nerve cell survival and quicker recovery in locomotion [21-23]. The potential of employing ADSCs to treat acute liver failure was assessed in experimental settings involving mice, rats, and dogs.

Additionally, spinal cord injuries, bone abnormalities, articular cartilage, mucosal ulcerations, induced urine incontinence, muscle dystrophies and diabetes have all been addressed in research with auto- and allogeneic stem cells. Numerous studies employing xenogeneic stem cells have been reported. In several studies on rabbits, mice, rats, dogs, and baboons [20], Adipose tissue stem cells have so far been effectively used in veterinary medicine, primarily to treat musculoskeletal illnesses, bone abnormalities, ocular and kidney ailments, and damage to tendons and joints in horses and dogs [24]. Prospects for ADSC therapy in the future have been thoroughly investigated in veterinary and human medicine. These include investigating

hydrogel and scaffold-based techniques and cell-free therapeutics (including secretome and exosome) that utilise the bioactive compounds produced by ADSCs [25]. It is necessary to address several issues related to cell-free therapeutics involving exosomes and secretomes, including large-scale manufacturing and standardization of their characterization and separation processes, before they may be used in clinical settings [26].

Even while veterinary regenerative medicine has advanced significantly in recent years, there are still many unsolved questions in the area, and more research is needed before clinical patients may be proven [27]. Regulatory bodies have little oversight on the clinical use of ADSCs in veterinary patients as they are in human populations. The approaches must be more uniform, and basic preliminary testing is essential. A unified law governing the use of stem cell therapy in veterinary medicine is still lacking, and each member of the European Union regulates this field independently, despite some basic principles for stem-cell-based treatments for animals being proposed by the European Medicines Agency's (EMA) Committee for Medicinal Products for Veterinary Use (CVMP) [24, 27]. When it comes to the uniformity and efficacy of treatments, variables like the age of the donors, genomic stability, tumorigenicity, differentiation potential, senescence and microbiological contamination should be evaluated [17, 24].

Conclusion

ADSCs hold significant potential for improving the health and welfare of livestock species. Their versatility, abundance, and immunomodulatory properties make them valuable tools in veterinary regenerative medicine. While challenges remain in standardization, cost, and regulatory approval, ongoing research and technological advancements will likely overcome these hurdles, paving the way for the widespread adoption of ADSC therapy in livestock. The future of veterinary medicine will undoubtedly benefit from integrating these innovative cell-based therapies, ultimately enhancing animal health and productivity.

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ORAL PRESENTATION

OP_4.1: Analysis of Chitinase-3-Like Protein 1 Gene Expression in Buffalo (*Bubalus bubalis*) Cumulus-Oocyte Complex

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Buffalo (*Bubalus bubalis*) productivity is critically impacted by reproductive efficiency, which can be improved through advanced reproductive technologies such as in vitro maturation (IVM) of oocytes. This study investigates the expression of Chitinase-3-Like Protein 1 (CHI3L1) in buffalo cumulus-oocyte complexes (COCs) during in vitro maturation. CHI3L1, a member of the glycoside hydrolase family 18, is involved in tissue remodeling and inflammation processes, which are crucial during oocyte maturation. COCs were retrieved from buffalo ovaries collected from a local abattoir. The COCs were graded based on their morphology and subjected to IVM in TCM 199 media supplemented with FSH, LH, and estradiol. Total RNA was extracted from both immature and mature COCs, and CHI3L1 gene expression was quantified using qRT-PCR. The fold change in CHI3L1 expression between immature and mature COCs was calculated using the ΔΔCt method. The results indicated a 1.3-fold increase in CHI3L1 expression in mature COCs compared to immature COCs. This suggests that CHI3L1 may play a significant role in the cytoplasmic and nuclear maturation of oocytes, potentially influencing their developmental competence. To our knowledge this is the first report on the expression of CHI3l1 in buffalo oocytes. CHI3L1 plays a crucial role in tissue remodeling and inflammation, processes essential for the successful maturation of oocytes. Its increased expression in mature oocytes highlights its importance in ensuring the developmental competence of oocytes. Understanding the role of CHI3L1 in oocyte maturation can lead to improve in vitro maturation protocols and enhanced reproductive efficiency in buffalo. The increased expression of CHI3L1 in mature buffalo oocytes highlights its potential as a biomarker for oocyte maturity. Further research is needed to elucidate the specific mechanisms by which CHI3L1 influences oocyte maturation and its possible applications in improving reproductive efficiency in buffalo.

OP_4.2: *In vitro* Embryo Production with Oviductal Exosome based Co-Culture and Its Evaluation

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Achievement of successful pregnancy and high-quality offspring(s) with *in vitro* embryo production system always demands to recreate a physiological environment. In the present study, oviductal exosomes was supplemented during *in vitro* embryo culture to improve the developmental competence and quality of buffalo embryos. Being a bioactive cargo, exosomes help to provide the earliest maternal signal between the oviduct and the developing embryo. The embryos so produced were comparatively evaluated for their developmental competence, viability and for characteristic transcriptomic signature. It was found that the cleavage rate on Day 2 and morula/early blastocyst (EB) yield on Day 5 (was significantly higher in the exosome-treated group with a concomitant improvement in viability rate of morula/EB stage embryos, when compared to the control group. Further molecular support was established by a relative analysis of the candidate transcripts of the embryo conditioned medium. Investigation on the relative abundance of mRNA transcripts (RT-PCR) showed significant upregulation of the candidate genes: PLAC8, CX43,

SCD, GPX and BCl_2 in the conditioned medium of exosome treated embryos, supporting the findings of the morphological evaluation. This study recommends that, co-culture with oviductal exosomes could significantly improve the developmental rate, viability, and beneficial gene expression pattern of bovine embryos, by providing the conducive maternal environment required for early embryonic development.

OP_4.3: Assessing the impact of supplementing nano forms of copper and cobalt on milk production and methane emission in dairy cows

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This study was undertaken with objective to synthesize nano copper sulphate and nano cobalt sulphate, characterize them, optimize their level along with nitrogen and to evaluate their effect on methane emission and milk production in dairy cows fed with rice gruel-based diet. Nano copper sulphate and nano cobalt sulphate were synthesized by physical method using planetary ball mill. The average particle size (nm) of nano copper sulphate was 84.80, 22.85, 74.53 and 11.21 when samples were analysed by particle size analyzer, XRD, UV-Vis spectroscopy and TEM, respectively. The average particle size (nm) of nano cobalt sulphate was 93.50, 80.22, 54.04 and 15.17 when samples were analysed by particle size analyzer, XRD, UV-Vis spectroscopy and TEM, respectively. The zeta potential of nano copper sulphate and nano cobalt sulphate was (-18.00) mV and (-13.90) mV respectively and they are stable in nature. The shape of both nano copper sulphate and nano cobalt sulphate was found to be spherical, which was confirmed by TEM. The XRD analysis revealed that the surface characteristics of nano copper sulphate and nano cobalt sulphate were similar to that of the standard. The FTIR study revealed presence of (-OH) and ($-SO_4$) functional group in both nano copper sulphate and nano cobalt sulphate. Further these characterization results revealed that there was no impurity in synthesized nano copper sulphate and nano cobalt sulphate. *In vitro* cytotoxicity assay of nano copper sulphate and nano cobalt sulphate carried out using BHK-21 revealed safe level of inclusion as 50 and 10 mg / kg of feed, respectively. *In vitro* gas production study was carried out to optimize the level of nano copper sulphate and nano cobalt sulphate along with nitrogen on rice gruel-based ration based on the result of microbial biomass production and methane emission. At the end of trial, it was found that the level of copper at 12.22 mg / kg, cobalt at 0.25 mg / kg and urea at 7.5 g / kg of rice was optimum in both mega and nano forms for further validation. *In vivo* trial was conducted to evaluate the optimized level of nano copper sulphate and nano cobalt sulphate along with nitrogen on methane emission and milk production in Gir cows. The addition of both mega and nano mineral supplement has improved resistance in pH, ammonia nitrogen, VFA profile and milk production. Though the enteric methane emission did not vary significantly due to supplementation of both mega and nano forms of copper sulphate and cobalt sulphate along with nitrogen, on an average 7.5 – 8.5 % reduction in methane emission was observed in supplemented cows. Ruminal bacteria were significantly ($p<0.05$) improved in nano mineral supplemented group compared to other groups, but ruminal protozoa did not reveal any increase in count. The gene expression study for the milk fat (SCL2A8) gene was significantly ($p<0.05$) upregulated in nano mineral supplemented group. Whereas, the milk protein gene (CSN2) revealed no significant difference among different treatment groups. The abundance of *Methanobrevibacter sp.* was significantly ($p<0.05$) reduced in the nano mineral supplemented group. The total methanogen population did not differ significantly among different treatment groups. However, total methanogen population was non-significantly reduced in the nano mineral supplemented group. From this present study it was concluded that, supplementation of critical nutrients like copper sulphate and cobalt sulphate, in both mega and nano forms along with nitrogen

in rice gruel-based diet have impact on mitigation of methane emission and improved the milk production in Gir cows.

OP_4.4: Probiotics as a growth promoter in rural poultry of Andaman Nicobar Island, India

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To replace antibiotics as a growth promoter, a multi-strain probiotic was tested in a rural poultry variety, reared at Andaman and Nicobar Islands, India. A total of 120 newly hatched Vanaraja chicks were selected, 30 chicks were randomly divided into 4 groups for 60 days. Negative control (NC) received only basal diet. Positive control (PC) received basal diet + antibiotic growth promoter (AGP). One test group (T1) received basal diet + 0.1% of multi strain commercial probiotics. Another test group (T2) received basal diet + 0.3% of multi strain same commercial probiotics. Results revealed that the mean body weight of chicks supplemented with 0.1% (T1) and 0.3% (T2) multi strain probiotics was significantly higher ($p \leq 0.05$) than that of control groups. There was also a significant increase in feed conversion ratio (FCR) among the test groups on different intervals of time. Both T1 and T2 showed significant changes ($p \leq 0.05$) in biochemical parameters such as total protein, albumin, globulin, blood urea nitrogen (BUN), glucose, bilirubin on different intervals of time than control groups. There was a significant decrease ($p \leq 0.05$) in T1 and T2 groups than that of control in the levels of triglycerides, total cholesterol, HDLc, LDLc, superoxide production, lipid peroxidation in different intervals of time. There was a significant increase ($p \leq 0.05$) in the levels of IL-2, IL-4, HSP-70, and lymphocyte proliferation in treated groups T1 and T2 than that of control groups. A significant increase ($p \leq 0.05$) in the villi height (µm) and crypt depth (µm) of duodenum and jejunum of experimental birds were observed in the groups supplemented with 0.1% (T1) and 0.3% (T2) of multi-strain probiotics than that of control groups upon histomorphological analysis. In short, multi-strain probiotics showing improved growth performance, immunomodulatory activities and better intestinal morphology may be recommended as an alternative to commercial antibiotic growth promoter.

OP_4.5: A Study to Evaluate the Microbial load of Vedic and Enriched Panchagavya Preparations from Cross Bred Jersey and Gir Cattle.

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Panchagavya was used in conventional rituals by Hindus and recognized for its medicinal importance in Ayurvedic treatment and is termed as 'Cowpathy'. Panchagavya is a living elixir of many microorganisms and basic elements with known and unknown growth promoting factors, micronutrients, trace elements, antioxidants and immunity enhancing factors. In this study Vedic panchagavya was prepared by mixing five

ingredients viz., cow dung, urine, milk, curd and ghee. In addition to this jaggery, tender coconut water, ripened poovan banana and water is mixed for preparing Enriched panchagavya. Both were evaluated individually. The raw material for the preparation was collected from two different breeds i.e. Cross Bred Jersey and Gir. The shelf life and growth promoting factors were studied at stipulated intervals. For the bacterial isolation five different types of agar media was used , viz., Mac Conkey, Ken Knight, Rose Bengal, Jensen and Kings Medium. Based on Gram's staining majority of the bacterial genera fell into the Gram -ve category while Gram +ve slender Bacillus spp was also isolated. The total count of each bacterial type was estimated as per the spread plate method on Nutrient agar. The mean minimum countable range was between 10^{-8} to 10^{-15}/ 100 µl / plate. The filtrate from the two Panchagavya preparations was collected at regular intervals and stored as stock solutions. For spraying on the fodder a 3% solution was prepared. Pot culture technique was followed to grow the maize fodder and evaluate the quality of Panchagavya with suitable control groups. Between the two preparations, the enriched panchagavya had a better effect than the vedic preparation, of both the breeds on the growth performance of maize crop as estimated by its microbial load.

OP_4.6: Characterization of Major Histocompatibility Complex II DRB 3.2 polymorphisms of Punganur cattle in association to disease resistance

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We sought to investigate the association of DRB3.2 allelic polymorphisms in BoLA-DRB3 exon 2 with disease resistance or susceptibility of Punganur cattle a dwarf humped breed native to Andhra Pradesh. The cattle (n=54) were screened for Brucellosis, Johne's disease, Foot and Mouth disease and Infectious Bovine Rhinotracheitis using antibody detection ELISA kits. Seropositivity to FMD was 77% which indicates that animals had previous exposure to FMD infection. The herd was found to be free of Brucellosis infection. Seropositivity for IBR was also high (37/54, 68.5%) in the farm. Ten animals (18.5%) were confirmed as (ELISA) positive for MAP infection indicating potential risk of Johne's disease.

The DRB3 exon2 sequences of the 21 Punganur animals were analysed by using manual sequence-based typing and blast search in IPD-MHC data base. Among 21, one animal (P17) is homozygous with allele 25:01:01 and all other are heterozygous at the DRB3.2 locus. These alleles formed 20 different genotypes. The genotype 28:02/11:04 is observed in 2 animals (P578, P516), all other genotypes are observed only once. This indicates high degree of polymorphism in the Bovine MHC class II DRB3.2 locus of Punganur cattle. In 21 animals 27 different MHC II DRB3.2 alleles were identified**.** The 25:01:01 allele is with highest frequency (detected in five animals out of which one is homozygous for this allele) in the population with frequency of 0.143. The allele *100:01 was highly polymorphic with nucleotide sequence variation at many base positions and it was phylogenetically distant from the other 26 alleles in the present study.

The allele 25:01:01 is considered to be associated with disease susceptibility as out of five animals that possessed the allele 25:01:01, all are FMD positive, four are IBR positive and 2 are JD positive. The animal

with the allele 3:01:01 is negative for all the diseases. However, the association of any DRB3.2 alleles with susceptibility or resistance of Punganur cattle to diseases is statistically insignificant p>0.05 (Fisher's exact test) as most of the alleles were found only once in the study group.

OP_4.7: Impact of FMD Vaccination on Bull Seminal Plasma Proteome alters the abundance of Fertility-Related Proteins

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Artificial insemination (AI) in livestock breeding relies on high-quality semen, but mandatory vaccinations, like for FMD, can temporarily reduce semen quality. The molecular mechanisms behind this decline, particularly changes in seminal plasma proteins, remain unclear. This study aims to investigate these protein alterations to understand the temporary reduction in semen quality post-vaccination. This study, conducted at the Artificial Breeding Research Centre, ICAR-NDRI, Karnal, Haryana. Five Sahiwal bulls were received single dose of Raksha OVAC trivalent FMD vaccine @ 2 ml via deep I/m. Seminal plasma samples were collected in two phases: before vaccination (14 days prior) and after vaccination (on the 3rd, 7th, 12th, 21st and 35th days post-vaccination), with two ejaculates taken from each bull on each sampling day. LC-MS/MS technique was used to study the protein profile of seminal plasma to determine their differential abundance before and after FMD vaccination. Following vaccination, alterations in fertility-related proteins were observed: Proteins involved in fructose binding, fructose transport (GLUT5) acrosomal binding proteins binding (ACR, SPESP1, ACRBP, SPACA3) proteins of sperm with piece sheath and end piece (ATP1A4, RAP1A, AKAP4) were down regulated up to 21 days during post vaccination. Conversely, proteins involved in pH reduction (ATP6V1D, ATP6V1F) glycolysis (ENO3, BPGM) cellular oxidant detoxification (S100A9, HBA, HBB) cholesterol transport (APOA1, APOA2, APOM) were upregulated up to 21 days after vaccination. Functional annotation of differentially abundant proteins reveals that, Citrate cycle (TCA cycle) was found to significantly downregulated pathways from day 7 to 21 days after vaccination. These changes suggest that vaccination induces stress in epididymis and accessary sex glands, prompting significant alterations in seminal plasma proteome. Such alterations may adversely affect sperm function, motility, and overall fertility by altering ATP production pathways and compromising mitochondrial function under vaccination stress conditions.

TECHNICAL SESSION - V

NANO-BIOTECHNOLOGY IN ANIMAL HEALTH AND PRODUCTION

Lead Paper -1

Advancing Healthcare Nanosytems: Prioritizing and Productizing Bioactive Nanomaterials for Tissue Regeneration

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Bioactive materials, highly esteemed in the medical field, are used in scaffolds, foams, hydrogels, thin films, and more due to their significant health benefits. Advances in production technologies have enabled patients to recover from implantations and routine replacements of fracture plates, screws, sutures, and other components. Biomaterials are categorized into bio-inert, bio-resorbable, and bio-active types. Bioactive scaffolds, made from hydroxyapatite and bioactive glasses like 45S5 Bioglass®, support osteogenesis and tissue regeneration. They release ions like Na, Ca^{2+}, and PO_4^{3-} in biological environments, aiding bone and tissue regeneration. Hydroxyapatite, making up 70% of bone weight, is crucial for bone regeneration. Effective technologies that develop biomaterials mimicking natural bone with high compatibility are pivotal in healthcare.

The function of biomaterials, especially hydroxyapatite and bioactive glass, depends on factors like size, morphology, phase formation, facet setting, pH, room temperature, and precursors. Combeite ($Na_2Ca_2Si_3O_9$), a stable but challenging high-temperature phase in bioactive glass, and Monetite, a pH-dependent phase in hydroxyapatite synthesis, are notable examples. Research highlights the benefits of these phases and their impact on bioactivity. Bioactive materials with an equimolar Ca:P phase, subjected to various sintering temperatures, show improved bioactivity. Studies also evaluate the necessity of monovalent modifier cations in bioactive glass[1]. Scaffolds and fibers made from these materials, blended with polymers, show dependency on the phase and structure of the bioactive materials[2–4].

Biomaterials represent a significant medical advancement, enhancing recovery and regeneration of bone and tissue, leading to better patient outcomes. This talk covers the notable results achieved on biomaterials sol-gel synthesis and various techniques and also enhanced biomaterials used in dentistry, orthopedics, ophthalmology, drug delivery, wound healing, and tissue regeneration.

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Lead Paper -2

Microfluidics as a molecular platform for integration of padlock probing-based rolling circle amplification towards precision diagnostics of hypervariable viruses

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Background: Highly sensitive and specific amplification of nucleic acid sequences become important for achieving the required detection milestones in molecular diagnostics. Though PCR is the gold-standard technique used till date, the inherent temperature ramping, involving multiple rounds of denaturation, primer annealing and extension pose heavy limitations to the bioanalytical versatility of this technique. In this context, various isothermal amplification methods, also including Rolling Circle Amplification (RCA), become interesting and important for specific detection of nucleic acid sequences pertaining to infectious diseases. RCA is based on the mechanism of rolling circle replication evident naturally in bacteria and viruses, which give rise to a long single stranded molecule (containing thousands of concatenated copies of the template strand) resulting from the unidirectional amplification of a circular nucleic acid template. Such generation of a single stranded molecule per amplification event, with high sensitivity and specificity, forms the fundamental disruptive feature using RCA in the context of pathogen diagnostics.

Padlock Probing: Padlock probes (PLPs) are linear oligonucleotides whose end sequences are designed in a way complementary to the target of interest. In addition, they also feature a backbone sequence that is custom-designed for various functionalities such as binding to detection or restriction oligonucleotides enabling precise detection. The nature of nucleic template (RNA/DNA), its composition (GC-content, melting temperature) and length of the sequences hybridizing to the target determine the optimal performance of a PLP-based bioassay. Hybridization of the end sequences of the PLP to the complementary regions in the target generates a nick between the two end sequences. Following hybridization, use of an appropriate ligase such as T4 DNA ligase or thermostable *Tth* ligase seals the nick, in turn enabling target-specific probe circularization, where the PLPs become topologically linked to the target.

Rolling Circle Amplification: The circularized PLPs are amplified via RCA, using *Phi29* DNA polymerase, possessing good processivity and strand displacement activity, thereby serving as a high fidelity enzyme for diagnostic applications. In about an hour, around 1000 complementary copies of the circularised probe are generated as a long DNA concatemer. After amplification, the RCA products collapse in order to fold into a random coil-like conformation. Presence of salt influences this structural collapse, exploited for easy microscopy observation as spatially-discrete distinct bright spots when labelled with suitable fluorophore tagged detection oligonucleotides. Whenever a higher sensitivity is required, the products from a first round of RCA can be subjected to a second RCA by including a second primer allowing restriction of the RCPs followed by circularization of the fragments. When this is done as two or more controlled rounds of RCA, it is termed as circle-to-circle amplification (C2CA), while an uncontrolled exponential reaction often producing circle-independent amplification byproducts is termed as hyperbranched RCA.

PLP-RCA: One of the key features of PLP-RCA is to provide an intrinsically digital detection of the target molecules upon amplification in a homogeneous solution. Besides the quantitative merits of a digital detection, i.e., high signal-to-noise ratio, calibration-free quantitative data, high sensitivity and high precision, such type of digital detection allows a remarkable degree of multiplexing to parallel detect multiple targets. Thus, the combination of RCA with PLPs has led to the development of bioanalytical assays that combine the high multiplexing capability and specificity of PLPs, with the isothermal amplification mode of RCA. The applications of PLP-RCA are very wide in the area of molecular detection, ranging

from the detection of antimicrobial strains in bacteria and detection of hypervariable virus mutants to precise veterinary diagnostics and understanding of *in situ* pathogenesis. Detailed notes on the need, scope, molecular mechanism, applications and perspectives of PLP-RCA on their massive multiplexing ability for pathogen diagnostics and genotyping can be found in the review article cited here (1).

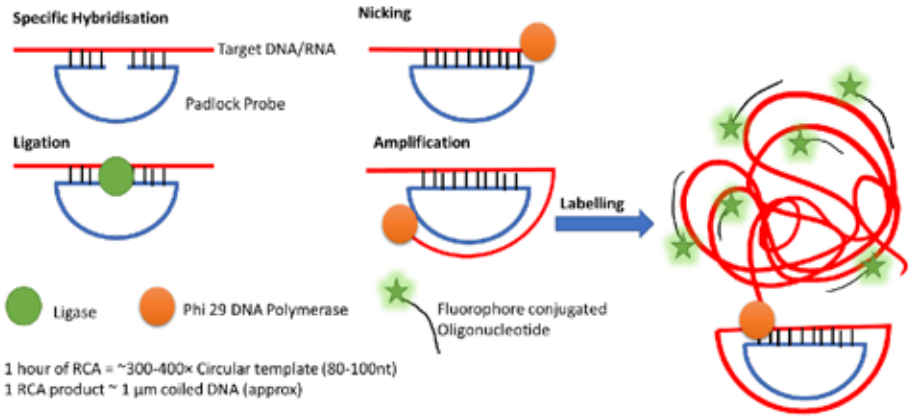

Figure: Schematic representation of padlock probing and rolling circle amplification assay for sensitive and specific pathogen diagnostics.

Probe Design Strategies for RCA

- The probe design is relatively simpler and straightforward in RCA compared to other isothermal applications. The selection of pathogens of interest is the first step.
- Sequence construct: The two end sequences of the PLPs which are also referred to as the hybridization arms are made complementary to the target region. Additionally, the 5' end of the PLP is phosphorylated for the ligation event to occur. The backbone sequences are functionality-dependent and thus can be modulated to suit the applications. For instance, the backbone sequence can contain a region complementary to oligonucleotides for capture and labelling/detection applications. The backbone sequence can also be modified to contain restriction sites for performing C2CA for achieving lower sensitivity limits. Degenerate bases maybe included, wherever required, in order to avoid the increased number of probe constructs, especially for hypervariable strain detection.
- Sequence curation: The target region may be selected using relevant databases such as the National Center for Biotechnology Information (NCBI). Pathogen-specific or disease-specific databases can also be used wherever appropriate. The conserved regions for probe sequence selection may be screened from the gene of interest using multiple sequence alignment tools.
- Sequence filtering and clustering: The selected probe can then be checked using alignment tools for ensuring cross-hybridization binding with other potential sequences. Phylogenetic tree may be constructed wherever essential, in order to handle diverse genotypes / serotypes. It also needs to be ensured that the PLPs do not contain any secondary structures. This may be analyzed using tools such as mFOLD.
- The short-listed filtered probe candidates are taken for experimental validation. Multiple probes can be used for targeting the gene of interest in order to enhance the sensitivity and targeting potential for precise molecular identification.

In order to overcome the challenges associated with the complexities involved in shortlisting the target regions of hypervariable viruses for probe design, an integrated AutoPLP pipeline for automation of probe design process for a diverse pathogen panel of interest has been developed for ready use (2).

RCA in Veterinary Diagnostics

- A rapid PLP-RCA with microarray readout was used to differentiate foot-and-mouth disease virus, swine vesicular disease virus and vesicular stomatitis virus, with serotyping information (3).
- The same combinatorial PLP-RCA-microarray platform was extended to develop a subtyping assay for detecting hemagglutinin and neuraminidase of Avian Influenza Virus (4).
- Recently, a mutation tolerant PLP design for multiplexed detection of hypervariable RNA viruses was established, where differential detection of poultry RNA viruses like Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV) and AIV, along with genotyping of NDV strains was demonstrated (5).
- Bovine papillomavirus type I has been detected using multiply primed RCA for subsequent cloning and sequencing (6).
- Viral DNA replication, both *in vitro* and *in vivo,* has been demonstrated using randomly primed RCA to detect Porcine Circovirus type 2 (PCV2) infections in pigs (7).
- Multiply-primed RCA has been employed to amplify PCV2 genomes and construct infectious clones from tissues of pigs with signs of post-weaning multisystemic wasting syndrome (8).

Microfluidics for Diagnostic Point-of-Care Assays

The rapidly emerging domain of microfluidics and microfluidic biosensors can be readily made available to the patients as bedside diagnostics, thus providing answers to medical diagnostic questions even far from laboratory facilities. The generic advantages of microfluidic devices include, but are not limited to, their miniaturization leading to smaller sampling size, enhanced sensitivity, high throughput, and scope for automation and multiplex modules (for parallel analysis). In addition, they do not require robust development of any heavy instrumentation for final use, thereby proving beneficial especially at the resource-limited settings (Tier-2 and Tier-3 cities in countries like India). These have clearly changed the face of disease diagnostics for a variety of end applications, thanks to the associated features such as mass production, cost-effectiveness, portable instrumentation with adequate information processing capabilities and disposable/ reusable nature, as required (9).

Microfluidic Technologies: The process flow of fabrication and use of microfluidic devices for point-of-care applications are simple and straightforward (10). One such strategy involves prototyping using standard polydimethylsiloxane (PDMS) soft lithography (full PDMS or PDMS-glass/polymer hybrid devices) to facilitate device replication, and to provide simple and versatile fluidic connections owing to its elastomeric properties. The surface functionalization of the microfluidic device materials is usually tested in either 2D (inner surface of the microchannels) or 3D (nano/microporous beads packed inside the microchannels or nano/microporous materials embedded in the device) confirmations for manipulating the concentrations of solutions and to obtain enhanced signal per unit volume. Sample preparation methods for microfluidic studies can fall under a variety of categories including physical filtration, hydrodynamic methods and molecular affinity-based techniques. Similarly, the readout units for the detection of various biomarkers, including proteins and nucleic acids, can be coupled to optical, electrical, magnetic, electrochemical or mass-based methods. Such emerging microfluidic modules have facilitated effective manipulation and interrogation of tissues and fluids including blood, saliva, urine, etc. for addressing a variety of biomedical questions including identification of pathogens, molecular signatures and host response to various conditions. Advances in mobile communications and the developed synergies thereof have leveraged the potential deployment of microfluidic point-of-care sensing technologies in a relatively widespread fashion.

Microfluidic RCA: The combinatorial potential of RCA and microfluidics has been adequately exploited in pathogen diagnostics for the sensitive and specific detection of nucleic acid amplicons. A PLP-RCA assay for detection of Ebola virus, together with multiplexing of other tropical viruses including Zika and Dengue, has been demonstrated on a pump-free microfluidic chip after amplicon enrichment (11). PLP-RCA with an electrochemical readout platform integration based on glucose-oxidase enzyme could serve as an effective alternative to ELISA and PCR in decentralised settings (12). Sub-attomole detection of HIV-1 was achieved by combining PLP-RCA with microfluidic affinity chromatography enrichment (micro-ACE) technique, wherein the RCA products were dual-labelled with a fluorophore and biotin, thereby enabling simultaneous specific capture and sensitive detection in the microfluidic platform (13). The same micro-ACE strategy was extended for the detection of Zika fever, however employing two rounds of RCA, namely C2CA, along with microfluidic molecular analysis of anti-flaviviral drug efficacy (14).

All of these approaches inherently enable the translational relevance of such microfluidic tools for the establishment of lab-on-chip systems for biomolecular detection. With proper exploitation of the miniaturisation merits of microfluidic systems, together with appropriate integration strategies bringing together various stages of microfluidic operations including fluid handling, sample prep, molecular recognition, signal transduction and digital readout (15), these lab-on-chip systems can be transformed into point-of-care devices deployable at resource-limited settings.

Future Trend: Government and other organizations have started supporting R&D and scale-up (including manufacturing and market validation) for microfluidics-based technologies during the recent years, more specifically for accelerating their path from bench to bedside. Implementation of microfluidics, an emerging technology platform in the development of point-of-care devices, can directly reduce the burden on our healthcare system, thanks to the spatio-temporal resolution and advantages it offers. Ready sample-to-answer analytical systems, wherein using either a raw or a minimally processed biological sample matrix would yield qualitative or quantitative assessment of one or multiple analytes of interest, are the desired technological tools in taking molecular diagnostics from bench-to-bedside for effective patient care. These can directly impact the rapid and timely diagnosis of diseases, which is a striking factor for impactful clinical outcome and public health.

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OP_5.1: Chitosan nanoparticles coupled inactivated Infectious laryngotracheitis (ILT) Vaccine for chickens

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Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory tract infection in chickens that cause a huge economic impact on the poultry industry all over the world. The present study aimed to assess the immunogenicity of chitosan nanoparticles coupled inactivated ILTV. The infected chorioallantoic membranes with pock lesions after second passage was cultivated in bulk, purified and titrated. The purified virus was quantified and inactivated by formaldehyde at the final concentration of 1200 µg/ml at 37^0C for 24 hr. The coupling of inactivated ILTV at the concentration of 5mg/ml with chitosan nanoparticles were carried out by ionic gelation method. The particle size of empty and ILTV antigen loaded chitosan nanoparticles were observed to be 7.1 nm and 260 nm respectively. The zeta potential of empty and ILTV antigen loaded chitosan nanoparticles were observed to be +8.8 mv and +19.1 mv respectively. The shape of chitosan nanoaparticles was spherical and smooth by electron microscopic examinantion. The immunogenicity of CS-ILTV was assessed along with commercial ILTV live and *in house* prepared inactivated vaccines through immunization trail in chickens. The cell mediated immune response was assessed by IFN-γ ELISA which was significantly higher in live group seven days after booster dose. The humoral immune response was either poor or weak in CS-ILTV groups. Challenging with field ILTV after booster immunization showed mortality in control (40%) followed by inactivated (20%) and no mortality and clinical signs due to ILTV in other groups. It is concluded that chitosan nanoparticles coupled inactivated ILTV showed protective against ILTV infection.

OP_5.2: Study on the Inhibitory effects (bacteriostatic and bactericidal kinetics) of Beta cyclodextrin-Gentamicin encapsulated Zinc oxide Nanoparticles-based Film forming Gel against Antibiotic Resistant Bacteria Causing Bovine Mastitis

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In this study an emulsion method was used to encapsulate gentamicin with zinc oxide nanoparticles. The gentamicin-coated zinc nanoparticles were about 80–90 nm in size. This was utilized as nano-antibiotic to study the anti-microbial inhibitory effects. The characterizations of the plain and gentamicin conjugated particles (Zn+ GEN) was confirmed using FTIR, Raman, SEM, and DLS methods under first order kinetics. Antibiotic-resistant bacteria isolated from bovine mastitis milk was used for the anti-microbial investigations. The nano antibiotic possessed efficient biofilm inhibition properties to different degrees against both antibiotic-resistant *E. coli* and *S. aureus*. This nano-antibiotic (Zinc conjugated with Gentamicin (Zn+ GEN) was included in the formulation of a film-forming gel-based spray for field applications as it forms a protective layer on udder skin and is quite effective in treating bovine mastitis.

OP_5.3: Study on the Inhibitory Effects of OTC encapsulated zinc oxide nanoparticles to Combat Bio-film forming bacteria causing Bovine Mastitis

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Zinc oxide nanoparticles were utilized to encapsulate oxy tetracycline (OTC) as per an emulsion technique. The technique employed OTC-coated zinc nanoparticles, with a size of approximately 80-90 nm, as nano-antibiotics. FTIR, Raman, SEM and DLS techniques were used to validate the particle characterizations. The release kinetics suggested it under first order kinetics. For field applications, this nano antibiotic was combined with a hybrid gel based film of Gelatin and Chitosan which formed a film. Characterization by EDX and FTIR analysis of the hybrid gel film confirmed the presence of OTC-coated nanoparticles. For the anti-microbial studies antibiotic resistant bacteria isolated from bovine mastitis milk was tested. The results demonstrated that the nano antibiotic (Zn+ OTC) in the hybrid film was highly effective against both antibiotic-resistant S. aureus and E. coli as per the bio-film inhibition assay and could eradicate biofilm formation to various degrees after multiple applications as a novel way to treat bovine mastitis in future and prevent recurrence.

OP_5.4: Fluorescent Silica Nanoparticles combine with Fourier-transform infrared spectroscopy -based *E. coli* detection

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This study used a new emulsion approach to create fluorescent silica nanoparticles. The synthesis required mixing *Tinospora cordifolia* leaf extract with β-cyclodextrin and generating an emulsion medium using triton x-100 and ethanol. Tetra ortho ethyl silicate and tris(bipyridine) ruthenium (II) chloride were then introduced. The nanoparticles produced were approximately 75-80 nm in size, and their characterisation was performed using SEM, FTIR, XRD, and a spectrofluorometer. For the *E. coli* detections, anti-*E. coli* antibodies were conjugated with fluorescent silica nanoparticles and then bacteria was detected using FTIR, fluorescent spectral changes and fluorescent microscope based counting. The detection methods showed lowest detection limit is 1×10^2 CFU/ml and the detection time is 45 min. Finally, the new approach described here is suitable for bacterial detections, and functional fluorescent nanomaterials based imaging, clinical and environmental applications.

OP_5.5 : Immunogenicity and protection studies of a bivalent intranasal Nanovaccine against Newcastle disease and Infectious bronchitis

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Newcastle disease (ND) and avian infectious bronchitis (IB) continue to be major threats for the poultry industry due to loss of productive performance of both egg and meat-type chickens. Both live attenuated and inactivated viral strains of ND and IB are being used for immunisation against the two potent pathogens- Avian Orthoavulairus-1 (AOAV1) and Avian Corona virus (AvCoV). The recent emergence of newer circulating viral strains, has triggered the need for continuous development of improved or novel

vaccines against both the diseases. The present study involved the use of well-characterised, indigenous field isolates of AOAV1/D58 and AvCoV/ B17 for a combined chitosan-based nanovaccine suitable for mucosal immunization in chicken. Water soluble chitosan nanoparticles encapsulated with live/inactivated viruses (AOAV1 and AvCoV) were prepared by ionic gelation method. *In vitro* characteristics of these virus-encapsulated nanoparticles identified them as spherical particles with an average size of 228.6±1.6nm. The encapsulation efficiency was 95% and the burst release of each of the viruses occurred at 50 hours, followed by sustained release upto 120-144 hours. The formulation was found to be safe and stable based on *in vitro* and *in vivo* studies. An *in vivo* experimental immunization trial with the virus-encapsulated chitosan nanoparticles in SPF birds revealed significant humoral and mucosal immune response ($P<0.0001$) against both antigenic targets, in comparison to the conventional vaccines. The nanovaccine also offered 100% protection against virulent AOAV-1 challenge by an additional support at the mucosal surface. This study provides an insight for development of chitosan nanoparticle-based combined inactivated vaccine using the same genotype circulating in the field and other prevalent avian viruses for a better mucosal immune response in addition to humoral response.

OP_5.6: Bioactive Glass Bilayer Scaffolds for Effective Repair of Mandibular and Femoral Bone Defects in Tissue Engineering Application

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The study aimed to create a bilayer scaffold using alginate, gelatin, and bioactive glass and evaluate its potential for tissue engineering and regeneration, particularly in bone defect repair. Bilayer scaffolds were fabricated using combinations of alginate/gelatin (AG), alginate-actenol/gelatin (AGD), alginate-actenol/gelatin-45S5 BG (4AGD), and alginate-actenol/gelatin-59S BG (5AGD) through freeze-drying. Structural and morphological characterization was performed using powder X-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM). The scaffolds were further evaluated for hemocompatibility, porosity, swelling behaviour, biodegradation, drug release, and antibacterial properties. Bone defects remain a significant challenge in healthcare. This study developed a bilayer scaffold combining alginate, gelatin, and bioactive glass (BG) for tissue regeneration and drug delivery. XRD confirmed amorphous and crystalline properties, while FTIR showed molecular changes in 4AGD and 5AGD scaffolds. The porous structure promoted tissue integration, with 45S5 BG causing faster degradation and 59S BG enabling controlled breakdown. The scaffolds exhibited high water absorption, good hemocompatibility, and sustained Actonel release over 72 hours. In vivo, 5AGD promoted significant bone regeneration with a 20-25% increase in new bone area. Nano-CT confirmed enhanced osteogenesis, suggesting potential for treating critical bone defects. Further large-scale studies are recommended to evaluate biomechanical performance and bone regeneration capacity.

OP_5.7: Rapid green synthesis and characterization of silver Nanoparticles and their inhibitory activity against human Pathogens and liver cancer cell lines

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The basic biological macro-molecules and active structures are provided by the nature as a boon to develop modern medicine. From ancient times, silver is known to have good antimicrobial activity which is a base for the synthesis and application of Silver Nano Particles (SNPs) against microbial pathogens.

SNPs are also having unique properties in effective drug delivery for cancer treatment. Aqueous extract of *Leucas aspera* is used for the bio reduction synthesis of the SNPs. The colour change in colloidal solution, justify the reduction of Ag+ into Ag0. The confirmation of SNPs was read spectrophotometrically, the intense peak was observed at 447nm. Size and surface morphology of the particles were analyzed by Scanning Electron Microscope (SEM), which showed spherical shaped SNPs and the average size is 45nm. The presence of functional bio-molecules involved in synthesized SNPs was identified by Fourier-transform Infrared (FTIR) spectroscopy. The size and the crystal lattice of the SNPs were determined by X-ray diffraction (XRD) measurements. The disc diffusion method was performed to evaluate the antimicrobial activity of SNPs which showed potential activity against multiple clinical pathogens namely *Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella dysentriae, Salmonella paratyphi, Escherichia coli* and *Acinetobacter*. Cytotoxicity assay was carried out against PLC/PRF/5, HepG2 and HUH-7 cell lines at various concentrations and IC50 values were 31.33µg/ml, 44.48µg/ml and 22.86µg/ml respectively. The present study proved that green synthesis can be employed as a method to develop SNPs as natural alternative to conventional medicines against clinically isolated pathogens and to treat liver cancer.

OP_5.8: Enhancing Bioactive Materials for Mandibular Bone Regeneration: Converting Fibrous Mats into 3D Matrix Cotton for Superior Shape Stability and Rapid Hemostasis

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The study aimed to transform fibrous mats composed of Polycaprolactone (PCL) and Pluronic F-127 (PF-127) with varying concentrations of monetite calcium phosphate (MCP) into a three-dimensional (3D) scaffold, referred to as matrix cotton, using a novel gas foaming technique. The objective was to assess the biomimetic properties, hemostatic potential, and bone regeneration capabilities of these 3D matrix cottons. Fibrous mats of PCL/PF-127 with different percentages of MCP were fabricated via electrospinning technique and rapidly converted into 3D matrix cottons using gas foaming technology, producing customizable shapes such as squares and hollow tubes. The 3D matrix cottons exhibited a uniform distribution of monetite particles and highly porous structures, mimicking the extracellular matrix. Our thorough investigations into the structure, water/blood absorption capacity, hemolytic potential, and hemostatic properties revealed promising results, particularly with PCL-MMC15%, which exhibited superior absorbent capabilities, excellent cell viability, and rapid hemostasis. In-vitro studies further demonstrated the potential of PCL-MMC15% in supporting periodontal cell viability and promoting spheroidal formation in co-culture studies involving NIH-3T3 and MG-63 cells. Most notably, the in-vivo rabbit study on mandibular defects confirmed the effectiveness of PCL-MMC15% in promoting new bone formation, thereby resolving mandibular defects. These findings underscore the significant promise of the 3D matrix cotton, especially PCL-MMC15%, in applications related to mandibular bone regeneration and hemostasis. This research indicates that the 3D matrix cotton (PCL-MMC15%) holds significant potential for applications in both mandibular bone regeneration and hemostasis.

TECHNICAL SESSION - VI

ONE HEALTH PROBLEMS AND SOLUTIONS

Lead Paper - 1

Bacteriophages as bio-control agent against multidrug resistant Salmonella species

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Typhoid epidemic out breaks happen almost every year throughout the world not only in under developed or developing countries but also in developed countries. Globally, 11.7 million typhoid cases were reported in a single calendar year including 216000 deaths. The standing solution to this problem is antibiotics. Chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole, streptomycin, sulfonamides, tetracycline, quinolone or third-generation cephalosporin are some of the potent drugs commonly used in case of typhoid. Apart from the outbreaks, the drug resistance is an emerging threat that needs to be taken under serious consideration. Microbes are called multi drug resistant if they are resistant to first line antibiotics. Salmonella is emerging as drug resistant pathogen and it is a grave threat to mankind. Hence, there is a dire need to come up with a new alternative and effective solution to circumvent the infection.

Bacteriophages are the viruses which very specifically kill bacteria. Generally, phages are species specific but, in some cases, they have also been found to infect closely related species. Since their discovery, phages have been considered for therapeutic purposes in case of infectious diseases by various pathogenic bacteria. Due to the discovery of antibiotics, phage research and phage therapy application was cornered to very limited areas of the world such as Russia, Georgia and Poland. However, of late, researchers are emphasizing once again on uses of phages as bio-control agents including western world. Bacteriophages have been studied and reported in various fields to control pathogenic and resistant bacteria such as poultry, fishery, agriculture, phage therapy and veterinary. Here, in this study we propose a lytic, potential bacteriophage against *Salmonella typhi* with bio-physiochemical characteristics and genome analysis that could be used as a bio-control agent.

Bacteriophage isolation and purification

The bacteriophage under this study was isolated from the waste water sample collected from a slaughter house. The bacteriophage (3A_8767) formed clear, large and round lytic plaques of size 13mm after overnight incubation at 37 ºC on the agar plate. The phage lysate was concentrated to 10^{10} pfu/mL using PEG6000 and NaCl. The high titer phage lysate obtained after precipitation was further subjected to purification with ion exchange chromatography.

One-step growth, Lytic kinetics, Lytic spectra and Morphology

The infectivity of the bacteriophage 3A_8767 was evaluated by one-step growth experiment by infecting the host bacterial strain in its exponential phase at multiplicity of infection (MOI) 0.1. The experiment depicts a latent period of 30 min for the phage, which is the time period from absorption to lysis of the host bacterial cell. The burst size for the phage 3A_8767 was 142 particles The rise period for the phage obtained from the experiment was 25 min, which was between end of the latent period and start of stationary phase. The lytic kinetics of the phage was determined at different MOI 10, 1, 0.1, 0.01 and 0.001in broth medium inoculated with host bacteria. The absorbance was on increase till 3 h and then decreased in 4th h till 9 h observation. The infectivity of bacteriophage 3A_8767 was tested against many different bacteria such as *Salmonella, E. coli, S. aureus, P. aeruginosa, Klebsiella, Brucella* and *Bacillus*. It was observed that the phage could infect one strain of *Salmonella typhi 8767* and *E. coli* out of the tested strains. It was found that the phage

sensitive strains were resistant to more than three antibiotics. The transmission electron microscope (TEM) was used to investigate the phage 3A_8767 morphology which revealed the phage particle as icosahedral in shape and the head diameter around 56nm with a stubby short tail. This morphology description implies that the phage 3A_8767 could tentatively be placed under Podoviridae family.

Stability at different pH, temperature and salt

The phage stability was assessed in different pH buffers ranging from 3 to 11 with 4hours incubation. The optimal pH range for the stability and survival of the phage 3A_8767 was found between pH 5 to 9 where 90 % of the phages were active. Bacteriophage 3a_8767 was found to be stable at wide range of temperature ranging from 4 ºC to 60 ºC. Thermal stability study till 4hs showed that the phage at 50 ºC was stable for 1 h, but at 60 ºC significant reduction in pfu count was observed. The Osmo tolerance of the phage was analysed at different salt concentration (1M, 2M, 3M and 4M) and found that the phage show no significant reduction in pfu count up to 4hours.

Cytotoxicity assay

Treatment of different cell lines with varying concentration of phage particles was evaluated for toxicity. From the absorbance at 549nm of formazan amount produced during treatment, it was found that there is no significant reduction in cell viability. Phages with approximate concentration from 10^5 to 10^{10} pfu / mL were used for the study and it was found to possess no momentous toxicity in comparison to control.

In vivo efficacy experiment of phage in *Bombyx mori* model

To assess the in-vivo therapeutic efficacy of the phage 3A_8767 against *Salmonella, Bombyx mori* larvae were used. Bombyx mori larvae selected for the study were in 4th instar stage and maintained on fresh leaves of mulberry at room temperature. Larvae were divided into 4 groups: 1, control (SM buffer), 2, phage, 3, salmonella bacteria (10^8 CFU/mL), 4, and treatments (10 MOI) each containing 10 larvae. All the larvae received the 20uL of doses and observed at 6h intervals till 48h. We observed the effect of control solution and phage solution which showed the survival rate not affected. Whereas the group 3rd infected with bacteria showed 90% mortality. The treatment group post infection injected with phage 3A_8767 (10 MOI) showed significant survival rete up to 60% in 48 hr study.

Bacteriophage DNA extraction and genome sequencing

Purified bacteriophage was subjected to genomic DNA isolation. The DNA library was prepared using QIAseq FX DNA Library kit. Next generation sequencing (NGS) of the bacteriophage genome was done on Illumina platform (2 x 150 bp chemistry) and 1.5Gb data was generated. From sequencing data comparison using the BLASTn tool, the phage 3a_8767 genome was found to be highly related to T7 phage with 92.84% identity and 92% query coverage from which it could be inferred that the phage 3A_8767 had a linear genome. The size of the whole genome was confirmed as double stranded 38821 bp with G + C (49.25 %) content.

Proteomics of structural proteins

Purified phage particles after subjected to SDS page and in-gel trypsin digestion followed by mass spectrometric analysis revealed the presence of at least 11 structural proteins ranging from 6kDa to 158kDa. There was no presence of the toxic protein which makes the phage a good candidate for the various applications.

Conclusion

We have isolated, purified and characterized a potential lytic bacteriophage 3A_8767 which infects the multi drug resistant *Salmonella typhi* as well as *E.coli* with defined characteristics. Based on the appealing characteristics such as lytic nature, non-toxic, stability at various physiochemical parameters, the phage would be taken further for its use as bio-control agent. Moreover, the genome similarity and phylogenetic study suggests the phage belongs to T7-likephage under Podoviridae. Further, animal model studies would provide with better understanding of phage host interaction and effects of application when move from lab to field in order to develop and extend the range of practices.

Lead Paper - 2

Zero by 2030: Challenges and Opportunities from the Animal Health Perspective

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Rabies is a fatal viral infection and its association with dog bites has been recognized by humans since ancient times. The disease is largely maintained and transmitted through the bites of Free-Roaming Dogs (FRD) (also referred to as stray dogs/ community dogs) and is widespread in countries that either do not have legislation regulating the movement and ownership of dogs or do not implement them strictly. The increased incidence of Rabies in many countries is due to a considerable increase in the stray dog population and an estimated 59,000 human deaths globally have been reported to be dog-mediated rabies and 90% of them are children living in rural areas. India accounts for 36% of the global deaths due to rabies and 65% in the Southeast Asia region (WHO, 2016). As per the reports from the Integrated Disease Surveillance Plan- Integrated Health Information Platform, there is a considerable increase in the reports of dog bites across India from 17.01 lakh cases in 2021 to 30.43 lakh cases in 2023 (IDP-IHIP, 2024). The Ministry of Health and Family Welfare, GOI has been implementing the National Rabies Control programme since the 12th Five Year Plan with support being extended to the state governments for canine anti-rabies vaccination under the ASCAD and RKVY schemes. The Animal Welfare Board of India (AWBI) has also been financially assisting registered NGOs for the ABC programmes to aid in dog population management and dog vaccination.

Given the significance of the disease, the World Health Organization (WHO) in 2018 undertook a coordinated effort to offer suitable guidance and encouragement to nations that began their efforts to eradicate dog-mediated rabies. The current Global Action Plan (GAP) supported by WHO, FAO, WOHA and the Global Alliance for Rabies Control (GARC) (also called 'United Against Rabies (UAR)) also focuses on a global strategy to fight against dog-mediated rabies with the common objective of eliminating the disease by 2030. To align with global efforts, the National Action Plan for Dog-Mediated Rabies Elimination (NAPRE) was brought out by the government of India in 2021 with all the key stakeholders. The action plan targets two key component systems namely (i) the human health component primarily to focus on timely access to post-exposure prophylaxis (PEP) to all animal bite victims and creating a well-responsive public health system and (ii) the animal health component directed to achieve at least seventy percent anti-rabies vaccine coverage among dogs in defined areas annually for three consecutive years. The invited lecture for the VIBCON-2024 will focus on a few important challenges and opportunities for the implementation of animal health components as one of the contributors to the NAPRE.

CHALLENGES:

a) Framework of GDREP: The global dog rabies elimination pathway (GDREP) is divided into three phases mainly based on their dog vaccination rates to reach the required 70% dog population vaccination coverage. There is expected to be variation between and within countries for the implementation and scaling-up of national dog vaccination campaigns.

Implementation Phase:	**Phase I: Preparation**			**Phase II: Scale-up dog vaccination**			**Phase III: Sustained 70% dog vaccination**						
Program year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Expected dog vax coverage:	<18% (current rate)			18% - 35%	35% - 53%	53% - 70%	≥70%						
Activities achieved	Field studies Workforce training Strengthening lab capacity			Pilot implementation Scaling-up vaccination coverage Logistical improvements Operational equipment			Mass vaccination of dog Surveillance to establish disease burden and assess progress						
Cost estimates:	Current vaccination coverage Infrastructure improvements*			Expected vaccination coverage Infrastructure improvements*			Vaccination of 70% of the dog population						

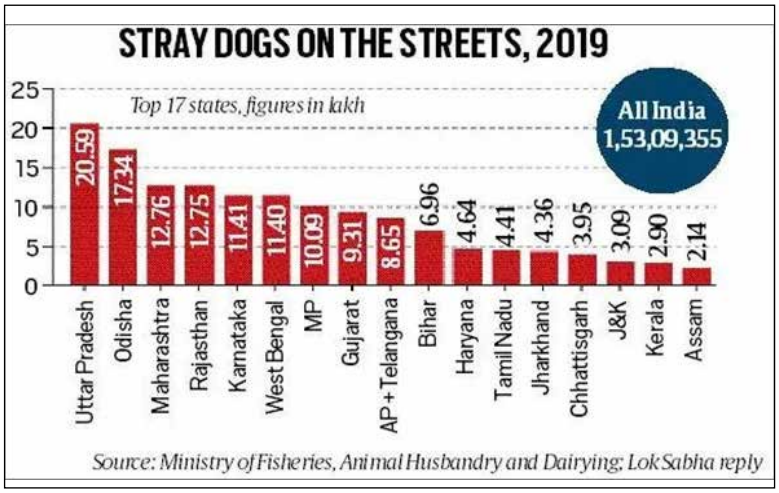
*(Source: Wallace et al., 2017; * estimated to be equivalent to the cost of vaccination for 10% of the country's unvaccinated dog population)*

Two important criteria that need consideration include the (i) cost to vaccinate a dog & (ii) dog vaccine production.

(i) *Cost to Vaccinate Dogs*: The cost to vaccinate a dog is variable and may differ from country to country and this includes the vaccine costs including disposables, cold chain maintenance, vaccinators, transportation, awareness among the stakeholders, etc.

(ii) *Dog vaccine production*: There is expected a large gap in the current supply of vaccines in dog-endemic countries based on the vaccine production potential in the second and third phase of the GDREP when the different countries will be crossing the 18% coverage and trying to reach the expected 70% coverage

b) The stray dog crisis with specific reference to our country: The multiple incidents of dog attacks on children and their deaths in some places of the country have made people raise concerns about the increasing stray dog population and the attitude of dog lovers. *(i) Stray dog population:* The number of dogs on India's streets declined from 1.71 crore in 2012 to 1.53 crore in 2019. The Stray dogs (also referred to as community dogs) are protected under the Prevention of Cruelty to Animals Act, 1960, and rules established under Section 38 of the Act, including the Animal Birth Control (Dogs) Rules, 2001; the Indian Penal Code, Sections 428 and 429; and Article 51A (g) of the Constitution. Kerala is the only state to have a committee constituted on the orders of the top court, headed by S Siri Jagan, to decide compensations for cases of community dog bites.



(ii) Guidelines for an individual for feeding dogs: It is legal to feed stray dogs and threatening dog feeders from carrying out their essential obligation to feed the stray dogs is prohibited under Article 51A(g) of the Constitution of India. In addition, interfering or harassing anyone who chooses to care for and feed community dogs is criminal intimidation, as defined by Section 503 read with Section 506 of the Indian Penal Code.

OPPORTUNITIES:

a. Rabies Antibody Levels Vs Protectivity: The acceptable cut-off antibody level post-vaccination that is claimed to be protective is 0.5 IU / ml. A minimum level of 0.5 IU / ml indicates seroconversion (in samples collected 4 weeks after the end of the vaccination series) and in addition, this 0.5 IU / ml is associated with protection in dogs and cats (by challenge studies). However, in humans, 0.5 IU/ml indicates seroconversion (not protection as they cannot be correlated with challenge studies in humans)

b. Rabies vaccine: Human vs Animal Vaccines contrasting features: The rabies vaccines that are used are usually qualified based on the potency of the final product (1 dose of the vaccine usually contains at least 2.5IU of the rabies antigen determined by the mouse potency test or by estimating the G protein by ELISA which employs monoclonal antibodies directed against site III or site II of the G protein) thereby ascertaining that the quantity of the rabies antigen provides optimal amounts of rabies

glycoprotein to locations, rich in antigen-presenting cells (APC) to induce production of sufficient rabies virus neutralizing antibodies (RVNA), as well as memory B and T cells, for prevention of rabies. The vaccines available and in use for canines are different from that of the human vaccines in the following context:

Human Rabies Vaccines	Animal Rabies Vaccines
Vaccines are used commonly for post-exposure prophylaxis and in limited situations as prophylactic	Vaccines are commonly used for prophylaxis and post-exposure prophylaxis (following the human schedule) even though it is not an approved protocol
Are inactivated highly purified vaccines	Are inactivated vaccines and not highly purified
The formulation is a freeze-dried purified rabies antigen, free of cellular DNA (<10ng), and quantified for the glycoprotein content of each dose	The formulation is usually a liquid vaccine as they are cost-effective and generally clarified to remove the cellular substrate used for propagation of the virus and quantified for the antigen content at least >1.0 I.U potency
Mostly non-adjuvanted vaccines	Mostly adjuvanted vaccines – with aluminum hydroxide
Multiple dosing (one dose on the following days – 0, 7th, and 28th day) for a prophylactic schedule	Primary vaccination: The common recommendation by manufacturers and also the common clinical practice is to vaccinate the puppies at 3 months of age The alternate recommendation by some manufacturers is to vaccinate at 4 weeks of age and a booster dose is given at 3 months of age
A necessary booster after one year	Despite immunity claimed for a minimum period of 2 years by most of the manufacturers, the dogs are boosted annually

c. ***Oral rabies vaccine: a new strategy to be explored:*** The delivery of a rabies vaccine through bait is much quicker, easier, and more practical than capturing dogs with nets to give them rabies injections. The use of oral rabies vaccine (ORV) has been a suggested strategy for wild-life rabies control and the same has been in place for the past 30 years. The WHO and OIE, strongly advocate for the operational assessment of ORV in dogs within rabies-endemic areas as a complementary approach to parenteral vaccination methods. Two types of ORV are commercially available (i) modified attenuated live vaccine derived from the rabies strains Street Alabama Dufferin (SAD) isolated in 1935 by CDC that has been passaged in several cell types and also thermal stabilization and (ii) vector-based vaccines created with the insertion of the major antigenic gene of rabies virus the Glycoprotein (Vaccinia and Adenovirus have been the delivery vectors). The success story of the ORV for dog-mediated rabies control is evident from feasibility and effectiveness studies in Thailand (in four of the provinces). With the study targeting around 2000 dogs across 338 sites, 65.6% of the free-roaming dogs could be vaccinated by the ORV. A significant increase in vaccination coverage within a short period through the use of ORV will not only break the rabies transmission cycle but also a practical opportunity to achieve the goal of eliminating dog-mediated human rabies in Thailand by 2030.

The lecture will discuss the opportunities and challenges in detail for the implementation of the NAPRE from the animal health perspective

Lead Paper - 3

Vectors and vector borne pathogens of Zoonotic importance

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Vector-borne zoonoses (VBZ) are diseases caused by a range of pathogens that affect animals and humans. Vectors, such as mosquitoes, ticks, fleas, phlebotomine sand flies, lice, and kissing bugs, may transmit numerous bacteria, protozoa, helminths, and viruses to animals and humans.

Among the vector borne disease, ticks are the major vector transmit many pathogens to human beings. Worldwide, there are 900 valid species of ticks (Guglielmone *et al*. 2010), of which, 283 tick species were recorded on humans. Out of that only 25 ticks reported in India and only 2 ticks were found parasitizing human beings in Tamil Nadu (Guglielmone and Robbins 2018). In humans, ticks can cause paralysis, toxicoses, allergy, and irritation and transmit infectious diseases (Estrada-Pena and Jongejan 1999). Tick-borne infections of humans, farm, and companion animals are essentially of wildlife animal reservoirs (Baneth 2014).

Tick infestation on human beings

In India, only few reports on prevalence of tick infestation in human beings (Soundararajan *et al* 2017; Ajith Kumar *et al* 2018; Soundararajan *et al* 2018b; Soundararajan *et al* 2019)). In Tamil Nadu, *R. haemaphysaloides* and *R. sanguineous* and *Argus persicus* parasitizing human beings in Kanchipuram and Namakkal district of Tamil Nadu (Soundararajan *et al*. 2018a & c) whereas *Otobius megnini*, *H,isaaci*, *Haemaphysalis spinigera, H. megalaimae*, *Haemophysalis* Spp., *Anomalohimalaya* Spp. *Amblyomma varanense* and *Amblyomma* spp,, in Nilgiris hills of Western Ghats (Soundararajan *et al*. 2018c; Soundararajan *et al* 2019).

Haemaphysalis longicornis and *H. megaspinosa* tick bite in man was reported in Japan (Maeoka *et al*. 1990; Seishima *et al*. 2000). Many engorged *Ixodes holocyclus* ticks were collected from face, cheek, scalp, neck, back, and limbs of 48-year-old man in Australia (Miller 2002). *Amblyomma americanum* was the most common tick reported to bite human beings in U.S.A (Brian *et al*. 1994; Childs and Paddock 2003). *Rhipicephalus sanguineus* was collected on the limb and scalp of a male and female in Brazil (Dantas-Torres *et al*. 2006; Mentz *et al*. 2016) and US Air Force personnel (Brian *et al*. 1994), on the neck and groin regions of children and in an adult female in Nigeria (Okoli *et al*. 2009), on the right shoulder of a woman in Israel (Uspensky 2009) and on a girl in Argentina (Guglielmone *et al*. 1991).

The most common tick species on humans in Sri Lanka were *Dermacentor auratus* and *Amblyomma testudinairum*, while *Haemaphysalis intermedia*, *Rhipicephalus microplus* and *Rhipicephalus sanguineus.* Humans and domesticated animals were mostly infested by the nymphal stages while adult ticks were found on wild animals. Wild animals play a significant role as a reservoir of many tick-borne infections which can easily be spread to domesticated animals and then to humans via tick infestations (Liyanaarachchi *et al.,* 2015). Rodriguez-Vivas *et al*. (2016) reported *Amblyomma mixtum* and *A. parvum* in humans in Mexico and concluded that domestic animals and wildlife play important roles as reservoirs of zoonotic pathogens that are transmitted to humans by ticks.

Bursali *et al* (2011) observed 26 ixodid tick species infesting humans in Turkey of which *Hyalomma marginatum (18.6%), Rhipicephalus bursa (10.3%), R. sanguineus (5.7%), R. annulatus (2.2%), Dermacentor marginatus (2.5%), Haemaphysalis parva (3.6%), and Ixodes ricinus (1.6%)* were the most

prevalent species. *Dermacentor marginatus* occurs on domestic animals (cattle, sheep and goat), pet animals (dogs) and human beings (Walker *et al*., 2003).

Tick borne diseases in human beings

Ticks are obligatory hematophagous ectoparasites of vertebrates and are responsible for vectors or reservoirs in the transmission of pathogenic fungi (Dermatophilosis), protozoa (Babesiosis and Theileriosis), viruses (Crimean–Congo hemorrhagic fever, Powassan encephalitis, Kyasanur forest disease (KFD), Louping ill, Colorado tick fever, Omsk hemorrhagic fever (OHF), West nile virus (WNV), African swine fever virus (ASFV) and other tick-borne encephalitis (TBE), rickettsial (Rocky Mountain spotted fever, Brazilian spotted fever, Boutonneuse Fever or Mediterranean spotted fever, African tick bite fever, Cowdriosis, Human granulocytic anaplasmosis, Human monocytic ehrlichiosis, other ehrlichiosis and anaplasmosis) and other bacteria (lyme disease, tularemia, Q fever, relapsing fever and borreliosis) during their feeding process on the hosts (Sonenshine and Roe 2014).

Four major tick-borne diseases reported in India

1. Kyasanur forest disease (KFD)
2. Crimean-Congo haemorrhagic fever (CCHF)
3. Ganjam disease.
4. Indian tick typhus (ITT)

Among these, KFD is one of the major tick-borne viral hemorrhagic fevers that affects both monkeys and humans.

1. Kyasanur Forest disease (KFD) :

Kyasanur Forest Disease (KFD, locally better known as Monkey fever) is a fatal viral disease. It is a Zoonotic disease which is being reported from Karnataka and other few contiguous States of Western Ghats in Southern India. It occurs through tick bites or contact with infected animals. Among the various ticks, *Rhipicephalus haemaphysaloides* is a three-host tick affecting cattle, sheep, goat, and human has also been incriminated in the transmission of Kyasanur Forest disease (KFD) in human (Bhat *et al* 1978; Zhou *et al*.2006; Soundararajan *et al*. 2018a). KFD has been reported also from Tamil Nadu and Kerala (John *et al* 2014). In humans, KFD causes high fever, frontal headache, myalgia and bleeding from the nasal cavity, throat, gingivae and gastrointestinal tract (Dobler 2010). In humans, KFD transmitted by *Haemaphysalis. spinigera.* Holbrook (2012) reported that the KFD was transmitted by the nymphal stage of *Haemaphysalis spinigera* and *Haemaphysalis turturis*.

Kyasanur forest disease (KFD) and Crimean-Congo haemorrhagic fever (CCHF), tick borne diseases found in India with high mortality rate (Ghosh and Nagar 2014). John *et al*. (2014) reported that KFD was an emerging zoonotic viral tick-borne disease affecting mainly monkeys (black-faced langurs and red-faced bonnet monkeys). In enzootic areas, the KFDV was maintained and circulated in small mammals (rodents, shrews, ground birds) and ticks. KFD was endemic in 5 areas of Karnataka, India mainly Shimoga, Chikkamagalore, Uttara Kannada, Dakshina Kannada, and Udupi. KFD has been reported also from Tamil Nadu and Kerala. KFD transmitted by *H. spinigera* (Mourya and Yadav (2016) occurs in Karnataka, Kerala, Tamil Nadu and Goa.

2. Crimean–Congo haemorrhagic fever (CCHF)

Crimean-Congo hemorrhagic fever is a viral disease and caused by an arbovirus of the Bunyaviridae. Ticks act as a vector as well as a reservoir and transmitted transovarially and transstadially (Mertens *et al*., 2013). CCHF transmitted by *Hyalomma* spp tick has been reported in Gujarat (Karale *et al*. 2017). *Hyalomma isaaci* is a two-host tick occurs mostly on cattle, buffalo, sheep, goat and on human (Geevarghese

et al. 1997; Soundararajan *et al*. 2018a)) and considered to be an important vector for Crimean–Congo haemorrhagic fever (CCHF) (Mourya *et al*. 2016). In India, KFD was reported in human beings in Karnataka and Kerala (Mourya *et al* 2013; Mourya et 2014; DHS Kerala 2015) with 2 to 10 per cent morbidity in south India (Gould and Solomon, 2008) while CCHF reported in Kerala and Gujarat (Shanmugam *et al* 1976; Yadav *et al* 2013). *Hyalomma* ticks (*H. marginatum*, *H. detritum*, *H. turanicum*, *Hyalomma anatolicum*, and *H. aegyptium*) were the most common vectors of CCHFV in Turkey. They confirmed CCHF virus in tick pools (28%) by visualizing 536 bp of RT-PCR products amplified using primers specific for CCHFV (Bursali *et al* (2011).

3. Ganjam disease

Ganjam disease or Nairobi sheep disease virus (NSDV) or Ganjam viral disease is a tick-borne virus which causes a severe disease in sheep and goats. Ganjam virus, also known to cause human infection, is transmitted through *Hyalomma* species of ticks. *Rhipicephalus appendiculata* in Africa and *Haemaphysalis intermedia* in Orissa (India). 33 strains of GANV have been isolated from India, mainly from *Haemaphysalis* ticks. The presence of this virus in India was isolated from the tick species *Hyalomma anatolicum* and also from a mixture of *Hyalomma* and *Boophilus* species collected in Pakistan. Ganjam virus (GANV), a member of genus Nairovirus of family Bunyavirdae is of considerable veterinary importance in India. Though, predominantly tick borne, GANV was also isolated from mosquitoes, man and sheep.

4. Lyme disease

Lyme disease is caused by the bacterium *Borrelia burgdorferi* sensu stricto (a tick-borne spirochete) and rarely, *Borrelia mayonii.* It is transmitted to humans through the bite of infected black-legged ticks (*Ixodes scapularis*), colloquially known as the deer tick (Burgdorfer, 1984).

In North America and parts of Europe, Lyme disease transmitted by several species of *Ixodes* ticks was a main cause of human morbidity, surpassing any mosquito-borne disease. Lyme disease was responsible for more than 90% of all vector-borne disease cases in the United States (Radolph *et al*., 2012) and it was estimated that it may be responsible for disease in 255,000 persons annually world-wide, mostly in Europe and North America (Rudenko *et al*., 2011). Lyme disease, the leading vector-borne ailment in the U.S., annually affects an estimated 476,000 individuals, predominantly in the Northeast and Upper Midwest (Burgdorfer, 1984). Lyme disease was also prevalent in northern China where 30,000 persons were estimated to acquire this disease annually (Wu *et al*., 2013).

5. Tularemia

Tularemia is caused by infection with the bacterium *Francisella tularensis* (highly infectious gram-negative organism). People can become infected by tick bite and deer fly bites, and contact with infected animals (especially rodents, rabbits, and hares). *Amblyomma americanum*, *D. andersoni*, *D. marginatus*, *D. reticulatus, D. variabilis*, *H. concinna* and *I. ricinus* were responsible for transmission of Tularemia. (Foley and Nieto 2010).

6. Indian Tick Typhus (ITT)

Indian Tick Typhus (ITT) is a tick-borne rickettsiosis prevalent in India. It is a rickettsial spotted fever (SF) similar to rocky mountain SF (RMSF) and is caused by *Rickettsia conorii*. *Rhipicephalus sanguineus, Haemaphysalis* and *Hyalomma*. Affect the travelers to endemic areas. In India, they are reported from Maharashtra, Tamil Nadu, Karnataka, Kerala, Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Rajasthan, Assam, and West Bengal. Common symptoms include fever, headache, tiredness, muscle aches, a rash, and swollen glands near the tick bite. In acute cases of QTT, the affected person can have a fever of up to 41°C. The rash can appear as early as 24 hours after a tick bite.

7. Skin lesions and other damage

The person infested with *R. haemaphysaloides* revealed erythematous papule (2 mm size) and inflammatory lesion up to 16 days whereas, the people infested with *H. isaaci* showed continuous itching and irritation for [6 months and wound formation (0.5 cm) at the biting site. *Otobius megnini* is a one host tick found on dogs, cattle, sheep, goat, horse and on human (Soundararajan *et al*. 2018b).

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Lead Paper – 4

Rabies Elimination by 2030 – Problems and Solutions

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Rabies is a neglected tropical zoonotic disease transmitted through the bite of rabid animal mostly by dogs, it is almost hundred percent fatal yet hundred percent preventable by timely vaccination. Global Alliance for Rabies Control (GARC) – have joined forces, as the United against Rabies collaboration, and determined to reach the global target of "Zero human deaths due to dog-mediated Rabies by 2030".

In India, Rabies is endemic in all States except Andaman and Nicobar and Lakshadweep Islands. Even though Rabies affects people of all age groups, children are the most vulnerable and it constitutes about 40% of people exposed to dog bites in Rabies-endemic areas.

Government of India is implementing the National Rabies Control programme throughout the country since the 12th Five Year plan. The "National Action Plan for dog mediated Rabies Elimination from India by 2030" (NAPRE) was conceptualized in the year 2018 by the Division of Zoonotic Disease Programme at NCDC.

Prevailing Rabies situation in Tamil Nadu:

In Tamil Nadu as per IHIP- IDSP data for the year 2024 (Till August) the total dog bite cases reported were 3,16,064. More than 10,000 dog bite cases reported from Salem, Thanjavur, Tiruchirappalli, Pudukkottai, Sivaganga, Virudhunagar, Ramanathapuram, Villupuram & Kanniyakumari. Minimum of 1672 dog bite cases reported in Nilgiris. In the year 2024, 25 Rabies deaths were reported in Tamil Nadu, Out of which 19 patients were not vaccinated, and four patients has not completed the ARV schedule.

Rabies Elimination approach in Tamil Nadu:

The objectives of the National Rabies Control Program is to prevent human deaths due to Rabies by capacity building, mandatory of anti-rabies vaccine by ID route for Rabies and PEP (Post-Exposure Prophylaxis), increasing awareness in the general community, strengthening surveillance of animal bites and Rabies cases and strengthening Rabies diagnostics and inter-sectoral coordination. The program is being continued with an expanded vision & goal of elimination of Human Rabies by 2030 with dedicated financial and technical support to the State Governments.

Challenges to combat Rabies elimination:

Only a few countries (Japan, the United Kingdom, Australia, New Zealand, Andaman, and the Nicobar Islands) have managed to completely eradicate the disease, by their geographical isolation and excellent governmental initiatives to control rabies. They have recognized it to be a public health concern and acted upon it with long-term and sustained investment. Following are the challenges to combat Rabies Elimination:

1. Lack of Awareness:

- One of the reasons attributed to the high number of rabies deaths is the lack of awareness in the population about this fatal disease and its prevention.

- A major part of rural India is still lacking awareness and basic knowledge regarding the appropriate management of animal bite wounds and vaccine administration.
- After a dog bite, their first aid measures seem to be worse, as there are myths and misconceptions about the initial home management of wounds, such as the application of red chili, lime, tobacco leaves, or the visit to a tantric or local temple to perform some or other religious customs.
- Lack of awareness is largely responsible for such tragic deaths, as people mostly ignored pet bites.
- Only 47% of dog bite victims receive the ARV.
- Incomplete vaccination schedule

2. Uncontrolled Canine Population:

In India, according to a report (Rabies on the Indian Subcontinent) published in 2013, there was a dog for every 36 people, with only 20% of these dogs being pets. Stray dogs, are commonly seen across urban and rural areas in Tamil Nadu. An estimated population of Stray dog in India is 60 million. Incidents of dog bites are common in areas with a large stray population, leading to injuries, infections, and psychological trauma. The lack of effective sterilization programs among the dog population results in large numbers of stray dogs which exacerbating the existing problem. Stray dog plays major role in rabies transmission.

3. Laws and their enforcement:

The legal framework for handling dog bite incidents in India is often insufficient:

- Prevention of Cruelty to Animals Act, 1960: This law is primarily focused on preventing cruelty to animals. It does not provide a comprehensive framework for dealing with dog bites or compensating victims.
- Limited Control over Pet Owners: Although pet owners are required to control their dogs and prevent them from becoming a public nuisance, enforcement of these rules is lax, especially in cases of pet dogs that bite or cause harm.

4. Lack of collaboration between stakeholders:

The stakeholders include Public Health sector, Department of Animal Husbandry, Dairying under Ministry of Fisheries, Ministry of Environment, Forest and Climate Change, Municipal Corporations, Non-Government Organizations and Private sectors. Delayed Action on Dog Bite Reports, leaving victims vulnerable. In addition, there is delay in timely interventions like dog capture, treatment of victims and rabies vaccination drives.

Vision for the Future:

- *Strengthening access to health care:* Improving access to timely medical care can significantly reduce the risk of death from rabies and other consequences of dog bites.
- Improve Availability of Rabies Vaccines and Immunoglobulins.
- *Stock Management:* Health facilities need to have robust systems for managing stock of vaccines and RIGs with regular updates on inventory levels to avoid shortages.
- *Training healthcare workers* at the PHC level on how to handle dog bites and administer post-exposure prophylaxis (PEP) is critical.

- *Public Awareness Campaigns*: Public education campaigns on the importance of seeking immediate medical treatment after a dog bite are essential.
- *First Aid Education:* Educating people on basic first aid for dog bites, such as washing the wound thoroughly with soap and water, and the importance of going to a hospital immediately for rabies vaccination, can help reduce complications.
- *Focus on High-Risk Populations:* Campaigns should specifically target high-risk populations, including children and those living in areas with large stray dog populations, to ensure they understand the urgency of seeking treatment.
- *Training and Capacity Building* on Dog Bite Management: Medical professionals, including doctors, nurses and paramedics should receive specialized training on how to manage dog bites, including wound care, tetanus vaccination, rabies post-exposure prophylaxis (PEP), and the administration of RIGs.
- *Tracking Dog Bite Incidents:* a centralized system for reporting and tracking dog bite cases, treatment could help healthcare providers, and policymakers understand the scope of the issue and ensure better resource allocation.
- *Inconsistent Implementation of the Animal Birth Control (ABC) Program:* The ABC program, which mandates sterilization and vaccination of stray dogs, is not uniformly implemented across cities and states. This inconsistency in the program's execution is due to a lack of fund. At least 70% of stray dog population should be sterilised within a period of 6 months, before the next reproductive cycle begins.
- *Multisectoral Approach:* A multisectoral approach involving the Public Health, veterinary departments and NGOs can ensure both preventive measures (like dog sterilization and vaccination) and proper healthcare for victims addressed in a coordinated manner.
- *Legislation and Policy Framework:* Mandatory Reporting of Dog Bites: Establishing a legal framework that makes reporting dog bites mandatory can ensure timely intervention and treatment. This will also enable authorities to keep track of rabies outbreaks and allocate resources effectively.

ORAL PRESENTATION

OP_6.1: Identification of Nontuberculous mycobacterium from bovines: Emerging public health threat

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The present study was undertaken to reveal the occurrence of mycobacterium infection with special reference to non-tuberculous mycobacteria from bovines. The study collected a total of 695 samples, comprising 154 nasal swabs, 167 faecal samples and 156 milk samples, randomly from the live animals and 218 tissue samples from slaughter houses from bovines. The diagnostic tests employed were staining, Isolation and identification of acid-fast bacilli (AFB) by LJ slants and Middlebrook medium and species identification by sequencing of *hsp*65 gene & MALDI-TOF. The overall prevalence identified by AF staining performed directly from samples was found to be 0.72% (5 out of 695 samples). In this study, out of 695 samples processed, nine samples 1.29 % (9/695) had positive growth in both LJ slants and Middlebrook medium. All the nine isolates were subjected to PCR targeting *hsp*65 gene (439 bp) confirming all the isolates were belonged to mycobacterial genus. Species identification was carried out by sequencing of *hsp*65 gene and identified species were M*ycobacterium kyorinense (*Nasal swab*), Mycobacterium fortuitum,*(Faeces) *Mycolicibacterium smegmatis* (Milk)*, Mycobacterium fortuitum* (Milk)*, Mycobacterium asiaticum* (Tissue)*,* and *Mycobacterium kansasii* (Tissue) remaining 3 were assigned uncultured *Mycobacterial* spp. MALDI TOF identified 3 isolates (*M. fortuitum, M. kansasii & M. smegmatis,*) with high confidence (score > 9) and 2 isolate (*M. asiaticum & M. fortuitum*) with medium confidence (score 7 -9). MALDI TOF identified one isolate *(M. kyorinense*) with low confidence (score 5.7). In conclusion, this study has reported the occurrence of NTM at 1.2% for the first time in southern part of India. Along with commonly identified NTM species *a* new NTM species *M. kyorinense* was also first time reported in animals. The results were reconfirmed with MALDI TOF MS and WGS. This study highlights that NTM species from bovines identified in this study are already known to be a serious public health threat in humans. Hence, this study concludes that occurrence of similar NTM species in human and animals indicates chances of spillover of pathogens who shares a common environment warranting one health approach.

OP_6.2: Phenotypic and genotypic AMR pattern of *Klebsiella pneumonia* isolates from Jaipur

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Klebsiella pneumoniae is an important ESKAPE pathogen, in which most of the anti-microbial genes were first detected before their spread to clinically important pathogens. This organism can live in wide environmental niches including soil, aquatic environment, human and animals. The high genetic diversity of organism and high plasmid load will also contribute to its role as AMR trafficker from environment to pathogens. In the present study *Klebsiella pneumonia* were isolated from human animal environment interface. A total of 344 samples collected from animals and environment and obtained 73 isolates which were confirmed by cultural, biochemical characterization followed by PCR. Then the isolates were subjected to Kirby Boer disc diffusion method to characterize the antimicrobial resistance pattern. All the isolates were resistant to penicillin, ceftazidine and vancomycin and 97.56% were resistant to cefazolin

and cefotaxim. Most of the isolates were resistant to multiple groups of antibiotics like cephalosporins, quinolones, macrolides, carbapenem and aminoglycosides. Two isolates were categorized under MAR category I and were resistant to almost all the antibiotics used in the study and have a MAR index of 0.9545. The high MAR index of the organisms points out that the organism have the ability to acquire resistant gene from the human animal environmental interface. 35 isolates were phenotypically confirmed for ESBL production. In genotypic characterization all the 35 isolates have blaTEM gene some isolates carried blaSHV or blaCTX-M gene. One isolate carried blaKPC and blaNDM. The presence of extended spectrum beta lactamase gene and carbapenemase gene in human animal environmental interface is very important one health issue, appropriate approach is essential to tackle development of AMR in Klebsiella organism.

OP_6.3: Antimicrobial Resistance Profiling of Bacterial Isolates from Wastewater and Environmental Samples Collected from Pharmaceutical Industries in South India

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The study was aimed to determine the phenotypic and genotypic antimicrobial resistance in the isolated bacteria from the influent (25), effluent (15) and surface, ground water (15) surrounding the pharmaceutical industries located in south India. Forty-eight isolates out of 55 samples were obtained viz. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Corynebacterium sp.*, *Acinetobacter sp.*, *Aeromonas punctata*, *Ralstonia picketti*, *Staphylococcus aureus, Stenotrophomonas maltophillia*, *and Citrobacter freundii.* The phenotypic profile of resistance through antibiotic susceptibility test was carried out against sixteen different antibiotics. Standard PCR technique was used for the detection of 12 genes encoding carbapenems, quinoline, aminoglycoside, β-lactam belonging *blaOXA-58 blaOXA-22, qnrA, qnrB, aac(6)-Ib-cr, aac (3)-XI, mec A, qepA,* aadB, *blaVIM*, *blaOXA-48* and *blaNDM*. *Pseudomonas aeruginosa* (1: TN/I/2020) showed presence of 3 resistance genes. *qnrB* (489 bp) gene was present in a maximum of 7 isolates while *blaVIM* (196 bp) gene was present in 6 isolates. The resistant gene *blaNDM* (621 bp) was present in three different isolates; *aac (X):6)-lb-cr* (482 bp), *qepA* (495 bp), *aadB* (500 bp), *blaOXA-58* (843 bp) resistant genes were present in two different isolates each among the bacterial isolates obtained in this study. In phenotypic resistance profiling by AST method, a maximum of 14 antibiotics were resistant among 16 tested. Similarly, in genotypic resistance profiling, among 12 resistant genes tested, a maximum of three resistant genes were noticed in *Pseudomonas aeruginosa.* There were positive and negative correlations observed between phenotypic and genotypic resistance among different antibiotics and their resistance genes indicating the variations in the resistant gene expression.

OP_6.4: Seroprevalence of Leptospirosis from Andhra Pradesh

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Leptospirosis is a zoonotic disease of global importance with significant mortality& morbidity. However, the disease is frequently overlooked& neglected & under diagnosed. It is caused by pathogenic Leptospira in livestock, pet animals, wild life and humans throughout the world. The losses are due to reproduction problems in livestock & mortalities in humans. Outbreaks usually occurs due to contact with infected animals and contaminated soil & water. The study aimed at bringing in evidence of laboratory confirmed Leptospira cases to know the seroprevalence of Leptospirosis. The sero-epidemiological study was

conducted at Leptospira lab, SLDL, SVVU, Tirupati during 2016 to 2023 with Microscopic Agglutination Test (MAT), the gold standard test for Leptospira as per the method of RMRC, Port Blair with certain modifications. A panel of 10 serovars namely *L. autumnalis, L. ballum, L. canicola, L. grippotyphosa, L. hardjo, L. hebdomedis, L. icterohaemorrhagiae, L. javanica, L. pomona and L. patocnonpathogenic* serovars were used in the MAT. A total of 942 Bovine,315 Ovine,372 Canine, 59 Wild animals and 68 human suspected for leptospira were screened against leptospiral antibodies with MAT. Bovine with 8.39%(79), Ovine with 26.67% and Canine with 41.12%(153) sero- positivity were reported. Similarly, out of 59 sera samples screened from wild animals 21 samples found positive for leptospiral antibodies with 35.59% positivity. According to the serovar prevalence highest prevalence of *L. canicola* with 31.65% (100) followed by *L. hardjo 26*.58% (84), *L. icterohaemorrhgiae* 14.87%(47), *L. grippotyphosa* 7.6%(24), *L. pomona* 7.28%(23), *L. hebdomedis* 6.33%(20), *L. autumnalis* 2.21%(7). *L. ballum* 2.21% (7) *and L. javanica* with 1.26% (4) were recorded. In wild animals highest prevalence of *L. pomona* with 42.87% (9) followed by *L. javanica* 33.33%(7) , *L. hardjo* 14.28%(3) and *L. icterohaemorrhgiae* with 9.52%(2) were reported. Out of 68 clinically suspected cases of human 21 samples were found positive with 30.88% sero-positivity. *L. hardjo* serovar with 33.33%(7) was found to be dominating followed by *L. canicola* 23.81%(5), *L. pomona* 23.81%(5), *L. ballum* 9.52%(2) and *L. grippotyohosa* with 9.52%(2). The presence of Leptospira in domestic and wild animals indicated the threat to the risk group especially livestock /pet owners, Vets /para vets , zoo attendants and public because of zoonoses and immediate action is needed for the treatment and control as it causes production loss in livestock and mortality in Wildlife including human.

OP_6.5: A study on Antimicrobial Resistance (AMR) among the isolates from Asiatic Lions (*Panthera leo persica*)

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Antimicrobial resistance (AMR) among human beings is one of the major threats being faced by medical professionals in treating cases. However, among wild animals especially in Asiatic lions very little data is available regarding AMR. A study for antimicrobial resistance was carried out among nine adult (six females and three males) Asiatic lions (*Panthera leo persica*), under ex-situ condition on the basis of culture and sensitivity tests done at pathological laboratories at different periods, by analysing the data available at Etawah safari park, Etawah, India. Samples used for the tests included urine culture, whole blood, pus swab, wound swab, vaginal swab, anal swab and oral swab. In most of the cases *Staphylococcus* spp., *Enterococcus* spp. and *E. coli* were identified based on specific growth pattern on selective medium and with biochemical tests. Almost all adults showed resistance against most of the antimicrobials commonly used including amikacin, cefotaxime, ceftriaxone, penicillin, azithromycin and amoxicillin plus clavulanic acid whereas ciprofloxacin was found to be the most resistant antimicrobial. However, antimicrobials such as ampicillin plus sulbactam, cefotaxime plus sulbactam, erythromycin, were found to be sensitive in increasing order and linezolid, the synthetic oxazolidinone was the most sensitive among all animals. However, clindamycin, doxycycline and gentamicin were found to be sensitive and resistant for around 50 percent of lions. Usually, wild animals have minimal exposure to pathogens of anthropogenic origin as they live in the environment rarely affected by anthropogenic activities. Therefore, suspected acquired AMR under ex-situ condition among wild animals may be hypothetically an indicator of anthropogenic pollution due to their proximity to the human environment. But the study needs extensive data related to AMR among captive as well as wild animals in natural habitat especially in Asiatic lions in order to establish exact scenario and causes of AMR.

OP_6.6: Sero-Survey of pigs in wet lands: A Pragmatic One Health Approach to Japanese Encephalitis Epidemic Preparedness and Spillover Prevention

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Japanese encephalitis virus (JEV) is a major zoonotic cause of encephalitis in Southeast Asia. Natural JEV ecology involves Ardeidae birds as reservoir host pigs, pig as amplifying host and mosquito as vector. Pig plays a central role in JEV transmission and spillover. Tamil Nadu, a state located in the southern part of India, boasts the highest number of wetland sites protected under Ramsar convention. Despite reporting JE cases regularly, there is a lack of epidemiological data on JEV in the context of wetlands. This study aims to assess the prevalence of JEV in pig populations across various wetland ecosystems in Tamil Nadu, with a focus on spillover risks to humans. In the cross-sectional study, a total of 489 pigs reared in 83 farms across 26 villages of 12 taluks in 6 districts of Tamil Nadu were sampled using multistage cluster random sampling. Serum samples were analysed for their JEV status using inhouse recombinant NS1 based Immunoglobulin M and Immunoglobulin G Enzyme-Linked Immunosorbent Assay. Data on human spillovers were extracted from the national portal of Integrated Disease Surveillance Program. Information on types of wetlands ecosystem were collected from Tamil Nadu State Wetland Authority. The apparent and true JEV seroprevalence at animal-level were 60.4% (95% CI: 56.8% – 64.0%) and 70.1% (95% CI: 57.0% – 73.2%) respectively. Coastal wetlands had the sero-prevalence of 71.1% (95% CI: 55.6% – 83.6%) while river deltas 76.4% (95% CI: 50.1% – 93.2%) and mangrove swamps had 60.1% (95% CI: 53.1% – 66.4%). The corresponding human spillover rates were 9.2%, 16.5%, and 18.4%, respectively, in these regions. The current study identifies coastal wetlands, river deltas and mangrove swamps as high-risk landscapes for spillover. One Health approach, fostering collaboration between animal and public health authorities to review veterinary data and plan human control measures, is essential for controlling Japanese Encephalitis in this region.

OP_6.7: Molecular detection of ESBL-producing *Escherichia coli* from thoroughbred horses, horse personnel and stable environments in Southern India

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India has witnessed several devastating outbreaks of infectious diseases in equines over the past decade, such as equine influenza, glanders, and equine rhinopneumonitis. To prevent potential loss, antibiotics are often used excessively in the treatment of horses. Due to antibiotic resistance, antimicrobial medications lose their effectiveness, making the treatment of bacterial infections challenging or even impossible. Other common conditions such as cellulitis, diarrhea, respiratory diseases, laminitis, and abscess (capped elbow/hock) also warrant the use of antibiotics for these animals. The extensive use of beta-lactam antibiotics has been recognized as a major contributing factor to the emergence and spread of extended-spectrum beta-lactamase (ESBL)-mediated resistance among gram-negative bacterial pathogens, particularly *E. coli*. Therefore, the study was designed to detect *E. coli* and their associated antimicrobial resistance (AMR) genes in thoroughbred horses, horse personnel, and stable environments in Southern India by Real-time PCR assay. A total of 324 samples were collected from apparently healthy horses (nasal, rectal, and skin

swabs), their stable environments (feed trough swabs, soil, and water samples), and horse personnel (stool samples, nasal and hand swabs). The DNA extracted from the samples, using a magnetic bead-based method, was tested for the presence of *E. coli*. This was done using the TaqMan probe-based real-time PCR assay which targets the *E. coli* specific uspA gene. Following this, the samples were screened for AMR genes including TEM, SHV, and CTX-M using a SYBR green-based qPCR assay. Results showed that the overall *E. coli* prevalence of 50.9%, with the highest positivity in horse personnel samples (64.8%) followed by 52.8% from horses and 35.1% from stable environment samples. The *E. coli* were also screened for ESBL genes. The TEM gene showed maximum positivity in rectal swabs of horses (41.9%), the SHV gene in skin swabs of horses (60%), and the CTX-M genes in stool samples of humans (71.4%). The presence of AMR genes differed across cities, with significant variations in the distribution of genes. Furthermore, 23 samples showed the co-occurrence of methicillin resistance and ESBL genes, highlighting the complexity of AMR dynamics. This study highlights the need for ongoing surveillance of antimicrobial resistance in horse settings with a one health approach.

OP_6.8: Molecular Investigations for Assessment of the Prevalence of Thermophilic *Campylobacter* among Wild Animals from Three Different States of India

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Being an important enteric bacterial pathogen causing foodborne diarrhoeal illness associated with contaminated animal products, *Campylobacter* emerged as a serious zoonotic threat among wild and domestic animals. Due to increased deforestation and urbanization, the interface between humans and wildlife is decreasing which causes man-wildlife conflict. Hence, the current study investigated the molecular epidemiology of the thermophilic *Campylobacter* by assessing its presence in fecal samples of wild animals from various zoos/sanctuaries/national parks of Uttarakhand, Uttar Pradesh, and Chhattisgarh provinces of India. The isolation of Campylobacter using blood-based media Columbia Blood Agar (CBA) and blood-free Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) is followed by biochemical and molecular confirmation using multiplex PCR targeting *CadF, hipO and aspK,* and finally subjected to partial genome sequencing. The overall positivity of *Campylobacter* isolates was reported to be 11.90% and 11.70% through biochemical and multiplex PCR. Sanger's partial genome sequence analysis revealed 98-100% identity and 97-100% query coverage and showed 47 -79% evolutionary distances with sequences already submitted in the NCBI database.

OP_6.9: Concurrent Evaluation of Rabies Vaccine and Its Post Immune Response in Naive and Immunized Dogs

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Vaccination of dogs against rabies is considered as fundamental for National rabies control programmes since the dog is the main reservoir and transmitter of the disease in India, where more than 20000 human deaths are reported only due to rabies. Though the young puppies are generally primed with rabies vaccine when they attain three months of age, there were few cases of puppies aged below 2 months old presented to rabies surveillance Unit, Madras Veterinary College Teaching Hospital, Chennai and showed positive by fluorescent antibody test for rabies. By considering this contrast with traditional practice, the study was

designed to assess maternal derived antibody status of puppies from vaccinated and unvaccinated bitches (Group I and II). Each group comprises of 18 healthy dogs which were vaccinated with inactivated anti-rabies vaccine and blood samples were collected in day 0, 21, 90 and 180 post vaccination and the serum neutralizing rabies antibody titres were assessed by Rapid fluorescent antibody test (RFFIT). A significant difference ($P<0.01$) was noted between MDA in puppies of both vaccinated and unvaccinated bitches where 15 puppies born to unvaccinated bitches had a titre below 0.5 IU/mL at the age of 45 to 60 days.

Antigenic content (Glycoprotein) of commercially available inactivated anti-rabies vaccine was quantified by *in-vitro* Single Radial immuno-Diffusion Test (SRID) and three commercial rabies vaccines were found to be complying the minimum required potency (1IU) per dose as stipulated by Indian Pharmacopoeia and this test may be employed as expedite cost effective method to assess the potency of rabies vaccine where the facility is unavailable to carry out NIH potency test in Swiss Albino mice.

Since the level of maternal derived antibodies even in puppies born to vaccinated bitches are insignificant at 10 to 12 weeks of age, the immunization strategy against rabies in puppies warrants the advancement of priming age with one booster vaccine at a suitable interval of 3 to 4 weeks to achieve the population immunity in view of effective containment of rabies spread.

OP_6.10: Molecular prevalence of zoonotic nematode parasite *Calodium hepaticum* in household Rat population in Chennai city, India

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Calodium hepaticum (syn. *Capillaria hepatica*) is a zoonotic nematode parasite of mammals occurring commonly in liver of rats which act as a primary reservoir host. In the present study, 42 household rats in Chennai, Tamil Nadu, India were trapped and screened for *C. hepaticum* infection in liver. A parasite specific semi-nested PCR was developed to amplify partial *18S rRNA* gene of *C. hepaticum* from the infected household rat liver samples. The limit of detection of the semi-nested PCR test was 15 aM. All the collected rat liver samples were subjected to macroscopic examination and screened by the semi-nested PCR. Rat liver samples with yellowish white patch lesions were confirmed for the presence of bi-operculate and ellipsoidal shape eggs by histopathological examination. All the liver samples were screened by semi-nested PCR targeting partial *18S rRNA* gene of *C. hepaticum.* The amplified PCR product was sequenced and confirmed as *18S rRNA* gene sequence of *C. hepaticum.* This report is the first molecular confirmation of *C. hepaticum* in rats in India. Among 42 household rats screened by semi-nested PCR, 14 rats were found to be positive for *C. hepaticum*. The percentage prevalence of *C. hepaticum* in Chennai was found to be 33.33%. This study highlights the high level of prevalence of *C. hepaticum* in household rat population and the risk of zoonotic transmission of hepatic capillariasis to human beings living in close proximity to the infected rats.

OP_6.11: A flow cytometry-based molecular detection of nucleic acid biomarkers for multiplexing of Zika and Rabies viral targets

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OP_6.12: Tick infestation on human beings in Kyasanur Forest Disease (KFD) endemic zone of the Nilgiris hills of Tamil Nadu

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Ticks are hematophagous parasite and act as a vector to transmit varieties of pathogenic organisms to human beings. A total of 108 human beings (52 male and 56 females) were examined for the presence of tick infestation in Kyasanur forest disease (KFD) endemic at Gudalur, the Nilgiris district of Tamil Nadu from January 2018 to December 2023. The collected ticks were identified as larval and nymphal stages of *Haemaphysalis spinegera*, nymphal stage of *Amblyomma varanense* and nymphal stage of *Anomalohimalaya lama*. by stereo zoom and also by scanning electron microscope. Ticks were also collected from the premises by tick drag and tick flag methods and identified as larval and nymphal stage of *H. spinegera.* Persons infected with ticks showed fever and vomition until removal of ticks and reluctant to work, severe itching, tiredness, inappetance and the skin lesions persist from a week to more than two years. After healing of the wound it causes scar on the skin. Erupted, thickened brownish gray scar size ranged from 2 to 5 mm in chest region and wide spread multifocal lesions in the back from the neck to hip region, abdomen, shoulder and hands were seen. Few affected persons showed dark pigmentation of the nodular eruptions and surroundings. The affected persons working in the reserve forest region revealed multifocal erythematous nodular eruptions in the leg and thigh region. Women workers of tea estate revealed lesions on the lower abdomen and inguinal regions.

POSTER PRESENTATION

PP_6.1: Evaluation of anti-rabies antibodies in pet dogs following single-dose vaccination, in Thrissur, Kerala

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Dog-mediated rabies is a global zoonotic threat, causing about 60,000 human deaths annually. Parenteral vaccination is the basis for eradicating rabies in dogs. Determining anti-rabies antibody levels is essential for implementing effective control measures. The immune response from pre-exposure vaccination helps to understand early protection, guiding vaccination strategies. This study assessed anti-rabies antibodies in pet dogs vaccinated with a single dose of six different commercially available anti-rabies vaccines using iELISA in Thrissur district, Kerala. Blood samples from 69 pet dogs, aged 2 months to 8 years, were collected within a year of prophylactic vaccination. Anti-rabies antibody levels were measured using iELISA (Platelia™ Rabies II kit Ad Usum Veterinarium, Bio-Rad). Results showed that 72.4% samples (50/69) had antibody titre level ≥0.5 Equivalent Units/mL (EU/mL), unit equivalent to international units by seronuetralisation, with 26.08% (18/69) having titres >4 EU/ml. Samples were categorized based on the time since pre-exposure vaccination: Group 1(1-3 months, n=27), Group 2 (3-6 months n=15), Group 3 (

6-9 months, n=5), and Group 4 (9-12 months, n=22). Protective antibody levels were: Group 1 (81.48%, 22/27), Group 2 (60%, 9/15), Group 3 (60%, 3/5) and Group 4 (72.72%, 16/22). The protective level was highest within the first three months after vaccination, declining over time. Variation in antibody response may be due to the type of vaccine used and age at vaccination. The overall seroconversion rate with the different vaccines used varied from 60 % to 86.6 %. Seroconversion failures were observed in 26.08% dogs under one year and 30.5% of dogs over two years. Based on the results of the study, yearly booster vaccination should be strictly adhered to maintain optimal protection against rabies. Similar studies on stray dog population are necessary to understand the impact of vaccination effectiveness across different dog populations.

PP_6.2: Standardization of sensitive and specific multiplex PCR for rapid detection of *Escherichia coli* O157:H7

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E. coli O157:H7 is recognized as one of the most significant food borne pathogen relating to public health. Conventional cultural method using Soyabeen bile broth as enrichment medium and Sorbitol Mac Conkey agar as the selective and differential medium is a time consuming method to diagnose *E. coli* 0157:H7 in food borne outbreaks. And also the specificity is very less. In this research work, a multiplex PCR method for the detection of the pathogen *E. coli* O157:H7 was standardized using primer pairs targeting six specific virulent genes of *E. coli* O157:H7; *fliCh7*, *eaeA, rfbE, hly, stx1,* and *stx2.* The multiplex PCR produced species-specific amplicons of the six targeted genes of the size; 625 bp, 397bp, 296 bp, 166 bp, 210 bp and 484 bp, respectively. The threshold sensitivity was found to be 0.01 CFU/ml. The specificity was evaluated by subjecting *Staphylococcus aureus* culture for the standardized multiplex PCR as negative control. None of the six bands was found in the PCR product of negative control emphasizing high specificity of the method**.** The results obtained show that the standardized multiplex PCR protocol is a rapid, sensitive, species-specific and reliable method for the detection of the pathogenic *E. coli* O157:H7 and could be used for further research work, during emergencies of biological war and investigation of suspected food and water borne outbreaks.

PP_6.3: Antimicrobial resistance profile and prevalence of efflux pump genes in multi drug resistant *Staphylococcus aureus* isolated from bovine mastitis

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Mastitis, a significant economic concern in cattle, arises from various infectious pathogens. Staphylococcus aureus (S. aureus) stands out as a major culprit, having evolved into a multidrug-resistant and zoonotic pathogen of public health concern. Our study aims to assess the prevalence of S. aureus in clinical mastitis cases, analyze their antibiotic resistance patterns, and investigate the prevalence of efflux pump genes among multidrug-resistant S. aureus isolates. We collected 120 milk samples from clinical mastitis cases and identified bacterial pathogens through cultural characteristics, Gram staining, and biochemical tests. Among the 25 suspected isolates, 19 were confirmed as S. aureus through nuc gene amplification.

Alarmingly, all 19 isolates demonstrated resistance to at least four or more antibiotics, underscoring the severity of antimicrobial resistance in mastitis pathogens. We further assessed methicillin resistance (mecA) and the presence of efflux pumps (norA, norB, norC, mdeA, and mepA) in all isolates. Our findings revealed that 84.21% of isolates harbored the mecA gene, associated with methicillin resistance, while 17 isolates exhibited one or more efflux pump genes, as indicated by ethidium bromide cartwheel assay. Specifically, the prevalence of norA, norB, norC, mdeA, and mepA was 42.10%, 73.68%, 57.89%, 68.42%, and 42.10%, respectively. These results offer crucial insights for implementing antimicrobial stewardship programs at the field level. By understanding the prevalence and mechanisms of antimicrobial resistance in mastitis-causing pathogens, we can develop more effective strategies to combat this pressing issue and safeguard both animal and public health.

PP_6.4: Emerging threats: Unveiling antimicrobial resistance, virulence and biofilm-forming capabilities of ESBL-producing Enterobacteriaceae in ducks and their ecosystems in West Bengal, India

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To explore the antimicrobial resistance patterns in ESBL producing and biofilm-forming Gram-negative *Enterobacteriaceae*, sourced from duck and their surrounding environments, cloacal and tracheal swabs, and environmental samples (soil, water and feed) were collected (n=462) from some representative districts of West Bengal, India. Among the total isolates, 58.48% *E. coli*, 42.20% *Salmonella* spp., and 62.11% *Klebsiella* spp. were identified as ESBL producers. The prevalence of ESBL genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, and bla_{AmpC}) in these isolates were abundant, bla_{AmpC} being the most prevalent. Biofilm formation genes (*csgA*, *sdiA*, *rpoS*, and *rcsA*) were also prevalent in the isolates, with *E. coli* showing high rates. In *E. coli*, *csgA*, *sdiA*, *rpoS*, and *rcsA* were prevalent in 508, 490, 499, and 478 isolates, respectively. In *Salmonella* spp., *csgA*, *sdiA*, *rpoS*, and *rcsA* were found in 238, 229, 350, and 278 isolates, respectively. Among *Klebsiella* spp. isolates, *csgA*, *sdiA*, *rpoS*, and *rcsA* were exhibited by 542, 524, 591, and 547 isolates, respectively. Highly significant association ($p < 0.001$) was found between the presence of biofilm genes and the studied bacterial species. In *E. coli*, 266 isolates (48.01%) showed presence of virulence genes. *Salmonella* spp. isolates exhibited 32.34% (141 out of 436) prevalence of the *invA* gene, while a small subset (4 isolates) showed positivity for the *sefA* gene. However, none of the *Klebsiella* spp. isolates were found to carry the *rmpA* gene. The high prevalence of resistance genes and virulence factors warrants strict surveillance and mitigation of multidrug-resistant bacteria in this region.

PP_6.5: Prevalence of Virulent and Antibiotic-Resistant *Pseudomonas aeruginosa* in Meat from Retail Markets in Chennai: A Public Health Concern

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Pseudomonas aeruginosa, an opportunistic pathogen associated with meat spoilage, was screened in a study involving 210 samples of chicken, mutton, and pork from retail markets, stalls, and slaughterhouses in Chennai. Meat samples, stored both aerobically and under frozen conditions, were analyzed using cetrimide agar for isolation, followed by biochemical tests and molecular confirmation via PCR assays targeting 16S rRNA and *rpo*B genes. Of the 22 isolates identified, frozen meat showed higher isolation rates, with pork

having the highest occurrence (12.8%). The isolates harbored virulence genes, such as *plc*H, *alg*D, and *Las*B, with higher prevalence in frozen meat. Virulence genes were detected in pork meat at 100% for *plc*H and *alg*D, indicating a greater risk for contamination and potential human infection.

Antimicrobial resistance was also assessed, revealing multiple antibiotic resistance (MAR) in all isolates, with resistance to 3–5 antibiotics. The MAR index ranged from 0.4 to 0.62, signalling contamination from sources with frequent antibiotic use. The presence of extended-spectrum beta-lactamase (ESBL) genes, such as *bla*TEM (83.3%–100%) and *bla*CTX-M (50%–100%), and carbapenemase genes like *bla*NDM, *bla*OXA23, and *bla*KPC, further underscores the threat posed by these multidrug-resistant strains. Tetracycline resistance (*tet*B gene) and the absence of *qnr*A and *qnr*B fluoroquinolone resistance genes were noted.

This study highlights the potential risk of virulent, antibiotic-resistant P. aeruginosa in meat, necessitating improved hygienic practices, proper handling, and stringent surveillance of antimicrobial use in animal husbandry to mitigate the public health threat posed by multidrug-resistant pathogens in the food chain.

PP_6.6: Development of a Point-of-Care Lateral Flow Assay to Detect Anti-Brucella Antibodies

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Brucella spp. are intracellular bacteria that infect both humans and animals, leading to brucellosis, a zoonotic disease with a broad range of clinical manifestations. The diagnosis and monitoring of brucellosis traditionally rely on antibody-based diagnostic tests, along with clinical symptoms and radiographic findings. Brucella's lipopolysaccharide (LPS) has been identified as a key antigen used in assays to detect anti-Brucella IgM/IgG antibodies. The present study aimed at optimizing point of care diagnostic kit to detect brucellosis. We developed a rapid lateral flow assay (LFA) to detect anti-Brucella antibodies employing the LPS extracted from B. abortus S99. A positive result is indicated by a distinct cherry-red colour in the test line, while the absence of this colour indicates a negative result. A cherry-red control line consistently appears to confirm the assay's functionality and it is suitable for multi-species screening. The results of LFA were compared with a monoclonal antibody based competitive ELISA (cELISA) kit. In total, 114 sera samples of cattle, sheep, goats and buffaloes were analysed in this study. LFA identified 44 positives and 73 negatives whereas 25 positives and 89 negatives were noticed in cELISA thus yielding a Kappa value of 67%, indicating substantial agreement. Test line intensities showed a linear correlation with ELISA titters. Further optimization is needed to improve the specificity of the LFA. This point-of-care test provides rapid detection of anti-Brucella antibodies, offering healthcare providers real-time results, which could significantly enhance the diagnosis and management of Brucellosis.

PP_6.7: Effect of meloxicam on *Escherichia coli* biofilm formation and its impact on antibacterial effect of ceftriaxone

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Ceftriaxone is a commonly used antibacterial in farm animals. In practice, meloxicam is commonly administered while treating infections and its associated inflammatory conditions. However, the interactions

between the two compounds are less understood. Inhibition of biofilm is one of the key mechanisms of antibacterial activity. The present study is undertaken to understand the antibiofilm activity of meloxicam and its influence on the efficacy of ceftriaxone in isolates of *E. coli* from goats. Microtiter plate assay was performed for meloxicam (2.5mg/ml), ceftriaxone (4mg/ml) and the combination of the two drugs. Meloxicam and ceftriaxone at MIC value concentration were evaluated separately for their inhibitory potential against bacterial cell attachments. Inhibition of biofilm was assessed by serially diluting meloxicam in a broth with *E. coli* culture adjusted to 0.5 Mc Farland. Similarly testing for biofilm inhibition by ceftriaxone and the combination of meloxicam and ceftriaxone. After incubation, wells were stained with crystal violet, destained and finally absorbance measured at 595 nm using microtiter plate reader. Meloxicam seemed to possess antibiofilm activity in part of the isolates. Ceftriaxone was found to inhibit biofilm as shown by reduced OD value in all isolates. When combined, meloxicam was found to further reduce OD values a synergism in half of the isolates tested. Meloxicam exhibits antibiofilm activity against *E.coli* and was found to be in synergism with ceftriaxone. Combination of the two can be of value clinically, particularly when biofilm related resistance poses a therapeutic challenge.

PP_6.8: Assessment of point of care rapid diagnostic test for detecting leptospiral infection

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Leptospirosis is a quintessential one health disease of humans and animals, febrile worldwide zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. The majority of leptospirosis is subclinical or mild self-limiting systemic illness. To obtain optimal health for people, animals and environment and one health approach was initiated for screening the leptospires. Rapid and accurate diagnostic test for the detection of leptospires is essential to prevent the disease progression from the acute, non-severe illness to potentially fatal infection. The **aim** of our study is proving leptospiral infection with simple rapid commercially available diagnostic tests since conduct of MAT, a series of serovars ,expertise needed and for culture it needs two months, Dark Field Microscopic identification needs a costliest microscope, molecular methods, needs a DNA cycler, Gel electrophoresis, Gel documentation instrument and cooling centrifuge etc., but on-site rapid diagnostic tests supports clinical decisions even in poor diagnostic resource settings. Samples such as blood and urine were collected and subjected to Microscopic Agglutination Test (MAT), Dark Field Microscopic test (DFM) and molecular techniques (PCR) and also rapid diagnostic test (LEPTOCHECK TEST) both for animals and humans. All tests are consistent but point of care test was rapid easily commercially available. We conclude **LEPTOCHECK** test as rapid Point of care test in clinical diagnostics.

PP_6.9: Developing a visual detection system for the rapid diagnosis of Leptospirosis based on Loop-mediated isothermal amplification (LAMP) in acute infection

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Leptospirosis is one of the worldwide zoonotic diseases caused by pathogenic *Leptospira* species. Microscopic agglutination (MAT) test is the gold standard test for the diagnosis of leptospirosis in clinical samples that can be done only in well-established laboratories. Apart from its interpretation being subjective

and continuous maintenance of reference leptospiral strains is required. PCR-based assays are very sensitive and its variant Loop-mediated isothermal amplification (LAMP) assay is an isothermal assay which is widely used in molecular diagnostics. In the present study, a LAMP assay has been developed as a point-of care assay with visual detection system for the diagnosis of leptospirosis, an alternative to traditional PCR. The present LAMP assay consists of 5 sets of primers targeting LipL32 gene for visual colorimetric detection of pathogenic *Leptospira* in blood samples. This approach utilizes a pH indicator dye Phenol red to detect drop in pH from nucleotide hydrolysis during nucleic acid amplification. Further its sensitivity and specificity were also analysed to be utilized for its clinical application. The LAMP is more sensitive, rapid, economical, and faster assay than PCR. Our study suggests that LAMP has better diagnostic sensitivity and specificity for routine diagnosis of leptospirosis.

PP_6.10: Ticks and mite infestation on human beings in Tamil Nadu

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A total of 86 human beings were examined for the presence of ticks and mite infestation at Namakkal, Kancheepuram and The Nilgiris districts of Tamil Nadu from 2020 to 2023. Ticks and mites were collected from the human beings. Ticks collected from human beings were identified as *Haemaphysalis spinigera, Amblyomma varanensis, Rhipicephalus sanguineus* and *Argus persicus*. The mite was identified as *Ornithonyssus bacoti.* The persons infested with *A.persicus* showed irritation, inflammation, itching and ulcer at the site of biting. The person infected with *R.sanguineous* showed erythema and irritation. The persons infected with *A. varanensis* showed swelling with central opening at the site of bite with severe irritation from one weeks to two years. Persons infected with *H. spinigera* showed intermittent fever and vomiting until removal of ticks and reluctant to work, severe itching when stand more than 10 minutes in sunlight and the skin lesions persist from a week to more than two years. After healing of the wound, it causes scar on the skin. The persons infected with mites showed pin head sized erythema and itching sensations. No ticks were observed those persons applied with a mixture of palm oil, tobacco leaves and salt.

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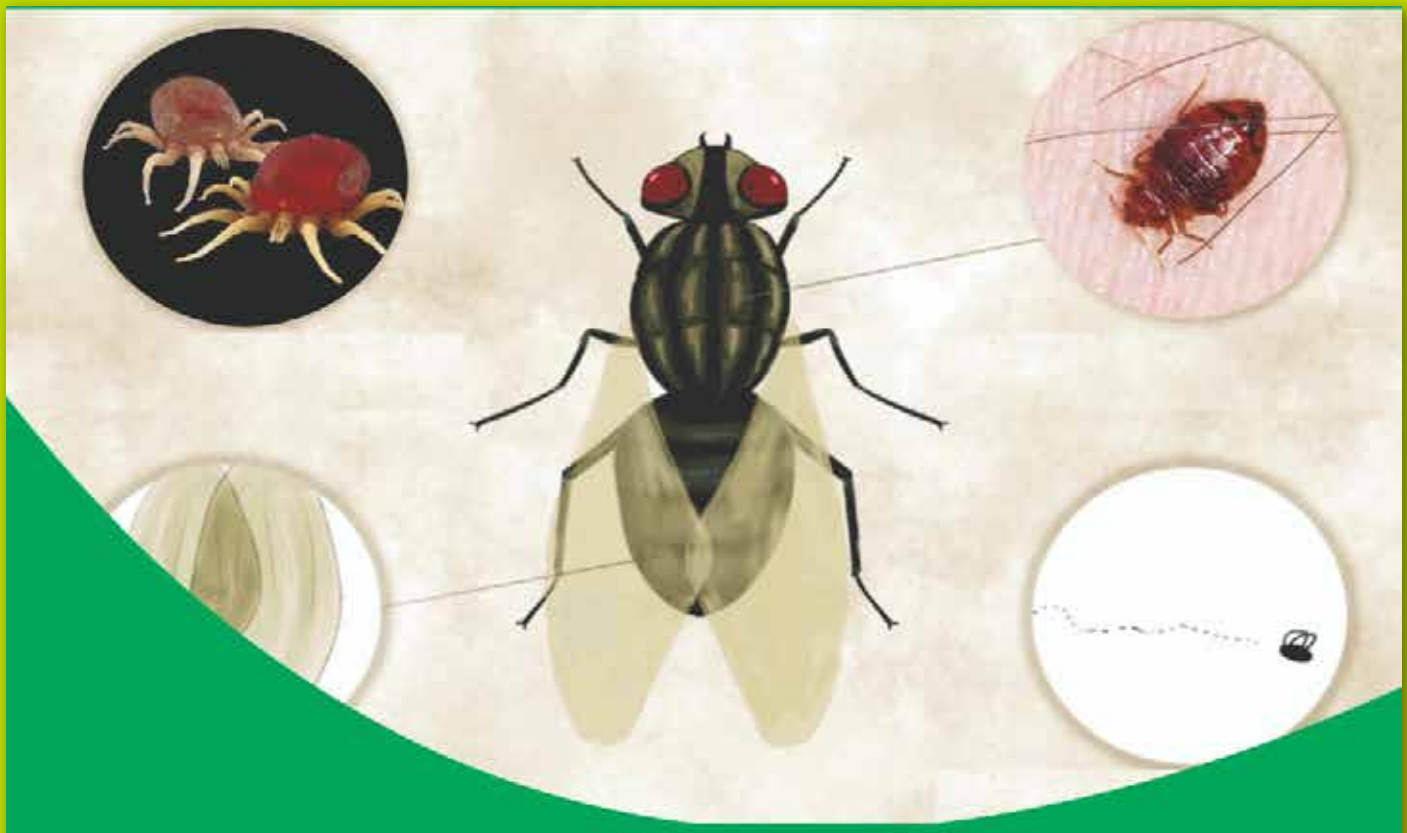

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