



Souvenir-Cum-Compendium

VIBCON-2023

XXVIII Annual Convention

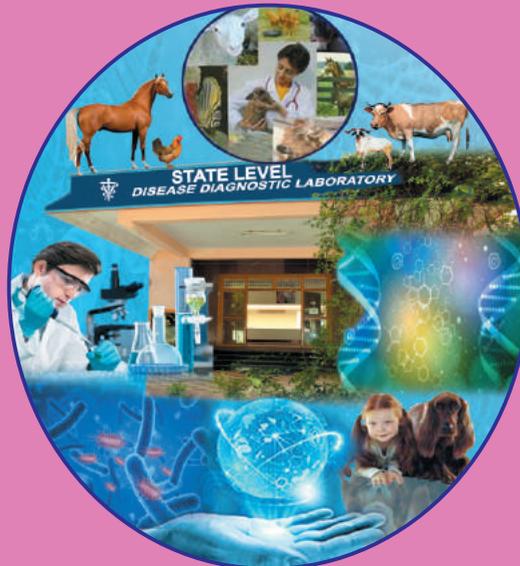
of

**Indian Society for Veterinary Immunology and
Biotechnology & National conference**

On

**“ADVANCED CONCEPTS, STRATEGIES IN
IMMUNO-BIOTECHNOLOGY
FOR DISEASE DIAGNOSIS AND CONTROL”**

12-14 December - 2023



Organized by

**STATE LEVEL DIAGNOSTIC LABORATORY
SRI VENKATESWARA VETERINARY UNIVERSITY**

TIRUPATI-517 502, ANDHRA PRADESH



*The organizing committee is
immensely thankful to the
DST-SERB
for funding the conference*

Thank you
For your support!



**XXVIII Annual Convention of Indian Society of Veterinary
Immunology and Biotechnology & National conference
on**

**ADVANCED CONCEPTS, STRATEGIES IN IMMUNO-
BIOTECHNOLOGY FOR DISEASE DIAGNOSIS AND CONTROL**

VIBCON-2023

December 12 - 14, 2023

SOUVENIR CUM COMPENDIUM

Editor

Dr. D. Rani Prameela

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Dr. M. Sravanthi

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Dr. L. Lahari

Organized by

**STATE LEVEL DIAGNOSTIC LABORATORY
SRI VENKATESWARA VETERINARY UNIVERSITY**

Tirupati-517502, Andhra Pradesh

**ISVIB thankfully acknowledges the financial support provided
by the following organizations for holding this event**

- ▶ DST SERB
- ▶ Sri Venkateswara Veterinary University
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Sri Gopal Krishna Dwivedi, IAS
Vice-Chancellor,
SVVU, Tirupati



Message

I am very much delighted that State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati and Indian Society for Veterinary Immunologists and Biotechnologists are jointly organizing the 28th Annual Convention of ISVIB and National Conference on "Advanced Concepts, Strategies in Immuno-Biotechnology for Disease Diagnosis and Control" from 12-14th December, 2023 at College of Veterinary Science, Tirupati, Andhra Pradesh.

India has vast Livestock Resources and its economy majorly depend on livestock. The livestock sector provides livelihood for rural people. However, the livestock sector is threatened by various infectious and contagious diseases and hampering the animal health and production. To overcome the health issues and the challenges in animal/poultry sector in the present scenario, I hope this conference will be a good platform for discussions and deliberations on newer technologies in the improvement of Health and Production for diverse groups from all over the country.

The National Conference is focusing on advanced molecular and innovative Immuno-Diagnostics, Immuno-Prophylaxis, Nano-Biotechnological interventions and current trends in Vaccinology. This is a very good opportunity for Academicians, Researchers, Scientists, Industrialists and students to share and gain knowledge & expertise on advanced technology in Disease Diagnosis and Vaccines so as to formulate strategies/ action plan in prevention and control of emerging, re-emerging, zoonotic and trans boundary diseases.

I wish that the conference will be a grand success.

GKD
4/12/2023

GOPAL KRISHNA DWIVEDI

Prof. (Dr.) K. Sarjan Rao
Director of Research
SVVU, Tirupati



Message

I would like to congratulate the Professor and Head, Organizing Secretary and scientific team of State Level Diagnostic Laboratory, SVVU, Tirupati for organizing the "XXVIII Annual Convention of ISVIB and National conference on "ADVANCED CONCEPTS, STRATEGIES IN

IMMUNO-BIOTECHNOLOGY FOR DISEASE DIAGNOSIS AND CONTROL" from 12-14 December, 2023. This conference provides a platform to bring academia together with the policy makers and bio industry for the collaborations and networking for new discoveries and then translating them into products and services to the welfare of livestock and for the livelihoods of farming community.

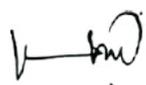
Immuno biotechnology represents a synergy of two powerful domains — immunology and biotechnology. By harnessing the body's immune system and integrating it with the precision of biotechnological tools, scientists are pioneering new ways to combat diseases, enhance diagnostics, and revolutionize therapeutic interventions. One of the most significant contributions of immuno biotechnology lies in the development of novel vaccines and immunotherapies. From mRNA vaccines that have transformed our response to viral threats to personalized cancer immunotherapies, the field is at the forefront of ushering in a new era in medicine. What sets immuno biotechnology apart is its emphasis on precision and personalization. Tailoring treatments to an individual's unique immune profile holds immense promise for more effective and targeted interventions. This level of customization has the potential to maximize therapeutic outcomes while minimizing adverse effects.

Immuno biotechnology is also reshaping diagnostics. Rapid and accurate detection of diseases, including infectious agents and cancers, is being achieved through sophisticated immunoassays and biomarker identification. These advancements contribute to early diagnosis and better disease management. As we navigate global health challenges, immuno biotechnology emerges as a key player in our collective resilience. The development of robust and adaptable immune responses through vaccines, along with ongoing research into antiviral and antibacterial strategies, positions the field as a pivotal force in safeguarding public health.

Immuno biotechnology stands as a testament to human ingenuity and the limitless possibilities that arise when science and technology unite. It is an exciting journey of exploration, with the potential to transform the landscape of healthcare and improve lives on a global scale.

It is a great pleasure to welcome all delegates and participants to this conference, coming from near and a far. I hope you will enjoy Temple city Tirupati's outstanding sculptures and exotic sights. I would like to congratulate State level Diagnostic laboratory for their commitment and superb drive in organizing this conference. I wish them all the success.

Best wishes...


(K. SARJAN RAO)

Prof. (Dr.) R.K. Singh
Former Director
ICAR, IVRI
Izatnagar, Bareilly



Message

I am delighted to note that the 28th Annual Convention of the Indian Society of Veterinary Immunology and Biotechnology (ISVIB) and National Conference on “**ADVANCED CONCEPTS, STRATEGIES IN IMMUNO-BIOTECHNOLOGY FOR DISEASE DIAGNOSIS AND CONTROL**” from 12-14th December, 2023 are being organized at State Level Disease Diagnostic Laboratory of Sri Venkateswara Veterinary University, Tirupati which would be attended by the academicians, scientists, faculty, field veterinarians, and students.

India has vast resource of livestock, poultry, companion and wildlife population. Due to climate change and cross-border movement of animals and human beings, the emerging and re-emerging animal diseases are on an increasing trend in our country. Being infectious in nature, these diseases are affecting large population of animals and human being leading to wide spread adverse impact on health and economy. Such a situation entails envisioning Nextgen diagnostics, vaccines, and therapeutic approaches for global health security and national and global economies.

The theme of the conference aims at providing the unique opportunity in the light of creating awareness and discussing the recent advances in application of biotechnology and immunology tools in rapid and [precise diagnosis of infectious animal diseases including zoonoses and transboundary animal disease. In addition, this conference will be a good platform for budding veterinarians to have interactions with the renowned professionals and scientists to improve their knowledge, skills and to identify the need-based problems pertaining to strategizing the animal health management under field conditions.

I congratulate the organizing committee for their efforts in organizing this special event and hope that this conference will benefit the participants in updating their knowledge and skills.

I wish the conference a “Grand Success”.


(RAJ KUMAR SINGH)

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



Message

Animal husbandry activities provide significant income to rural households. Contribution of livestock sector to national GDP is around four percent. Outbreak of animal diseases leads to direct and indirect losses to the farmers. Prevention and control of infectious diseases is important for sustaining profitable farming. Rapid and cost effective diagnostic tests are required for early diagnosis of diseases. Safe and effective vaccines are also required for disease prevention. Molecular methods are increasingly being used for the development of diagnostics and vaccines. Real time PCR and isothermal amplification methods are applied for disease diagnosis. Virus vector and mRNA based vaccines expressing specific antigens are being employed for disease prevention. Bioinformatics tools are used for prediction of B and T cells epitopes for effective vaccine design. In this context it is appropriate to hold a National conference on "Advanced Concepts, Strategies in Immuno-biotechnology for disease diagnosis and control"

I hope the scientific interactions in the national conference will help scientists to form interdisciplinary teams and formulate research projects for developing new diagnostics and vaccines. I appreciate the efforts made by the organizing committee for holding the ISVIB convention and National conference in the State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh.

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

I convey my best wishes to all. I wish the event a grand success.

A. Thangavelu
5/12/2023

(A. THANGAVELU)

Prof. (Dr.) D. Rani Prameela
Organizing Secretary
State Level Diagnostic Laboratory,
Sri Venkateswara Veterinary University
Tirupati.



Message

I am pleased to inform that State Level Disease Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati is organizing the XXVIII Annual Convention of Indian Society of Veterinary Immunologists and Biotechnologists and national conference, VIBCON-2023 on “Advanced concepts, Strategies in Immunobiotechnology for Disease Diagnosis and Control from 12-14 December, 2023. The theme is appropriate for disease diagnosis and control for holistic development of Livestock sector strengthening animal health and production.

In recent years, several latest technologies are available for rapid diagnosis and efficient immune-prophylaxis of zoonotic, transboundary and infectious animal diseases. To combat these infections, it requires interdisciplinary and cross sectorial approaches in disease diagnosis, prevention, surveillance, monitoring and control in mitigation as well as environmental conservation. Presently, inter collaborative research is very essential to mitigate the problems involving veterinary, medical experts, public health professionals and epidemiologists. The trainees will have unique opportunity for multi-disciplinary interaction on various facets of animal health under single umbrella.

I am confident that this occasion will be able to provide the platform to strengthen ourselves in knowledge sharing and dissemination.

I am thankful to Hon'ble Vice-chancellor, SVVU, Sri. Gopala Krishna Dwivedi, IAS, Director of Research, SVVU, Dr. K. Sarjan Rao and executive committee of ISVIB for their constant encouragement and support. Finally, I would like to thank all sponsors for their financial assistance is greatly acknowledged. Further, I am sure that State Level Diagnostic Laboratory, going to be versatile in future to serve the farmers and to support the researchers and students.

Best Wishes

(D. RANI PRAMEELA)

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**XXVIII Annual Convention of Indian Society for
Veterinary Immunology & Biotechnology and National Conference**

on

**ADVANCED CONCEPTS, STRATEGIES IN IMMUNO-
BIOTECHNOLOGY FOR DISEASE DIAGNOSIS AND CONTROL**

VIBCON-2023

December 12-14, 2023

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ABOUT SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI

The establishment of Sri Venkateswara Veterinary University was the culmination of efforts during the period, 1955 to 2006 to strengthen Education and Services in the fields of Veterinary Science, Dairy Technology and Fishery Science in the State of Andhra Pradesh. The establishment of three Veterinary Colleges, the first at Rajendranagar during 1946, the second at Bapatla during 1955 and the third at Gannavaram during 1998 paved the way for strengthening Veterinary education in the State. The Veterinary College at Bapatla was shifted to Tirupati during December, 1957. The Dairy Technology Programme was started at College of Veterinary Science, Tirupati during 1983 and was elevated to college status during June, 2009. The establishment of Veterinary University was announced by the then Hon'ble Chief Minister of Andhra Pradesh, Dr.Y.S. Rajasekhara Reddy on 30th September, 2004 at the inaugural function of the Golden Jubilee Celebrations of College of Veterinary Science, Tirupati. The State Cabinet at its meeting held on 04-03-2005 resolved to establish the Veterinary University at Tirupati in the State Legislative Assembly on 30-3-2005.



SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI

Accordingly, the Sri Venkateswara Veterinary University was established vide Act No: 18 of 2005 and G.O. M.S. No: 29 dated: 21-06-2005 of Animal Husbandry, Dairy Development and Fisheries (AH II) Department. The Hon'ble Chief Minister of Andhra Pradesh Dr.Y.S. Rajasekhara Reddy inaugurated Sri Venkateswara Veterinary University on 15-07-2005 and it started functioning from ANGRAU, Hyderabad and with Tirupati as its headquarters from 01-04-2006. A total of 40 (27 constituents and 13 affiliated) institutions are functioning under the university to serve the state and its stake holders in the domain areas of education, research and extension activities. The University and all the constituent colleges were accredited by the Accreditation Board, ICAR, New Delhi for another five years in 3rd cycle from 01-04-2022 to 31-03-2027. The University was listed in UGC under Section 12 (B) of the Act 1956.

STATE LEVEL DIAGNOSTIC LABORATORY, SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI

State level animal disease diagnostic laboratory was established under RKVY project during the year 2009 in the premises of college of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati for diagnosis of various animal diseases and diseases of zoonotic importance with the primary objective of improving animal health & production and further extending the facility to human health.

Objectives

- To provide diagnostic facilities for early & accurate diagnosis of animal & poultry diseases.
- To cater the needs of the farmers.
- To attend the disease outbreaks, collection and processing of samples, diagnosing the diseases and to suggest control measures.

Facilities available

The laboratory has been established full-fledged with the following facilities:

- Bacteriology lab: Culture and sensitivity of clinical samples, characterization of isolates and total microbial count of clinical samples.
- Molecular diagnostic laboratory: For fast, early and accurate diagnosis of animal & poultry diseases by PCR and real time PCR.
- Pathology: Analysis of hematological parameters, histopathology, cytological examination & Immuno-histo-chemical studies.
- Biochemical analysis of serum & urine samples.
- *Leptospira* lab: BSL-II facility, unique facility for the diagnosis of *leptospira* in the state from animals and humans.
- BSL-III facility: This is an unique facility in Tirupati to deal with the diagnosis of virulent organisms like Brucella, Anthrax, Rabies, Covid-19 etc.
- The laboratory is fulfilling the objectives by providing diagnostic facilities for various animal, poultry and human diseases.
- The laboratory is also providing facilities for PG & PhD students to carry out their research activities in different fields.

Significant Research Outputs

1. Standardized the methodologies for isolation, detection and Sero-grouping of *Dichelobacter nodosus* from field samples.
2. Standardized the methodologies for molecular diagnosis of malignant catarrhal fever (MCF).
3. Standardized the methodologies for isolation, sero and molecular diagnosis of Leptospirosis in both animals and humans.

4. Standardized the protocol for the analysis of soil, water, feed & food materials for bacteria & fungi.
5. Standardized the protocol for *invitro* sensitivity of chemotherapeutic agents, disinfectants.
6. Standardized the protocol for cyto-chemical & immuno histochemical studies.
7. Developed Trivalent Inactivated whole cell vaccine against Ovine Foot Rot and transferred technology to Indian Immunological Pvt. Ltd, Hyderabad for commercialization.
8. Standardized the molecular techniques for the detection of Mastitis pathogens of *S. aureus*, *S. agalactiae*, *E.coli*, *Pseudomonas aeruginosa* and *K. Pneumoniae* from milk samples.
9. Evaluated on farm milk culture system for the identification of Mastitis pathogens in Chittoor district.
10. Autogenous vaccine was prepared against mastitis of *S.aureus* origin and evaluated in Chittoor district.
11. Standardized the molecular techniques for the detection of *Bacillus anthracis* from tissues, blood and soil samples.
12. Standardized the molecular techniques for the detection of Bovine papillomas from the wart lesions. Autogenous vaccine was prepared and evaluated successfully for treating the bovine papillomas.
13. Standardized the molecular techniques for the detection of *Theileria* and *Babesia*, *E.canis* blood protozoan's from blood samples in Bovines.
14. Standardized the molecular techniques for the detection of Lumpy skin disease in cattle.

COVID -19 LAB TESTING AT SLDL, SVVU, TIRUPATI

A total of 3,023 samples were tested for COVID-19 using BSL-III facility from 09-04-2020 to 16-09-2021 and uploaded the results through SVGRR portal to ICMR

RESEARCH ON LEPTOSPIRA

Significant research outcome

1. Standardized MAT for sero-diagnosis of *Leptospira* in animals and humans.
2. Standardized molecular diagnosis of *Leptospira* in animals and humans.
3. Developed trivalent inactivated whole cell vaccine against Bovine Leptospirosis.
4. Developed recombinant subunit vaccine against Bovine Leptospirosis through reverse vaccinology with immune-informatics approach.
5. Development of multi epitope vaccine against canine Leptospirosis through reverse vaccinology/genetics with Immuno-informatics approach.

On-going Research

- ▶ Development of rapid, field recombinant protein based diagnostic kit for Bovine Leptospirosis.
- ▶ Development of rapid, field recombinant protein based diagnostic kit for Canine Leptospirosis.
- ▶ Evaluation of recombinant vaccine against Bovine, Canine Leptospirosis.

RESEARCH ON FOOT-ROT

New Research findings

- ▶ Molecular techniques were standardized for rapid diagnosis of Foot rot cases
- ▶ A, B and I sero-groups were found to be prevalent sero groups on Isolation and characterization studies and selected as vaccine candidates.
- ▶ A trivalent inactivated whole cell vaccine was prepared and standardized.
- ▶ Vaccine was evaluated in Lab and Farm level.
- ▶ Efficacy of the vaccine was studied and found to be protective up to 120 days of post vaccination.
- ▶ Vaccine technology had been transferred to Indian Immunological Private Limited, Hyderabad for commercialization.

Technology transferred

Development of a Tri-valent inactivated whole cell vaccine against ovine foot rot and Transfer of Technology to Indian Immunological Private Limited, Hyderabad.

RESEARCH ON MASTITIS

Research findings

- ▶ Developed autogenous vaccine against S. aureus mastitis and evaluated its efficacy in field.

RESEARCH ON LUMPY SKIN DISEASE (LSD)

Research findings

- ▶ Standardized techniques for molecular detection of LSD in Bovines.
- ▶ Isolated the LSD virus isolates from clinical cases from outbreak areas.

On-going Research

- ▶ To study molecular epidemiology in Andhra Pradesh
- ▶ Development of cell culture vaccine against LSD.

RESEARCH ON BOVINE PAPILLOMA

- ▶ Standardized molecular techniques for typing of Bovine papilloma virus.
- ▶ Development of autogenous vaccine against Bovine Papilloma virus type 5 and 3 and evaluated successfully at field level.

RESEARCH WORK ON ANTHRAX

Anthrax is a disease of herbivorous animals and humans incidentally acquire the disease by handling infected dead animals and their products. Anthrax is endemic in southern states like Andhra Pradesh, Kerala and Tamil Nadu. In Andhra Pradesh especially Chittoor, Kurnool, Kadapa, Anantapur, Guntur, Prakasam, Nellore districts are the known endemic areas for animal and human anthrax. During recent years tribal hamlets in Araku valley of Visakhapatnam district more human cutaneous Anthrax cases were also reported. A number of outbreak in remote areas may also go unreported mainly due to lack of adequate veterinary coverage. Due to environmental contamination by *B. anthracis* spores results in recurrence of outbreak in some of the districts of the state and also there is a need to identify the strains of *B. anthracis* and related strains involved in repeated outbreaks in particular geographical areas of Andhra Pradesh. As outbreaks are reported time to time from different areas, there is a great need of early diagnosis of the disease and effective vaccine to save animals as well as human. Hence the work has been initiated with the following objectives.

Research Findings

- ▶ Standardized the methodologies for molecular diagnosis of anthrax from clinical and environmental soil samples.
- ▶ Standardized the methodologies for isolation of *Bacillus anthracis* from clinical and environmental soil samples.

On-going Research

- ▶ Field evaluation of diagnostic kit for the detection of *Bacillus anthracis* in livestock species.
- ▶ Sero monitoring of vaccinated livestock against anthrax in the field.
- ▶ To study the molecular epidemiology of the anthrax in the state.
- ▶ To assess risk analysis in the risk groups

Completed Research Projects (RKVY)

1. RKVY Project entitled “Epidemiological surveillance, Prophylactic & Control Measures against foot rot disease in sheep” to be operated at State Level Diagnostic Laboratory, SVVU, Tirupati for the year 2016-17.
2. RKVY Project entitled “Strengthening of SLDL for fast and accurate diagnosis of Live stock Diseases” to be operated at State Level Diagnostic Laboratory, SVVU, Tirupati for the year 2016-17
3. RKVY project entitled “Development of novel vaccine against Leptospirosis” to be operated at State Level Diagnostic Laboratory, SVVU, Tirupati for the year 2017-18

ON-GOING PROJECTS

I. One Health Approach for Molecular Epidemiology and Risk Assessment of *Bacillus anthracis* in India – Penn State University

Financial Outlay : Rs. 207 Lakhs

Duration : 3 Years

II. DBT Network Programme on Anthrax diagnosis and Control in India

Financial Outlay : Rs. 77.05 Lakhs

Duration : 3 years

III. RKVY: Potential up gradation of State Level Diagnostic Laboratory (SLDL) for Advanced Disease Diagnosis, Forecast and Training

Financial Outlay : 400 Lakhs

Duration : 2 Years

Training Programs Conducted

S. No.	Title of the programme	Duration	Sponsoring Agency	No. of participants
1.	Training programme on “Laboratory Diagnosis of Animal Diseases”	30.10.2017 to 03.11.2017 & 19.12.2017 to 23.12.2017	SVVU, Tirupati	20 per batch
2.	Training programme on “Early and Accurate Diagnosis of Animal Diseases”	25.10.2018 to 27.10.2018 & 11.12.2018 to 13.12.2018	SVVU, Tirupati	20 per batch
3.	Hands on training programme on “Molecular Techniques in Disease Diagnosis” from 19-10-2022 to 22-10-2022 to the PG Students (18) of CVSc, Tirupati.	Four days	ICAR	20
4.	Recent molecular approaches in the diagnosis of Livestock and Poultry diseases to the faculty of State agricultural universities	21 days	ICAR	18
5.	Advanced Diagnostic and Therapeutic Approaches of Emerging, Re-emerging and Metabolic diseases of Livestock, Poultry and Companion animals to the faculty of State agricultural universities	21 days	RKVY	18

CONTENTS

Code	Title of Papers and Authors	Page No.
AWARD SESSION		
1	ISVIB Young scientist award	1
2	ISVIB mid-career scientist award	3
3	ISVIB Scientist award	5
4	GADVASU women scientist award	6
5	ISVIB Dr Linghard Memorial award	8
KEY NOTE ADDRESS		
1	Molecular diagnostics - Current research and Applications <i>Dr. Kumanan Kathaperumal</i>	9
Technical Session I EMERGING AND REEMERGING ZOOSES- ONE HEALTH		
Lead paper 1	Integrating animal rabies diagnosis and control into one health initiatives <i>Dr. Shrikrishna Isloor</i>	13
Lead paper 2	National Animal Disease Control Programme on Brucellosis: National Guidelines and review of progress <i>Dr. Rajeswari Shome</i>	17
Lead paper 3	Biosecurity with regard to viral diseases- threats and control strategies revisited <i>Dr. Arnab sen</i>	20
ORAL PRESENTATIONS		
OP_1.1	Development of hybridization chain reaction probes for the detection of pathogenic leptospires in canine <i>K. Amuda, T.M.A. Senthilkumar, M. Parthiban, B. Samuel Masilamoni Ronald and M. Vijaya Bharathi</i>	21
OP_1.2	Pilot study on anthrax outbreaks in different geographical regions of Karnataka <i>Shivaraj, B.M, Manohar Raju, V.M., Kavya, M. Bhavana, Keerthana, R., Sripad.K and Raveendra Hegde</i>	21
OP_1.3	Brucellosis outbreaks in sheep and its diagnosis <i>Shivaraj,B.M, Shivashankar,B.P., Shankar,B.P., Sripad, K. and Raveendra Hegde</i>	22
OP_1.4	Seroprevalence of leptospirosis in small ruminants in Tamil nadu <i>K.Manimaran, T.M.A.Senthil kumar, and A.Raman C.Sourdrarajan</i>	23
OP_1.5	Comparative evaluation of serum and whole blood samples in a 16s rRNA-based PCR assay for the diagnosis of brucellosis in goats <i>Mukartal S Y, Arun Kharate, Sreedhara J N, Malatesh D S and Shivanagouda Patil</i>	24

Code	Title of Papers and Authors	Page No.
OP_1.6	SARS-COV-2 full-length spike ectodomain elicits production of neutralizing monoclonal antibodies with affinity to conformational epitopes <i>Pratik M Kulkarni, Suresh Basagoudanavar, Shreya Gopinath, Harshita Patangia, P.K. Gupta, BP Sreenivasa, Senthil Kumar, Rahul Sharma, Sandeep Bhatia, Gaurav Kumar Sharma, V Bhanuprakash, G Saikumar, Pragya Yadav, Aniket Sanyal, and M Hosamani</i>	24
POSTER PRESENTATIONS		
PP_1.1	Hematobiochemical profile of dogs affected by leptospirosis: a comprehensive analysis <i>Mukartal SY, Arun Kharate, Sreedhara J N, Malatesh D S and Shivanagouda Patil</i>	25
PP_1.2	Prevalence, virulent gene profiling and antimicrobial resistance pattern of emerging zoonotic <i>Clostridium difficile</i> from foods of animal origin, environment and humans <i>Nagarjuna Reddy.N, Srinivasa Rao. T, Bindu Kiranmayi. CH, Sudha Rani. CH</i>	26
PP_1.3	Characterization and evaluation of biocontrol agents to combat <i>Listeria monocytogenes</i> in foods of animal origin <i>Chinta Siva Swetha, Porteen Kannan, Elango A., Ronald B.S.M. and Senthil Kumar T.M.</i>	27
PP_1.4	Sero prevalence of brucellosis in animals from Andhra Pradesh <i>Dr. D. Rani Prameela, Dr.M. Sravanthi, Dr.L.Lahari</i>	28
PP_1.5	Seroprevalence of leptospirosis and distribution of <i>leptospira</i> serogroup specific antibodies in buffaloes, in Telangana state, India <i>Prajakta P. Bokade, K. Vinod Kumar, Apsana R., Archana Pal, M. Swathi, V. Bharat, D. Hemadri, B. R. Gulati, V. Balamurugan</i>	29
PP_1.6	Isolation and characterization of <i>Bacillus anthracis</i> from endemic areas of Anthrax in Andhra Pradesh <i>Budiredla Gireesha, Devalam Rani Prameela and Bollini Sreedevi</i>	30
Technical Session II ADVANCED TECHNOLOGIES IN HEALTH AND REPRODUCTION		
Lead paper 1	Power of scaffolding the animal stem cells for regenerative therapies <i>Dr. G. Taru Sharma</i>	31
Lead paper 2	Bacteriophage therapy for mastitis: current status <i>Dr. Sashikanta Parida, Dr. Nagendra R Hegde</i>	33
Lead paper 3	Reproductive biotechnologies for genetic improvement of cattle and buffaloes <i>Dr. Mutha Rao M</i>	36

Code	Title of Papers and Authors	Page No.
ORAL PRESENTATIONS		
OP_2.1	Correlation of fowl adenoviral load and pathology in experimentally infected chicken <i>Chitradevi S, Sukumar K, Suresh P, Balasubramaniam GA, Kannan D</i>	42
OP_2.2	Digital infrared thermal imaging of ocular region (body temperature): a novel non-invasive technology to monitor calving process in murreh buffaloes (<i>bubalus bubalis</i>) <i>Teja Allu, Jeyakumar. S, Kumaresan. A, Vedamurthy GV, Mukund AK, Sivaram M, Das DN, Ananda Rao K, Varma CG, Narayanan K</i>	42
OP_2.3	Effect of growth hormone at different time intervals on development of in vitro preantral follicles in sheep <i>L.S.S.Varaprasad Reddy, B.R.Naik, A.V.N.Sivakumar, B.Punyakumari and J.Suresh</i>	43
OP_2.4	<i>In vitro</i> study of acetyl-coa carboxylase beta (ACACB) gene silencing in chicken myoblast cells <i>G. Sushma, Tarun Kumar Bhattacharya, P. Jaya Laxmi, S.T. Viroji Rao, R.M.V. Prasad, M. Kanakachari and Ch. Shiva Prasad</i>	44
OP_2.5	Development of CRISPR/Cas 12 based diagnostic test for rapid detection of African swine fever (ASF) virus <i>Senthilkumar D, K. Rajukumar, G. Venkatesh, Fateh Singh, Gopal Sarkar, Jashwant Patel, Rohit Sahu, Nourin Khan, Suman Mishra, Vijendra Pal Singh and Aniket Sanyal.</i>	44
OP_2.6	Does antral follicle count (AFC) and serum AMH levels have relation in parous in Ongole cattle (<i>Bos indicus</i>) – a preliminary study <i>M Praveen Kumar, N. R. Srikanth, K. Sunny Praveen, T.Sreemannarayana and M. Mutha Rao</i>	45
OP_2.7	Adverse drug interactions in Chlorpyrifos toxicity <i>Kalyani P, Padmaja K, Eswara Prasad P Srinivasa Rao G, Ramani Pushpa RN</i>	46
POSTER PRESENTATIONS		
PP_2.1	Molecular characterization, phylogenetic analysis and viral load quantification of fowlpox virus in chicken <i>Sanganagouda Koppad, Sabha Kounin, Nagaraja K, Basavaraj Sajjanar, H.K Muniyellappa, Shivaraj Murag, Pavithra B.H, Sanjeevakumar Lalasangi, Arun Kharate, Anjan Kumar K R and Raveendra Hegde</i>	47
PP_2.2	Association of doe's vaginal microbiota with reproductive hormones using 16s rRNA sequencing <i>Krishna M. S. R., Suthar V. S., Suthar B. N., Nanavati B., Gohil P., and Joshi C.G.</i>	47
PP_2.3	Isolation studies of lumpy skin disease virus in bovine from Andhra Pradesh <i>Dr. Ranjith Reddy R, Dr. Rani Prameela D, Dr. VijayaLakshmi S, Dr. N. Sailaja, Dr. L. Lahari and Dr. B. Rajendra Babu</i>	48

Code	Title of Papers and Authors	Page No.
Technical Session III MOLECULAR DIAGNOSTICS AND APPLICATIONS		
Lead paper 1	Molecular diagnostics-promises and possibilities <i>Dr. K. Vijayarani</i>	49
Lead paper 2	Molecular characterization of <i>Clostridoides difficile</i> isolates from animals with special reference to typing by PCR-ribotyping and PFGE <i>Dr.I. Hussain and Dr. P. Borah</i>	52
ORAL PRESENTATIONS		
OP_3.1	Molecular detection of <i>mycobacterium avium subspecies paratuberculosis</i> in sheep milk using IS900 polymerase chain reaction <i>Mukartal S Y, Arun Kharate, Sreedhara JN, Malatesh D S and Shivanagouda Patil</i>	55
OP_3.2	Comparative molecular characterization of non-cerebral and cerebral coenurosis in goats of Assam, India <i>Deepa Lahkar, B. C. Das; S. Islam; P. Borah; J. B. Dutta; G. Mahato; D. Kalita, A. Saleque and N. K. Deka</i>	55
OP_3.3	Study of histopathological changes and molecular detection of ILTV from respiratory infections of poultry in Andhra Pradesh <i>Nagendra Reddy T Sreedevi B, Vinod Kumar N, Srilatha CH, Madhava Rao T</i>	56
OP_3.4	Epidemiology and diagnosis of lumpy skin disease in Karnataka <i>Viveka Prabhu, B., B.M. Veeregowda, G. B. Manjunatha Reddy, D. Rathnamma, P. T.Ramesh, Leena, G And D. M. Basavarajaiah</i>	57
OP_3.5	Development of multiplex PCR and molecular characterization analysis of prevalent viral pathogens of RDC in Nagpur. <i>Shubhnagi Warke Mehak Tikoo & Prashant Sonkusale</i>	58
OP_3.6	Outbreak of novel parapox virus infection among buffalo calves in an organized farm <i>S. Jaisree, G. Kalaiselvi, N. Jayanthi, N. Babu Prasath, R. Ramya, P. Azhahianambi, G. Balakrishnan, and C. Soundararajan</i>	59
OP_3.7	Isolation and molecular characterization of lumpy skin disease virus from Tamil Nadu, India <i>Prabhu, M., Malmarugan, S., Rajagunalan, S., Balakrishnan, G., Lakshmi Prasanth, T and Ganapathi, P</i>	59
OP_3.8	A novel multiplex PCR assay for detection and differentiation of <i>Mycobacterium orygis</i> <i>K. Karthik, S. Saraswathi, M.V. Priyadharshini M, G. Dhinakar Raj</i>	60
OP_3.9	Molecular analysis of classical swine fever virus associated field infections in Tamil Nadu evidences novel CSFV subgenotype <i>S.Parthiban, B.Kowsalya, M.Parthiban, A.Ramesh, P.Raja, K.Gopal, S. Jaisree, and R.Thangathurai</i>	61

Code	Title of Papers and Authors	Page No.
OP_3.10	Molecular and pathological evidence of duck viral enteritis in manila duck (<i>Cairina moschata</i>); First report in Southern India R. Durairajan, S. Parthiban, J. Ramesh, R. Ramya, B. Jayanthi and K.Karthik	61
OP_3.11	Occurrence of classical swine fever in an organized farm of Madurai, Tamil Nadu. M.S.Murugan, V.Palanichamy and A.Ramesh	62
OP_3.12	Incidence of porcine circovirus-2D genotype in the indigenous pigs under extensive system of management in Tamil Nadu P. Ponnusamy, P.Balachandran, S.Saravanan, G.Senthil Kumar, A.Thangavelu, K.Sukumar and M.Selvaraju	62
OP_3.13	Molecular report of <i>Haemoproteus sp.</i> , in a rescued rat snake (<i>Ptyas mucosa</i>) M. Veeraselvam, A. Latchumikanthan, K. Karthika, R. Velusamy, M. Saravanan, P.K. Ramkumar and R. Jyothi Priya	63
OP_3.14	Molecular detection and confirmation of sheep associated malignant catarrhal fever (SA-MCF) in buffalo and cattle from northwestern zone of Tamil Nadu Ramya.K, K.Sukumar, Jayalakshmi.K. TakkoliNavadeeepa and Kalaiyarasu.S	64
OP_3.15	Molecular detection of <i>Mycoplasma mycoides</i> cluster organisms in respiratory infections of goats in andhra pradesh Raja Deepika, Vijayalakshmi.S, Nagendra Reddy. T, Siva swetha.C	64
POSTER PRESENTATIONS		
PP_3.1	Detection of haemoprotozoa and haemoreticidal organisms from naturally infected dogs in south india using multiplex pcr assay K. Jalajakshi, C. Sridevi, V.C. Rayulu, N. Nalini Kumari and Rani Prameela	65
PP_3.2	Prevalence of <i>pasteurella multocida</i> type a in organized turkey farms of Tamil Nadu G Kalaiselvi, G Balakrishnan, R Ramya, S Jaisree, R Saheethya and C Soundararajan	66
PP_3.3	Molecular detection and their biogram patterns of clinical isolates from bovine mastitis Nagendra Reddy T	66
PP_3.4	Detection and molecular characterization of beak and feather disease virus from rose ring necked parakeet (<i>psittacula krameri manillensis</i>) and cockatiel (<i>nymphicus hollandicus</i>) S. Jaisree, M Palanivelrajan, R Ramya, R Sridhar, G Balakrishnan, C Sreekumar and C Soundararajan	67
PP_3.5	Molecular detection and characterization of Lumpy skin disease virus from recent outbreaks in Andhra Pradesh, India (2023) Saikumar G, Ramani Pushpa RN, Supriya AR	68
PP_3.6	Molecular detection of <i>mycoplasma</i> species from respiratory infections of poultry Pavan Kumar K, Babu Naik K, Sanath K S, Sravani G, Srivani M and Deepthi B	68

Code	Title of Papers and Authors	Page No.
PP_3.7	Molecular detection of Streptococcus from mastitis milk samples <i>S Revathi Reddy, B. Pavan Kumar Reddy, T. Nagendra Reddy</i>	69
PP_3.8	Concurrent infection of <i>fowl adenovirus</i> and <i>reovirus</i> in commercial broilers <i>Pranay. M, K. Sukumar, K. Ramya and A. Balasubramaniam</i>	69
PP_3.9	PCR-based identification of mycoplasma from respiratory infection in turtles <i>Ingle S, Missal T, Nagargoje R, Majee S, Thorat V and Pharande R</i>	70
PP_3.10	Molecular detection of mycoplasma infections in Kadaknath poultry <i>B. Pavan Kumar Reddy, K. Venkata Prathap Reddy, S. Revathi Reddy, T.Nagendra Reddy</i>	70
PP_3.11	Molecular detection of <i>mycoplasma ovipneumoniae</i> infection and its associated bacterial pathogens in pneumonic sheep <i>Guru Srinath Reddy. M, Vijayalakshmi. S, Rani prameela. D, Sailaja.N</i>	71
PP_3.12	Molecular characterization of <i>Ornithobacterium rhinotracheale</i> in commercial poultry farms of Andhra Pradesh <i>Shobha Priya K, Nagendra Reddy T, Vinod Kumar N, Sujatha K</i>	72
PP_3.13	Detection of <i>mycoplasma bovirhinis</i> from clinical mastitis case <i>M. Madhava Reddy, S. Vijayalakshmi, K. Lakshmi Kavitha and A. Nasreen</i>	73
PP_3.14	Molecular characterization of the circulating Marek's disease virus isolates in South India <i>Kamani Dinesh Reddy, Rajalakshmi Shanmugham, Ramya Rajendra, Porteen Kannan, Raja Paramasivam and John Kirubaharan John</i>	73
Technical Session IV		
GENOMICS AND PROTEOMICS IN INFECTION AND IMMUNITY		
Lead paper 1	Diagnostic approaches of cancers in animals <i>Dr. Ch,Srilatha</i>	75
Lead paper 2	Postbiotics: Concepts to applications in veterinary medicine <i>Dr. K. Adilaxmamma</i>	80
ORAL PRESENTATIONS		
OP_4.1	Transcriptomic signatures of African Swine Fever naturally infected porcine kidney tissues <i>Rajib Deb, Gyanendra Singh Sengar, Joyshikh Sonowal, Seema Rani Pegu, Pranab Jyoti Das, Arutkumaran Selvaradjou, Nitin Attupurum, Swaraj Rajkhowa and Vivek Kumar Gupta</i>	86
OP_4.2	Adaptation and genetic analysis of porcine circovirus 2 (PCV2) in non-porcine origin cells <i>S.Parthiban, R.Rajwin, C.Sathish, M.Parthiban, P.Raja, A.Ramesh, G.Sathish, C.Yamini and G.Dhinakar Raj</i>	86

Code	Title of Papers and Authors	Page No.
OP_4.3	A study on prevalence of antimicrobial resistant (AMR) genes in predominant staphylococci and <i>escherichia coli</i> associated with bovine mastitis in Karnataka state. Saritha, N.S., Shrikrishna Isloor, D. Rathnamma, R. Sharada, K.L. Phaniraj, S.M. Byregowda, M.L. Satyanarayana, and Nagendra R. Hegde	87
OP_4.4	Isolation, whole genome sequencing and comparative genomics of <i>clostridium perfringens</i> isolated from different animal species in Tamil Nadu K. Karthik, S. Anbazhagan, M. Latha Mala Priyadarshini, R. K. Sharma, S. Manoharan	88
OP_4.5	Emergence of colistin resistance among the isolates of <i>Escherichia coli</i> Dr.T.V.Padmavathi, Dr.P.Ramya, Dr.C.S.Swetha and Dr.D.Raniprameela	88
OP_4.6	First report on <i>l-meq</i> gene detection and pathogenic analysis of virulent Marek's disease virus serotype 1 field outbreak of bv380 coloured layer hens in Pudukottai district of Tamil Nadu South India B.Puvarajan, G.Balakrishnan, S.Jaisree and S.Meenakshisundaram	89
OP_4.7	Epidemiological and Molecular studies of MRSA and ESBLs from livestock, animal handlers and environment in Bengaluru Rural district, Karnataka Shivasharanappa N, Kavya P, Sangeetha T R, Ranjitha G, Praveen Kumar A M and Rajeswari Shome	90
OP_4.8	Salmonellosis outbreak in a private <i>japanese quail</i> farm and it's mitigation strategy M. Latha Mala Priyadarshini, S. Manoharan, G.Kalaiselvi, K. Padmapriya and C. Soundararajan	91
OP_4.9	Comparative genomics of lumpy skin disease virus from different waves of outbreaks in India spanning from 2019-2023 Kalyani Putty, Madhuri Subbiah, Vishweshwar Kumar Ganji, Lakshman Pachineella, Kavitha Kandimalla, Harsha Manthani, Prashanthi Potta	92
OP_4.10	Serotyping and virulence pathotyping of <i>Escherichia coli</i> isolated from zoo herbivores. Jayalakshmi R, Lakshmi Kavitha K, Chaitanya RK and Amaravathi P	93
OP_4.11	Study of molecular variations in common respiratory bacterial infections of poultry in Rayalaseema region of Andhra Pradesh Swetha.P, Nagendra ReddyT, Vijaya Lakshmi.S and Anand kumar A	93
POSTER PRESENTATIONS		
PP_4.1	Molecular characterization and pulsed-field gel electrophoresis (PFGE) analysis of <i>listeria monocytogenes</i> isolates from sewage and vegetation Shubhangi Warke, Sumedha S. Boabde and Sujata Bhatkar	94
PP_4.2	Isolation and characterization of lumpy skin disease virus in embryonated chicken eggs: insights into pathogenesis via histopathological analysis Saikumar G, Ramani Pushpa RN, Supriya AR, CH. Sudha Rani Chowdary	95
PP_4.3	Studies on antibiogram pattern of bacterial isolates from bovine and bubaline semen of breeding bulls in Karnataka Nithyananda, V. C. Shivaraj Murag, Chandrashekhar, K.M., Shivashankar B.P, Isloor., S and Rathnamma D.	95

Code	Title of Papers and Authors	Page No.
PP_4.4	Molecular confirmation of pneumonic Pasteurellosis and <i>Tachygonetria</i> sp., in a star shelled tortoise <i>E. Tamileniyan, M. Jeevitha, M. Veeraselvam, A. Latchumikanthan, K. Karthika, M. Saravanan, P. K. Ramkumar and R. Velusamy</i>	96
Technical Session V NANOTECHNOLOGY & BIOINFORMATICS		
Lead paper 1	Immunoinformatic approaches in vaccine design <i>Prof. Amineni umamaheswari</i>	97
Lead paper 2	Nano-Ig Y technology: Applications <i>Dr. D. Sreenivasulu</i>	99
ORAL PRESENTATIONS		
OP_5.1	Polymeric meloxicam nanoparticles mitigate mono iodoacetate-induced osteoarthritis in Wistar rats by modulating NF-kb and <i>nrf2</i> pathways <i>Muralidhar Y, Prakash N, Sravanthi M and Prasad TNVKV</i>	105
POSTER PRESENTATIONS		
PP_5.1	Study of antimicrobial effect of silver nanoparticles against the clinical isolates of ovine pneumonia <i>Nagendra Reddy T and Dilip Reddy</i>	105
PP_5.2	Quercetin mediated zinc oxide nanoparticles: Synthesis, characterization and <i>in-vitro</i> evaluation of anticancer activity against Hela cervical and MDA-MB 231 breast cancer cell lines <i>Sravanthi M, Muralidhar Y and TNVKV Prasad</i>	106
Technical Session VI INNOVATIONS IN IMMUNODIAGNOSTICS AND THERAPEUTICS		
Lead paper 1	Translating innovations in diagnostics: challenges and opportunities <i>Dr. G. Dhinakar Raj</i>	107
Lead paper 2	Immunotherapy in oncology <i>Dr. Nageshwar Reddy</i>	111
ORAL PRESENTATIONS		
OP_6.1	Pregnancy induced modulation of humoral immune response to foot-and-mouth disease vaccination in the HF cow <i>Kishor PV. Sughra Banu, Tamil Selvan RP, Dechamma HJ, Priyanka Mahadappa, Hollabasappa Teggi, Narayanan Krishnaswamy, Mukesh Singh and Manjunatha Patel BH</i>	114
OP_6.2	Ornithobacteriosis in layer chickens of namakkal region/Tamil Nadu – a comprehensive report <i>Sukumar K and Ramya K</i>	114
OP_6.3	Development and evaluation of indirect-ELISA for detection of <i>pasteurella multocida</i> specific antibodies in bovines <i>Prajapati A, Manish Chandan S, Yogisharadhya R, Chanda MM, Hemadri D and Shivachandra SB</i>	115

Code	Title of Papers and Authors	Page No.
OP_6.4	Evaluation of the effect of TLR3 and TLR7 agonists in duck peripheral blood mononuclear cells <i>Sariga AS, Bindu S, Ramakrishnan Saravanan and Satyabrata Dandapat</i>	115
OP_6.5	Diagnostic evaluation of immunodominant LSDV095 recombinant core protein for detection of lumpy skin disease virus antibodies <i>Prabhu M, Malmarugan S, Rajagunalan S, Balakrishnan G, Lakshmi Prasanth T and Yogisharadhya R</i>	116
OP_6.6	Towards eradication of PPR: seroprevalence of ppr in sheep and goats in northern states of India before implementation of PPR-EP vaccination <i>Rakshit Ojha, Swathi M, K Vinod Kumar, Annett Helcita Dsouza, Archana Pal, Asha A, Prajakta Prashant Bokade, Suhail Ashraf, Harshitha, S. K, Ramachandra Deshpande, K. P. Suresh, G. Govindaraj, Bhopinder Singh, S. Rizwan Qureshi, Devendra Sharma, Vishal Dhawan, Chandra Sekar, D. Hemadri, B. R. Gulati and V. Balamurugan</i>	117
OP_6.7	Development of whole and recombinant antigen based indirect ELISAs for serological detection of Anatid alpha herpesvirus -1 infection in ducks <i>Priyanka S, Manoharan S, Aravindh Babu R. Parthiban, Ramya R and Barman, N. N</i>	118
POSTER PRESENTATIONS		
PP_6.1	Evaluation of immune response to live attenuated goat pox vaccine in goats <i>Omkar MR, Amitha Reena Gomes, Chandranaik, Shrikrishna Isloor, Sanganagouda Koppad, Shivshankar BP, Sudha G, Raveendra Hegde, Rathnamma D</i>	119
PP_6.2	Molecular and sero-surveillance of reticuloendotheliosis in layer chickens <i>K.Sukumar, Takkoli Navadeepa and Ramya.K</i>	119
PP_6.3	Development and immunological evaluation of cpg adjuvanted kysanur forest disease vaccine in mice <i>Bharath TL, Chandranaik BM, Amitha Reena Gomes, Rathnamma D, Byregowda SM, Shrikrishna Isloor, Jagadish Hiremath, Suguna Rao, Venkatesha MD, Apsana R, Raveendra Hegde</i>	120
PP_6.4	Rescue and characterization of foot-and-mouth disease virus (FMDV) mutants in BHK-21 cell <i>Padmaja SD, Subhadra E, Sindhutai BN, Manoj Kumar G, Priyanka M, Narayanan Krishnaswamy, Tamil Selvan RP, Umapathi V and Dechamma HJ</i>	120
PP_6.5	Growth kinetics of foot-and-mouth disease virus (FMDV) nonstructural proteins 1-protease and FMDV 3a mutant in the BHK-21 cell <i>Sindhutai BN, Padmaja SD, Manoj Kumar G, Lekshmi JD, Priyanka Mahadappa, Narayanan Krishnaswamy, Umapathi Vijayapillai and Dechamma HJ</i>	121

Code	Title of Papers and Authors	Page No.
PP_6.6	Studies on the estimation of haemagglutination inhibition titre values in broilers vaccinated with new castle lentogenic strains <i>Sravani G and Ganeshan P</i>	122
PP_6.7	Immunohistochemical evaluation of cytokeratin in various uterine pathologies of domestic felines <i>G. Swetha, A. Nasreen, A. Anand Kumar, K. Rajesh and S. Bharathi</i>	122
PP_6.8	Sero-monitoring of <i>Brucella abortus</i> S19 vaccine at different intervals of post-vaccination under NADCP brucellosis control program <i>G. Shanmugam, Skariah Somy, Mohandoss Nagalingam, K.P. Suresh and Shome Rajeswari*</i>	123
PP_6.9	Study on P1 ^{A1} /P1 ^{A2} glycoprotein IIIa polymorphism in myocardial infarction by Dual Anti-Platelet Therapy (DAPT) <i>N. Dhanush</i>	124
Technical Session VII CURRENT TRENDS IN VACCINOLOGY, VETERINARY PHARMACEUTICS AND DRUG DELIVERY		
Lead paper 1	Biosecurity from one health perspective: vaccines and crucial need for immune modulation <i>Dr. K. G. Tirumurugan</i>	125
Lead paper 2	Challenges in the bovine mastitis vaccine development: The way forward <i>Dr. Manoharan S, Karthik, K. and Latha Mala Priyadharshini, M.</i>	135
ORAL PRESENTATIONS		
OP_7.1	Evaluation of an autogenous Snuffles vaccine for rabbits under field condition <i>S. Manoharan, M. Latha Mala Priyadharshini K. Padmapriya, P. Balamurugan and C. Theophilus Anand Kumar and G. Dhinakar raj</i>	138
OP_7.2	Design of multi-epitope subunit vaccine against infectious laryngotracheitis virus using immune-informatics approach <i>P. Ponnusamy, K. Sukumar, A.Raja, S. Saravanan, P. Srinivasan, K. Ramya and A. Thangavelu</i>	139
OP_7.3	Exploring the proteomes of <i>Leptospira interrogans canicola</i> to identify potential vaccine candidates against canine leptospirosis <i>Balumahendiran Manoharan, Rani Prameela Devalam, Sreedevi Bollini, Vaikunta Rao V and Jagadeesh Babu A</i>	139
OP_7.4	Evaluation of immunogenic potential of <i>streptococcus suis</i> phage lysate as vaccine candidate <i>Lahari Laddika, Shumaila Malik and Salauddin Qureshi</i>	140
POSTER PRESENTATIONS		
PP_7.1	Development of recombinant fimbrial vaccine against ovine footrot <i>Vijayalakshmi.S, Raniprameela.D and Sreenivasulu.D</i>	141



AWARD SESSION





ISVIB YOUNG SCIENTIST AWARD

DEVELOPMENT AND EVALUATION OF SAFETY, EFFICACY ALONG WITH WHOLE GENOME ANALYSIS OF AN INDIGENOUS CELL CULTURE-BASED VACCINE FOR CONTROL OF DUCK PLAGUE IN INDIA

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Duck plague caused by Anatid herpesvirus-1, is the most significant contagious viral disease of ducks reported worldwide, causing huge economic losses. In India, the DP vaccine is produced by using a foreign strain, through a cumbersome process of egg inoculation, which is not convenient for industrial-scale production. To address these limitations, the present study was planned to develop a cell culture-based DP vaccine using an Indian field isolate, including an evaluation of its safety, efficacy and whole genome analysis. A virulent duck enteritis virus (DEV/India/IVRI-2016), was isolated and characterized with its whole genome sequencing (MZ824102). The isolate was used to develop a live attenuated DP vaccine (DPvac/IVRI-19) by serial propagation in primary chicken embryo fibroblast cell culture. The vaccine has been released by ICAR and this technology has a patent application no. 202211014041. However, to confront certain limitations in the primary cell culture system, this vaccine candidate was further adapted to the DF1 cell line. The cell line adapted vaccine candidate (DPvac-DF1/IVRI-23) has passed the safety test and afforded 100% protection against the virulent challenge infection. It induced a significantly higher blastogenic response of lymphocytes and neutralizing antibody titer ($2^{4.3}$). Significant induction of antiviral cytokines (IFN- α and IFN- β), IFN- γ and IL-4 expression provided insights into induction of humoral and cell-mediated immunity. Further, post-vaccination and post-challenge gross pathological study, histopathology, viral shedding, and viral load estimation substantiate the vaccine's safety and efficiency. Comparative whole genome analysis of the DPvac/IVRI-19 (MZ911871), DPvac-DF1/IVRI-23 (OR571472), with the field isolate (DEV/India/IVRI-2016) (MZ824102) revealed nucleotide mutations in various genes, which might have resulted in attenuation of the virus. This indigenous DF1 cell line-adapted DP vaccine is highly potent and suitable for industrial-scale production with a precisely pure form of the vaccine and uniform titer from batch to batch, thus can replace the existing vaccine being prepared from a foreign strain.

INTERFERON-STIMULATED GENE 15 (ISG15) EDITED BHK-21 CELLS ENHANCE THE REPLICATION OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) SEROTYPE O

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India aims to eradicate FMD by 2030 through regular biannual vaccination of cattle, sheep, goat and pigs using inactivated FMD vaccine. However, the vaccine requirement exceeds the current production capacity. We hypothesized that editing the antiviral genes in BHK-21 cells would favour



the replication of FMD virus and would increase the titer as well as the yield of inactivated antigen. To test the hypothesis, GeneArt CRISPR nuclease vector was used for cloning sgRNA for ISG15 gene editing in BHK-21 cells. ISG15 is among the earliest and most abundant proteins induced upon interferon signaling, indirectly inhibiting the viral lifecycle by altering the host immune response. The ISG15 edited BHK-21 cells were evaluated for replication of FMDV serotype O by absolute quantification of viral transcripts by real-time PCR, plaque assay, and end-point dilution assay. The results revealed that ISG15-edited BHK-21 cells produced a higher copy number of 1.83×10^{10} FMDV transcripts than the parent BHK-21 cells at 16 hours post-infection. Similarly, the number of PFU/mL was higher in the ISG15 edited cells and plaque morphology was comparable with that of parent BHK-21 cells. Further, the virus titer ($\log_{10}TCID_{50}/mL$) was higher by 0.5 in ISG15-edited BHK-21 cells. It is concluded that ISG15-edited BHK-21 cells favour the growth and replication of FMDV serotype O. This study provides new insight into the interaction between FMDV and ISG15 and enriches understanding of the biological function of ISG15 in virus replication.

EVALUATION OF IMMUNOGENIC POTENTIAL OF *STREPTOCOCCUS SUIS* PHAGE LYSATE AS VACCINE CANDIDATE

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Streptococcus suis serotype 2 infection is considered as a major pathogenic disease worldwide both in humans and swine. In the present study, *S. suis* vaccine development was aimed with main criteria is to induce heterologous protection by both humoral and cell mediated immune response and to reduce the mortality of piglets by reducing the bacterial load and prevent the carrier animals in herd by immunotherapeutic approach of using phage lysate vaccine preparations. In the present study, the objectives were to isolate and characterize bacteriophage against *Streptococcus suis* serotype 2, to standardise preparation of phage lysate bacterin against *Streptococcus suis* serotype 2 and to evaluate the immunogenic potential of lysate against the direct challenge with *Streptococcus suis* serotype 2. Four different lytic phages SS1, SS2, SS3 and SS4 were isolated belong to the family myoviridae under order caudovirales. Phages SS1, SS2 and SS3 were having broad host range lytic activity to other serotypes of *S. suis*, to other streptococcal species and MRSA. Upon chemical characterisation, increased phage titre was observed on incubation with 10% Phenol and 1% SDS. 0.1 MOI of phage bacteria ratio shown to have high phage titre and is selected for Phage lysate vaccine preparation. Upon Lymphocyte transformation test, heightened Cell mediated immune response was observed for adjuvanted phage lysate vaccine group on 28th day post immunisation.



ISVIB MID CAREER SCIENTIST AWARD

DESIGNING AND DEVELOPMENT OF NOVEL MULTI EPITOPE BASED VACCINE CONSTRUCT AGAINST CANINE LEPTOSPIROSIS IN IMMUNOINFORMATICS

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Leptospirosis causes a huge impact on the health of companion and farm animals causing serious economic losses. *Leptospira* infected pet and stray dogs and poses major threat to public health. Currently, no effective vaccine is available against pathogenic leptospira and requires an effective stimulation of both the humoral and cell-mediated immunity of the host to defend leptospirosis. Immunoinformatics is now revolutionary in the field of vaccine development utilizing genome and proteome-based information in prediction of potential vaccine candidates. Here we used immunoinformatics tools, molecular docking and simulation methods to predict and screen the antigenic epitopes of *Leptospira interrogans canicola*, to design a multi-epitope vaccine containing B- and T-cell epitopes against canine leptospirosis. Proteome-wide comparison of whole genome data base of 20 *Leptospira* serovars with *L.canicola* yielded 18 protein candidates on high throughput screening. Of these, only 10 proteins showed epitopes having a threshold values based on certain crucial parameters. Finally four proteins with five potential MHC Class II epitopes having overlapping B cell and MHC Class I binding residues were used for constructing the chimeric multi epitope vaccine using HEYGAEALERAG, GPGPG and KK linkers. Canine beta defensin 103 and Pan HLA DR-binding epitope (PADRE) sequences were added as adjuvant. A soluble and non-allergic protein with a molecular weight of 40.436 kDa was obtained on *insilico* analysis. Physico-chemical properties, secondary and tertiary structures, stability, intrinsic protein disorder, solubility, and allergenicity of this vaccine construct were assessed by applying immunoinformatics. Model validation tools PROCHECK, ProSA and Ramachandran plot validated the stability and *insilico* immune simulation studies revealed the ability of the epitopes to induce B-cell and T-cell mediated immune response. Therefore, immunoinformatics analysis showed that the multi epitope vaccine construct can properly stimulate the both T and B cells immune responses and could potentially be used for prophylactic or therapeutic usages.



COMPREHENSIVE STUDY ON RECENT OUTBREAKS OF THE LUMPY SKIN DISEASE VIRUS IN CATTLE: AN EPIDEMIOLOGICAL AND GENETIC ANALYSIS

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Lumpy skin disease is an emerging, reemerging and transboundary disease caused by the lumpy skin disease virus (LSDV) belonging to the Capripoxvirus genus and Poxviridae family in cattle. The recent episodes of disease outbreaks in the last 4 years from 2019 to 2023 caused huge socio-economic loss. In the present study, clinical samples from LSD outbreaks were collected from different districts of Karnataka, India for virus isolation and molecular characterization. Preliminary confirmation of LSD virus was performed by targeting envelope protein P32 (192 bp) gene by conventional Polymerase chain reaction (PCR). Real-Time qPCR was developed for absolute quantification of viral copy numbers in the infected samples. The viral copy number Log₁₀ (Mean ± SD) in the majority of the representative samples ranged from 4.962 to 12.432. The partial genes of GPCR and P32 were sequenced and submitted to GenBank. Phylogenetic analysis was performed by comparing sequences with other LSDV, Sheep pox virus (SPPV) and Goat pox virus (GTPV). The results indicated that our isolates have clustered together with 100% similarity to the LSDV sequences from Nepal, Bangladesh, Botswana, Egypt, Kenya Turkey Saudi Arabia and India fall within the same clade. While Vietnam, China and Thailand have clustered together with different clades. Our results highlight the necessity of ongoing surveillance and molecular characterization of circulating LSDV strains required for perspective development of a suitable LSD vaccine to control the disease.



ISVIB SCIENTIST AWARD

EMERGENCE OF NOVEL PORCINE CIRCOVIRUS 2 GENOTYPES IN SOUTHERN INDIA

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A total of 200 samples from Porcine circovirus 2 suspected (n = 112) and healthy (n = 88) swine populations collected from different districts of Tamil Nadu, south India were used in this study. The samples comprising of serum (n = 124), swabs from natural orifices (n = 52), and postmortem tissues (n = 24). All the samples were processed and subjected to the screening and detection of the PCV2 genome by a specific PCR assay. PCV2 genomes from positive samples were further subjected to genotyping with specifically designed primers for the full-length amplification of the ORF2 gene which codes for capsid protein (Cp) and serves as an epidemiological marker. Randomly, 13 amplified ORF2 genes were sequenced and the aligned sequences were subjected to signature motif analysis and phylogeny in MEGA X. The molecular prevalence of PCV2 infection in Tamil Nadu is 10.5% (n = 21). Signature motif and phylogenetic studies of 13 samples revealed 38.5% (n = 5) presence of each PCV2b intermediate 1(IM1) and PCV2b genotypes, followed by 15.4% (n = 2) PCV2d-2 and 7.7% (n = 1) PCV2d genotypes. The PCV2b-IM1 genotype has a 99.43% sequence homology with Vietnam isolate (JX506730). PCV2b genotypes showed 99.72% sequence identity with Chinese isolate (KX068219). PCV2d-2 genotypes reported in this study have 100% sequence identity with Taiwan isolate (MF169721). PCV2d genotype showed 97.87% sequence identity with Thailand isolate (MF314293). Amino acid analysis of all the 13 full-length ORF2 gene sequences revealed specific mutations in the immune reactive domains of A, B, C, and D. Capsid protein of three PCV2b and five PCV2b IM1 isolates had extra amino acid residue lysine (K) at 234 position of ORF2 similar to PCV2d. For the first time in South India, PCV2b IM1 and PCV2d-2 genotypes are reported. This study evidences the genetic shifts of PCV2 isolates in India and it is analogous to that of global genotypic shift.

Citation: S P, A R, Karuppanan AK, G DR, J JR, S H, S J, K S, D B, M P, Ghadevaru S. Emergence of novel Porcine circovirus 2 genotypes in Southern India. *Transbound Emerg Dis.* 2022 Jul;69(4):1804-1812. doi: 10.1111/tbed.14158. Epub 2021 Jun 4. PMID: 34008351.



GADVASU WOMEN SCIENTIST AWARD

BOVINE VIPERIN RESTRICTS THE *IN VITRO* REPLICATION OF FOOT AND MOUTH DISEASE VIRUS

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In the early stage of virus infection, the pattern recognition receptor (PRR) signaling pathway of the host cell is activated to induce interferon production, activating interferon-stimulated genes (ISGs) that encode antiviral proteins that exert antiviral effects. Viperin is one of the innate antiviral proteins that exert broad-spectrum antiviral effects by various mechanisms. FMDV is a single stranded, positive sense RNA virus belonging to *Aphovirus* genus of *picornaviridae* family. In the present study, the expression patterns of PRRs (TLR-3,7, RIG-I and MDA-5), Interferons (IFN α , β , γ and λ_3) and Interferon stimulated genes (viperin, OAS1, ISG-15, Mx1, PKR, Ch25H and ZAP) were analyzed by Real time PCR based quantification of mRNA levels in MDBK cells following experimental infection with FMD Asia 1 virus strain. The results indicated that viperin, OAS1 and ISG-15 are the principal ISGs expressed during FMDV infection. Further, we investigated the *in vitro* antiviral activity of viperin in FMD Asia I virus infection. Following transient overexpression of the viperin in MDBK and BHK-21 cells, there was a consistent 1-log difference in both the viral titres and copy numbers between viperin overexpressed cells and normal cells. The viral structural proteins in the western blot experiments yielded identical outcomes. The antiviral activity of the viperin was further confirmed by knock down of viperin expression in the cell lines by siRNA. Our results highlight a potential mechanism whereby viperin is able to inhibit FMDV replication and play an important role in antiviral innate immunity.

DEVELOPMENT AND IN-VITRO EVALUATION OF NANO-ANTIBIOTICS TO COMBAT ANTIMICROBIAL RESISTANCE IN BOVINE MASTITIS PATHOGENS

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Antimicrobials are still the basis for treatment of infections. But AMR/ABR is a rapidly emerging threat that seeks multifaceted attention. Bovine mastitis is a global problem with no easy solution and relies heavily on antibiotic treatment. In this study, a total of 1539 milk samples was collected from six districts of the North Eastern regions of Tamil Nadu and were screened by TANUCHECK SCC Kit (TRPVB-TANUVAS) to segregate as subclinical (643) and clinical mastitis (443) and positive samples were (1086). The positive samples were subjected to isolation and 698 isolates were obtained (438 Nos. –Staphylococcus spp.; 260 Nos. *Escherichia coli*).

Among Staphylococcus spp. 252 Nos. from SCM and 186 Nos. from CM was got and 61 was *coa* + ve (CoPS) and 191 was *coa*– ve (CoNS) in SCM while 29 was *coa* +ve and 157 was *coa* –ve in



CM. Cultural, biochemical and molecular characterization of all bacterial isolates was done. Further phenotypic (15-20 antibiotic used) and genotypic (ABR genes- *tetA*; *tetB*; *tetK*; *tetO*, *sul-I*, *msrA*, *tetM*; *tetO*; *sul-II* and *aad*) characterization was done for all the isolates by AST and PCR methods respectively. Among the 74 resistant *Staphylococcus* spp., 26 isolates were multidrug resistant for more than 2 or 3 *abr* genes and 48 isolates carried single *abr* gene.

Through this study, five different antibiotics viz. Ampicillin, Cefotaxime, Erythromycin, Gentamicin and Tetracycline were selected and used to prepare nano-antibiotics by coupling with Zinc and Chitosan nanoparticles (NPs) by chemical synthesis method and both the plain as well as antibiotic coupled NPs were characterized for its particle size, zeta potential and by FTIR, XRD pattern and SEM imaging. Cell viability and toxicity tests was done for both NPs by the MTT assay at a concentration of 5mg/ml which was non-toxic by 100% to BHK-21.

The characterized nano-antibiotics were assessed for its in vitro efficacy by AST method with required controls like respective plain and coupled NPs, positive (sensitive) and negative (resistant) antibiotics and solvents used.

The results for resistant *Staphylococcus* spp isolates showed that the percent of efficacy after conjugation with ZnO NP was highest for the resistant drugs Tetracycline (66.66%), followed by Erythromycin (11.76%) and Gentamicin (4.54%), Ampicillin and Cefotaxime after conjugation with ZnO NPs had lesser efficiency of 8.7% and 8% respectively.

The AST results for resistant *E.coli* isolates, using (ZnO+GEN) NPs conjugated with Gentamicin showed 50% and ZnO NP conjugated Erythromycin (ZnO+E) had 20% efficacy respectively.

The conjugated Ampicillin (ZnO +AMP) had 15% and conjugated Cefotaxime (ZnO +CTX) had 13.05% lesser efficacy than the sensitive drug.

For the drug conjugated Chitosan NPs against two resistant *Staphylococcus* spp isolates, the most efficacious drug was Tetracycline (92.30%); followed by Erythromycin (46.15%), Gentamicin (40%), Ampicillin (66.66%) and Cefotaxime (40%). The efficacy of drug conjugated Chitosan NPs was highest for ampicillin (CHT+AMP)-100% followed by Chitosan + Erythromycin (CHT+E) (75%), Tetracycline (38.88%) and Gentamicin having (10.52%) against the resistant *E. coli* isolates.

Irrespective of the resistant isolate used, the efficacy of antibiotics after conjugation with Chitosan was highest for Tetracycline (92.30%); followed by Ampicillin (66.66%); Erythromycin (46.15%) and Cefotaxime (40%).

The ZnO NPs conjugated antibiotics had lower efficacy towards resistant bacterial isolates.

In comparison to both the nano particles, antibiotic conjugated Chitosan NPs exhibited synergistic and higher anti-bacterial effect against the resistant isolates of both *Staphylococcus* spp and *E. coli*. Being bio-compatible, Chitosan based nano-antibiotics can be considered as cutting-edge solution to clinical infections.

The study concludes that the nano-antibiotics could be an alternative source of therapy to combat antimicrobial resistance which is the burning problem across the globe.



ISVIB DR LINGHARD MEMORIAL AWARD

PRODUCT NAME: INACTIVATED OIL ADJUVANT VACCINE AGAINST RIEMERELLOSIS

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Riemerellosis, well-known earlier as "new duck disease", is a contagious septicemic bacterial disease caused by *Riemerella anatipestifer*, primarily infect ducklings' upto 8 weeks of age. It is a Gram-negative, non-sporulating, non-motile bacillus belonging to the family *lavobacteriaceae*. Since the first report of an outbreak in 2008, it has been well documented as a cause of considerable economic loss to the duck industry of the state. On characterisation and serotyping of the isolates obtained from different regions of the state, all were grouped under a single serotype, though they revealed molecular level variation. The prevalent serotype obtained from Kuttanad area has been named as RA 1 with whole genome sequencing accession number (PPEC0000000) and MTCC patent deposit number (MTCC 25439). As there is little or no cross-protection among different serotypes, an ideal vaccine incorporating prevalent serotype at that locality is the only solution to provide broad-spectrum protection. Hence, an extensive research work on development of inactivated oil adjuvant vaccine against riemerellosis was executed. A vaccine emulsion was prepared comprising of an aqueous phase of bacterin of *R. anatipestifer* with a concentration of 3×10^9 CFU/ml to 15×10^9 CFU/ml and an oil phase of liquid paraffin and lanolin at a ratio of 5:0.5 to 10: 1.5 as adjuvant. The prepared vaccine was found to be sterile, safe and no untoward reaction was noticed among vaccinated ducklings. It was administered to ducklings at 2 weeks and booster at 5 weeks of age. Humeral and cell mediated immune responses were assessed by in-house developed indirect ELISA and lymphocyte proliferation assay (LPA), respectively. Statistical analysis revealed that the vaccinated group differed significantly from control group on days 7 to 120 and rOmpA based ELISA was highly sensitive and better choice than crude Omp based ELISA. Maximum proliferation of lymphocytes was achieved on 14th and 28th days post-vaccination by LPA among the vaccinated birds. Challenge studies recorded 90 to 95% protection. It was concluded that the prepared vaccine was potent and effective in controlling the disease based on challenge studies and estimation of immune responses. Hence, it is desirable to advocate the use of inactivated vaccine in field condition. Patent was granted for the product with number 447336 on 25-08-2023.



KEY NOTE ADDRESS





MOLECULAR DIAGNOSTICS - CURRENT RESEARCH AND APPLICATIONS

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The success of any animal husbandry practice depends on good managerial and effective disease control programs. Control of livestock and poultry diseases has become very challenging due to intensification of farming activities and the emergence of new diseases. With the ‘One Health’ approach getting more attention, zoonotic diseases, re-emerging diseases and transboundary diseases also need to be kept in mind while evolving diagnostic procedures. Precise and rapid diagnosis is very important for the effective implementation of control and prevention programmes of any disease. Though conventional methods like clinical signs, necropsy lesions and serological techniques are the early tools of diagnosis, limitations in the form of differential diagnosis and time involved do exist. Another very important issue will be differentiating the infected animals from the vaccinated (DIVA) ones.

In recent times, several molecular diagnostic techniques have come in handy to address the concerns associated with the conventional methods of livestock disease diagnosis and some approaches have even been helpful in the DIVA strategy.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction is one of the most commonly and widely used molecular diagnostic method for pathogen detection and characterization. Since its invention by Kary Mullis in 1985, PCR has undergone several modifications to suit scientific needs. Reverse-Transcription PCR, Real time quantitative PCR, Multiplex PCR, Nested PCR, ARMS PCR and *in-situ* PCR are the few to mention. PCR and its modifications are useful in pathogen detection and quantification, genus/strain differentiation, mutation detection, gene cloning, cancer detection, etc, PCR is a rapid, sensitive and cost-effective technique.

Gene sequencing and phylogenetic analysis

Establishing the sequence of DNA is key to understanding the composition of a genome and function of genes. Currently, several different methods are available for DNA sequencing, which are grouped as First, Second, Third and Fourth generation sequencing. Sanger sequencing is classified as first-generation sequencing and this method involved sequencing by synthesis of radioactively labelled DNA strand complementary to the template strand by dideoxy chain termination technique. Resulting fragments are then assessed by Polyacrylamide gel electrophoresis. Second generation sequencing is also known as Next generation sequencing (NGS) or High throughput or Parallel sequencing. This approach works without separating the sequencing reactions into lanes, capillaries, or tubes. It allows



billions of sequencing processes to be happened simultaneously in parallel on a slid surface, either glass or beads. Third generation sequencing is also known as Single Molecule Sequencing and employs a single template approach. The fourth-generation sequencing integrates nanopore technology into Single Molecule Sequencing. This technology performs real-time sequencing without amplification and repeated cycles by eliminating synthesis. This method is also known as *in situ* sequencing.

Phylogenetic analysis is one of the best tools for understanding the origin, evolution and relatedness of microbes. It can also provide insights in to the spread of pathogenic microbes. Phylogenetic analysis and generation of a phylogenetic tree can be done with DNA sequence for genes and amino acid sequence for proteins. A phylogenetic tree can be rooted or un rooted, implying directions corresponding to evolutionary time.

Genotyping

Genotyping methods relies on genetic markers. These include Restriction Fragment Length Polymorphisms (RFLP), Tandem repeats (TRs), and Single Nucleotide Polymorphisms (SNPs) which tend to cluster isolates by phenotypically identified species. RFLP patterns are the result of restriction-digested DNA from a pure sample or an amplified gene. The restriction fragments are electrophoresed to generate a ladder of fragment sizes from large (top) to small (bottom) on a gel or membrane. The resulting pattern is unique for each restriction enzyme. Analysis is best accomplished using computer software specific for the RFLP genetic analysis. This method generally can genotype isolates and differentiate between closely related species.

Tandem repeat is defined as a series of end-to-end duplications of a core DNA sequence, ranging from 2 to 60 or even more nucleotides. Tandem repeats are also known as simple sequence repeat (SSR), short tandem repeat (STR), simple sequence length polymorphism (SSLP), and variable-number tandem repeat (VNTR)). These are widely distributed throughout the genomes of most eukaryotic and prokaryotic organisms. The number of repeats in a particular locus can vary between species and strains, allowing their typing by conventional PCR or Multiple Locus Variable Number assays. SNPs are the most common type of genetic variations in the genomes of all living organisms and used for strain differentiation.

Multilocus sequence typing (MLST)

This technique targets fragments of multiple conserved genes (housekeeping genes) which contain different polymorphic sites. Based on the sequence data for these targets microbes can be assigned to designated sequence types. This typing is characterized by concatenation of the different target fragment sequences into a single sequence for further phylogenetic analysis.

Multiple locus VNTR analysis (MLVA)

MLVA is commonly used for genotyping of bacteria. This technique assesses the number of repeats within multiple VNTR loci. MLVA is similar to the standard PCR with endpoint product detection by gel or capillary electrophoresis. Various fluorescently labelled primers are used to provide



amplicon sizing using capillary electrophoresis. Currently, MLVA genotypes can also be assessed by *in silico* analysis of whole-genome sequences.

DNA Barcoding

DNA barcoding is a powerful taxonomic tool to identify and discover species. DNA barcoding utilizes one or more standardized short DNA regions for taxon identification. With the emergence of new sequencing techniques, such as Next-generation sequencing (NGS), ONT MinION nanopore sequencing, and Pac Bio sequencing, DNA barcoding has become more accurate, fast, and reliable. Rapid species identification by DNA barcodes has been used in a variety of fields, including forensic science, and disease understanding.

Metabolomics

Metabolomics is the study of metabolites and a promising tool in cancer research. There are two approaches to study the metabolism, targeted and untargeted. The general approach is that metabolomic data are interpreted by bioinformatics tools correlating with metabolomic databases to obtain significant findings. With the use of specific analysis tools, such as nuclear magnetic resonance and mass spectrometer combined with chromatography, metabolic profile or metabolic fingerprint of various biological specimens could be obtained. Metabolomics data are used to identify potential cancer biomarkers.

The developments in the area of molecular diagnostics are dynamic and newer techniques are being evolved every other day. Biosensor, CRISPR and Aptamer based diagnostics are going to play a major role in the future.



TECHNICAL SESSION I

**EMERGING AND REEMERGING
ZOOZOSES- ONE HEALTH**





LEAD PAPER-1

INTEGRATING ANIMAL RABIES DIAGNOSIS AND CONTROL INTO ONE HEALTH INITIATIVES

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Rabies is a deadly zoonotic disease that affects mammals, including humans and there by poses a significant public health threat globally, nationally and locally. It causes human deaths with over 99% of cases resulting from the bite of an infected dog. Although dogs are the major vectors, nevertheless, other species of animals including cats and wild carnivores could also play role in transmission. It is primarily transmitted through the bite of an infected animal reservoir to humans and other animals including livestock. As part of the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, addressing animal rabies with special reference to dogs becomes a crucial component in preventing human cases. Thus, One health approach has emerged as a pivotal framework for addressing complex health challenges. This encompasses surveillance, vaccination programs, and collaborative efforts to mitigate the risk of transmission from animals to humans and other animals. Recognizing the intricate links between human, animal, and environmental health, the World Health Organization (WHO) and World Organization for Animal Health (WOAH), so also Government of India have embraced the One Health approach to combat rabies. This write up not only explores the various facets of animal rabies-related activities with reference to it's diagnosis, surveillance and control within the context of the One Health framework but also India's specific initiatives, guidelines, and programs related to animal rabies control within the broader One Health framework.

One Health Approach to Animal Rabies Control

One Health is an interdisciplinary approach that recognizes the interdependence of human, animal, and environmental health. In the context of animal rabies, this approach involves collaborative efforts between human health professionals, veterinarians, ecologists, environmental scientists, and other stakeholders. One Health initiatives focus on integrated strategies to control and prevent rabies at the human-animal-environment interface. Furthermore, one Health strategies for animal rabies control extend beyond conventional veterinary measures. Surveillance and monitoring involve not only tracking the incidence of rabies in animals but also assessing environmental factors that contribute to its spread. Advanced data analytics enable the identification of high-risk areas, facilitating targeted interventions such as mass vaccination campaigns and public awareness initiatives.



Surveillance and Monitoring

One Health emphasizes the importance of surveillance and monitoring systems to track the prevalence of rabies in animal populations. By collecting and analysing data from both human and animal health sectors, authorities can identify hotspots and implement targeted interventions. The Integrated Disease Surveillance Programme (IDSP) plays a crucial role in monitoring rabies cases. By integrating human and animal health data, the IDSP contributes to the early detection of potential outbreaks and facilitates a coordinated response.

Vaccination Programs

Vaccination programs form the cornerstone of One Health interventions. These programs, often executed in collaboration with veterinary and public health agencies, aim to achieve herd immunity in domestic animal populations, breaking the cycle of rabies transmission. One Health advocates for comprehensive rabies vaccination programs in domestic animals, particularly dogs, which are major vectors for transmitting the virus to humans. These programs not only protect animal populations but also serve as a barrier to the transmission of rabies to humans. Advances in vaccine development and novel delivery methods, such as baited vaccines for wildlife as well as free roaming dogs, contribute to the effectiveness of these programs. The National Rabies Control Program (NRCP) and National Action Plan for Elimination of dog mediated Human Rabies (NAPRE) guidelines launched by the Government of India, focus on eliminating human rabies by 2030. It emphasizes mass dog vaccination (MDV), responsible pet ownership, and awareness campaigns. The program encourages collaboration between the Ministry of Health and Family Welfare and the Ministry of Animal Husbandry, Dairying and Fisheries.

Community Engagement

Engaging local communities is vital for the success of rabies control programs. Educating communities about responsible pet ownership, the importance of vaccination, and how to respond to potential rabies exposures empowers individuals to actively participate in disease prevention. Cultural beliefs, socioeconomic factors, and accessibility issues influence community participation in vaccination campaigns and reporting of potential rabies cases. Tailoring awareness programs to address specific community needs and concerns is essential. Recognizing the importance of community engagement, the government implements various awareness programs with special reference to ‘Dos’ and ‘Donts’. Such initiatives aim to empower communities to actively participate in rabies prevention.

Research and Innovation

Investing in research and innovative technologies can enhance the efficiency of rabies control. This includes the development of cost-effective vaccines, improved diagnostic tools, and novel strategies for mass vaccination campaigns. Research and innovation are pivotal for overcoming existing challenges. Ongoing efforts to develop more cost-effective vaccines, improve diagnostic tools, and explore innovative strategies for mass vaccination campaigns contribute to the sustainability and



scalability of rabies control initiatives. Integrating technology, such as mobile applications for Dog population enumeration, rabies reporting and tracking rabies cases, enhances the efficiency of surveillance systems. In this context, The Indian Council of Agricultural Research (ICAR), Indian Council of Medical Research (ICMR), Department of Biotechnology (DBT), KVAFSU-CVA Rabies Diagnostic Laboratory, WOAHP Reference Laboratory for Rabies in India, Veterinary College, KVAFSU, Bangalore and other research institutions / organizations contribute to the development of cost-effective vaccines and diagnostic tools through the expertise available in Immunology, Biotechnology and Virology in these organizations. Ongoing research projects explore innovative strategies for effective rabies control, considering the diverse socio-cultural and ecological landscape of the country.

Collaboration, Communication, One Health Governance and Global co-operation

Effective communication and collaboration between human and animal health professionals are essential for a successful One Health approach. Sharing information on cases of rabies, vaccination coverage, and control measures ensure a coordinated response to the disease. Joint efforts ensure a more nuanced understanding of the disease landscape, leading to better-informed decisions on preventive measures. Cross-disciplinary training programs that foster communication skills among professionals from diverse backgrounds further enhance collaboration.

Strengthening the governance structure for One Health initiatives is crucial. Coordination between the Ministry of Health and Family Welfare, Ministry of Animal Husbandry, Dairying and Fisheries, Ministry of environment, Forest departments, Civic bodies, Vaccine manufacturers, Veterinary Universities, Diagnostic Laboratories, other relevant bodies and importantly the community needs to be streamlined for a more effective, integrated response to rabies.

Global cooperation is indispensable in the fight against rabies as rabies knows no borders, and is essential to address the disease comprehensively. Sharing knowledge, experiences and resources on a global scale can contribute to the development of standardized approaches for rabies surveillance and control that align with the principles of One Health. Collaborative research projects, sharing best practices, and coordinated responses to cross-border rabies threats strengthen the One Health approach on a global scale. International organizations play a crucial role in facilitating knowledge exchange and resource mobilization. Furthermore, as rabies is a trans boundary disease, collaboration with neighbouring countries and international organizations is vital. The World Health Organization (WHO), the World Organization for Animal Health (WOAH, founded as OIE) and Food and Agriculture Organizations (FAO) play key roles in facilitating global cooperation and knowledge exchange.

Challenges, Resource Constraints and Future Directions

Despite notable progress in animal rabies control through One Health initiatives, several challenges persist in the integration of animal rabies control into One Health initiatives. Limited resources in many regions hinder the implementation of robust surveillance and vaccination programs,



inadequate infrastructure especially in remote / rural areas, poses barriers to effective data collection, vaccine distribution and comprehensive implementation of rabies control programs. Furthermore, gaps in cross-sectoral collaboration hinder the comprehensive implementation of integrated programs. Additionally, the involvement of communities and public awareness campaigns are crucial for the success of these initiatives. Addressing these challenges requires continued investment in infrastructure, training and outreach. Additionally, rapid urbanization contributes to the increase in stray dog populations. Efforts to manage and vaccinate stray dogs are essential to the success of rabies control programs. The Animal Birth Control (ABC) program, which involves the sterilization and vaccination of stray dogs and exploring the usage of Oral Rabies Vaccine in free roaming dogs are steps in this direction.

Conclusion

Integrating animal rabies control into One Health initiatives is paramount for preventing human cases and achieving a holistic approach to health and there by a sustainable approach to address this deadly disease. By addressing the disease at the human-animal-environment interface, we can create a more resilient and sustainable system that protects both human and animal populations from the threat of rabies. This approach enables us to work towards a future where rabies is no longer a major public health threat, emphasizing the importance of a united global effort in achieving this goal.

In conclusion, India's efforts in integrating animal rabies control into One Health initiatives reflect a commitment to addressing complex health challenges. While progress has been made, ongoing collaboration, innovation, and community involvement are essential to achieving the goal of eliminating rabies and ensuring a safer and healthier future for all.



LEAD PAPER-2

NATIONAL ANIMAL DISEASE CONTROL PROGRAMME ON BRUCELLOSIS: NATIONAL GUIDELINES AND REVIEW OF PROGRESS

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Introduction

India's livestock wealth (534.54 million) includes 192.48 million cattle, 109.85 million buffaloes, 148.88 million goats, 74.26 million sheep and 9.06 million pigs. India is the largest producer of milk globally with production of 187.74 million MT (2019). Yet, prevalence of animal diseases is a serious impediment to the growth of the Livestock Sector. Losses due to some of these e.g. Foot & Mouth Disease (FMD), brucellosis, etc. are humongous and often beyond estimation.

Brucellosis is a reproductive disease of cattle and buffaloes caused by bacterium *Brucella abortus*. The disease induces abortion at the last stage of pregnancy, infertility, delayed heat, interrupted lactation resulting in loss of calves, loss in production of meat and milk. Brucellosis has an adverse impact on human health as it is zoonotic (transmissible to humans) and serious occupational hazard. Affected humans may exhibit undulating fever, night sweats, body pains and aches, poor appetite, weight loss and weakness. Bovine brucellosis is endemic in India and appears to be on the increase in recent times, perhaps due to increased trade and rapid movement of livestock. The nature of livestock rearing, especially in rural India, is conducive to spread of infection from livestock to humans due to close contact with animals.

Hence, control of brucellosis will have a double impact - in both human health and livestock health, besides rich economic gains to the animal owners / farmers. In the absence of any treatment for brucellosis in bovine animals, the disease can be prevented by vaccination. Control of brucellosis can be achieved by a once-in-a-lifetime vaccination of female bovine calves (4–8 months old). This will not only make animals healthy but will also result in better productivity and acceptability of our animal products worldwide. Finally, efforts in this direction would further contribute towards doubling farmers' income. Hence, intensive Brucellosis Control Program in animals was envisaged for controlling brucellosis, which will result in effective management of the disease, in both animals and in humans.

Brief guidelines of the program

- ❖ National Animal Disease Control Program for brucellosis (NADCP) is a Central Sector Scheme where 100% of funds shall be provided by the Central Government to the States / UTs. The major activities include



- ❖ Mass screening of cattle and buffaloes to know exact incidence of the disease in an area / villages / block / district of state,
- ❖ Once in lifetime vaccination of all female calves between 4-8 months using *B. abortus* S- 19 strain vaccine (*any alternative vaccine may replace the existing one in future*),
- ❖ One time grant to strengthen one ELISA laboratory in a State/UT, consumables for ELISA Laboratories,
- ❖ Remuneration to privately engaged vaccinators in absence of sufficient manpower, publicity and awareness campaigns at national, state and block level, including orientation of the state functionaries for implementation of the program and online monitoring & data management
- ❖ Brucellosis in animals is highly zoonotic, therefore handling of live attenuated vaccines and vaccinating animals need extra care. Any accidental exposure to it may result infection to the personnel engaged in vaccination. The manpower physically engaged in vaccination of animals need personal protective equipment (PPE) gumboots, goggles, gloves and masks, etc. and also requires proper training for handling of vaccines and performing vaccination of the animals.
- ❖ **Conducting sero-monitoring at National Level:** ICAR-NIVEDI was assigned for conducting sero-monitoring (pre-vaccination and post-vaccination sera samples), confirmation of doubtful cases, training of laboratory personnel, state-wise sampling plan and technical support to State/UT Governments for screening outbreak samples etc.

Table: Details of work plan with technical indicators for NADCP – BCP

S. No.	Items of Work Plan	Indicator
1	States to be covered	All States and Union Territories in the Country.
2	Animals to be vaccinated	100% vaccination coverage of bovine female calves of 4-8 months of age
3	Vaccination	Once in a life-time calf-hood vaccination
4.	Vaccine	Brucella S 19
	<ul style="list-style-type: none">➤ Brucella vaccine shall be kept constantly at a temperature between 2°C and 8°C➤ Use only the diluent provided with the vaccine for its reconstitution➤ The dose of vaccine used shall be as per the manufacturer’s instructions which is at present 2 ml each for female cattle and buffalo calves Route — sub-cutaneous (<i>Care must be taken not to vaccinate the rump region of the animal</i>)	



BRUCELLOSIS VACCINATION AS PER INAPH UPTO NOVEMBER -2023

Monthly data of countrywide brucellosis vaccination was extracted from INAPH (Information Network for Animal Productivity & Health) website of NDDB (National Dairy Development Board) to correlate with sero-monitoring. As per INAPH data, overall vaccinations carried out were 18.5 crore in the country and 11.5 crore farmers were benefited under NADCP program. West Bengal performed highest brucellosis vaccinations (3.2 crore) and in all, brucellosis vaccination was carried out in 22 states and 5 UT's of the country.

BRUCELLOSIS POST VACCINATION SERO-MONITORING STATUS TILL NOVEMBER-2023

A total of 44,476 serum samples received under phase-I, II and III vaccinations were tested by indirect ELISA (Indian Patent No. 335659 with a sensitivity of 95% and specificity of 96%, respectively) to detect antibodies to S19 vaccination. Overall sero-conversion post brucellosis vaccination was found satisfactory (70.15%). In phase-I, sero-conversion >70% was recorded in 14 states and five UT's. In phase-II, >70% sero- conversion was recorded in five states and two UT's. Similarly, in phase-III, >70% sero- conversion was recorded in only one UT and till processing of the samples is continuing.

SUMMARY

The post-vaccinal antibody response in sera of 4-8 months old female calves vaccinated with *Brucella* S19 vaccine under NADCP program implemented by Department of Animal Husbandry and Dairying (DAH&D), Government of India. The antibody response was extremely good in three states [Chandigarh, Karnataka and Goa]. Higher antibody response was observed during 21-45 days post-vaccination (DPV) which indicated this period as appropriate for sampling to assess the antibody response. The knowledge acquired with respect to the post-vaccination sero-monitoring in this study will help the ongoing brucellosis control program under the flagship program of Government of India launched in 2019 as National Animal Disease Control Program (NADCP). Countrywide coverage will facilitate control of brucellosis in the country by 2025 and beyond.

Further reading: <https://dahd.nic.in/schemes/programmes/nadcp>



LEAD PAPER-3

**BIOSECURITY WITH REGARD TO VIRAL DISEASES- THREATS AND
CONTROL STRATEGIES REVISITED**

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Viruses are thus adapted to extremely diverse niches. Zoonotic viruses are spectacular examples of emergence and re-emergence resulting from innocent environmental manipulation or natural environmental change. Important aspects of ecological change and their relation to emerging viral life cycles are: 1) Population movements and the intrusion of humans and domestic animals into new arthropod habitats, particularly tropical forests; 2) Deforestation, with development of new forest-farmland margins and exposure of farmers and domestic animals to new arthropods; 3) Irrigation, especially primitive irrigation systems, which are oblivious to arthropod control; 4) Uncontrolled urbanization, with vector populations breeding in accumulations of water (tin cans, old tires etc.) and sewage; 5) Increased long distance air travel, with potential for transport of arthropod vectors; 6) Increased long-distance livestock transportations, with potential for carriage of viruses and arthropods (especially ticks); and 7) New routing of long-distance bird migration brought about by new man-made water resources. Rapid globalization leading to increased trade and thus continuous movement of humans and animals, climate change, increased concentration of animals and humans pose an ever increasing threat of infectious diseases crossing the borders and leading to huge outbreaks. Outbreaks of exotic viral and bacterial diseases have become a real threat to animal populations worldwide in recent years. Outbreaks of FMD in UK in 2001 and 2007, Blue tongue in 2007, equine influenza in Australia in 2007 and India in 2008-09, highly pathogenic avian influenza (H5N1) are some of the glaring examples of the amount of devastation they can cause in terms of economic losses and damage to the industry. The scars from these outbreaks are stark reminders which make us sit and probe our capabilities to work towards creating technologies and garner our scientific knowledge in pursuit to overcome these infections. Theoretically, sealing our borders and stopping the movement of all human and animals can help us achieve the task but its sheer magnitude and enormity certainly make us ponder upon some better scientific means to find a sensible solution to this enigmatic problem of transboundary diseases.



ORAL PRESENTATIONS

OP_1.1 : DEVELOPMENT OF HYBRIDIZATION CHAIN REACTION PROBES FOR THE DETECTION OF PATHOGENIC LEPTOSPIRES IN CANINE

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Leptospirosis is a globally important zoonosis caused by the pathogenic *spirochaetes* of the genus *Leptospira* which belong to the family *Leptospiraceae*. The clinical presentation of leptospirosis is neither specific nor consistent and misdiagnosis is common. Different antigen detection tests have been developed but none of them is sensitive enough to be routinely used. Whereas antibody detection methods possess low sensitivity at the acute stage of the disease. Molecular detection involves enzymes, which are expensive and require other conditions such as pH, temperature and ion concentration. Development of DNA detection methods with high sensitivity and specificity are extremely important in clinical diagnosis. The hybridization chain reaction (HCR) does not rely on enzymes and other harsh conditions to detect DNA. The method is compatible with other techniques and expensive equipment is not needed. HCR relies on the use of a pair of complementary DNA hairpins to propagate the chain reaction of hybridization events. In the present study, a pair of complementary DNA hairpins were designed for propagation of chain reaction of *Leptospira* targets using NUPACK, an online web tool for hybridization chain reaction which aids in the early diagnosis during the acute stage of the disease. Blood (n=48) and urine (n=4) samples were collected from the dogs suspected of leptospirosis. The products of HCR were visualized by colorimetric detection based on gold nanoparticles and agarose gel electrophoresis. For efficient HCR, parameters such as hybridization buffer and hybridization temperature were optimized. HCR was carried out using the clinical samples and reference leptospiral strains to analyze the performance. HCR- AuNP detection and HCR-Agarose gel electrophoresis detection were subjected for sensitivity evaluation and compared those with the sensitivity and specificity of conventional PCR.

OP_1.2 : PILOT STUDY ON ANTHRAX OUTBREAKS IN DIFFERENT GEOGRAPHICAL REGIONS OF KARNATAKA

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7. Director, IAH&VB.

In India, outbreaks of Anthrax are regularly reported (Kumar *et al.*, 2000), particularly in states such as Karnataka, Andhra Pradesh, Jammu & Kashmir, Odisha, and Tamil Nadu (Pattnaik *et al.*, 2022). Between 1997 and 2016, the state of Karnataka recorded a total of 206 villages, where anthrax was detected at various times (Chanda *et al.*, 2023).



In the present study, earpiece/ blood samples from suspected animals were collected from endemic villages of Bellary, Tumkur, Chikkaballapura, and Vijayanagara districts. In Chikkaballapur district Myalaganahalli village of Gauribidanur Taluk has reported several laboratory confirmed outbreaks during 2022-23. Other endemic places where laboratory confirmed Anthrax cases were, Varadapur / Ankasamudra villages of Hagaribommanahalli Taluk, Vijayanagara district and Bevinahalli / Badamanganahatti villages of Sira Taluk, Tumkur district.

The samples were streaked onto Blood agar and PLET agar plates. After 24-48 hours of incubation Grey to white coloured colonies with medusa head-like appearance were found on both Blood and PLET agar plates. The colonies on Blood agar plate did not exhibit any hemolysis. The colonies were examined with smear examination after staining with Gram's stain and Polychrome (McFadyen's) Methylene Blue for the confirmation of *Bacillus anthracis*. On microscopic examination, the organisms were observed to be in chains resembling boxcars.

Then they were subjected to PCR confirmation with published primers targeting pXO1 (Protective antigen- 747 bp) and pXO2 (Capsule antigen- 264 bp) and Ba813- 152bp. The whole genomes were sequenced and used for comparative analysis between these isolated strains. The soil samples from the above endemic villages were collected and analysed for different factors like pH, Moisture content, Calcium content etc., to ascertain the role of soil contents in survival of Anthrax organisms / Spores leading to the repeated outbreaks in the same villages/ areas.

OP_1.3 : BRUCELLOSIS OUTBREAKS IN SHEEP AND ITS DIAGNOSIS

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Brucellosis, a bacterial disease caused by members of the genus *Brucella*, an important zoonosis and a significant cause of reproductive and economic losses in animals. Brucellosis is usually caused by a Gram negative, facultative intracellular coccobacillus or short rod in the family *Brucellaceae*. *B. abortus*, *B. melitensis*, *B. suis* and *B. canis* are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal and are associated with a high morbidity rate in native herds, and a much lower morbidity rate in chronically infected herds. In the present study, two different cases from sheep herds of Mysore and Mandya Districts were sent for Brucellosis diagnosis, with a history of repeated abortions (abortion storms), with retention of placenta in many cases.

The clinical materials received at laboratory were aborted fetal stomach contents, placenta with cotyledons (Two) one each from Mysore and Mandya district. Apart from this serum samples from the closely associated animals (five each from above two districts) were also received. The aborted materials (Fetal contents and placenta were streaked onto the *Brucella* selective agar with anaerobic incubation (10% CO₂- enrichment). After 5-6 days of incubation at 37°C, growth of pinpoint pure culture colonies resembling brucella were noticed on the agar plates. On Gram's staining, gram-



negative coccobacilli organisms resembling brucella were observed. Further the culture and original aborted materials were subjected to conventional PCR after DNA extraction using Qiagen kit with the genus specific published primers. Forward 5'- TCG AGC GCC CGC AAG GGG-3' and Reverse 5'- AAC CAT AGT GTC TCC ACT AA -3' (905 bp). The tissue samples and culture from both the districts were found to be positive for Brucellosis. The serum samples (10) were subjected to Rose Bengal Plate Test and Indirect ELISA (IDEXX Kit). All the ten serum samples have shown seropositivity with varying OD values. The above findings have clearly indicated that both the herds were infected with Brucellosis. Necessary suitable advisory for disease control were given to both the herds.

OP_1.4 : SEROPREVALENCE OF LEPTOSPIROSIS IN SMALL RUMINANTS IN TAMIL NADU

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Leptospirosis is a common Zoonotic disease of Man and all Farm animals. Humans are most commonly infected through occupational, recreational or domestic contact with the urine of carrier animals either directly or via contaminated water or soil. Although most leptospiral infections in sheep and goats are asymptomatic, they may play an important role in the epidemiology of the disease by spread of leptospira. This study was carried out to evaluate the role of sheep and goats in the epidemiology of Leptospirosis. Serum samples were collected from 228 sheep and 210 goats of Thiruvannamalai district of Tamil Nadu state with the clinical signs of loss of appetite, reduced feed and water intake, blood tinged milk, abortions and weakened offspring. To detect leptospira antibodies, sera samples were tested with a panel of 12 serogroups representing pathogenic leptospire by Microscopic Agglutination Test (MAT). The result of MAT showed that 58% of sheep and 31% of goats had antibodies against atleast one or more examined serogroups. Among the various serogroups, the dominant reacting serogroups were *L. australis*, *L. autumnalis*, *L. canicola* and *L. pomona* in both animal species. Based on the age groups, 13.1%, 18.4%, 28.5% and 40% had MAT positive titre in less than 2 years, 2-3 years, 3-4 year and more than 4 years aged groups, respectively in sheep and 16.2%, 19.5%, 26.2% and 38% had MAT positive titres in less than 2 years, 2-3 years, 3-4 years and more than 4 year aged goats, respectively. However, it was observed that in adult sheep and goats (more than 3 years age group) were more sero-positive than the young animals. Highest prevalence of Leptospira was noticed during rainy season (68.45%) than the summer season (31.5%). The occurrence of leptospirosis was observed in both sexes without bias. It is concluded from the observation that infected sheep and goats also can excrete the leptospira and would transmit the disease among sheep and goats, other animals as well as the animal handlers.



OP_1.5 : COMPARATIVE EVALUATION OF SERUM AND WHOLE BLOOD SAMPLES IN A 16S rRNA-BASED PCR ASSAY FOR THE DIAGNOSIS OF BRUCELLOSIS IN GOATS

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This study aimed to assess the diagnostic efficacy of a Polymerase Chain Reaction (PCR) assay targeting a 1412 bp segment of the 16S rRNA sequence of *Brucella melitensis* in suspected cases of brucellosis in goats. Specifically, we investigated the performance of the assay when applied to both serum and whole blood samples. A total of 42 suspected brucellosis cases were analyzed. Results indicated a notable difference in sensitivity between serum and whole blood samples. The PCR assay exhibited a higher sensitivity of 90% when using serum samples, in contrast to a sensitivity of 80% observed with whole blood samples. Furthermore, the specificity of the serum PCR assay was determined to be 100%, signifying its precision in correctly identifying negative cases. In conclusion, the findings strongly advocate for the preferential use of serum samples over whole blood in the context of brucellosis diagnosis in goats employing the 16S rRNA-based PCR assay. This recommendation is underlined by the significantly enhanced sensitivity observed in serum samples, reinforcing the diagnostic utility and accuracy of this methodology. These insights contribute to the optimization of diagnostic protocols for brucellosis detection in goat populations, with potential implications for veterinary practice and research endeavors.

OP_1.6 : SARS-COV-2 FULL-LENGTH SPIKE ECTODOMAIN ELICITS PRODUCTION OF NEUTRALIZING MONOCLONAL ANTIBODIES WITH AFFINITY TO CONFORMATIONAL EPITOPES

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Recent evidence of human to animal as well as animal to animal transmission of SARS-CoV-2 has necessitated development of diagnostics for detection of antigen or antibody in different animal species for population surveillance. The spike glycoprotein of SARS-CoV-2 involved in viral entry is highly immunogenic. In this study, spike protein of SARS-CoV-2 (Delta variant) was expressed and monoclonal antibodies (mAbs) to the recombinant protein were produced for development of



diagnostic tools for sero-monitoring of the viral infection in animals. Seven mAbs were generated using the insect cell expressed full-length spike protein (S1/S2 ectodomain). In addition, 8 mAbs were generated against a mammalian cell expressed S1 of the Wuhan isolate with D614G mutation. Characterization of both the panels of mAbs revealed that the mAbs produced against S1/S2 antigen had affinity towards conformational epitopes of the spike glycoprotein while those against S1 antigen had affinity towards linear epitopes only. In virus neutralization test, among the S1/S2 mAbs, one mAb (3B8) neutralized only the Delta variant while two mAbs (3H3 and 3E10) neutralized both Delta and Omicron BA.1 variant of SARS-CoV-2, respectively. Among the S1 mAbs, only the Omicron BA.1 variant of the virus was neutralized by one mAb (7D12). In summary, the S1/S2 antigen, induced a panel of monoclonal antibodies that were diverse in their specificity to epitope repertoire with an ability of cross-variant neutralization, when compared to the S1 antigen. The recombinant proteins and the panel of mAbs developed against the protein could potentially be utilized for the development of robust diagnostics for specific detection of COVID-19 infection in different host species. In addition, the mAbs can also help us in understanding the effect of mutations that enable immune evasion of SARS-CoV-2 variants as the virus constantly evolves in the host under immune pressure leading to antigenic drift.

POSTER PRESENTATIONS

PP_1.1 : HEMATOBIOCHEMICAL PROFILE OF DOGS AFFECTED BY LEPTOSPIROSIS: A COMPREHENSIVE ANALYSIS

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Leptospirosis is a zoonotic disease affecting various species, including dogs, with potentially severe consequences. This study investigated the hematological and biochemical alterations in dogs diagnosed with Leptospirosis. Conducted at the Veterinary College Hospital Hebbal Bengaluru, 47 dogs exhibiting Leptospira-positive titers (MAT \geq 100) were categorized into four groups based on serovars: *L. grippityphosa* (Group I, n=16), *L. canicola* (Group II, n=15), *L. icterohaemorrhagiae* (Group III, n=9), and *L. pomona* (Group IV, n=7). A control group (Group V) included ten healthy dogs. Hematological parameters, including hemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, and differential count, were assessed alongside biochemical analyses, encompassing blood urea nitrogen, serum creatinine, alanine aminotransferase, total protein, albumin, cholesterol, sodium, potassium, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, glucose, and inorganic phosphorus. Results demonstrated a marked difference in hemograms between control and clinical groups. All clinical groups exhibited profound anemia, suggesting a significant impact on red blood cell parameters. Leukocytosis characterized by prominent neutrophilia and lymphopenia was



evident across all clinical groups, indicating potential inflammatory and coagulatory responses to Leptospirosis. Biochemical analyses revealed significantly elevated levels of blood urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total and direct bilirubin, total protein, albumin, globulin, glucose, cholesterol, sodium, potassium, and phosphorus in clinical groups compared to the control. These findings indicated hepatic and renal involvement, highlighting the systemic impact of Leptospirosis on vital biochemical pathways. In conclusion, this study provides comprehensive insights into the hematological and biochemical changes associated with Leptospirosis in dogs. The observed alterations underscore the complexity of the disease's impact on various physiological systems, emphasizing the need for a thorough understanding of these changes for effective clinical diagnosis and management in veterinary practice.

PP_1.2 : PREVALENCE, VIRULENT GENE PROFILING AND ANTIMICROBIAL RESISTANCE PATTERN OF EMERGING ZONOTIC *CLOSTRIDIUM DIFFICILE* FROM FOODS OF ANIMAL ORIGIN, ENVIRONMENT AND HUMANS

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Clostridium difficile is a recently emerging zoonotic pathogen causing life threatening diseases such as antibiotic associated diarrhoea and pseudomembranous colitis. Globally, over the past few years, there is an upsurge in *C. difficile* food borne outbreaks due to consumption of contaminated food products. The main objective of the current study was to examine meat samples and faecal samples for the isolation, molecular characterization, and antimicrobial resistance pattern of *C. difficile*. A total of 400 samples viz chicken (50), pork (50), mutton (50) and cattle (25) samples and faecal samples of food animals (125), humans (25), soil (25), environment (25) were collected randomly from Krishna and Prakasam districts of Andhra Pradesh. and processed for further examination. Briefly, samples were subjected to enrichment in fluid thioglycolate broth and then streaking on selective CCFA (full name) with 5% sheep blood agar incubated anaerobically. The presumptive isolates showing off white colored colonies and confirmed biochemically. Further the isolates were confirmed by using PCR targeting *tpi* and *gluD* (species specific) genes. Of the 400 samples, *C. difficile* was present in 11 samples including, pig faecal swabs 4% (2/50), cattle faeces (4%, 1/25), chicken 8% (4/50), mutton 2% (1/50) and, humans 8% (2/25), environment 4% (1/25). Finally, the confirmed isolates were subjected to virulent gene profiling using multiplex PCR. 80% of isolates carried toxin A and toxin B and 20% isolates carried only toxin B, 60% isolates carried binary toxin. On antibiogram study with 12 different antibiotics, 100% isolates showed resistance to metronidazole, moxifloxacin, gentamicin, and pencillins. 50% of isolates showed resistance to ciprofloxacin and clindamycin, signifying the increasing of antibiotic resistance, against common drug of choices in animal and human medicine. In conclusion, this study reveals or (provides information on) the importance of meat and meat products of animal origin as a potential source for *Clostridium difficile* infection in humans and animals.



PP_1.3 : CHARACTERIZATION AND EVALUATION OF BIOCONTROL AGENTS TO COMBAT *Listeria monocytogenes* IN FOODS OF ANIMAL ORIGIN

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The present study has been contemplated to characterize genotype and serogroup of *L. monocytogenes* recovered from different foods of animal origin, environmental interface and to determine the serotype, virulence by molecular assay, to study antimicrobial patterns by both phenotypic and genotypic methods, characterization and testing of selected essential oils of plant origin were for the antimicrobial activity against *L. monocytogenes* isolates. Out of 474 samples (274 milk and 50 each of soil, feed, sewage and beef) screened, ten *L. monocytogenes* isolates (milk=8, soil=1 and beef=1) were confirmed by PCR targeting *hlyA* gene and found to belong 1/2a, 3a serogroup and falls under type II lineage. Virulence gene profiling studies has revealed that all the ten *L. monocytogenes* isolates were harboured *iap* gene while *plcA* and *plcB* genes were observed in seven (70%) and eight (80%) isolates respectively. Six isolates from milk were found to group in the same cluster by ERIC and RAPD fingerprinting suggesting that both these methods are efficient molecular typing tools for *L. monocytogenes*. Further, antimicrobial resistance pattern studies by phenotypic method had shown variable resistance for commonly used antibiotics listeriosis treatment. Genotypic characterization of AMR genes revealed that seven isolates were positive for *tetM*, five for *mefA*, four for *msrA* and one for *lnuA* genes while none of the isolate shown *tetK*, *ermA*, *ermB*, *lnuB* genes. On characterization of the essential oils by Gas Chromatography-Mass Spectrometry (GC-MS), it was noticed that the cinnamaldehyde (74.76%) was the major compound in Cinnamon bark oil, Eugenol (78.413%) in Cinnamon leaf oil, Eugenol (77.004%) and Caryophyllene (10.688%) in Clove bud oil, diallyltrisulphide (37.144%), diallyldisulphide (28.478%) and diallylsulphide (5.741%) in Garlic oil. Among the four essential oils (Cinnamon bark oil, Cinnamon leaf oil, Clove bud and Garlic oil) screened for antibacterial efficacy against *L.monocytogenes* isolates, cinnamon bark oil has showed widest zone of inhibition (27 mm), followed by cinnamon leaf oil (17 mm), clove bud oil (14 mm) and garlic oil (12 mm) respectively. The Minimum Inhibitory Concentration (MIC) studies found that cinnamon bark oil has only shown antibacterial activity against *L. monocytogenes* at the concentrations of 2% and 1% and it has shown of MIC value 1% (v/v) among four different concentrations (2%, 1%, 0.5% and 0.25%) of essential oils. This study has identified the occurrence of antimicrobial resistant *L. monocytogenes* isolates in foods of animal origin (milk and beef) and environmental samples (soil, feed and sewage samples) and also made an attempt to employ essential oils as antimicrobial agents in food system as an alternative to control food borne pathogen as well as to curb the antimicrobial resistance in foods of animal origin. It is concluded that, further research on the application of varying



concentrations of essential oils will be immensely helpful in enhancing the efficiency of these essential oils as bio-control agents to facilitate eco-friendly and environmentally safe agents in food systems which eventually leads to strategies to combat this, foodborne and antimicrobial resistant *L. monocytogenes*.

PP_1.4 : SERO PREVALENCE OF BRUCELLOSIS IN ANIMALS FROM ANDHRA PRADESH

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Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals caused by the member of the genus *Brucella*. It stands first in the list of zoonotic bacterial diseases and 5,00,000 human cases are reported annually in endemic regions. It is an occupational disease, occurring most often in veterinarians, farmers, stock inspectors, abattoir workers, Laboratory personnel & butchers. The WHO classify it as one of the world's neonatal zoonotic diseases because of more disease burden on low-income countries. The geographical distribution of brucellosis is constantly changing, with new foci emerging or re-emerging. In livestock, the disease results in significance economic losses due to reproductive impairment caused by abortion, still birth & weak calves and neonatal mortality & infertilities. In humans, *Brucella* species causes a fertile illness to highly fatal nervous signals. The present study revealed the sero prevalence of Brucellosis in animals from different livestock farms of A.P. screened at SLDL, SVVU, Tirupati.

A total of 9,287 sera samples were screened against Brucellosis during the period from 2015 to 2023 October using Rose Bengal Plate Test (RBPT) & Standard Agglutination Test (STAT). Out of 9,287 sera sample screened 1001 Sera samples were found Positive for *Brucella* antibodies on RBPT with 10.78 % sero positivity and 620 sera samples with 6.68% sero positivity on STAT respectively. In Bovine, out of 6,372 sera samples, 982 sera samples positive on RBPT with 15.41% sero positivity and 561 sera samples positive on STAT with 8.80% sero positivity on STAT followed by Ovine's of 2,662 Sera samples 6 samples were positive on RBPT with 0.23% sero positivity & 4 samples with 0.15 % sero positivity on STAT. Similarly in porcine, out of 231 sera samples with 3.46% (8 No's) sero positivity on RBPT & 2.16% sero positivity (5 No's) on STAT. Whereas 22.73% (5 No's) sero positivity on RBPT & 13.64% (3 No's) sero positivity on STAT was reported in human out of 22 sera samples screened.

Due to zoonotic significance Govt. of India has initiated & Implemented National control programme on Brucellosis. Test and slaughter of infected animals played a crucial role in stamping out of the disease. Further, effective veterinary services, periodical surveillance, disease awareness & education to the farmers improved managemental precautions, Restricted movement of infected ones, sanitary & Bio-safety measures at farm & lab levels are essential component in effective control of the disease. However, in recent years, a very important approach in control of Brucellosis is one health approaches to control & prevent human and animal Brucellosis.



**PP_1.5 : SEROPREVALENCE OF LEPTOSPIROSIS AND DISTRIBUTION
OF *LEPTOSPIRA* SEROGROUP SPECIFIC ANTIBODIES IN BUFFALOES,
IN TELANGANA STATE, INDIA**

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Leptospirosis is an emerging, re-emerging, and neglected anthroponosis with a worldwide distribution in a wide range of animal hosts and is caused by different serovars of the pathogenic spirochetes belonging to the genus *Leptospira*. Among the livestock hosts, buffaloes play an important role in maintaining and transmitting the disease as a carrier host after cattle. Despite its severity, leptospirosis in water buffaloes is often neglected due to a lack of awareness about the extent of the problem. The objective of the present study is to determine the prevalence of anti-leptospiral antibodies and the frequency distribution of *Leptospira* serovars in buffaloes in enzootic states, namely Telangana, in India. A serosurvey was conducted from March to December 2022 to establish the prevalence of anti-leptospiral antibodies in buffaloes in Telangana (collected serum samples through state animal husbandry department through ICAR-NIVEDI's NADEN centre). The working hypothesis of the study was the homogeneous occurrence of anti-leptospiral antibodies in the epi-units in the target population. A stratified two-stage random sampling procedure was adopted to collect sera from different states' epi-units in the studied region. A total of 915 serum samples were received from 29 districts of the Telangana state of India. The serosurvey covered 62 taluks/blocks and 65 villages and was tested at a 1:100 dilution in a microscopic agglutination test using five to seven-day-old cultures at a concentration of $1-2 \times 10^8$ organisms/ml of 21 reference *Leptospira* serovars as live antigens. An overall seroprevalence of 21.42% was observed. The district-level analysis showed variability in prevalence rates, with the highest positivity recorded in Kamareddy at 87.5%, Rangareddy at 79.2%, Peddapalli at 75%, and Siddipet at 70.8%. The predominant leptospiral antibodies were determined against the frequency of distribution of the serovars Pomona (58.2%), Tarassovi (47.4%), and Autumnalis (19.9%). This study indicates an alarmingly seroprevalence of leptospirosis in buffaloes in the state of Telangana. The above prevalent serovars in buffaloes and the major prevalent serovars in other livestock species in the particular region may be of use in the reference panels of *Leptospira* antigens in MAT in humans and animal disease diagnostic laboratories for providing an accurate diagnosis of leptospirosis. Further, understanding the epidemiology of leptospirosis would help achieve adequate surveillance and accurate diagnosis, which, in turn, helps in planning and mitigating the appropriate control measures and avoiding public health problems.



PP_1.6 : ISOLATION AND CHARACTERIZATION OF *BACILLUS ANTHRACIS* FROM ENDEMIC AREAS OF ANTHRAX IN ANDHRA PRADESH

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Bacillus anthracis is the etiological agent for the dreadful and fatal disease of Anthrax and is responsible for high mortality rates in livestock and zoonotic impact in humans from endemic districts of Andhra Pradesh. The proposed work had aimed with the objectives to collect, isolate and detect and detect *Bacillus anthracis* in the soil and clinical samples from outbreak/endemic areas of Anthrax in Andhra Pradesh. In present study, A total of 15 clinical earpiece samples; cattle(8), sheep(6) and goat(1) & 48 environmental soil samples from outbreak/endemic sites of Andhra Pradesh were processed for cultural isolation, 15 out of 15 clinical earpiece samples & 16 out of 48 soil samples were shown ground glass appearance on blood agar and showed square cut ‘Bamboo stick appearance’ on methylene blue and gram’s staining methods were confirmed morphologically as *B. anthracis*. On biochemical examination, all the 31 isolates were positive for *B. anthracis* by gelatin stab inoculation and Pencillin susceptibility test (except two earpiece isolates of Srikakulam Sr6 and Kurnool K12 shown resistance to penicillin). 31 *B. anthracis* isolates recovered were subjected to molecular diagnosis with PCR. A total of 22 *B. anthracis* isolates were confirmed as *B. anthracis* genus amplifying PA gene of PXO1 and 10 *B. anthracis* isolates were confirmed as *B. anthracis* species amplifying CAP gene of PXO2. All the 31 *B. anthracis* isolates were confirmed as bacillus amplifying *rpoB* gene and *B. anthracis* (species) chromosomal amplifying *Ba813* gene. However, Gene sequencing followed by molecular diagnosis confirmed that all the 31 isolates were confirmed as *Bacillus anthracis*. On nucleotide analysis with genus specific *rpoB* gene of earpiece and soil isolates shown 99-100% identity, whereas nucleotide analysis with species specific genes; PA of PXO1, CAP of PXO2, *rpoB* and chromosomal *Ba813* gene shown 98-100% identity with their respective reference strains of gene bank. On phylogenetic analysis, earpiece isolates and soil isolates from endemic districts of Andhra Pradesh shown close evolutionary relationship with each other but earpiece isolates of Srikakulam Sr6 (Accession No: MK310254) and Nellore N3 shared ancestral relation with global reference strains of USA (Accession No: CP012730) and Japan (Accession no: AP014833) respectively. Similarly soil isolate of Visakhapatnam VM9 also shared ancestral relation with global reference strain of Japan (Accession no: AP014833). This phylogenetic analysis deciphered that there is no strain variation among isolates of *B. anthracis* identified in endemic regions of different districts from Andhra Pradesh.



TECHNICAL SESSION II

ADVANCED TECHNOLOGIES IN HEALTH AND REPRODUCTION





POWER OF SCAFFOLDING THE ANIMAL STEM CELLS FOR REGENERATIVE THERAPIES

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Uses of scaffolds enriched with mesenchymal stem cells (MSCs) has emerged as a promising strategy for repairing or replacing damaged or diseased cells, organs or tissues, one of the major goals of regenerative medicine. We have been experimenting different types biological as well as non-biological scaffolds for the regenerative therapies of livestock and pets. To create appropriate 3D microenvironments, these scaffolds are made to match the structural properties of a particular tissue/s and organ/s. Once they are augmented with the adult stem cells, these scaffolds dramatically enhance the shape and performance of sick and injured tissues and organs when they are loaded with MSCs.

Synthetic scaffolds such as:

- i) **Polymeric Scaffolds:** These are primarily employed in the creation of biodegradable scaffolds. Polycaprolactone (PCL), polyglycolic acid (PGA), polylactic acid (PLA), polyethylene oxide (PEO), polyethylene glycol (PEG), polyvinyl alcohol (PVA) and polyurethane are among the synthetic polymers.
- ii) **Porous Scaffolds:** These scaffolds have been utilized to create tissues like bone, cartilage, ligaments, skin, vascular tissues, neural tissues, and skeletal muscle as well as to transport MSCs, medications and proteins.
- iii) **Hydrogel Scaffolds:** These cross-linked, water-swelling polymeric networks were created by straightforward reactions between just one or two monomers. The closest material class to biological tissues is hydrogels.
- iv) **Fibrous Scaffolds:** These have a high porosity and are constructed of fibers. They are utilized in applications that demand materials with a large surface area and small pore diameters.
- v) **Microsphere Scaffolds:** These are spherical particles, typically between 1 and 1000 μm in diameter. Numerous organic and synthetic materials can be used to make microspheres.
- vi) **Metal Scaffolds:** These are frequently applied in orthopedic applications, where the materials must bear significant mechanical loads.
- vii) **Composite Scaffolds:** These scaffolds have the advantageous characteristics of each component as well as unique properties that come from the combination because they are formed from two or more different types of materials, are considered the good ones.



Whereas, different types of **bio scaffolds**,

- i) **Periosteum**: In the engineering of bone tissue, due to its similarity in structure to the original tissue, the periosteum replicates the natural microenvironment. This bio scaffold is essential in creating the mechanical and physical milieu that cells require to survive and grow.
- ii) **Decellularized Matrix Scaffolds**: These are made by removing all the cells from a tissue or organ and then using the extracellular matrix, which serves as a scaffold, to rebuild the tissue or organ.
- iii) **Amniotic membrane (AM)**: AMs are mainly used to regenerate skin/wounds. They are known for their biocompatibility and mechanical stability.
- iv) **Bio mesh**: It is a biomaterial applied mainly in surgery, primarily for varieties of hernia repair. Bio mesh should specifically have low infection risk, biocompatibility and longevity and facilitates wound healing.
- v) **MSCs-loaded scaffolds** are a promising approach in regenerative medicine. They offer a different approach to treating refractory and incurable diseases and have moved from fundamental research towards clinical translation as a potential future therapeutic option.

During my talk, I will be sharing the details of our work using the above scaffolds with or without the stem cells, exosomes or it's conditioned media.



BACTERIOPHAGE THERAPY FOR MASTITIS: CURRENT STATUS

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India ranks first in the world in bovine population as well as in milk production and consumption. The value of dairy products in India is more than that of rice and wheat combined. Besides egg, milk is the most easily available and budget-friendly protein source for human consumption. Therefore, the demand for milk is ever increasing owing to population growth, changes in demography (urbanization), and higher income and lifestyle. Consequently, milk production is dependent on high milk yielding breeds. However, such animals are also more susceptible to intramammary infection (IMI), which results in mastitis. Mastitis is a widespread and costly disease, and the annual loss to India was 71.6551 billion two decades ago.

Mastitis is mainly caused by bacteria, and it is not uncommon to isolate multiple species from different genera from the same animal showing signs or symptoms. The most common bacterial species include *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus* and *Escherichia coli*. Mastitis has historically been treated with antibiotics. Due to compounded reasons of farmer not willing to lose productivity, time taken to obtain results of antibiotic susceptibility testing, and patient compliance issues, the treatment often involves using higher classes of antibiotics. This injudicious use has been blamed to be one of the factors associated with multi-drug resistance of mastitis pathogens as well as emergence and/or maintenance of antimicrobial resistance (AMR) in nature, with a cascading consequence on infections of other animals as well as humans. Most intractable cases of mastitis are often owing to AMR, and frequently involve *Staphylococcus aureus*, one of the most serious human pathogens. Thus, mastitis and its treatment with antibiotics has wide ranging implications not only for livestock productivity but also for public health.

Healthcare costs as a consequence of deaths and disability owing to the estimated burden of AMR are projected to run into USD one trillion (10^{12}) annually by 2030, if corrective measures are not undertaken. Addressing AMR will need multiple approaches. Besides anti-microbial stewardship programs, emerging areas are targeting outcome-oriented research into alternatives to antimicrobials, and are being pursued to the extent of personalized and precision medicine. These include drugs and nanoparticles; phytochemicals and other natural compounds; biologics such as vaccines, bacteriophages and their products, bacteriocins, antimicrobial peptides and probiotics.

Ideally, alternatives to antibiotics need to be directed against a broad genus of bacteria so that multiple agents can be targeted at once, and yet focused not to affect normal microbiota. One such alternative is the use of bacteriophages. Although used for more than a century, rise in AMR has reinvigorated research and development into the use of bacteriophages. Crude preparations were



initially attempted for curing bacterial infections, in Soviet Republics and Eastern Europe, immediately after the discovery of bacteriophages. Several studies have also showed the therapeutic effects in experimental animal models. However, the first clinical trials were not carried out till 2009. While bacteriophages have been found to be safe, clinical efficacy has been found to be variable, depending on various factors. On the other hand, bacteriophages have been used to counter bacteria during food preservation, particularly the frozen and ready-to-eat kind. There are now several cases of saving lives and curing people from bacterial infection through compassionate use of bacteriophages, notably in cases where all antibiotics have failed.

We have begun to systematically search for phages to target pathogens causing bovine mastitis. With the intent to target mixed infections as well as to initiate treatment even before knowing the pathogen, we isolated 25-30 phages each against *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli*. This was followed by host range studies where 25-30 strains of each of the bacterial species were tested in spot assays. Few bacteriophages specific to *E. coli* were selected through further characterization, based on morphology (electron microscopy), growth kinetics (burst size, latent period), pH and temperature stability, and genetic characterization (restriction fragment length polymorphism). We then elucidated the genome sequence of six bacteriophages specific to *E. coli*. Similar work is in process for bacteriophages specific to *Streptococcus agalactiae* and *Staphylococcus aureus*.

A few studies have reported is the isolation and characterization of bacteriophages which can lyse bovine mastitis-associated bacteria *in vitro*. Some reports have also shown the effect of combinations of different bacteriophages as well as bacteriophages and other biologics such as probiotics on target bacteria *in vitro*. On the other hand, there have been very few reports on broad spectrum bacteriophages that work simultaneously against a mixture of bovine mastitis pathogens, bacteriophages or bacteriophage-derived enzymes that can disrupt biofilm formed by such bacteria. More work is needed in this direction as well as to evaluate if any of these can be used in combination with antibiotics or solely to replace antibiotics in treating bovine mastitis. Ultimately, we envisage that bacteriophages could replace or at least drastically reduce the application of antibiotics in the treatment of mastitis.

Bacteriophages and their products have several advantages over antibiotics. They are very specific and kill only target bacteria, and in many cases, even specific strains. Although one can argue that there could be some effect on microbiota, the effect may not be on all strains or on all subjects. Antibiotics on the other hand, kill pathogenic as well as beneficial bacteria, which sometimes can cause serious secondary complications. Thus, bacteriophages may avoid dysbiosis or decrease secondary infection. Secondly, bacteriophages can replicate at the site of infection and be available where it is required, whereas antibiotics sometimes metabolize and are cleared out from the body and are not always available at the target site. Third, bacteriophage therapy has not been shown to cause any serious immediate or long-term side-effects, whilst antibiotics can result in allergy, secondary infection, intestinal disorder etc. Fourth, although there isn't sufficient data yet, available evidence suggests that



bacteriophages can degrade bacterial biofilms, whereas antibiotics, by themselves, are poorly effective or ineffective against biofilms.

However, given the modern regime of drug substance production and control, clinical development and application of bacteriophages has been slow, in spite of the fact that no serious or life-threatening adverse events have been observed in multiple experimental animal challenge models or the few instances of treatment of humans. This includes the absence of sepsis as a result of the release of endotoxins following bacteriolysis. In addition, immune responses have rarely been observed against the injected bacteriophages, providing some level of confidence in developing bacteriophages for therapy. On the other hand, production facilities and bioprocess development conforming to good manufacturing practice are under operationalization in some places. These could pave the way for phage therapy playing a prominent role in the fight against bacterial infections as well as AMR.



REPRODUCTIVE BIOTECHNOLOGIES FOR GENETIC IMPROVEMENT OF CATTLE AND BUFFALOES

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Manipulation of animal reproduction is probably as old as domestication itself. Since man started keeping animals in captivity, he exerted profound influence on the natural behavior and reproduction in domestic animals. Assisted reproductive technologies have been successfully employed in augmenting farm animal productivity during the last one hundred years. Artificial insemination and Embryo transfer immensely contributed to disseminating the best male and female genetics across continents. For about 30 years from 1970, the Embryo Transfer Technology (ETT) was extensively applied in developed countries to produce superior bulls and bull mothers. However, due to its inherent limitation in terms of number of embryos produced, the conventional method of embryo production (*in-vivo*) is gradually being replaced by the laboratory production (*in-vitro*) of embryos (Blondin, 2015). The *in-vitro* embryo production technology revolutionized the speed at which generations of embryos could be produced thereby enhancing the rate of female reproduction and thus reducing the generation interval. In recent times, production of calves of desired sex, by development of sexed semen technology, is slowly gaining momentum to further augment livestock productivity with quantum jump in heifer calf production. India is consistently undertaking genetic improvement and breed conservation programmes by employing various assisted reproductive techniques since last seven decades. The present article discusses about the policy and scientific interventions that the country has undertaken for genetic improvement of cattle and buffalo in the country for the past 60-70 years.

Artificial Insemination: This is the first and the most efficient reproductive technology that has transformed the livestock breeding strategies including import and export of germ plasm overcoming the geographical barriers. The primary driving force behind AI is its potential to increase the rate of genetic gain in livestock populations by widespread use of sires with elite genetic merit.

In India AI was introduced in the early forties and Dr. Sampat Kumaran is considered to have performed the first AI at Mysore palace dairy farm. The focus of AI had been crossbreeding with exotic breeds. However, currently stress is being given to the conservation and propagation of indigenous breeds using superior HGM native sires. In the first (1951-56) and second five year plans (1956-61) GOI started key village centers to improve cattle and buffaloes in the country. The National Project on cattle and buffalo Breeding (NPCBB), initiated in the year 2000 focused on genetic up-gradation of bovines and streamlining AI services and support system. The National Dairy Plan -1 (NDP-1) started in 2012, focused on producing High Genetic Merit (HGM) bulls of different breeds through Progeny Testing (PT) and Pedigree Selection (PS). These bulls were used to produce disease free high quality



semen at A and B graded semen stations of the country. As per Animal Husbandry Department, Govt. of India annual report 2016-17 there are presently 49, ISO certified (A and B Graded) Semen Stations in India producing around 95 million Frozen Semen Doses (FSDs) to cater the need of Artificial Insemination in the country. The Animal husbandry department initiated the Rashtriya Gokul Mission since 2014 for conservation and development of indigenous breeds of livestock in India. The 2 important objectives of this mission are up-gradation of nondescript cattle using elite indigenous breeds like Gir, Sahiwal, Rathi, Deoni, Tharparkar, Red Sindhi and distribution of disease free high genetic merit bulls for natural service. National Mission on Bovine Productivity (NMBP) will be implemented with following four components: (i) Pashu Sanjivni: an Animal Wellness Programme encompassing provision of Animal Health cards (Nakul Swasthya Patra) along with UID identification and uploading data on National Data Base. (ii) Advanced breeding Technology: including Assisted Reproductive Techniques- IVF/MOET and sex sorted semen technique to improve availability of disease free high genetic merit female bovines. (iii) Creation of “E-Pashu Haat” an e-market portal for bovine germplasm for connecting breeders and farmers (iv) National Bovine Genomic Centre for Indigenous Breeds (NBGC-IB).

At present there are 56 grade A and B semen stations in the country maintaining about 2700 HGM bulls and producing 120 million semen doses. The AI coverage in India, in contrast to western countries, is only 30-35% and in some states like UP it is only 19% due to large breedable bovine population in the state. Some of the bottlenecks that need to be addressed are - lack of sufficient number of progeny tested / pedigree tested bulls, limited AI network and delivery services, small herd size and no initiative to form breed societies, Problems associated with heat detection and lack of door step AI and other extension services. Most Govt. AI centres were stationery due to deficiency of manpower and transport facility. Further, it is imperative that all the states in the country enact stringent laws by adopting “Bovine Breeding Act” and follow Minimum Standards (MSP) for frozen semen production, AI service providers and AI training centres. Also periodic evaluation and grading / accreditation of semen stations and AI training centres need to be carried out.

Embryo Transfer: A specialized technique of breeding mainly used to increase the rate of reproduction in females thereby ensuring production of more offspring per cow per unit time. Thus, it helps in faster multiplication of rare, genetically superior dams. Also sire evaluation can be done more accurately on the basis of sib and pedigree records. The technology can further be used to protect rare species from the risk of extinction. Following the birth of first ET calf (Wilmot et al., 1951) and subsequent successful freezing of cattle embryos (Wilmot and Rowson, 1973), the technology has grown into an on farm activity since 1970 onwards and has become a potential genetic improvement activity for production of elite bulls and bull mothers. Also embryo cryopreservation has simplified the concept of animal transport across borders.

Genesis of ETT in India: Initial work on ET and related technology was done by certain Agricultural Universities and research institutes like NDRI and IVRI and it resulted into birth of first calf at CVSC, Tirupati in 1986 (Ramakrishna and Bose, 1986). In 1987, The DBT, GOI launched a



project entitled "cattle herd improvement for increased productivity using ETT". The main objective of the project was to develop infrastructure for research, training and practice of ETT in order to improve the productivity of cattle and buffaloes. The project was implemented in 2 phases; phase I (1987-1992) and phase II (1993-1996). During this period 3188 embryos were collected from 1958 elite donor cows and buffaloes. Transfer of 1875 embryos resulted in 296 recorded births. ETT was successfully integrated for implementation of Open Nucleus Breeding System in which the merit of males was judged by the performance of full sibs and half sibs within the herd. Many of the ranked males born out of ET have been mounted on country's AI net work for genetic improvement. The ETT project has resulted in creation of excellent infrastructure and to some extent skill pool in the country.

In-vitro Embryo Technology: During the last 10 years the focus is shifted from conventional method of embryo production involving superovulation technique to oocyte aspiration by trans-vaginal ultrasound guided technique (commonly known as Ovum pick up - OPU) and then subjecting those oocytes to in-vitro fertilization (IVF) and in-vitro culture (IVC). Almost a fivefold increase in embryo yield can be achieved from a given donor in a unit time through OPU-IVF method over the conventional method of embryo production. Thus a technology is now available to produce more and more in-vitro derived embryos in the place of in-vivo embryos. However, the only limitation with IVF embryos is more vulnerability of IVF embryos to cryo shock and reduced pregnancy rate in surrogate mothers. In 2018, Dept. of AHD&F, Ministry of Agri., GOI conceived a new project titled "ET & IVF technologies for Bovine breeding" as part of Rashtriya Gokul Mission (RGM) for productivity enhancement of indigenous breeds of cattle. A total of 31 IVF labs have been established in the country and about 1800 calf births were already recorded between 2018 and 2021 through IVF. Under accelerated IVF programme, it is now envisaged to produce 46000 IVF pregnancies in the country by involving Private Service Providers, which is a programme implemented under PPP mode. The country is poised to produce huge stock of genetically superior indigenous cattle utilizing IVF technology in the coming few years.

World scenario: When India is still in its infancy in applying ET for breed improvement, several countries in the world are way ahead and successfully integrated this technology for production of elite animals. The last 80 years have seen great innovations in the field of Assisted Reproductive Technologies (ART) thereby rendering farming and other agricultural businesses more profitable. The technology is now commercialized and export and import of embryos has become reality. The bovine industry is greatly benefitted by Assisted Reproductive Technologies and IVF has emerged as a major game changer in ET business. The world production of embryos in 2015 was reported to be 12,72,930 (48.1% in-vitro embryos and 51.9% in-vivo embryos) and USA is currently the global leader (5,06,626 embryos produced in 2015) followed by Brazil (3,91,805 embryos produced in 2015).

Reasons for low uptake of ET in India include lack of infrastructure including non-availability of hormones (FSH), equipment etc., inadequate trained man power, lack of regular funding (break in continuity) and lack of commercial companies engaged in ET. Also some misconceptions about ET which need to be shattered are - ET is a modern technology and not fit to be adopted, ET should replace



AI as a field activity, requires vigorous training for long period, is a highly expensive activity and donors become infertile / sterile.

Three Pronged approach to implement ET/IVF in the country:

- I. ET programme in a breeding farm: Top 5-10% animals should only be used as embryo donors and use all low pedigree cows available on the farm as surrogates. Establishment of infrastructure, training of man power and skill pool development should be done on priority.
- II. Use of high pedigree animals available with farmers as donors to produce males & replacement heifers by paying incentives. Also use of low pedigree farmers cows as surrogates by offering incentives.
- III. Establish regional ET centers to utilize cryopreserved embryos to produce elite bulls and bull mothers.

Sex sorting of Semen: Sex is the total morphological, physiological and psychological differences that distinguish a male from female. In mammals female is homogametic (XX) and male is heterogametic (XY) and in nature the primary sex ratio is always 1:1. Sexed semen is semen in which fractions of X-bearing (female) Y-bearing (male) sperm are sorted based on difference in DNA content by a procedure called flow cytometrical cell sorting (Seidel, 2014).

In the 1980s, a breakthrough in semen-sexing technology was achieved by USDA and the patents for this technology were licensed to XY Inc., Fort Collins, Colorado which performed a considerable amount of research to optimize efficiency of sperm sorting procedures. Commercialization of sexed semen in the United States was initiated in 2003 for which license was granted to Sexing Technologies (ST). Sexing Technologies Inc. was created with the goal of producing Sexed Sorted Semen around the globe. In 2003 they acquired the license and in 2004 the first lab started operations in Navasota, TX. In 2007 Sexing Technologies acquired XY Inc.; this is a major step towards the improvement of the technology. The great impact of the technology as well as the successful results in the field has made Sperm Sexing Technology to grow at a steady pace, opening facilities in many countries around the world such as USA, Brazil, UK, Netherlands, Italy etc. In USA many companies and cooperatives are selling sexed semen whose volume is about 2 million doses representing about 5% of total semen sales volume.

The primary goal of producing sex sorted semen is to produce calf of a desired sex so that herd replacement and herd extension can be done quickly. Due to availability of sufficient number of replacement heifers, the breeders can do away with the practice of procuring outside heifers thereby avoiding bio security issues. As per 20th Livestock census (2019), of the 301 million bovine population in the country, about 57 million are males (47 million cattle males and 10 million buffalo males). Due to mechanization of agriculture and transport, oxen or bulls are no longer required and only female calves are retained by farmers for milk production. Beef is generally produced from male calves of buffalos or cows. Farmers sell them at low prices or sometimes leave them orphan. This creates an



animal welfare issue and moral compunction among animal lovers. Many religious sects also prohibit slaughter of bulls which has resulted in communal tensions. Extensive use of sex sorted semen in AI programme can address the issue of unwanted male calf production in the country. Also Sexed semen has great utility in IVF programme where in one dose of sexed sperms can be used to produce many embryos of desired sex. And combination of superovulation and insemination with sexed semen further increases desired calf crop.

In India there are seven semen labs producing sexed semen of various purebred, cross bred and indigenous cattle under the licence of ABS Genus, USA and Sexing Technologies, USA. These labs are at BAIF, Pune; ULDB, Rishikesh; CSS, Bhopal; ABS, Chitale, Maharashtra; GLDB, Patna; Jagudan semen station, Mehasana, Gujarat and UPLDB, Babugarh, UP. Govt. of India has embarked upon an ambitious Accelerated Breed Improvement Programme by using sex sorted semen for getting assured pregnancy. Under this programme it is proposed that sex sorted semen doses will be used for getting 51 lakh assured pregnancies, leading to birth of 45 lakh female calves.

However, the technology has its own limitations which include - Sperm sorting into X (which produces female calf) & Y (which produces male calf) is a very slow process. Currently it takes approx. 9 min. for a single sorter to produce one straw of sexed sperm, which is approximately 7 straws/h with 2 million sperms / dose. Sperm are sexed serially (one by one), rather than sexing multiple sperms simultaneously. Sexing works best with fresh semen, so sorters usually are located near the bulls and sperms are cryopreserved after sexing process. The equipment is fairly complicated and expensive (USD 3,50,000). Also it is expensive to install & maintain the sorters and requires skilled operators. Low sorting efficiency, Low pregnancy rates and high cost per dose (Rs.1200) limit the wide spread application of this technology among dairy cattle.

Expanding the horizon of AI technology including sex sorted semen for production of large number of replacement heifers, using ET and IVF for production of superior bulls and bull mothers and integrating the technology with breed improvement programme will go a long way in genetic improvement of cattle and buffaloes in the country. The projects that are being implemented by the Govt. of India as part of Rashtriya Gokul Mission such as National AI programme, Implementation of ET & IVF under National Mission on Bovine Productivity and Accelerated Breed Improvement Programme using sex sorted semen for getting assured pregnancy will help augmenting the productivity of indigenous cattle and buffaloes and also conserve the rare native genetic resources.

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ORAL PRESENTATIONS

OP_2.1 : CORRELATION OF FOWL ADENOVIRAL LOAD AND PATHOLOGY IN EXPERIMENTALLY INFECTED CHICKEN

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Fowl adenoviruses (FAdV) have been associated with many disease conditions in chicken including inclusion body hepatitis (IBH), Hydropericardial syndrome (HPS), gizzard erosion and pancreatitis. The aim of the present study was to correlate the fowl adenoviral copy numbers and pathology from different tissues viz., bursa of Fabricius, caecal tonsil, kidneys, liver, spleen and thymus of experimentally infected chicken. To assess the FAdV load in different organs, ninety-eight numbers of one day old commercial broiler chicks were grouped into three and inoculated with 0.5 ml ($10^{6.5}$ TCID₅₀/ml) FAdV serotype 11 via oral and intramuscular route except control group. The birds were sacrificed on 3, 5, 7, 10, 14 and 21 dpi and DNA was extracted from various tissues viz., bursa of Fabricius, caecal tonsil, liver, kidney, spleen and thymus and SYBR Green based real time PCR was carried out using 590 bp FAdV specific primers. In orally infected groups, the higher FAdV copy numbers were seen in caecal tonsil at 5th dpi followed by bursa of Fabricius and liver. In intramuscularly infected birds, the higher FAdV copy numbers were seen in caecal tonsil at 7th dpi followed by bursa of Fabricius and liver and the FAdV copy numbers were seen up to 21 dpi in all the organs. The FAdV copy numbers were high in caecal tonsil and bursa of Fabricius in both the groups but the gross and histopathological lesions could not be observed throughout the study. Spleen and thymus showed moderate to minimum FAdV copy numbers but no major lesions could be noticed. Liver showed massive gross and histopathological lesions with constant FAdV load throughout the study.

OP_2.2 : DIGITAL INFRARED THERMAL IMAGING OF OCULAR REGION (BODY TEMPERATURE): A NOVEL NON-INVASIVE TECHNOLOGY TO MONITOR CALVING PROCESS IN MURRAH BUFFALOES (*BUBALUS BUBALIS*)

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Prediction of calving time is a key element in livestock farming. Approximately 50% death of the dam/calf loss which occurs due to dystocia could be prevented by giving timely correct obstetrical assistance. As the calving approaches there is distinguish temperature variation of the dairy animals. These changes can be used to predict approximate time of calving. The ocular region temperature as monitored by infrared thermal imaging is considered as an indicator of core body temperature of animal. Hence, in the present study infrared thermographic profile of ocular region associated with



calving process has been established and explored its possibility to monitor/ predict the calving process in the buffalo. Infrared thermal imaging of eye in 28 multiparous pregnant Murrah buffaloes were recorded at six hourly intervals for a period of 96 hours before the expected date of calving, at the time of calving and 24 hours post calving continuously. Blood samples were collected at 24 h interval from 72 h prior to calving, on the day of calving and up to 48 h post calving for the estimation of progesterone (P₄). The results of the current study revealed that there was a significant decrease in body (eye) temperature from 48 h prior to onset of calving with a ΔT of 0.56 °C. The residual body temperature also followed a similar trend. P₄ concentration decreased from 72 h prior to calving and differed significantly between days to calving and reached 0.69 ng/mL at the time of calving. Thermogenic effect of P₄ concentration in circulation could have been a plausible reason for decline in body temperature. It is concluded that body temperature variation in relation to calving as monitored by digital infrared thermal imaging could be a promising potential, non-invasive, non-contact method for prediction of calving in buffaloes based on associated thermal signatures.

OP_2.3 : EFFECT OF GROWTH HORMONE AT DIFFERENT TIME INTRAVALS ON DEVELOPMENT OF IN VITRO PREANTRAL FOLLICLES IN SHEEP

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The present study was conducted to investigate the influence of addition of growth hormone on in vitro development of preantral follicles (PFs) in sheep. Preantral follicles isolated from the ovarian cortical slices using micro dissection method were cultured for six days in bicarbonate buffered tissue culture medium 199B (TCM 199B) or in a standard culture medium supplemented with growth hormone (1 m IU/ml) at different points during the culture period. COCs isolated from the follicles at the end of six day culture in different treatments were subjected to in vitro maturation for additional 24h. Supplementation of growth hormone during first two days of the culture supported better proportion of preantral follicles exhibiting growth and better average increase in diameter of preantral follicles at the end of six day culture. Supplementation of growth hormone to TCM 199B culture medium in early stages followed by standard medium alone in later stages supports better development of PFs *in vitro*. Following supplementation with growth hormone for the first two days (0-2 days) culture of PFs in standard medium appears to be advantageous for the *in vitro* development of preantral follicles in sheep.



OP_2.4 : IN VITRO STUDY OF ACETYL-CoA CARBOXYLASE BETA (ACACB) GENE SILENCING IN CHICKEN MYOBLAST CELLS

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In the poultry industry, abdominal fat is a major problem constituting up to 20% of the total body fat, which accounts for 2-3% of bird's live weight. Fatty acid metabolism controlled by the Acetyl-CoA Carboxylase (ACC) enzyme, which has two isoforms, ACACA and ACACB, each playing a crucial role. ACACB regulates the quality of meat and eggs by influencing the β -oxidation of fatty acids in chickens. The RNAi method is employed for silencing ACACB gene expression. Five shRNA constructs were designed, and the most efficient shRNA molecule was identified. shRNA 1 and 5 exhibited higher knockdown efficiency of 67 and 69% respectively, while shRNA 2, 3, and 4 showed a lower knockdown percentage range of 25-39%. When compared with the control, ShRNA 1&5 demonstrated higher knockdown efficiency due to the lack of secondary structure in their antisense strand. Furthermore, the expression of immune response genes, such as IFNA, IFNB, and BLB1 in control and ACACB knockdown myoblast cells was studied. No significant difference was found in the expression of immune response genes between control and knockdown cells. Using these knockdown myoblast cells also tracked the expression of key fatty acid metabolism genes and found that the down-regulation of the ACACA, FASN, SCD, and CPT1 genes and the up-regulation of ELOVL2. The observed downregulation of ACACA, FASN, SCD genes indicates the suppression of fatty acid synthesis, while the up-regulation of ELOVL2 indicates the enhancement of long-chain fatty acids formation. CPT1 is a rate-limiting enzyme, and its down-regulation indicates the blocking of β -oxidation to balance fatty acid synthesis and oxidation due to fewer fats accumulation in the tissues. Additionally, fats were measured in terms of cholesterol and triglycerides in control and ACACB knockdown myoblast cells. A significant difference was observed, suggesting that the silencing method may be useful in the development of leaner chicken.

OP_2.5 : DEVELOPMENT OF CRISPR/CAS 12 BASED DIAGNOSTIC TEST FOR RAPID DETECTION OF AFRICAN SWINE FEVER (ASF) VIRUS

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A two-step CRISPR/Cas 12a based diagnostic test was developed for rapid detection of African swine fever virus in samples of porcine origin. The first phase of the test involves recombinase polymerase based isothermal amplification of ASFV genomic region, followed by a CRISPR/Cas 12a based lateral flow assay. The recombinase polymerase assay (RPA) was optimized using plasmid DNA containing gene of ASFV and specific primers. The RPA was optimized at various concentrations of



each forward and reverse primer at various temperature and duration. Reporter cleavage conditions for the detection assay was optimized using RPA amplicon, two sgRNAs, one for the sense sequence and one for the antisense sequence, a single-stranded DNA (ssDNA) reporter with appropriate labels and recombinant Cas12a enzyme. Lateral flow assay (LFA) was optimized for visualization using commercial strips coated with streptavidin and anti-IgG antibodies with an incubation time of 4 to 5 min. to detect cleavage events involving FAM and Biotin labels. The test was evaluated using DNA extracted from 115 known ASFV positive (n=79) and negative (n=36) field samples. WOA recommended real time qPCR assay was taken as the gold standard test. The sensitivity was estimated to be 97.47% and specificity of 83.33%. The developed CRISPR/Cas 12 based diagnostic test will be useful for rapid detection of ASFV genome in field samples at peripheral diagnostic laboratories.

**OP_2.6 : DOES ANTRAL FOLLICLE COUNT (AFC) AND SERUM AMH LEVELS
HAVE RELATION IN PAROUS IN ONGOLE CATTLE (*BOS INDICUS*) –
A PRELIMINARY STUDY**

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The current study was aimed to investigate the relationship between the Antral follicle count (AFC) and serum concentrations of Anti-mullerian hormone (AMH) in Ongole breed cattle. Eighty parous Ongole cows aged 5-20 years with one to eleven lactations were selected and both ovaries were subjected for ultrasound examination at random stage of estrus cycle to determine the antral follicles count and recorded the overall mean \pm SE number of follicles as 30.38 ± 1.58 (range 3 – 63). Simultaneously, blood samples were obtained to estimate the levels of circulating AMH. The average serum AMH concentration was found to be 1.105 ± 0.116 ng/mL (range 0.407 to 7.171 ng/mL). Across all the animals a substantial ($P < 0.01$) drop in AFC and AMH was noticed with advanced age and parity. To draw comparison between AFC and AMH levels, all the animals were assigned into three groups based on their AFC as G-I (AFC: >30) (n=35), G-II (AFC: 16-30) (n=29) and G-III (AFC: ≤ 15) (n=16) and the mean \pm SE AMH levels recorded were 1.298 ± 0.216 , 1.028 ± 0.163 and 0.824 ± 0.151 in group-I, II and III, respectively but there was no significant difference among the groups ($P < 0.05$). AFC and AMH were shown to be positively connected both within and between each of the three groups. It was concluded that, given the positive correlation between blood AMH concentrations and AFC, these two attributes may be used as reliable indicators to select an oocyte donor for OPU-IVF-ET programme.



OP_2.7 : ADVERSE DRUG INTERACTIONS IN CHLORPYRIFOS TOXICITY

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The indiscriminate use of Chlorpyrifos pesticide in agriculture has led to widespread contamination of human food chain, with effects ranging from endocrine disruption to transgenerational genotoxic effects. Identifying new mechanistic modalities of Chlorpyrifos toxicity and designing ways to alleviate its undesirable effects is of significant research importance. The present work aims to study the toxic effects of Chlorpyrifos in non-target subjects using Sprague-Dawley rats as model animals, and how these toxic effects are modulated when exposed subjects are treated for inflammatory conditions using Dexamethasone. Sprague-Dawley rats, aged 6-8 weeks were purchased from National Institute of Nutrition, Hyderabad. All animals were maintained in standard housing and dietary regimen. Groups of 8 rats were each exposed to three different doses of Chlorpyrifos alone (IIa, IIb & IIc @ 1, 2 & 4 mg/Kg BW, respectively), three different doses of Chlorpyrifos (1, 2 & 4 mg/Kg BW) combined with standard dose of dexamethasone @ 0.5 mg/Kg BW (Groups IIIa, IIIb & IIIc) and dexamethasone alone @ 0.5 mg/kg BW (Group IV). Another group of 8 rats that received placebo were used as control (Group I). Chlorpyrifos toxicity was studied using measurements for oxidative stress (TBARS), antioxidant defense (SOD, Catalase), cytotoxicity (histopathological score), genotoxicity (apoptotic ladder assay) and regulation of efflux transporter via P-gp expression (western blotting). Chlorpyrifos treatment alone induced oxidative stress evident by significant increase in TBARS levels, marked cytotoxicity and genotoxicity. It also increased expression of P-gp indicating defense response from the host, although not optimal to protect. Dexamethasone combined with chlorpyrifos further increased oxidative damage and cytotoxicity, which may be due to immunosuppressive nature of dexamethasone as well as its inhibitory effect on P-gp expression, which might have contributed to increased chlorpyrifos uptake. However, dexamethasone treatment significantly decreased genotoxicity. The present study therefore reveals a novel interaction between Dexamethasone and Chlorpyrifos in rats, which reveals a yet unknown risk of Chlorpyrifos toxicity in individuals already under corticosteroid treatment.



POSTER PRESENTATIONS

PP_2.1 : MOLECULAR CHARACTERIZATION, PHYLOGENETIC ANALYSIS AND VIRAL LOAD QUANTIFICATION OF FOWLPOX VIRUS IN CHICKEN

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Fowl pox disease is highly contagious, re-emerging and endemic viral disease in India. Fowl pox virus (FPV) belongs to the *genus Avipoxvirus* and family *Poxviridae*. In the present study, infected skin scab tissue samples were collected from the bird suspected for the disease and investigated for the presence of Fowl pox virus. Polymerase Chain Reaction (PCR) amplification in combination with quantification of viral load by qPCR, sequencing and phylogenetic analysis are expedient and effective methods for molecular characterization and understanding the evolution of the Fowl pox virus. The primers were designed for both conventional and Real-Time quantitative PCR (RT-qPCR) for detection, quantifying and molecular characterization. The presence of the virus was detected by conventional PCR using the p4b gene with specific primers of amplicon size 415 bp. The virus was isolated from chicken embryonated eggs and confirmed by conventional PCR. The Fowl pox viral load in the infected bird was found to be 7.42 ± 0.041 (Log Mean \pm SD). Further, sequencing and phylogenetic analysis revealed that the fowl pox virus responsible for this infection clustered with the sub-lineage genotype clade of fowl pox virus isolated from Germany, Austria, Australia, South Korea, China, USA, and New Zealand. Similarly, the isolates from Canada, Hungary, South Africa and Australia share 100% identity and form a separate cluster with other avian pox viruses. Our, results emphasise the necessity of ongoing surveillance and molecular characterization of circulating FPV. The scope of the present study includes further exploring the patterns of virus evolution and perspective development of a new FPV vaccine strain that would effectively control and eradicate diseases.

PP_2.2 : ASSOCIATION OF DOE'S VAGINAL MICROBIOTA WITH REPRODUCTIVE HORMONES USING 16S RRNA SEQUENCING

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The success of livestock production is centered on the production of milk and meat that critically depends on reproductive performance. A number of factors affect reproductive success, likely including the microbial populations in the reproductive tract during copulation and pregnancy. To compare Doe's vaginal microbial communities, vaginal swabs were obtained from Twenty-three Mehsana does at the Livestock Research Station, Kamdhenu University, Goat farm, at different reproductive stages and grouped based on the hormone concentration ie., Progesterone dominance



(>4ng/ml) and Estrogen dominance (>13pg/ml). Estrogen and Progesterone concentration was determined by ELISA. Vaginal swab and blood samples were collected at various reproductive stages. The DNA from the vaginal swab sample was isolated using Qiagen DNA mini kit. The V3-V4 region specific 16S rRNA for Bacteria was amplified using PCR. The amplicon library was constructed and sequenced through Illumina Miseq. Annotation was carried out through Qiime2 with RNA database SILVA with 98% identity. Statistical, functional and integrative analysis of microbiome data was performed using online web-based platform Microbiome Analyst. The alpha diversity was higher in the Progesterone dominance group compared to the estrogen dominance group ($p < 0.05$). The beta diversity revealed significant ($p = 0.001$) difference between the groups. The Progesterone dominant group had Firmicutes as dominant phylum while, the estrogen dominant group had Proteobacteria as major phylum. On pairwise comparison Progesterone and estrogen dominant sample groups significantly ($p < 0.05$) differed from each other at Phylum, Class, Order, Family and Genus level. This study identifies The changes in vaginal microbial communities based on hormonal patterns.

PP_2.3 : ISOLATION STUDIES OF LUMPY SKIN DISEASE IN BOVINES FROM ANDHRA PRADESH

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Lumpy skin disease (LSD) is a contagious infection and emerging disease of cattle and water buffaloes listed by OIE. It is an economically important disease-causing reproductive problems of temporary and permanent infertility or sterility in males and females and abortions in females apart from reduced weight gain, reduced milk production and mastitis. There is no LSDV specific homologous vaccine against disease but goat pox vaccine is being used due to cross protection within the *capripox* genus. Hence, the present work was aimed for isolation and characterization of LSDV in clinically suspected cases from outbreaks across the state.

A total of 31 blood samples and 31 skin scabs were collected during the period 2021-22 from both adult and young calves of suspected cases of Lumpy skin disease showing clinical signs. All the samples were positive for PCR amplifying P32 gene at genus level and amplifying species specific GPCR, RPO30 and Ankyrin repeat genes at species level respectively using species specific primers.

The skin scab samples which were positive on PCR at genus level (amplifying P32 gene) and species level (GPCR, RPO30 and ankyrin repeat genes) were attempted for virus isolation through embryonated chicken eggs of 10-11 days old. All the seven isolates showed hemorrhages on chorio-allantoic membrane at second passage level on 5th day of post inoculation whereas, the three isolates showed characteristic pock lesions on 5th day of post inoculation at 5th passage level. Out of these three isolates, two isolates were subjected for adaptation in primary lamb testicular cell cultures and CPE was observed with shrinkage, cell rounding and cell aggregation between 48-72 hours post infection at first blind passage level.

The virus isolates recovered from both embryonated chicken eggs and through primary testicular cell cultures were further confirmed by PCR.



TECHNICAL SESSION III
MOLECULAR DIAGNOSTICS AND
APPLICATIONS





LEAD PAPER-1

MOLECULAR DIAGNOSTICS- PROMISES AND POSSIBILITIES

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Molecular diagnostics (MDs) could be used to assess an individual's/animal's health at the molecular level, analysing specific genetic sequences encoded in deoxyribonucleic acid, ribonucleic acid, or the proteins that are expressed from these gene sequences. The growth and developments in the field of MDs is enormous, and over the past two decades, as MDs are considered to be the quick and best way of confirmatory diagnosis. The importance of MDs was really felt even by the common man during the recent CoViD pandemic, since MDs assumed a larger global role than ever before. The stakeholders of MDs are many and include, Farmers, Clinicians-for rapid diagnosis, Diagnostic laboratories, Manufacturers and Academicians/Researchers - for understanding the pathogenesis.

Promises of Molecular Diagnostics

In the current scenario, Molecular diagnostics (MDs) have multiple levels of applications in the order of Prenatal testing, Disease predisposition, Disease diagnosis, Treatment schedules, Control strategies and Recurrence monitoring. Current day MDs are designed and developed to answer many of the challenges associated with diagnosis and could be of use in

- ▶ Determining the presence or absence of nucleic acids (DNA/RNA) of pathogenic organisms
- ▶ Quantifying the microbial load in infected animals to assess efficacy of treatment or control measures
- ▶ Detecting minimal residual disease, post therapy
- ▶ Identifying mutations associated with genetic diseases
- ▶ The potential advantages of MDs include,
- ▶ Accurate early diagnostic methods.
- ▶ Provide more information about the disease to allow for effective treatment resulting in improved efficacy and survival.
- ▶ Accurate methods to screen cancer.
- ▶ Reduced side effects from unnecessary treatments.

Nevertheless, validation and high cost involved are considered as impediments related to Molecular diagnostics.



Possibilities of Molecular Diagnostics

Molecular diagnostics encompasses several specific areas of molecular biology like, Transcriptomics, Metagenomics, Metabolomics, Pharmacogenomics, etc.,

Transcriptomics:

It is the study of the complete set of RNA transcripts (Transcriptome) or otherwise the sum of all of its RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell. High-throughput methods like Next generation sequencing or Microarray analysis can be performed to for transcriptome analysis. Comparison of transcriptomes allows the identification of genes that are differentially expressed in distinct cell populations, or in response to different treatments. RNA sequence analysis will be useful in identifying disease-associated single nucleotide polymorphisms, allele-specific expression, and gene fusions which play a major role in understanding variants of pathogenic microbes. Transcriptomics also identifies genes and pathways which respond to, and counteract abiotic and biotic stresses including infection with pathogens.

Metagenomics:

Metagenomics is the direct genetic analysis of genomes contained with an environmental sample. It helps in understanding the composition of functional genes of microbial communities. Metagenomic data provide better information than the phylogenetic surveys and often depends on diversity of one gene, like 16S rRNA gene. Typical genome-based metagenomics approach involves, Environmental sampling and processing, Sequencing, Assembling, Binning, Annotation and Analysis. Metagenomics is a best tool to address the fundamental question of microbial ecology, evolution and diversity and to evolve and test new hypotheses in disease diagnosis. This will go a long way in developing sensitive and specific molecular diagnostics for the existing and emerging pathogens.

Metabolomics:

It is the study of substrates and products of metabolism, which are influenced by both genetic and environmental factors. Metabolomics is a powerful approach since metabolites and their concentrations, will directly reflect the underlying biochemical activity and state of tissues or cells. In this context, metabolomics could very well represent the molecular phenotype. It could enable precise characterization of metabolic derangements related to a disease, discovery of new therapeutic agents, and discovery of biomarkers for disease diagnosis.

Pharmacogenomics:

Helps in understanding how genes impact an individual's response to medications, identifying medication responders and non-responders to avoid specific adverse effects and also to optimize drug dose. Pharmacogenomics will be an important tool in the field of 'Precision Medicine' for designing the treatment for each human or animal. It could give leads on whether the individual or group get a bad or expected reaction to the drug/treatment. This information will help the clinician in providing the best possible treatment.



Molecular Diagnostics in Oncology:

In cancer medicine, several molecular based assays are being used. This includes, testing for heredity cancer syndromes for identifying persons at risk and for systemic treatment. These techniques can be used to identify specific tumour phenotype and even residual tumour fragments. Molecular diagnosis of heredity cancer is possible by the identification of germ-line mutations in corresponding genes. Expression or over expression studies of molecular markers like Estrogen receptor, Epidermal growth factor receptor, etc., are useful molecular approaches in cancer detection.

Whatever developments that we are experiencing in MDs is only a beginning and tip of an iceberg. Considering the versatile utilities, the concerns associated with the widespread use of MDs need to be addressed and removed. To conclude, Molecular diagnostics will soon provide a golden path towards personalized health care, precision medicine and predictive health care.



LEAD PAPER-2

**MOLECULAR CHARACTERIZATION OF *CLOSTRIDOIDES DIFFICILE* ISOLATES
FROM ANIMALS WITH SPECIAL REFERENCE TO TYPING BY PCR-RIBOTYPING
AND PFGE**

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Clostridoides difficile (previously known as *Clostridium difficile*) is known as one of the important pathogens emerging as a common cause of nosocomial diarrhoea in humans. *Clostridoides difficile* infection (CDI) has also been associated with pseudomembranous colitis. In recent times, a remarkable rise in the rate of community associated CDI has been reported in human, the source of which is not clearly known. The pathogenic strains of *C. difficile* are found to produce two major toxins: toxin A, an enterotoxin, and toxin B, a cytotoxin (Voth and Ballad, 2005) encoded by *tcdA* and *tcdB* genes, respectively. A binary toxin, called *C. difficile* transferase (CDT), has also been reported to be an important virulence factor encoded by two separate genes, *cdtA* and *cdtB* (Stubbs *et al.*, 2000). The strains capable of producing CDT are more common in animals and are reportedly associated with more severe disease conditions in humans (Rupnik, 2007). CDT-producing strains have also been found to cause enteric diseases in animals (Songer and Anderson, 2006; Hammitt *et al.*, 2008; Silva *et al.*, 2013). Recovery of similar ribotypes of the pathogen from humans and domestic animals suggested that animals may possibly act as reservoirs of *C. difficile* for community-associated infections in humans (Arroyo *et al.*, 2005; Rupnik, 2007). In our study, we attempted to assess the prevalence and the molecular diversity of *C. difficile* in animals (cattle, sheep, goat, pig, dog, poultry, wild animals, and birds) in the North-Eastern region of India, and their possible genetic relatedness with the isolates from human patients.

In our study, *C. difficile* could be isolated from 18 (9.04%) of 199 human stool samples, 9 (4.89%) of 184 faecal samples from cattle, 29 (12.44%) of 233 samples from pig, 18 (14.75%) of 122 samples from dog and 23 (13.94%) 165 samples from poultry. The isolates were confirmed by detection of *C. difficile* specific *gluD* (glutamate dehydrogenase) and *tpi* (triose phosphate isomerase) genes. However, none of the samples from cow, sheep, goat, local chicken and wild animals and birds were found positive for *C. difficile*. The isolates were subjected to PCR for detection of toxin genes (*tcdA*, *tcdB* and binary toxins), toxinotyping, PCR ribotyping and pulsed-field gel electrophoresis (PFGE) using restriction endonuclease *Sma*I. The toxigenic isolates were also tested by cytotoxicity assay on Vero and HeLa cell monolayers.

Toxigenic *C. difficile* isolates could be obtained from human (8), cattle (1), pig (17), dog (12), and poultry (6). All the toxigenic isolates from cattle, dog and poultry carried both *tcdA*, and *tcdB*



(A⁺B⁺) while majority of the human and pig isolates had variant toxin genes (A⁻B⁺). The A⁻B⁺ isolates from pigs and one isolate each of A⁻B⁺ and A⁻B⁻ variants from human possessed binary toxin genes (*cdtA* and *cdtB*). Human toxigenic isolates belonged to two new toxinotypes proposed to be designated as XXXII and XXXIII. The A⁺B⁺ isolates from cattle, pig, dog, and poultry belonged to toxinotype 0 except for one isolate from poultry, which belonged to a new toxinotype proposed to be designated as XXXIV. Interestingly, the pig A⁻B⁺ isolates resembled with the human toxinotype XXXII. Human A⁻B⁺ and A⁺B⁺ isolates belonged to three ribotypes (045, 126 and ACD 019) and the A⁻B⁻ isolates belonged to seven ribotypes (SLO 002, SLO 028, ACD 017, ACD 018, ACD 020, ACD 021, and ACD 022). Cattle A⁻B⁻ isolates belonged to five ribotypes (014, SLO 138, ACD 007, ACD 008 and ACD 009) and A⁺B⁺ isolate belonged to ACD 010. Pig A⁻B⁺ isolates belonged to ribotype 126, while the lone A⁺B⁺ isolate belonged to ribotype ACD 011. On the other hand, the non-toxigenic pig isolates could be grouped into six ribotypes [046, 087, 084(CE), SLO 123, SLO 138 and SLO 182]. Dog A⁺B⁺ isolates revealed five ribotypes (012, 014, 046, ACD 005 and ACD 006), 012 being predominant among them, while the A⁻B⁻ isolates revealed five ribotypes (010, ACD 001, ACD 002, ACD 003, ACD 004). Six A⁺B⁺ avian isolates belonged to six different ribotypes (014, 087, SLO 134, SLO 160, ACD 012, ACD 014) while the A⁻B⁻ isolates belonged to seven different ribotypes [032(CE), 084(CE), SLO 002, SLO 131, ACD 013, ACD 015, ACD 016] with the predominance of ribotype 032(CE).

On pulso-typing, the toxigenic isolates from human, cattle, pig, dog, and poultry showed 3, 1, 2, 5, and 6 different profiles, respectively, which corresponded to the number of PCR-ribotypes identified among these isolates. All the toxigenic isolates showed cytopathic effect on Vero and Hela cell monolayers at 1:100 dilutions of cell free culture supernatants within 18-20 hrs of incubation. The toxins were also detected in the cell free culture supernatant of the isolates. Pulsed field gel electrophoresis analysis could not differentiate the isolates within a ribotype or toxinotype of *C. difficile* indicating that they belonged to the same clone. In conclusion, cattle, pig, and poultry also harbours toxigenic strains of *C. difficile* belonging to different ribotypes that are encountered in humans, and hence, may be a source of cross-infection between human and domestic animals.

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ORAL PRESENTATIONS

OP_3.1 : MOLECULAR DETECTION OF *MYCOBACTERIUM AVIUM* SUBSPECIES PARATUBERCULOSIS IN SHEEP MILK USING IS900 POLYMERASE CHAIN REACTION

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Mycobacterium avium subsp. paratuberculosis (MAP) is the etiological agent responsible for Johne's disease (JD) in ruminants, presenting significant economic implications for livestock industries. The potential zoonotic link between MAP and Crohn's disease in humans underscores the importance of effective detection methods. This study aimed to assess the efficacy of utilizing the IS900 polymerase chain reaction (PCR) for the molecular detection of MAP in sheep milk, evaluating milk as a non-invasive diagnostic matrix. Fifteen milk samples were obtained from lactating ewes, including seven samples from confirmed JD-positive sheep. The IS900 PCR assay demonstrated a sensitivity of 85.71%, successfully detecting MAP in six out of the seven known positive sheep milk samples. All remaining samples tested negative for MAP. These results underscore the potential of milk as a non-invasive diagnostic specimen for MAP infections in sheep. The study concludes that IS900 PCR is a reliable method for the sensitive detection of MAP in sheep milk, offering a promising tool for early diagnosis. The non-invasive nature of milk sampling provides a practical approach for screening and confirmation of MAP infections in sheep populations. This research contributes valuable insights into the development of effective diagnostic strategies, aiding in the management and control of Johne's disease in sheep, with potential implications for broader epidemiological studies. Further investigations are warranted to optimize and validate this approach across diverse sheep populations and settings.

OP_3.2 : COMPARATIVE MOLECULAR CHARACTERIZATION OF NON-CEREBRAL AND CEREBRAL COENUROSIS IN GOATS OF ASSAM, INDIA

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Taenia multiceps is an intestinal cestode parasite of dog whose larval or metacestodal stages known as *Coenurus gaigeri* infects the subcutaneous tissues or muscles causing non cerebral Coenurosis in goat. Another metacestode of *Taenia multiceps* that often invades the brain and spinal



cord of goat causing Cerebral Coenurosis. Both these metacestodes were morphologically similar to each other. In order to resolve the questionable relationship between these two metacestodes of *Taenia multiceps*, the present study was undertaken for molecular evaluation of both the larval forms of *Taenia multiceps* using the mitochondrial *COI* and *NAD1* genes as molecular markers for characterization. Polymerase chain reaction (PCR) was done using the molecular genetic markers, *COI* and *NAD1* of *Taenia multiceps* and the mitochondrial DNA sequences were subjected to phylogenetic analysis. The obtained sequences were submitted in NCBI GeneBank and the accession numbers (MZ713179, MZ713180, MZ713181 and MZ713182) were obtained for each of the isolates. The phylogenetic analysis of the mitochondrial DNA (*COI* and *NAD1*) sequences suggested that both the non-cerebral and cerebral metacestodes *i.e.* *Coenurus gaigeri* and *Coenurus cerebralis* respectively showed similar molecular characteristics with the available sequences of *Taenia multiceps* in the GeneBank and were monophyletic species. It can be concluded that *Taenia multiceps* is the single valid species and the origin of both non-cerebral (*Coenurus gaigeri*) and cerebral (*Coenurus cerebralis*) coenurus cysts in goats of Assam, India. The present molecular epidemiological data based on *COI* and *NAD1* sequences of both non-cerebral and cerebral coenurus cysts in goats was reported for the first time from India.

OP_3.3 : STUDY OF HISTOPATHOLOGICAL CHANGES AND MOLECULAR DETECTION OF ILTV FROM RESPIRATORY INFECTIONS OF POULTRY IN ANDHRA PRADESH

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The respiratory infections causing significant economic losses to poultry industry. Among the respiratory pathogens IBV, ILTV and MYCOPLASMA are the common pathogens causing severe respiratory infections and mortality. The control of these infections by vaccinations but ILT vaccines are not available in Indian markets. In spite of regular vaccinations of IBV and MYCOPLASMA still OBRs were recorded. To know the other etiological agent, ILTV, this study was designed with aim histopathological changes and molecular detection of ILTV in common respiratory infections of Poultry in AP. The 228 pooled Cloacal swabs, 228 pooled nasal swabs, 228 pooled tracheal swabs samples from the ailing birds and 152 pooled tracheal tissues, 152 pooled lung tissues and 76 pooled oviduct samples from the dead birds were collected from the suspected 19 poultry farms in A.P. and were labelled farm wise and specimen wise. The extracted DNA was screened for ILTV by targeting ICP4 gene and produced 635 bp PCR product in two farm samples. In ILT affected trachea revealed epithelial cells desquamation, intranuclear inclusions and syncytial cells. ILTV field isolate showed 94.28 to 100 per cent similarity with vaccine isolates and GenBank isolates. The variations of



nucleotides and amino acids observed by comparing the vaccine isolates and GenBank isolates and showed nucleotide variations at G56C and G111T positions in case of TSSVVU ILT field isolate and amino acid variations at C18S and insertion H35P in case of TSSVVU ILT field. The TSSVVU ILT field isolate was closely related with USA strain and the vaccine ILT isolate was closely related with Italian strain.

OP_3.4 : EPIDEMIOLOGY AND DIAGNOSIS OF LUMPY SKIN DISEASE IN KARNATAKA

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The Lumpy skin disease (LSD) a notifiable, exotic, vector borne transboundary disease caused by LSD virus affects cattle and buffaloes. The current study was conducted to know the incidence of LSD, to isolate and characterize LSDV and to record the genetic relatedness of LSDV isolates. Disease is characterized by fever, oro-nasal, lachrymal secretions, enlarged lymph nodes, anorexia, dysgalactia, depression, disinclination to move. The disease is transmitted mechanically by blood sucking arthropod vectors, during rainy and summer seasons which coincides with peak vector density. LSD was reported from all 30 districts of Karnataka. Highest morbidity and CFR were recorded in Belgaum division. Vectors implicated were; flies (46%), ticks (13%), flies and ticks (34%) and others (7%). Viral DNA was isolated from vectors undermining their role in transmission. Both crossbred and native zebu cattle were susceptible. The incidence was more in 2 to 4 years old animals. Common communal points had only 4 % influence on the incidence of LSD. Incidence was more in pre-existing animals (87%) than newly introduced ones (13%). Outbreaks were more in rainy and summer seasons. Decrease in milk yield in lactating animals and draught capacity in bullocks was noticed. Of 372 samples subjected for partial P32 gene-based PCR, 299 clinical samples and all 35 necropsy samples found positive, with an overall positivity rate of 89.78%. Among the clinical materials, Nasal /ocular / rectal swabs were preferred for the PCR detection of LSDV-DNA. Virus isolated using primary cell culture and cell lines, identified by PCR and Transmission electron microscopy. P32 gene sequences were submitted to GenBank. Phylogenetic analysis revealed isolates from different geographical regions having 100% nucleotide homology. Dot blot assay was standardized for serological diagnosis of LSD, 137 sera samples subjected to dot blot assay and only nine animals' sera were negative with a sensitivity of 93.4%, which were otherwise positive by PCR.



OP_3.5 : DEVELOPMENT OF MULTIPLEX PCR AND MOLECULAR CHARACTERIZATION ANALYSIS OF PREVALENT VIRAL PATHOGENS OF RDC IN NAGPUR

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This study aimed to developed multiplex PCR for rapid diagnosis of prevalent viral respiratory disease complex and their Molecular characterization analysis. Total 50 samples were collected from the different parts of Nagpur region and stored at -80°C till the processing. Multiplex PCR was developed and standardized for rapid detection of viral pathogens i.e. low pathogenic avian influenza (LPAI), Newcastle disease virus (NDV), Infectious bronchitis virus (IBV), Infectious Laryngotracheitis virus (ILTV) and singleplex PCR for Fowl adenovirus (FAdV) using 3-4 sets of primers. The mRT-PCR DNA products were visualized by agarose gel electrophoresis and consisted of DNA fragments of 244 bp for M gene of LPAI, 535 bp for F gene of NDV, 402 bp for S1 gene of IBV, 588 bp for P32 gene of ILTV, and singleplex 856 bp for Hexon gene of FAdV. Samples was further processed for Molecular characterization of different respiratory pathogens by sequencing. Results revealed that 3/50 samples positive for LPAI, 7/50 positive for NDV, 2/50 positive for IBV, 2/50 positive for ILTV and 4/50 positive by FAdV. Two isolates each were found positive for NDV + FAdV and NDV+IBV & three isolates were found positive for ILTV + IBV combinations using PCR assay. The sensitivity and specificity of mRT-PCR was determined and the test was found to be sensitive and specific for the detection of NDV and other poultry respiratory pathogens. Phylogenetic analysis of the 15 positive isolates was carried out. Phylogenetic analysis revealed that based on F gene of NDV strain belongs to XIII of class II, S1 sequence of IBV belong to G1 24 lineage., this strain is a nephropathic strain mainly damaging kidney. P32 gene of ILTV strain related to the CEO vaccine. FAdV strain belong to serogroup D. A mRT-PCR that can rapidly differentiate between the four pathogens will be very important for the control of disease transmission in poultry and in humans, along with the identification of four of the most common respiratory pathogens often seen as mixed infections in poultry, and hence economic losses will be reduced in poultry. The complete S1 gene data from these isolates indicate that IBV has consistently evolved through genetic recombination or mutation, more likely changing the viral pathogenicity and leading to larger outbreaks in chick populations, in Nagpur.



OP_3.6 : OUTBREAK OF NOVEL PARAPOX VIRUS INFECTION AMONG BUFFALO CALVES IN AN ORGANIZED FARM

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Parapoxviruses (PPVs) are contagious diseases of ruminants. The members of the genus Parapoxvirus includes Orf virus (OrfV), Bovine papular stomatitis virus (BPSV), Pseudocowpox virus (PCPV), Red deerpox virus, and Grey sealpox virus (GSEPV). Most of the parapox viruses except GSEPV and red deerpox virus, are zoonotic. PPV genomes are usually 130–140 kbp in size. This study reports the outbreak of novel parapox virus in buffalo calves that had happened during November 2022 to January 2023. The organized farm consists of 45 buffalo calves. 15 calves died over a period of 3 months. The calves were presented with papular growth / ulcers on gums, erosion on tongue with enteritis. Recovered calf showed alopecia. Post mortem examination revealed papular lesions around gums, ulcers on hard palate, abomasum and intestine; and enlargement of mesenteric lymphnode. Oral swabs, tissues, blood, serum and faecal samples were collected for the diagnosis. The samples were sent to ICFMD, Bhubaneswar and NIHSAD, Bhopal for FMD, BVD and LSD diagnosis. The samples were found negative for FMD, BVD, LSD. PCR was carried out at our lab for the diagnosis of buffalopox and Orf targeting *HA* gene and *GIF* gene, respectively. PCR was also carried out using parapoxvirus genus specific PCR primers targeting *B2L* gene of parapox virus which yielded 574 bp PCR product. The PCR product was gel purified and sequenced by Sanger sequencing method. Sequences were aligned and blasted in NCBI blast. The study sequence had high similarity with parapox sequence from Iran and has less similarity with *B2L* gene sequence of Orf, bovine papular stomatitis and pseudo cowpox viruses.

OP_3.7 : ISOLATION AND MOLECULAR CHARACTERIZATION OF LUMPY SKIN DISEASE VIRUS FROM TAMIL NADU, INDIA

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Lumpy skin disease (LSD) caused by LSD virus is a WOAHP notifiable, high-impact, transboundary poxviral disease of bovines. Since its first official report in India from 2019, several cases have been reported from many states including Tamil Nadu, a Southern state of India. The present study deals with isolation and molecular characterization of LSDV from Tamil Nadu during the period August 2020 to July 2022. Among the samples tested, 93.8 per cent of scabs (n=76) and 7.4% of unclotted blood (n=2) were found to be positive by PCR. LSDV was isolated in embryonated chicken



eggs (ECE) and BHK 21 cells and was characterized based on *P32*, *RPO30* and *GPCR* genes. The phylogenetic analysis revealed that Tamil Nadu isolates from India are closely related to other Indian strains, Kenyan strains and strains from neighboring countries such as Bangladesh, Nepal and Myanmar confirming the common exotic source for the transboundary spread across borders. The presence of unique signature of amino acid (aa) at specific positions (A11, T12, T34, S99 and P199) in the *GPCR* sequence confirmed the identity of LSDV. A twelve nucleotide (nt₉₄₋₁₀₅) insertion and corresponding aa (TILS) at 30-33 position was found in *GPCR* sequence and characteristic amino acid proline at 98 position (P98) in the *RPO30* gene sequence of our isolates was similar to strains from Bangladesh, Nepal and Myanmar. Further, dissimilarity of our isolates from Neethling like vaccine strains confirms the circulation of virulent field strains responsible for the outbreaks.

OP_3.8 : A NOVEL MULTIPLEX PCR ASSAY FOR DETECTION AND DIFFERENTIATION OF *MYCOBACTERIUM ORYGIS*

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Mycobacterium orygis a new member of MTBC has been reported to be the major cause of animal tuberculosis in South East Asia. Since the organism is closely related with *M. bovis*, a test that can clearly discriminate *M. orygis* is essential. Whole genome analysis indicated that certain genomic regions are absent in *M. orygis* while present in other MTBC members and vice versa. Hence, a multiplex PCR primer targeting different regions RD9, RD315, RDbovis, RD307 was designed. The primers are designed based on the specific regions that are absent which is the novelty of the assay. *M. tuberculosis* will have amplification at 446, 162, 359, 600 base pairs, *M. bovis* will have amplification at 162 and 600 bp while *M. orygis* will have amplification at 359 and 600 bp. The multiplex PCR was tested with the DNA from standard *M. tuberculosis* strain and 11 whole genome characterized *M. orygis* strains. The specificity of the assay was tested with *M. paratuberculosis* DNA. Similarly, DNA from tissue samples suspected for tuberculosis in buffalo and deer were also tested which showed amplification corresponding to *M. orygis*. The assay was also tested with 50 *M. tuberculosis* suspected DNA samples isolated from human. The assay could clearly detect *M. tuberculosis* and *M. orygis* while more *M. bovis* DNA is required for its optimization and discrimination. Hence the developed multiplex PCR can be used for detection and differentiation of *M. orygis* from tuberculosis suspected sample.



**OP_3.9 : MOLECULAR ANALYSIS OF CLASSICAL SWINE FEVER VIRUS
ASSOCIATED FIELD INFECTIONS IN TAMIL NADU EVIDENCES NOVEL CSFV
SUBGENOTYPE**

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Classical swine fever (CSF) is an endemic and major infection of Indian swine husbandry, contributing to great economic losses. Molecular detection and genotyping of CSF virus directly from field samples has great application in disease monitoring and control measures. A total of 57 porcine postmortem tissues (lymph nodes, spleens, livers, lungs, and kidneys) were collected from pigs suspected of systemic infections with live attenuated CSF vaccination history from different regions of Tamil Nadu. An NS5B gene specific PCR screening confirmed CSFV infection in 7% (4/57) of samples with a specific amplicon of 449 bp. Two PCR positive samples, TNI-4 and CHNL-2 sequenced and aligned contig sequences were subjected to BLAST homology searches and revealed that TNI-4 shared 99% sequence identity with Indian CSFV sequences of genotype 1 and CHNL2 shared 98% sequence identity with Indian CSFV sequences of genotype 2. Phylogenetic analysis of the TNI-4 and CHNL-2 sequences obtained in this study along with 38 published CSFV genotypes consisting of new genotypes and subgenotypes through the Maximum Likelihood tree (MLT) method in MEGA 11 revealed that TNI-4 clustered together with 1.7 subgenotypes and CHNL-2 clustered together with 2.2 subgenotypes. TNI-4 and CHNL-2 partial NS5B gene sequences obtained in this study deposited in the GenBank database under accession numbers of MW822568 and MW822569 respectively. The study is the first to report CSF infections associated with the 1.7 subgenotype in Tamil Nadu, southern India. It is possible that vaccination could affect the genetic diversity of the CSFV through recombination and point mutations for immune evasion.

**OP_3.10 : MOLECULAR AND PATHOLOGICAL EVIDENCE OF DUCK VIRAL
ENTERITIS IN MANILA DUCK (*CAIRINA MOSCHATA*); FIRST REPORT IN
SOUTHERN INDIA**

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This study documents the foremost incidence of duck viral enteritis (DVE) outbreak in free-range flock of 250 Manila ducks from Tamil Nadu, southern India. There was sudden 20% (n=50) mortality in duck flock with clinical findings of greenish and or blood strained diarrhea, anorexia,



ocular discharge, in-coordination and death. Postmortem examination of random 10 carcasses revealed blood in the body cavities, haemorrhages in heart, airsacs, proventriculus, liver and spleen, enlargement of kidney, spleen and liver with necrotic foci. Histopathological examination evidenced congestion and haemorrhages in proventriculus, heart, airsacs. Liver exhibited diffused congestion and hepatic steatosis. Spleen evidenced congestion, haemorrhages, and multifocal necrotic areas. Small intestine showed blunting and fusion of villi with mononuclear cells infiltration. Screening of pooled viscera evidenced no specific bacterial etiology and negative for NDV and DHAV genomes. PCR screening for DVEV genome targeting partial UL-31 and polymerase gene amplified 446 bp specific amplicon in all the 10 carcasses examined. Sequencing and alignment of PCR amplicon from one sample yielded 373 nucleotides encoding 94 amino acids and gene sequence submitted to the GenBank under the accession number of OR725707. BLAST homology search showed 100% sequence identity with published *Anatid alphaherpesvirus 1* genomes available in GenBank. Phylogenetic analysis revealed specific grouping along with published DVEV genomes of India, Bangladesh, China, USA and Germany. Further, deduced amino acid comparison with established DVEV AA sequences also showed 100% match to the published DVEV sequences.

OP_3.11 : OCCURRENCE OF CLASSICAL SWINE FEVER IN AN ORGANIZED FARM OF MADURAI, TAMIL NADU.

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Classical swine fever (CSF) is a highly contagious viral disease of swine causing huge economic losses due to heavy mortality and reproductive problems. The present study was a suspected outbreak of swine fever among cross breed pigs reared under organized farm conditions. Thirty three out of 220 pigs of all the age group died within two to three days period. History revealed that these animals were not vaccinated against CSF. The ailing animals showed clinical signs such as fever, staggering gait, huddling, conjunctivitis, severe respiratory distress and purpura of chest and abdominal region. Blood samples were subjected to reverse transcriptase polymerization chain reaction (RT-PCR) to confirm the classical swine fever virus genes of 5'UTR and E2. Both the genes were found positive in blood samples from ailing animals.

OP_3.12 : INCIDENCE OF PORCINE CIRCOVIRUS-2D GENOTYPE IN THE INDIGENOUS PIGS UNDER EXTENSIVE SYSTEM OF MANAGEMENT IN TAMIL NADU

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Porcine circovirus-2 (PCV2) is a widespread virus and presents sub-clinically in most of the swine herd. The present study aimed to investigate the causative agent of mortality in pigs reared under extensive system of management at Karur in the month of April'2022. The death was reported to be



acute in very young pigs. Samples like liver, lung, lymph node, heart swabs were collected from the necropsied cases in sterile container for molecular detection of Porcine circovirus and Classical Swine fever by Polymerase chain reaction. The samples were found to be negative for classical swine fever virus by RT PCR and found to be positive for porcine circovirus 2 by amplifying the ORF1 gene of PCV2 by using specific primers. For further confirmation, sequencing of PCR product of ORF1 gene of PCV2 was carried out. The sequences were closely clustered with previously reported PCV2 sequences available in the NCBI database. The sequences of ORF1 gene of PCV2 showed 100 % similarity with the other PCV2 sequences of various countries. For genotyping of PCR 2, PCR followed by sequencing of the ORF2 gene of PCV2 was carried out using specific primers. The ORF2 gene sequences of PCV2 showed more than 99 % similarity with 2d genotype of porcine circovirus sequences of various countries. The present study reveals the PCV2d genotype circulates in the indigenous pigs.

**OP_3.13 : MOLECULAR REPORT OF *HAEMOPROTEUS SP.*,
IN A RESCUED RAT SNAKE (*PTYAS MUCOSA*)**

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A female rescued rat snake (*Ptyas mucosa*) was presented with a history of dullness and reluctant to move. On clinical examination by manual restraining, ticks were present all over the body surface. Ticks and the faecal sample were collected for parasitological examination. Blood samples were collected from the ventral tail vein for haematological and serum biochemical analysis. Blood smear examination revealed intra-cytoplasmic gamonts of *Haemoproteus* sp. in the nucleated RBCs. The blood protozoan was molecularly confirmed and characterized by the ribosomal RNA (rRNA) genome of *Haemoproteus* sp., which was amplified using a genus-specific primer through polymerase chain reaction (PCR). The resulting amplicon, measuring 523 base pairs, served as a distinctive molecular marker for the presence of *Haemoproteus* sp. in the blood stream of the snake. Remarkably, this study represents, to the best of our knowledge, the inaugural documentation of a rat snake with a confirmed *Haemoproteus* sp. infection, strengthened by the utilization of molecular techniques. It is possibly first of its kind report and appeared to be no previous published reports on vector-borne blood protozoan parasites along with molecular confirmation using PCR in a rat snake.



**OP_3.14 : MOLECULAR DETECTION AND CONFIRMATION OF SHEEP ASSOCIATED
MALIGNANT CATARRHAL FEVER(SA-MCF) IN BUFFALO AND CATTLE FROM
NORTHWESTERN ZONE OF TAMIL NADU**

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Malignant catarrhal fever (MCF) is an acute, generalized and usually fatal disease of cattle and many species of *Artiodactyla*. Ovine gamma herpesvirus-2 (OvHV-2) is prevalent in domestic sheep as a subclinical infection and is the cause of sporadic MCF in species belonging to subfamily *Bovinae* and the virus is excreted only by the natural host, domestic sheep. The present study describes the occurrence of florid cases of MCF characterised by high fever, bilateral corneal opacity, profuse catarrhal discharges from the eye and nares, necrosis of the muzzle and erosion of the buccal epithelium in three buffaloes and two cattle. DNA was extracted from the blood samples collected from the ailing animals and subjected to hemi-nested PCR to amplify tegument gene using WOAHP recommended primers. The preliminary screening using the primary and secondary amplification with the respective primers at our lab yielded expected 422 bp and 238 bp amplicons specific for the tegument gene in all the five cases. The blood and DNA samples collected from the above animals have been submitted to NISHAD, Bhopal to distinguish OvHV-2 from Alcelaphine herpes virus-1 (AIHV-1) and were confirmed as OvHV-2. Further, the blood samples collected from the sheep reared in the infected farms to conclude the source of infection for the large ruminants revealed the presence of OvHV-2 in the domestic sheep as a subclinical infection. This investigation confirms the sporadic prevalence of sheep associated Malignant Catarrhal Fever among buffalo and cattle of North-western zone of Tamil Nadu.

**OP_3.15 : MOLECULAR DETECTION OF *MYCOPLASMA MYCOIDES* CLUSTER
ORGANISMS IN RESPIRATORY INFECTIONS OF GOATS IN ANDHRA PRADESH**

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The present study aimed at Molecular detection of *Mycoplasma mycoides* cluster organisms in respiratory infections of Goats. A total of 93 samples, including 56 nasal swabs, 33 lung tissues, and 4 pleural fluids from goats displaying respiratory symptoms, were collected for analysis. Among these samples, 52 (55.91%) goat samples tested positive for the *Mycoplasma* genus, producing a product of 280 bp in size. Specifically, only 6 goat lung tissues and 1 goat pleural fluid were found to be positive for *Mycoplasma mycoides* cluster. Upon further screening of seven positive samples within the



Mycoplasma mycoides cluster, 5 (5.37%) exhibited positivity for *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*), 2 (2.15%) for *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*), and 2 (2.15%) for *Mycoplasma mycoides capri* (*Mmc*) in goats. The respective product sizes were 316 bp, 192 bp, and 194 bp. The characteristic finding in this study is the identification of mixed infections involving *Mccp* with both *Mcc* and *Mmc* in various animals. This study marks the first reported instance of detecting *Mcc* in lung tissues in India and highlights the occurrence of mixed infections with *Mccp*. Furthermore, the gene sequences targeting 16S rRNA of *Mcc* were submitted to the Gene Bank, representing the first submission of such sequences from India.

POSTER PRESENTATIONS

PP_3.1 : DETECTION OF HAEMOPROTOZOA AND HAEMORICKETTSIAL ORGANISMS FROM NATURALLY INFECTED DOGS IN SOUTH INDIA USING MULTIPLEX PCR ASSAY

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The present study was carried out for the simultaneous detection of haemoprotozoa (*Hepatozoon canis*, and *Babesia* spp.) and haemorickettsial (*Ehrlichia canis*) organisms from naturally infected dogs in Andhra Pradesh, south India by multiplex PCR utilizing a total of 442 dogs blood samples. Blood smear examination could detect infection with either of these tick-borne pathogens in 21.27% of dogs, while multiplex PCR assay revealed 43.44% positivity indicating the higher sensitivity of the multiplex PCR to detect these pathogens during natural infections. The parasite specific multiplex PCR amplified 16S rRNA gene of *Babesia* spp. (619 bp), *H. canis* (737 bp) and VirB9 gene of *E. canis* (380 bp), without any non-specific amplification. DNA sequences of the multiplex PCR amplicons were subjected to BLASTn analysis and confirmed as *Babesia* spp., *E. canis* and *H. canis*. Infection with single haemoparasite species (30.77%) was frequent ($P < 0.01$) in infected dogs than co-infection (12.67%) with more than one haemoparasite. Number of dogs infected with *Babesia* spp. was more than dogs infected with *E. canis* and *H. canis*. Number of co-infections with *Babesia* spp. and *E. canis*, were more frequent. The living condition of the dogs (kennel dogs, stray dogs), age (young), gender (female), breed (mongrels), medium (urban dogs), and tick infestation were identified as risk factors for infection with these parasites by binary logistic regression models. The multiplex PCR assay could simultaneously detect natural co-infections in dogs emphasizing the need of the assay in epidemiological studies to reveal the true pattern of pathogens and selection of pathogen specific treatment protocols.



PP_3.2 : PREVALENCE OF PASTEURELLA MULTOCIDA TYPE A IN ORGANIZED TURKEY FARMS OF TAMIL NADU

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Pasteurella multocida type A is the etiologic agent of fowl cholera, a highly contagious and fatal disease of chickens and turkey. The present research work was performed for the isolation, identification and molecular detection of *P. multocida* Type A from turkeys. Samples were collected from organized turkey farms of Tamil Nadu. The *Pasteurella multocida* from the suspected samples were isolated, identified and characterized based on their colony morphology, staining, cultural, biochemical characters, histopathological study and Polymerase Chain Reaction (PCR) and sequencing. The *P. multocida* organism was isolated from 17 % (N = 17/100) samples. The organisms were gram negative, non-spore forming rod, non-motile, occurring singly or pairs in Gram staining, bipolar shaped organisms were observed in Leishman's stain. All the isolates were found positive for oxidase and catalase tests, produced indole, urease negative and negative for nitrate reduction and fermented glucose, mannitol and sucrose. Whitish necrotic foci in liver and congestion with hemorrhages in heart, lung, spleen, kidney, blood clots were found in the pericardium and a tear was visible in the left ventricular free wall. Vegetative aortic valvular lesions were observed on necropsy. In type specific PCR reaction, the organisms were confirmed as *P. multocida* Type A and sequence analysis shows 99% homology with other isolates. In conclusion, *P. multocida* type A is prevalent among turkey in the study undertaken regions particularly Tiruvallur, Chengulpattu, kanchipuram district of Tamil Nadu due to scavenging nature of turkey and strict biosecurity measures to be taken to control the disease.

PP_3.3 : MOLECULAR DETECTION AND THEIR BIOGRAM PATTERNS OF CLINICAL ISOLATES FROM BOVINE MASTITIS

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Mastitis is defined as an infection of the udder caused by bacteria entering the quarter through the teat end (Radostits, *et al.*, 2007), and according to the National Mastitis Council's current concepts of bovine mastitis. Milk production frequently falls short of meeting the nation's milk needs because of a variety of related factors and limitations, including poor management, insufficient management, low genetic potential, inadequate nutrition, poor reproductive performance, and various diseases, particularly mastitis, which is one of the most significant obstacles to the economic milk production. Bovine mastitis is a multi-etiological, complex, economical disease-causing inflammation of mammary gland and is a problem in many dairy cows. So, to know the common bacterial pathogens, the objective of this study was phenotypic and molecular detection of the common bacterial pathogens from bovine mastitis. A total of 24 mastitis milk samples were collected from the animals in and around Tirupati.



Initially confirmed by surf solution test (SST) then were cultured on different media and biochemical tests. Out of the 24 samples collected and examined all were positive for cultural isolation of bacterial species. Among the 24 isolates, 16 (*Staphylococcus aureus*), 8 (*Escherichia coli*) and 4 (*Pseudomonas aeruginosa*). Further all the 24 isolates the sensitivity pattern was studied for selection of drug to treat the animals. The molecular detection was done by targeting 16Sr RNA gene for *Staphylococcus aureus*, *Escherichia coli* and *aac3* gene for *Pseudomonas aeruginosa*. The high resistance of penicillin G, Methicillin, oxytetracycline, amoxicillin and oxacillin in mastitis in the present study were noticed. Based on the antibiotic susceptibility test and MIC break points, and MRSA showed maximum sensitivity to enrofloxacin, and ciprofloxacin followed by gentamicin and ceftazidime. Where as *Escherichia coli* and *Pseudomonas aeruginosa* isolates were found sensitive to gentamicin, enrofloxacin and ceftazidime. The present study revealed that both contagious and environmental microorganisms were responsible for bovine mastitis.

PP_3.4 : DETECTION AND MOLECULAR CHARACTERIZATION OF BEAK AND FEATHER DISEASE VIRUS FROM ROSE RING NECKED PARAKEET (*PSITTACULA KRAMERI MANILLENSIS*) AND COCKATIEL (*NYMPHICUS HOLLANDICUS*)

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Psittacine beak and feather disease (Pbfd) is a highly infectious disease of both old and new world psittacine. Pbfd is caused by Beak and feather disease virus (BFDV) belongs to the genus *Circovirus*, family *Circoviridae*. It is a highly mutable single stranded DNA virus of 1.7 to 2.0 kb in size. The clinical signs in Pbfd varies from mild subclinical infection to severe clinical infection. The clinical signs in severe clinical infection includes loss of tail and down feathers, feather dystrophy, beak deformities such as beak fracture, beak elongation and palatine necrosis. This study reports identification, isolation in Vero cells and molecular characterization of beak and feather disease virus from Rose Ring necked parakeet (*Psittacula krameri manillensis*) and Cockatiel (*Nymphicus hollandicus*). These birds brought for the treatment of alopecia at Madras Veterinary College pet bird medicine unit on August 2021 and September 2021. Blood and feather samples were submitted for the diagnosis of BFD. DNA was extracted from feather samples and PCR was carried out targeting replication associated protein (*rep*) gene. Two different Primer sets described by Ypelaar *et al.* (1999) and Ritchie *et al* (2003) were used separately in PCR due to high variability of the genome and avoid the mutation at primer binding sites. Parakeet sample was amplified with both the primers and yielded 717 bp and 610 bp *rep* gene amplicons respectively. Whereas, the cockatiel sample was amplified with primers described by Ritchie *et al.* (2003) alone and yielded 610 bp product specific to *rep* gene amplicons. PCR amplicons from both the sample were gel purified and sequenced by Sanger sequencing. Sequences were aligned and blasted with NCBI blast which showed 96 to 93.5% similarity with other sequences. Phylogenetic analysis revealed the Indian parakeet and cockatiel sequences have high similarity and forms separate clusters.



PP_3.5 : MOLECULAR DETECTION AND CHARACTERIZATION OF LUMPY SKIN DISEASE VIRUS FROM RECENT OUTBREAKS IN ANDHRA PRADESH, INDIA (2023)

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Lumpy skin disease (LSD) is a re-emerging transboundary disease with limited host range and currently restricted to cattle and water buffaloes. OIE makes it as a notifiable disease because of its potential for rapid spread and significant economic losses worldwide. Therefore, it is crucial to detect and characterize LSD in animals in order to lessen the financial burden on farmers. In the current investigation, a total of 81 samples (38 skin scabs, 21 nasal swabs, 18 blood samples and 4 faecal samples) from 54 clinically infected animals in different regions of Andhra Pradesh were collected. All these samples were used for the detection of Lumpy skin disease virus (LSDV) by polymerase chain reaction (PCR) targeting the p32 envelope protein gene (LSDV074) and A33R gene. Out of 38 skin scabs 34 samples (89.47%), 7 out of 21 nasal samples (33.33%) and 1 out of 4 faecal samples (25%) were found positive for both p32 and A33R genes by PCR assay. The coding region of A33R gene from three field isolates representing different regions of Andhra Pradesh was sequenced and the phylogenetic analysis showed that, they were clustered with isolates from Turkey, Serbia, Russia, and Kazakhstan. This new technique of amplifying LSDV A33R genome for the detection of LSDV in clinical samples is equivalent to the OIE-recommended PCR technique that amplifies the p32 gene.

PP_3.6 : MOLECULAR DETECTION OF *MYCOPLASMA* SPECIES FROM RESPIRATORY INFECTIONS OF POULTRY

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Chronic Respiratory Disease (CRD), caused by various *Mycoplasma* species, is widely prevalent in poultry flocks. Infected birds exhibit symptoms such as sneezing, rales, coughing, along with exudates from the nostrils and eyes. This study was aimed to detect the *Mycoplasma* infections in poultry through the Polymerase Chain Reaction (PCR) assay. During the course of this investigation, 25 Tracheal swabs were collected from dead birds across diverse flocks during necropsy procedures at disease investigation laboratories in Vijayawada, Andhra Pradesh. The 25 samples were targeted by PCR assay with genus-specific primers of *Mycoplasma* species. The resulting amplicon exhibited a consistent size of 270 base pairs, indicative of the presence of the targeted *Mycoplasma* species in the samples. Among the 25 samples analyzed, 13 (52%) tested positive for *Mycoplasma* infection, highlighting its prevalence. This study concluded that *Mycoplasma* infection could be rapidly and accurately diagnosed by PCR assay which is the predominant cause of mortality in the majority of the birds with respiratory infection. However, regular surveillance is essential for assessing the distribution of pathogen and implementing effective infection control measures in poultry farms.



PP_3.7 : MOLECULAR DETECTION OF STREPTOCOCCUS FROM MASTITIS MILK SAMPLES

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Mastitis is a wide spread mammary gland disease of dairy cows and causing economical losses to dairy industry. Different types of mastitis include clinical, subclinical and chronic mastitis. A clinical bovine mastitis is evident and easily detected by visible abnormalities, such as red and swollen udder, consistency, color of milk and fever in suffering animals. The mastitis is caused by many pathogens, among the bacterial pathogens, *Streptococcus* and *Staphylococcus* are the common gram-positive bacteria. By keeping in view this study was aimed to detect *Streptococcus* by targeting 16s rRNA gene by PCR. Collected about 25 milk samples from mastitis cases in and around Tirupati. The DNA was isolated from all the 25 milk samples by boiling method. The PCR test was standardized for *Streptococcus* by targeting 16s rRNA gene. All 25 milk samples were screening by PCR for 16s rRNA gene and found all were positive. In all positive samples, the amplicon produced the predicted 784 bp product. This study concluded that *Streptococcus* is the most common pathogen in and around Tirupati causing mastitis, so we can directly target *Streptococcus* to control the mastitis.

PP_3.8 : CONCURRENT INFECTION OF FOWL ADENOVIRUS AND REOVIRUS IN COMMERCIAL BROILERS

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An investigation was carried out in 20 commercial broiler flocks with a history of retarded growth, 10-15% mortality and increased feed conversion ratio. The post mortem examination of the dead birds revealed ascites, hydropericardium, mottling of liver and undigested feed in the intestine and samples such as heart blood, liver, spleen, pancreas, kidney and caecal tonsils were collected for bacterial and molecular detection of viral etiology. On cultural examination of the samples, no pathogenic bacteria of any etiological significance could be isolated. The molecular screening of the above samples for the viruses causing Newcastle disease, infectious bronchitis, inclusion body hepatitis (IBH), reoviral infection, chicken anaemia and avian encephalomyelitis revealed the presence of IBH (FAdV) and reovirus. Out of the 20 flocks, 9 (45%) flocks showed the presence of avian reovirus in liver and caecal tonsil. Additionally, concurrent infection with FAdV was detected in 4 (20%) flocks. 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 of FAdV. The findings of the investigation clearly demonstrate the prevalence of serotype 11 of FAdV in broilers as the etiology for IBH indicating the need for vaccinating the parent birds with serotype 11 of FAdV as well as reovirus to prevent the economic loss by these diseases in commercial broiler flocks.



PP_3.9 : PCR-BASED IDENTIFICATION OF MYCOPLASMA FROM RESPIRATORY INFECTION IN TURTLES

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Red Eared Slider turtles (*Trachemys scripta elegans*) are fresh water turtles native of southern United States and northern Mexico. The reptile is highly invasive in nature and can adapt to most environmental conditions. Consequently, it can now be found on most continents. Due to the compact size, ease of handling and ample availability at local aquariums, it has gained popularity as a common reptilian pet and is particularly popular among children. This association imparts the risk of transmission of various zoonotic infections viz., enteric and respiratory, to the individuals handling the animal. These reptiles are more prone to respiratory infections, of multiple aetiologies, due to improper management and care. Early and accurate detection of the infectious organism helps in planning control strategies and accurate treatment of the disease. Polymerase Chain Reaction is a promising tool for accurate detection of the pathogenic organisms directly from the clinical samples. This study aims to detect the presence of *Mycoplasma spp.* organisms from the choanal swabs of Red Eared Slider turtles by PCR targeting the genus specific 16S rDNA region of the organism. Choanal/ oral swabs were collected from 31 turtles showing signs of respiratory symptoms in MEM supplemented with 2% FBS and transported to the laboratory. The solution with swabs was directly subjected to extraction of DNA and further PCR. The genus specific primers used for PCR targeted the 16S rDNA region of *Mycoplasma spp.* and produced amplicons of ~710bp. A positive and negative control was always used while setting up the reactions to avoid false results. Out of 31 samples screened, four (12.9%) showed presence of *Mycoplasma spp.* organisms by PCR. Polymerase Chain Reaction can thus be employed to detect the presence of fastidious organisms which are difficult to cultivate and detect in artificial media. Also, early detection of zoonotic pathogens is facilitated so that timely control and treatment strategies can be planned.

PP_3.10 : MOLECULAR DETECTION OF MYCOPLASMA INFECTIONS IN KADAKNATH POULTRY

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The Present study was under taken to screen the common respiratory pathogen i.e. Mycoplasma infections in Kadaknath poultry by PCR, targeting 16s rRNA gene. A total of 148 samples were collected (Respiratory swabs) from 7 Kadaknath poultry farms located in and around Tirupati. The DNA was extracted by boiling method. The PCR test was carried out targeting 16srRNA gene. Out of 148 samples, five samples were positive for amplification of 16s rRNA gene yielding 280bp product



size confirming the mycoplasma among the respiratory pathogens. Hence, 16s rRNA PCR can be used for rapid field screening test at farm level and this will help to take necessary steps in the control as well as treatment aspect.

PP_3.11 : MOLECULAR DETECTION OF MYCOPLASMA OVIPNEUMONIAE INFECTION AND ITS ASSOCIATED BACTERIAL PATHOGENS IN PNEUMONIC SHEEP

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The present study was carried out to study the occurrence of *Mycoplasma ovipneumoniae* infections and its associated pathogens in sheep. A total of 148 sheep lung tissues were collected during postmortem examination and slaughter houses showing congestion, consolidation and hepatization. Out of 148 lung tissue DNA samples screened, 52 samples were found to be positive for genus *Mycoplasma* yielding 280 bp product. The occurrence of *Mycoplasma* in lambs below 6 months of age was found to be 57.14 percent and the lambs above 6 months and below 1 year was found to be 69.23 percent. The occurrence of *Mycoplasma* in sheep above 1 year of age was found to be 28.9 percent. The overall, occurrence of *Mycoplasma* in sheep lung tissue samples was found to be 35.1 percent. Out of 52 lung tissue DNA samples 28(53.8%) were found positive for *Mycoplasma ovipneumoniae* yielding 361 bp product. The occurrence of *Mycoplasma ovipneumoniae* in lambs below six months of age was found to be 35.7 percent and lambs aged between 6 to 12 months was found to be 30.76 percent. The 28 lung swabs collected from lung tissues which were positive for *Mycoplasma ovipneumoniae* were subjected for preliminary isolation of *Pasturella multocida*, *Mannhaemia haemolytica*, *Eschericia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus spp.* Out of 28 lung swabs processed for isolation of *Pasturella multocida*, 7(25%) and *Mannhaemia haemolytica* 5(17.85%) isolates were recovered in pure cultures. The isolates of *P. multocida* were confirmed by amplifying KMT1 gene by PCR which showed an amplified product of 460 bp. 7 isolates were positive in PCR yielding 460bp product size. NCBI- BLAST search of accession no sequence showed that these sequences has maximum homology 98-99 percent with 2 Genbank published sequences of KMT1 gene of *Pasturella multocida* strain NCTC10323 from Sanger institute, United kingdom, strain 375-A/15 strain from IVRI, India. The isolates of *M. haemolytica* were confirmed by amplifying PHSSA gene by PCR which showed an amplified product of 325 bp. 5 isolates were positive in PCR yielding 325bp product size. A total of 28 lung swabs collected in nutrient broth were subjected for preliminary isolation of other bacteria on nutrient agar, EMB, Mac conkey agar, MSA and Edwards medium. Bacteria isolated from nutrient broth. Out of 28 lung swabs processed for isolation *E.coli* 9 (32.14), *K.pneumonia* 6 (21.4%), *S.aureus* 10 (35.71%) and *Streptococcus* 7 (25%) isolates were recovered in pure culture.



PP_3.12 : MOLECULAR CHARACTERIZATION OF ORNITHOBACTERIUM RHINOTRACHEALE IN COMMERCIAL POULTRY FARMS OF ANDHRA PRADESH

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The present study was undertaken to screen the respiratory pathogen *Ornithobacterium rhinotracheale* in commercial poultry farms of AP by using molecular-based detection targeting the *16S r RNA* gene and *rpoB* gene. Samples were collected from 28 farms located in different districts of AP, which include tissues of the lung, liver, trachea, and air sacs, nasal swabs, oral swabs, tracheal swabs, exudates from infraorbital sinus, and heart blood. The DNA was extracted by boiling method. The PCR protocol was standardized for 16S r RNA and was found that 52°C annealing temperature was optimum for amplification. Similarly, the PCR protocol was standardized for the *rpoB* gene and found that 54°C annealing temperature was optimum for amplification. All the collected samples were screened for both *16S r RNA* and *rpoB genes* and found that tracheal swabs (85.84%), oral swabs (47.11%), nasal swabs (54.43%), exudates of infraorbital sinus (30.76%), heart blood swabs (75%), lung (34.92%), liver (47.61%), trachea (58.73%), air sac (47.05%) were positive. This study concluded that *Ornithobacterium* infection is common in commercial poultry farms which is leading to severe economic losses, high morbidity, and moderate mortality. To control the infection early detection is essential. This study helps us for rapid screening of *Ornithobacterium rhinotracheale* infection and based on this we can implement control strategies to reduce the infection in the commercial poultry industry.

PP_3.13 : DETECTION OF MYCOPLASMA BOVIRHINIS FROM CLINICAL MASTITIS CASE

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The present study was carried out to detect Mycoplasmal infections in mastitis cases of cows and buffaloes. A total of 200 clinical mastitis milk samples were collected, including 171 from cows and 29 from buffaloes. Culture enhanced isolation was carried out in PPLO broth, followed by DNA extraction and PCR for all inoculated samples. Out of the 200 samples, 43 tested positive for the genus *Mycoplasma*, yielding 280 bp PCR product size. Further, Genus-positive samples were screened for *Mycoplasma bovirhinis*, with 4 samples testing positive by PCR yielding 312 bp product. Confirmation was done through sequencing, verified by NCBI Blast, and the same sequences were submitted to Gene bank and assigned the accession number OR875372.



**PP_3.14 : MOLECULAR CHARACTERIZATION OF THE CIRCULATING
MAREK’S DISEASE VIRUS ISOLATES IN SOUTH INDIA**

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Marek’s disease is an oncogenic, lymphoproliferative disease of poultry wherein mortality rates have been significantly reduced by vaccination in the last few decades. However, in the recent past, an emergence of more virulent strains of Marek’s disease virus (MDV) despite vaccine-induced immunity has been reported in many parts of the world. Such an emergence of virulent MDV has also been reported in India over the last few years and this study adds more information on the evolution of the circulating MDV, especially in Southern India. The present study was laid with an objective of elucidating the molecular characteristics of the *Meq* oncogene in describing the virulent nature of the emerging MDV isolates. The investigations made in this study were from visceral tumors in both vaccinated commercial layers and native poultry flocks across five different States of South India. Virus isolation was carried out in primary CEF and DF-1 cell lines. Further identification of MDV was confirmed based on two copies of the 132-bp tandem repeats. Sequencing and phylogenetic analysis of the *Meq* gene and its encoded protein showed that the majority of the isolates belong to the “very virulent” pathotype of MDV. Point mutations in the Proline-rich region, interrupting the PPPP motifs were observed in the most of the virulent strains. The pathotypes of the field MDV isolates in this study were also assessed by the molecular characteristics of two other virulence-associated genes-namely, *pp38* and *vIL8*. Phylogenetic analysis revealed the clustering of the field isolates of this study with a few North Indian strains and virulent viruses of Nigeria and China. This study revealed that mutations continue to occur in the major virulence determinants of MD. These findings are mostly responsible for the evolution of newer virulent viruses due to the selection on MDV-host cell interactions.



TECHNICAL SESSION IV

**GENOMICS AND PROTEOMICS IN
INFECTION AND IMMUNITY**





DIAGNOSTIC APPROACHES OF CANCERS IN ANIMALS

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Cancer is becoming a major challenge in all animals including poultry and wild/zoo animals due to its increasing prevalence now a days. It is considered as most frequent cause of death in canines and felines. For diagnosis of cancer in animals one should have a comprehensive idea about different types of cancers in different sps. of animals and their probable etiological aspects for implication of different diagnostic aspects. Canine tumors are mostly similar to those of human cancers (mammary gland cancers, blood cancers and cancer of lymphatic system). Canine mammary gland tumors are comparable to human breast cancer. In bovines in addition to Bovine papillomas, eye cancer, horn cancer, even though uterine cancer established as a common tumor like woman, little attention being made in clinical diagnosis and attributing the cause of uterine cancer for infertility. In pigs the cancer is mostly of genetic origin whereas in poultry the viral oncogenesis is playing major role i.e Mareks disease and Lymphoid leucosis which are major threat to poultry industry instead of strict vaccination programme. Sheep also prone for cancers of viral origin mostly Jaagsiekte and Enzootic nasal adenocarcinoma. Cats are prone for lymphomas caused by Feline leukemia virus. Like farm animals, zoo animals are more prone for cancers especially aged carnivores i.e lymphosarcoma, Mesothelioma, hemangiosarcoma and adenocarcinoma etc. In deer fibroma and fibrosarcomas are common and are of viral origin i.e papilloma. In lab animals especially in rabbits uterine cancers and lymphosarcomas are very common. Rats and mice develop mammary tumors which are of genetic origin. The cause in animals can be majorly attributed to environmental pollutants as in case of human exposures and to biological and genetic causes to some extent.

Cancer diagnosis includes simple diagnostic techniques and complementary molecular techniques and starts with the identification of a lesion, a systemic dysfunction, adequate history and followed by clinical investigation coupled with laboratory examination. The early detection of precancerous changes significantly reduces the risk for the development of a tumor. According to veterinary cancer society, the warning signs of a cancer include abnormal progressive development of tumefaction in lymph nodes, improper healing of scars, hemorrhages or oral, nasal, vaginal, urinary or rectal secretion with putrid smell, difficulty in mastication, deglutition, difficulty in breathing, urination or defecation, loss of energy, loss of appetite and weight loss, persistent limping, difficulty in thoracic movement, difference in the sizes of testis. The diagnosis of cancer includes a complex examination starting with clinical and para clinical investigation but the diagnosis of certainty can be by histological and cytological examination. Diagnosis of cancer relies primarily on invasive tissue biopsy and the conventional histopathology based on light microscopy, complementing with ultrastructural, immunohistochemical and molecular diagnosis.



Development of advanced sophisticated techniques opened new avenues in cancer diagnosis, assessment of prognosis and therapeutics. Conventional diagnostic methods remained the standard diagnostic method. Recently conventional histopathology complemented with Immunohistochemistry, molecular diagnostic methods, oncological imaging assessment of hormonal receptor studies provides more precise diagnostic and prognostic information. The early detection of precancerous changes can significantly reduce the risk for the malignant development of the tumors and in turn helps in undertaking precocious therapeutic strategy and results into prolonged life expectancy. The detection of sub clinical cancer in animal is likely to improve the prevention of cancer related deaths and to reduce the morbidity associated with treating the animal with more advanced diseases. The main warning sign of cancer in animals can be loss of weight, absence of healing in the scar, chronic bleeding and discharges with putrid smell, (anal, vaginal, urinary and rectal), difficulty in mastication and deglutition, difficulty in breathing etc. Majority of the times little importance given to notice the preneoplastic syndromes which gives the suspicion for the cancer. The following diagnostics techniques can help in proper diagnosis and assessment of the cancer in animals.

Imaging techniques

Based on imaging information malignancies can be suspected. Radiological evaluation is the most common diagnostic technique in small animals. Advent of computed tomography and magnetic resonance imaging (MRI) structural and anatomic information can be obtained. Molecular imaging with magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) are recently used techniques in clinical practice.

Morphological diagnosis

It is based on the size, consistency and colour and offers precious data for subsequent correlation with histopathological examination. For example, the firm mass with grayish whitish consistency can be suspected for sarcomas, soft consistency in malignant lymphoma, oedematous or gelatinous in myxomas and myxosarcomas yellowish in leydig cell tumors, grayish black in melanotic tumours, red colour in hemangiosarcomas and green colour cholangiosarcomas. Any bleeding, ulceration and necrosis are to be noted. Always history should include detailed morphological aspects in cancer cases.

Cytology and biopsy techniques

Cytological examination is a simple diagnostic screening tool and extremely useful in cancer diagnosis. The samples can be obtained from biopsy, smears from excretions and secretions, as well as aspirations from cancer effusions scrapings from accessible mucosa and during cadaver examination. Cytological smear examination is quick to perform and process. It is less invasive and can be collected at regular intervals, The correlation between cytological and histological diagnosis exceeds 80% in majority of the cases. But cytological studies require good expertization.



Biopsy involves the surgical removal of tissue pieces from the living body for microscopic examination as an aid to diagnose the cancer. Diagnostic value of biopsy depends upon screening adequate and satisfactory specimen of tissue and its skillful histological value in the diagnosis of malignant tumors but also in determining their metastasis and prognosis. The technique varies with the tissue to be biopsied.

Biochemical studies

Most of the times little attention was given to observe the biochemical aspects in different types of cancers. For example, hypercalcemic can be the consequence of parathormone hypersecretion in case of parathyroid tumour, multiple myeloma and apocrine carcinoma. Hyperproteinemia is associated with neoplasms like multiple myeloma, chronic lymphoid leukemia. Blood examination results like anemia, hemorrhagic lesions appear in relation to coagulation disorders *i.e* petechiae, echymosis, bleeding, increased bleeding, clotting time and thrombocytopenia can give the suspicion for the cancers like hemangiosarcoma.

Molecular diagnosis of cancers

Molecular diagnosis helps in determining the best course of treatment and these diagnostics are fundamental component of precision medicine. The diagnosis is based on DNA, RNA, protein or related molecules. These tests are mostly run on samples of blood, saliva or tumor tissue. Molecular diagnostics are used to determine risk assessment, differential diagnosis, to assess prognosis, to predict treatment response to focus on pharmacokinetics, to monitor treatment response and to monitor recurrence of cancer in patients without symptoms of cancer.

Application of molecular biomarkers in cancer diagnosis

Tumor markers are biological or biochemical substances produced by tumors and secreted into body fluids or present in body tissues in higher-than-normal amounts. Molecular biomarkers are alteration in chromosomes, DNA, RNA, Protein and related molecules which indicate cancer. Molecular alteration can be inherited or acquired. Genetic, epigenetic, proteomic and imaging biomarkers can be used for cancer disease prognosis and epidemiological aspects. Tumor markers can be detected by ELISA, RIA, IHC, Immuno-scintigraphy and molecular genetic methods. Tumor marker levels reflect the stage of the disease and possible prognosis. A decrease or return to normal may indicate favorable response for the treatment. Samples assayed for the presence of biomarkers include urine, serum, tissue, cerebrospinal fluid and exhaled breath. The potential applications of biomarker identification in veterinary cancer patients include diagnosis, staging, determining prognosis and in monitoring the response to therapy.

Immunohistochemistry (IHC)

IHC is based on detection of specific antigen determinants present in the cells of the tissue by the use of polyclonal antibodies. In immunohistochemistry a sample which we are trying to identify a specific protein is placed together with antibodies that bind to that protein. It helps in identifying



metastatic tumours of unknown primary site. To determine estrogenic and progesterone receptor status, over expression of C-erbB2 oncoprotein, P53, PCNA, Ki67 etc in animal cancers IHC is more helpful.

Flow cytometry

Flow cytometry helps in diagnosing hematological malignancies. With this the DNA content in tumour can be measured and immunophenotyping can be done. FISH is used to look for the presence, absence, relative positioning and or number of specific DNA segments under fluorescent microscope.

FISH (*In situ* hybridization technique)

The technique involves the specific hybridization of a labeled nucleic acid probe to complementing genes sequence and subsequent visualization by immunocytochemical method in tissue sections, smears. Chromosomal abnormalities can be found in malignant cells.

PCR (Polymerase chain reaction)

The genetic and biochemical processes at the molecular levels can be detected by PCR. It helps in definite diagnosis and classification of the tumors based on unique molecular alteration that occur in specific tumor types. The change can be studied in DNA or RNA. PCR allows early diagnosis of malignant diseases such as Leukemia and lymphomas. PCR assay can be performed directly as genomic DNA sampling. Quantitative PCR methods allow the estimation of the amount of a given sequence present in a sample technique often applies to quantitatively determine the levels of gene expression. Real time PCR is very powerful and a accurate technique to examine expression pattern of different oncogenes, suppressor genes in different cancer conditions. PCR act as an important tool in the diagnosis of the virus induced tumours of the animals.

Real time PCR and Reverse transcription PCR

Real time PCR is similar to regular PCR except that the DNA is not only copied, but also detected or quantified in the same process. Reverse transcription PCR is similar to PCR except that it detects RNA instead of DNA. It can detect low levels of genes that are activated, and it is used to know the spread of cancers to distant organs

Cytogenetic testing

Cytogenetic testing involves examining the number and structure of chromosomes and is used for the typing of blood cancers such as leukemia. Conventional cytogenetic tests involve taking cells from a certain area of the body and growing of the cells in a test tube for one day or more. The cells that are dividing are then stopped or essentially "frozen" in the process of division. It is essential that the cells are dividing because only in this stage only the chromosomes can be seen by using a regular microscope.

Microarray Method

DNA microarray technology allows both qualitative and quantitative screening for sequence variations in the genomic DNA of cancer cells. Labeled DNA for analysis binds strongly only to those targets that are fully complementary to one of its sequences.



Mutational analysis

Detection of mutations in cancer is of importance for both understanding of the disease process and clinical practice. High density oligonucleotide assays are commonly used to achieve the purpose.

Polymorphism Genotyping

Single nucleotide polymorphism (SNP3) can be screened by a set of oligonucleotide probes.

Evaluation of gene expression

Microarray based expression indicates a panel of up or down regulated genes that are considered as molecular markers for cancer. Expression of genes differs in different type of tumors.

Tissue array

Relevance of cancer markers identified by genomic or proteomic analysis can be evaluated with tissue arrays. They are used in large sample screening of tissue specimens for the Insitu detection of DNA, RNA and protein targets.

Circulating tumor DNA as a biomarker in cancer diagnosis

ctDNA is an emerging research area and it is noninvasive biomarker that could be used to provide diagnosis and prognosis. It is a precision medicine biomarker to detect tumor heterogeneity and clonal selection. Cell free DNA circulates in plasma and other bodily fluids such as urine and saliva. ctDNA is released when tumor cell dies or through active secretions. Because ctDNA contains tumor-related genetics relevant to cancer development and it can be used as biomarker.

Majority of the times, it is a challenging to collect tumor tissue located in the deeper parts of the body. Liquid biopsy has become a promising approach including circulating nucleic acids such as circulating tumor (ctDNA), extra cellular vesicles (EVs), extra cellular RNA (exRNA), and circulating tumor cells (CTCs). The level of ctDNA is higher in a malignant disease patient than in healthy individual.

In conclusion the affective cancer diagnosis involves application of simple diagnostic methods to advanced molecular techniques and is essential for opting proper approach in treatment aspects and to assess prognosis.



POSTBIOTICS: CONCEPTS TO APPLICATIONS IN VETERINARY MEDICINE

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Animal efficiency is a key to supplying the growing population with sustainable agriculture. There is also a growing concern of antibiotic usage and resistances in animal agriculture, encouraging the assessment of alternatives to maintain animal efficacies. Probiotics, prebiotics and postbiotics are antibiotic alternatives that have demonstrated benefits on animal health and performance. Before discussing the role of these additives, we will outline the most recent definitions of each as determined by The International Scientific Association for Probiotics and Prebiotics 2021.

Probiotics: Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. eg. bifidobacterium, lactobacilli, bacillus and the yeast strains saccharomyces.

Prebiotics: A substrate that is selectively utilized by host microorganisms conferring a health benefit on the host. The most common prebiotics are fructooligosaccharide (FOS), inulin and fructans.

Postbiotics: A postbiotic is "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (1). This definition of postbiotic requires that the whole or components of inactivated microbes be present, with or without metabolic end products. Thus, postbiotics refers to the metabolic byproducts like enzymes, peptides, teichoic acid, peptidoglycan derived muropeptides, exopolysaccharides, cell surface and secreted proteins, bacteriocins and organic acids generated by a probiotic organism during the final or intermediate stage of its metabolic process (2,3).

Properties of Postbiotics

Postbiotics as nutritive patrimony that continue to improve the health of the host are secreted by probiotic organisms when eventually die. Postbiotics avoid risks linked with the administration of live microorganisms. They are believed to have probiotic effects without living cells. Contrary to probiotics, metabolites are relatively more stable in terms of handling and storage and are unlikely to transfer antimicrobial resistance traits to other bacteria (4). Postbiotics have advantage due to their clear chemical structure, safety dose parameters and longer shelf life which can influence the physiological function of host (5). Healthy postbiotics include nutrients such as vitamins B and K, amino acids, and antimicrobial peptides that help to slow down the growth of harmful bacteria. Other postbiotics such as short-chain fatty acids help healthy bacteria flourish.

Actions of Postbiotics

The important role of gut microbiota in promoting animal health and regulating immune function in livestock and poultry has been widely reported. Probiotics and postbiotics have been widely



developed as additives due to their beneficial effects in balancing host gut microbiota, enhancing intestinal epithelial barrier, regulating immunity, and whole-body metabolism. The action of postbiotics is multidirectional, but the most important is their immunomodulatory properties. For instance, proteins and peptidoglycans of lactic acid bacteria and lipoteichoic acid from Gram-positive bacteria can stimulate the immune system or inhibit the excessive response of monocytes. In addition, some postbiotic proteins can improve digestion by helping to regenerate the mucosa and intestinal walls. Postbiotics stimulate the gut microbiome and support the immune function of the gut. Indeed, they increase the expression of the gene responsible for intestinal mucin production, which protects the intestinal barrier from injury caused by lipopolysaccharide and tumor necrosis factor alpha (6).

Postbiotics, modulate immune response, gene expression, inhibit pathogen binding, maintain intestinal barriers, and help in controlling carcinogenesis and pathogen infections. Postbiotics have antimicrobial, antioxidant, and immunomodulatory properties with favorable physiological, immunological, neuro-hormonal, regulatory and metabolic reactions. Consumption of postbiotics relieves symptoms of various diseases and viral infections such as SARS-CoV-2. Postbiotics can act as alternatives for pre-probiotic especially in immunosuppressed patients, young and premature neonates. (7)

Application of postbiotics in animals and poultry

Ruminants

In ruminants, an in vitro study revealed the alteration of rumen fermentation and bacteria composition after supplementary of postbiotics from *L. plantarum* RG14, including elevated ruminal volatile fatty acid (VFA) and population of total bacteria, cellulolytic bacteria, and total protozoa (8). Izuddin et al. (9) concluded that postbiotic supplementation improved the weight gain of lamb by increasing feed consumption and nutrient digestibility. However, rumen ammonia N and butyrate concentrations were enhanced, whereas total VFA and pH were unaffected. Glucose, urea nitrogen, and complete protein in blood were higher in lambs fed postbiotics. The levels of triglycerides and cholesterol in the blood did not differ. Rumen protozoa and methanogens decreased after postbiotic treatment, but fiber-degrading bacteria improved. Dietary postbiotics reduced serum lipid peroxidation, enhanced serum and ruminal fluid antioxidant actions, and enzyme production of hepatic antioxidants in post weaning lambs

Swine

In post-weaning piglets, *Lactobacillus plantarum* could enhance growth performance, an environment of gut health, and digestibility of proteins when 0.5% metabolite combinations are administered in feed. In piglets, along with increasing parameters of growth performance, such as overall and average weight gain per day and birth weight, *L. plantarum* also reduced the occurrence of diarrhea. Beneficial effects of postbiotics were observed in swine for the growth promoter and regulation of the immune system. Strains from *L. rhamnosus* isolated from pigs were cultured and processed by heating at 80 °C for 30 min. Dietary inclusion of 1×10^9 CFU/g of this kind of product



could improve production performance, including growth rate, feed efficiency, and apparent total tract digestibility of dry matter in weaned pig. Feeding of 0.5% metabolites combination from strains of *L. plantarum* TL1, RG14, and RS5 isolated from Malaysian foods in the piglet diet could improve average daily gain and daily feed intake, as well as reduce diarrhea incidence in the post weaning piglets. What's more, lower Enterobacteriaceae (ENT), higher LAB counts and SCFA levels in the gut of piglets were observed (10).

Since early weaning usually induced atrophy of villous, oral administration of heat-killed and dried cell preparation of *Enterococcus faecalis* strain EC-12 led to the higher villous of jejunum in piglets weaned at 21-day-old, suggesting the postbiotics could protect the gut health and relief weaning stress in piglets (11). Oral administration of heat-killed *E. faecium* strain NHRD IHARA led to the increase in serum IgA production in weaned piglets, which showed similar effects with the administration of live cells. On the other hand, heat-killed strain *E. faecium* strain NHRD IHARA also showed beneficial effects on growth performance in pigs. Daily intake of heat-killed *L. plantarum* L-137 induced higher levels of IFN- β and gene expression in the whole blood cells of pigs, which might subsequently augment host defense against the virus infection (12). Spray-dried *L. plantarum* strain 22F, 25F, *Pediococcus acidilactici* 72 N isolated from pig feces exhibited beneficial effects in the nursery-finishing pigs, including a better feed conversion ratio, increase of Lactobacilli counts, decrease of Enterobacterial counts in the gut, which demonstrated the feasibility of substitute for antibiotics (13). The lipoteichoic acids of *L. plantarum* had the potential to induce IL-12, thereby stimulating innate immunity in the culture of splenic dendrites of mice. In a study of pig epithelial cell lines, lipoteichoic acid of *L. plantarum* was found to possess anti-inflammatory activity by down regulating the expression of IL-8, showing the potential of this component to modulate the immune response (14).

Poultry

Adding prebiotics and postbiotics containing inulin to broiler chicken rations improved the feed efficiency and total body weight of the birds and sustained growth factor1, mRNA expression of growth hormone receptors, and intestinal mucosal structure. Postbiotics enhance feed efficiency and reduce bird sickness and mortality, reducing the environmental impact of chicken production (15).

Heat-killed *Enterococcus faecalis* strain EC-12 also applied in newly hatched broilers from age of 3 to 14. Supplement with heat-killed *Enterococcus faecalis* strain EC-12 increased total IgA in the cecal digesta and total IgG in the serum, and reduced vancomycin-resistant enterococci (VRE) colonization in the intestine, suggesting this kind of postbiotics could stimulate the gut immune system and reinforce the immune reaction against the VRE challenge to accelerate its defecation in chicken (16)

The addition of metabolite combination of *L. plantarum* RS5, RI11, RG14, and RG11 strains could increase fecal lactic acid bacteria counts, villus height, and volatile fatty acids in broiler chickens. What's more, chicks fed with CFS of *L. plantarum* RI11 showed improvement of growth performance,



including higher final body weight, total weight gain and average daily gain than other groups, suggesting the *L. plantarum* RI11 could be used as an alternative antibiotic growth promoter. Also, supplementation of postbiotics improved the gut morphology, lowered ENT and *E. coli* counts and caecal pH value in the gut but showed limited effect on plasma IgA level (17&18). Anti-stress effects of postbiotics *L. plantarum* RI11 were also observed via regulation of antioxidant enzyme activity, gut barrier genes, and cytokine, acute phase proteins in broilers. Postbiotic metabolite combinations derived from *L. plantarum* strains RI11 also reduced fecal ENT levels, improved egg quality and increase hen-day egg production in laying hens. Apart from strains from *Lactobacillus*, postbiotics from *Bacillus subtilis* also showed beneficial effects in laying hens and broilers, including feed efficiency, egg quality, and immune response. Postbiotics product from a cocktail containing *Pediococcus acidilactici*, *L. reuteri*, *Enterococcus faecium*, and *L. acidophilus* could improve weight gain and alleviate the proinflammatory responses after the challenge of *Clostridium perfringens* in broilers(19).

Conclusion

Probiotics and postbiotics have considerable prospects in preventing various diseases in the livestock industry and animal feed and medical applications. The utilization of postbiotics has shown great potential and can be an alternative to antibiotics in animal production. However, despite the fact that the inanimate of postbiotics makes it more stable and safer than probiotics, the exact composition in postbiotics remains to be identified in the future, which would make it more capable and convinced in the application. Moreover, although studies have investigated the mechanism of a single factor in postbiotics, the complex interaction between diverse compounds and host can exist. Therefore, as an integration of various compounds, the exact mechanism of action of the postbiotics and their combinations is needed and to be further illustrated in future studies.

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ORAL PRESENTATIONS

OP_4.1 : TRANSCRIPTOMIC SIGNATURES OF AFRICAN SWINE FEVER NATURALLY INFECTED PORCINE KIDNEY TISSUES

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African swine fever (ASF) is a continual economical threat to the global piggery sector. The host immune evasion caused by African swine fever virus (ASFV) is well understood. However, the ASF virus's significance in oncogenesis is uncertain. In the present study, ASFV infected kidney tissue samples were subjected for Illumina based transcriptome analysis. A total of 2010 upregulated and 149 downregulated genes were identified to be differentially expressed (p-value < 0.05) in ASFV infected porcine kidney tissues. Review of literature survey revealed that the majority of the differentially expressed host genes in death animal tissue samples were related with oncogenic properties. Protein-protein network analysis identified that pathway associated with functional enrichment for basal cell carcinoma, breast cancer and gastric cancer. Host-viral interaction revealed that upregulated oncogenic RELA (p65 transcription factor) protein of *sus scrofa* can interact with A238L protein of ASFV. qRT-PCR experiments for different up and down regulated oncogenes, including MEX-3D, MAIP1, ZNF618, CCDC105, MOSPD2, FAM98B, FGFR4, GRKs, SPDYC, and SOCS, were done using H3F3A as the housekeeping gene to assess the correctness of RNA-Seq data. The levels of gene expression indicated by qRT-PCR were extremely similar to those determined by RNA-Seq. Differentially expressed host oncogene profiles in ASFV clinical symptoms may be a good indicator for the integration of additional repositories in the pathogenesis of ASFV. However, further research is needed to produce proof of concept for ASFV's oncogenic characteristic.

OP_4.2 : ADAPTATION AND GENETIC ANALYSIS OF PORCINE CIRCOVIRUS 2 (PCV2) IN NON-PORCINE ORIGIN CELLS

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Porcine circovirus 2 (PCV2) is the primary cause of Porcine Circovirus Associated Disease (PCVAD) with multiple clinical outcomes and it is one of the emerging, economically devastating and minimally explored infections in swine. PCV2 is routinely isolated from Porcine Kidney 15 (PK-15) cells and used in diagnostics and vaccine developmental studies across the globe. The non-cytopathic nature of PCV2 in PK-15 cells necessitates the requirement of specific immunofluorescence assay (IFA), PCR and other suitable assays for confirmation of virus growth. Further, non-pathogenic PCV1



contaminants from porcine cells may also frequently interfere with the isolation of PCV2. In this study, PCV2 was adapted in two non-porcine origin cells namely Vero and Raw-264.7 cells with origin from African green monkey and mouse macrophage respectively. A well-characterized PK-15 adapted PCV2b isolate from the field was blindly propagated in Vero and Raw-264.7 along with PK-15 infected and uninfected cell controls. The virus growth and stability were confirmed by PCV2-ORF2 gene based on specific PCR and sequencing. Passage 1, 2, and 3 cell culture harvests from all three Vero, Raw-264.7 and PK-15 cells yielded a specific amplicon of 802 bp confirming PCV2 propagation. Genetic analysis of PCV2b-ORF2 gene from both non-porcine origin Vero, Raw-264.7 cells and porcine origin PK-15 cells evidenced unaltered 702 nucleotides and 233 amino acid compositions. This is the foremost study in India, which evidences that PCV2b could be able to adapt to non-porcine origin cells without changing its genetic composition. There was no evidence of altered cell morphology in both the non-porcine origin cells. Further, the rate of viral replication in these cells needs to be assessed through IFA or qPCR based virus titration assays for the rate of virus multiplication

OP_4.3 : A STUDY ON PREVALENCE OF ANTIMICROBIAL RESISTANT (AMR) GENES IN PREDOMINANT STAPHYLOCOCCI AND *ESCHERICHIA COLI* ASSOCIATED WITH BOVINE MASTITIS IN KARNATAKA STATE.

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Bovine mastitis is an economically important and most significant disease seen in dairy farms worldwide resulting in the highest economic losses. As the major cause of bovine mastitis infections is by bacteria or other microorganisms, usage of antibiotics is the primary treatment approach. Unfortunately, despite the best possible antimicrobial treatments for mastitis, failures of bacteriological cure are common, and antimicrobial resistance (AMR) is considered to be one of the reasons. The present study was carried out with an objective to study the presence of AMR genes in *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* isolated from bovine mastitis cases from different regions of Karnataka. Out of 211 isolates, a total of 96 *S. aureus*, 50 *S. epidermidis* and 65 *E. coli* isolates were subjected for species specific PCR and 85 *S. aureus*, 23 *S. epidermidis* and 47 *E. coli* isolates were confirmed. On screening of 85 *S. aureus* isolates for AMR genes, *bla_Z* was detected in 61.17%, *mecA* in 3.53%, *aacA-aphD* in 4.7%, *tetM* in 16.4%, *tetK* in 3.52%, *strA* in 4.7%, *strB* in 7.05% and *ermC* in 4.7% of the isolates. Among 23 *S. epidermidis*, *bla_Z* was detected in 43.47%, *mecA* in 30.43% and *aacA-aphD* in 13.04%, *tetM* in 30.43%, *tetK* in 8.69%, *ermC* in 13.04%, *strA* in 4.34% and *strB* in 21.73% of the isolates. Among 47 *E. coli*, *bla_{TEM}*, *tetA* genes were detected in all isolates, whereas *bla_{SHV}* was detected in 31.9%, *bla_{OXA30}* in 12.76%, *tetM* in 2.12%, *tetB* in 2.12%, *strA* in 31.9% and *strB* in 51.06% of the isolates. The study revealed that as for the regional difference in the pattern of PCR based genetic profile of AMR genes is concerned, it was similar.



OP_4.4 : ISOLATION, WHOLE GENOME SEQUENCING AND COMPARATIVE GENOMICS OF *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM DIFFERENT ANIMAL SPECIES IN TAMIL NADU

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Clostridium perfringens infects different animal species and development of antimicrobial resistance among this pathogen is a major problem since this organism is present as a normal inhabitant in the intestine. A total of 162 *C. perfringens* isolates were obtained from various animal species from different parts of Tamil Nadu. Whole genome sequencing was performed for 4 *C. perfringens* type A isolates recovered from dogs (n=2), pig (n=1) and cattle (n=1) and the data was compared with 137 whole genome of *C. perfringens* available in the database. Whole genome phylogeny showed different phylogroups. Among the 4 genomes, 2 correspond to 2 different multi-locus sequence types namely 126 and 200 while STs were not assigned to other 2 genome which may be of new STs possibly. Except for the genome isolated from cattle, 3 genomes had 12 toxins predicted. All the four genomes were predicted with tetracycline resistance genes *tetA* (P) and *tetB* (P) which was also confirmed by PCR. One of the genomes (TANUVAS_BV4) was predicted with *lnu* (P) which corresponds to resistance against lincomycin. Comparative genomics showed that a genome (ZWCP210) from China was predicted with maximum number of 11 AMR genes that are responsible for resistance against erythromycin, aminoglycosides, tetracycline and lincomycin. Pangenome analysis showed that there were 1914 core genes, 1044 shell genes and 7149 cloud genes. Pangenome analysis also indicated that *C. perfringens* has an open pangenome structure. The study depicts that multidrug resistant *C. perfringens* is circulating in south India. Further, the open pangenome nature suggest that *C. perfringens* has the capacity to acquire additional AMR genes.

OP_4.5 : EMERGENCE OF COLISTIN RESISTANCE AMONG THE ISOLATES OF *ESCHERICHIA COLI*

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Emergence of antimicrobial resistant *Escherichia coli* infections is an increasing public health concern. As a part of endogenous microbiota, *E. coli* easily acquire resistance against many antimicrobials consumed by humans and animals. Pathogenic strains of *E. coli* cause colibacillosis inflict severe losses to the poultry industry and causes several extra intestinal diseases in humans like



urinary tract infections, neonatal meningitis and sepsis. The main objective of this study is to identify emergence of colistin resistance among the *E.coli* isolates. Keeping in view of above circumstances, in the present study, the isolation of *Escherichia coli* from poultry faecal samples was carried out by conventional methods. The antimicrobial resistance patterns of the confirmed isolates of *E. coli* were studied by using phenotypic methods. Further the presence of colistin resistance genes of confirmed *E. coli* isolates was done by targeting *mcr-1* and *mcr-2* genes using uniplex PCR. Out of 400 poultry faecal samples, 170 samples were found positive by cultural characteristics with an overall prevalence of 42.5%. Among the 170 *E. coli* isolates from poultry faecal samples, maximum resistance was observed for erythromycin (100 %) followed by Ampicillin (44.4%), Cefuroxime (30.7%), Gentamicin (25.3%), Colistin (16.8%), Amoxicillin (16.7%), Cefazolin (14.2%), Trimethoprim/ sulphamethaxazole (1%) and Meropenem (0.6%). Two sets of primers for *mcr-1* and *mcr-2* genes were targeted for the detection of colistin resistance among the confirmed *E. coli* isolates by using uniplex PCR. The desired PCR amplification of 309 bp for *mcr-1* gene and 567 bp for *mcr-2* gene was obtained for 1 (0.64%) and 9 (5.8%) isolates of *E. coli* respectively. In the present study, the colistin resistance among *E. coli* isolates detected was very low. It might be the consequence of a lack of selection of mobile colistin resistant (MCR) producing bacteria due to the lack of colistin use for the treatment of infections or may be due to high transfer of *mcr* like genes from animal or environment or human despite of colistin use. Though the prevalence was low, there is need for more thorough surveillance and control programmes to prevent further selection of colistin resistance.

OP_4.6 : FIRST REPORT ON L-MEQ GENE DETECTION AND PATHOGENIC ANALYSIS OF VIRULENT MAREK'S DISEASE VIRUS SEROTYPE 1 FIELD OUTBREAK OF BV380 COLOURED LAYER HENS IN PUDUKOTTAI DISTRICT OF TAMILNADU SOUTH INDIA

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The objective of this study was to identify the visceral type of Marek's Disease (MD) from suspected field outbreaks in a BV380 Coloured layer bird farms (4 nos) of 1500 capacity, unvaccinated against MD, aged 44 weeks reported a mortality of 8 -10 birds daily at farms of Kaatupatti, Annavalal, Iluppur and Kulavaipatti villages of Pudukottai District Tamilnadu. Marek's disease virus (MDV), an oncogenic, lymphoproliferative avian herpes virus, causing great economic losses to the poultry industry worldwide. MDV belongs to the family Herpesviridae, the subfamily Alphaherpesvirinae, and the genus Mardivirus (Marek's disease like viruses). On a diagnostic investigation to farms and after performing the necropsy, it was diagnosed as visceral form of MD by clinical history, gross and through samples such as feather follicles (FFE) liver, spleen kidney and heart etc., sent to TANUVAS Central University Laboratory Chennai and was confirmed serotype 1 MDV (L-meq gene based) on molecular detection and histological examinations. Grossly, visceral organs (heart, lungs, liver, spleen, kidneys and proventriculus) were enlarged with presence of focal to diffuse nodular growths. Cytological



examination of impression smears of heart, liver, and spleen during necropsy revealed a mixed population of pleomorphic lymphoid cells. Histopathology of these affected organs revealed diffuse sinusoidal and congestion with diffuse hepatocellular degeneration and necrosis and multifocal coalescing infiltration of pleomorphic lymphoid cells and pulpy reticular cells suggestive of MD. The nested PCR with meq open reading frame (ORF) was developed and standardised on amplification of the very virulent(vv) pathotype of Marek's Disease Virus in suspected birds by targeting 683bp fragment of the LTR region (mostly occur in 6-7week old young chickens) suggestive of latent MDV of meq and L -meq gene infection esp. as a latent phase which caused a devastating loss of severe drop in egg production and mortality. The sequencing of meq gene of 19 samples revealed that nucleotide sequences of the isolated vvMDV was different to the very virulent MDV, strain RB-1B.Hence in the present study, a developed and standardised polymerase chain reaction (PCR) was useful as a rapid and reliable assay for detection of MDV genome of serotype 1 of vvMDV (MDV-1) and was differentiated with herpesvirus of turkeys (HVT) using specific primers and first report in Tamilnadu of L-meq gene of MDV from field outbreaks in Tamilnadu of Cauvery Delta region.

**OP_4.7 : EPIDEMIOLOGICAL AND MOLECULAR STUDIES OF MRSA AND ESBLs
FROM LIVESTOCK, ANIMAL HANDLERS AND ENVIRONMENT IN
BENGALURU RURAL DISTRICT, KARNATAKA**

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Methicillin resistant *Staphylococcus aureus* (MRSA) and Extended spectrum beta lactamase (ESBL) *E. coli* are potential antibiotic resistant bacterial pathogens increasingly prevalent worldwide among humans, livestock and environment and posing significant public health risk. A cross-sectional study carried out during 2022-23 to know the prevalence of major AMR pathogens (ESBLs and MRSA) and their molecular signatures of antibiotic resistance. A multistage random sampling was done covering 16 villages in four taluks of Bengaluru Rural District of South Karnataka and collected 240 livestock, 120 animal handlers and environmental samples. Isolation and characterization and antibiogram profile of MRSA and ESBL *E. coli* was done as per standard methods. Multiplex PCR of ESBL genes in *E. coli* (TEM, SHV and CTX) and MecA of MRSA isolates was performed. The antibiotic resistance (AST) was determined by automated AST (BD Phoenix M50[®]) as per CLSI, 2022 breakpoints. The data was analysed using WHONET, 2022 software to know the resistance profile of isolates. A total of 196 *Staphylococcus* and 198 *E. coli* isolates were retrieved from livestock samples. The overall prevalence of MRSA was 6.63% (13/196) from livestock. There was evidence of horizontal gene transfer of mecA gene in MRSA and MRCoNS between livestock and animal handlers. The overall % prevalence of ESBL *E. coli* was 28.78% (67/198) and AmpC was %23.23 (46/198). The poultry samples revealed higher rate of ESBL and AmpC. Antibiogram of *Staphylococcus* (MRSA and



CoNS) in livestock-humans-environment revealed highest resistance to penicillin followed by cefoxitin, Linezolid, tetracycline, erythromycin, gentamycin, chloramphenicol Trimethoprim sulfamethoxazole and at least enrofloxacin. Similarly, antibiogram of *E. coli* (ESBL and AmpC) revealed highest resistance to ampicillin, nalidixic acid, amoxy-clavulanic acid, tetracycline, cefpodoxime, cefoxitin, cefotaxime, aztreonam, ceftriaxone, Imipenem, co-trimoxazole, chloramphenicol and least was found in enrofloxacin. The multiple antibiotic resistance index (MARI) was ranging from 0.2-0.6 in both MRSA and ESBLs. There was no evidence of MRSA in small ruminants. The study had significant understanding about the transmission of AMR pathogens between livestock, humans and environment.

OP_4.8 : SALMONELLOSIS OUTBREAK IN A PRIVATE JAPANESE QUAIL FARM AND IT'S MITIGATION STRATEGY

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Japanese quails are hardy and disease resilient game birds and have been hunted since ages for food. Since three decades *J. quails* are raised in semi-intensive and intensive farms for quail meat. But in intensive rearing, problems like occurrence of diseases, nutritional deficiencies etc., arises. *J. quails* are administered autogenous inactivated vaccine for Manhaemiasis to which quails are susceptible. But outbreaks of other bacterial diseases also occur. Salmonellosis outbreak in any poultry meat, meant for human consumption needs effective treatment and stringent control along with bio-security measures since it is a serious public health hazard. In this study, we discuss about the outbreak of Salmonellosis and its effective control measure in a private *J. quail* farm in south Tamil Nadu. Totally 3 Nos. of dead *J. quails* (106 days old) and ailing birds (03 Nos.) along with 3 packets each with 2 Nos. of femur was received with history of mortality reaching up to 85% and morbidity of 95% in affected birds. In pre-partum examination the ailing quails, exhibited clubbed toes due to soiled bedding and faecal material. Vent was soiled due to enteritis and the birds were debilitated. PM was done after blood collection and samples like liver, lung, spleen, trachea, femur, ileo-caecal junction and intestines etc., was collected for testing. Heart blood and tissue impression smears were stained with Giemsa. Bacterial isolation on different media was done for cultural characterization. Gram's staining exhibited Gram negative long slender rods. The isolates were subjected to panel of bio-chemical and the IMViC test. Production of H₂S in TSI slant indicated *Salmonella* spp tentatively. As per the AST, the isolate was sensitive to antibiotics like Fosfomycin, Ceftriaxone, Ciprofloxacin, Gentamicin, Co-trimazole, had an intermediate action to Erythromycin, Cefixime and Streptomycin and was resistant to Ampicillin, Bacitracin, Lincomycin, Vancomycin, Doxycycline Hcl. Differential diagnosis was done by molecular screening



for *Pasteurella* spp by PM-PCR for KMT gene and was negative for 460 bp. Toxino-typing and virulence genes screening was done by m-PCR for suspected *E. coli* (APEC) and was negative. PCR was carried out for liver tissue and intestines of *J. quails* suspected for Salmonella spp. using genus specific primers targeting *inv-A* gene and both were positive and amplified a product size of 284 bp confirming Salmonella spp infection. An autogenous inactivated vaccine incorporating the etiological agent was prepared and supplied to contain the disease.

OP_4.9 : COMPARATIVE GENOMICS OF LUMPY SKIN DISEASE VIRUS FROM DIFFERENT WAVES OF OUTBREAKS IN INDIA SPANNING FROM 2019-2023

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Lumpy skin disease (LSD) is a fast-moving transboundary viral disease infecting cattle and buffaloes and is a WOAHL-listed notifiable disease. It was first reported in India in 2019 and has since rapidly spread across the country. We had previously reported the first complete genome sequence (from South India) of a pathogenic LSD virus (LSDV) from India (LSDV/208/PVNRTVU/2020) obtained by direct sequencing of a suspected clinical sample using Illumina and Nanopore sequencing technologies. Here, we report the complete genome of LSDV (LSDV/IND-2022/NIAB-PVNRTVU) obtained by Illumina-based sequencing from an outbreak in Telangana State in October 2022. Comparative genomic analysis was done with the LSDV whole genome sequences (WGS) from previous outbreaks in India and worldwide. The complete genome sequence of LSDV/IND-2022/NIAB-PVNRTVU is 150763 bp long, codes for 158 putative genes and carries about 2000 bp inverted terminal repeats at either end. Variant calling analysis with complete LSDV sequences reported from India and other parts of the world revealed SNPs ranging from 25-1424, MNPs ranging from 1-161, insertions ranging from 9-42, and deletions ranging from 1-39. Phylogenetic analysis of the complete list of WGS of LSDV available in NCBI Genbank revealed that the current strain clustered separately from the previously reported LSDV sequences from India. This is in corroboration with variation in the clinical signs noticed in the different waves of LSD outbreaks reported during 2019-2023. Such variable rates of genetic changes warrant a routine and large-scale virogenomic survey for LSDV throughout the country. Since India presents a heterogeneous population of several breeds of cattle and buffaloes, as well as vector populations and weather conditions, all of which could potentially influence virus maintenance, transmission and evolution, comprehensive genomics studies will help understand the dynamics of disease outbreaks and aid in planning effective strategies for mitigating the spread and future epidemics.



**OP_4.10 : SEROTYPING AND VIRULENCE PATHOTYPING OF
ESCHERICHIA COLI ISOLATED FROM ZOO HERBIVORES.**

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A study was carried out on Isolation, molecular characterisation, and serogrouping of *E.coli* isolates from zoo herbivores. A total of 200 faecal samples were collected from different species of Zoo herbivores and were found 100% positive for *Escherichia coli* on cultural isolation. Whereas upon molecular diagnosis using 16s rRNA gene, 95% were positive for *E. coli*. The different serotypes observed in the isolated *E. coli* are O5, O7, O9, O11, O17, O18, O26, O63, O69, O76, O83, O84, O85, O86, O88, O98, O101, O107, O111, O119, O120, O126, O128, O149. The distribution of various serotypes in zoo herbivores is O84> O76> O11> 126≥ O26 with 8.57%, 7.61%, 6.66%, 3.80% and 3.80%. The percentage of rough serotypes is 16.19% and untypable is 20%. Virulence genotyping of *E. coli* isolates revealed the presence of STEC, EAEC as 12.10% and 0.5%, respectively and none were found positive for NTEC, and EPEC. The STEC genes identified are *stx1*, *stx2*, *eaeA* and *hlyA* and the EPEC gene *east* was also identified. To the best of our knowledge the study observed the occurrence of EPEC in Zoo Herbivores for the first time.

**OP_4.11 : STUDY OF MOLECULAR VARIATIONS IN COMMON RESPIRATORY
BACTERIAL INFECTIONS OF POULTRY IN RAYALASEEMA REGION OF
ANDHRA PRADESH**

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The most frequent causes of death and morbidity in chicken are respiratory diseases, which cause the poultry industry to suffer significant financial losses. we can decrease the losses by early detection of respiratory pathogens. So the present study was aimed to detect and differentiate the common respiratory bacterial pathogens of poultry. The samples were collected from 18 suspected poultry farms located in Rayalaseema region of Andhra Pradesh and were subjected to culture and molecular detection for the presence of *ORT*, Avian mycoplasmas (*MG* and *MS*) and *E.coli* by targeting *16S rRNA* gene for *ORT*, *mgc2* gene for *MG*, *vlhAgene* for *MS* and *16S rRNA* gene for *E.coli*. The overall incidence of all three pathogens, *ORT* with 77.77%, *E.coli* with 88.88%, *Mycoplasma* with 83.33%, *MG* with 11.11%, *MS* with 83.33% and the combinations of infections were *ORT+E.coli+Mycoplasma* with 66.66%, *ORT+ E.coli* with 5.55%, *E.coli +Mycoplasma* with 11.11% and *MG + MS* with 11.11% were observed. The *ORT* isolates showed 11 nucleotide substitution at G6C, C7G, T22, T27C, A30T, A32T,



G33C, A36G, G95A, T352C, and A728T. OP919604 isolate showed 4 nucleotide substitution at G3T, T4G, G6A and G33A. OP919610 isolate showed single nucleotide substitution at G33A. OP919613 showed 8 substitutions at G74T, G80A, A81G, T140C, A149G, A199T, C507T, and A723C. The MS isolates showed 7 nucleotide substitution at A10G, A11G, C14A, T22C, G230A, A242G & T243A and MG TP5 showed an additional 11 substitutions at C6T, C45T, A51G, T71C, T99C, G112T, A115G, A132C, C164A, A179C and G205A. The multiple sequence analysis of the published MG *mgc2* gene of the MG TP4 and MG TP5 isolates nucleotide substitutions were observed at A495G.

POSTER PRESENTATIONS

PP_4.1 : MOLECULAR CHARACTERIZATION AND PULSED-FIELD GEL ELECTROPHORESIS (PFGE) ANALYSIS OF *LISTERIA MONOCYTOGENES* ISOLATES FROM SEWAGE AND VEGETATION

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Listeria monocytogenes is an opportunistic and an important foodborne pathogen that is widely distributed in the environment. In developing countries, sludge from sewage is currently spread onto agricultural land without any sanitation procedure, and this entails some risk of introducing pathogenic agents into the soil. The present study is to characterize *L. monocytogenes* from sewage and vegetation growing around in Nagpur. Total of 170 samples were screened for the isolation of *L. monocytogenes*. The isolation was attempted by two-step enrichment procedure and confirmation on basis of the biochemical tests and haemolysis on sheep blood agar. Recovered isolates were tested for the virulence-marker genes namely *hly A*, *plc A*, *iap*, *act A* and *prf A* by PCR. Further these isolates were serovar grouped. The isolates were subjected to Pulsed-field gel electrophoresis (PFGE) analysis. Overall, prevalence of *Listeria* spp. in the present investigation was found to be 10%. Out of seventeen *Listeriae*, 12 (7.05%) were biochemically characterized as *L. monocytogenes* and 5 (sewage) and 7 (vegetation) isolates of *L. monocytogenes* were isolated. Serovar grouping of these isolates indicated seven isolates are of 1/2 a serovar group. The PFGE analysis revealed 12 *L. monocytogenes* isolate from *Apal* with genetic similarity. The PFGE discriminated the 12 isolates and displayed the same PFGE patterns (pulsotypes), and serovars within serogroups. In comparison majority of the strains showed location and serovar wise homology in PFGE pattern. The finding from this study revealed that sewage and vegetation growing around is a potent source of *L. monocytogenes* especially for spread of pathogenic agent and transmission of disease.



**PP_4.2 : ISOLATION AND CHARACTERIZATION OF LUMPY SKIN DISEASE VIRUS
IN EMBRYONATED CHICKEN EGGS: INSIGHTS INTO PATHOGENESIS
VIA HISTOPATHOLOGICAL ANALYSIS**

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Lumpy skin disease (LSD) is a re-emerging viral disease that has resurfaced in cattle. Since 2019, outbreaks of LSD were reported across the various states of India, with Andhra Pradesh as one of the main impacted areas where animals were severely suffered resulting in severe economic losses to farmers. Hence, a modest attempt has been made to study the LSDV isolates from Andhra Pradesh with regard to isolation and characterization. In the current study scab samples collected from the suspected cattle were subjected to PCR assay targeting LSDV specific fusion gene (LSDV117) for confirmation. These isolates were processed and inoculated on the chorioallantoic membrane (CAM) of 10-day old embryonated chicken eggs (ECE) to study the growth characteristics of isolates. After 3-4 serial passages, pathological findings observed were congestion, haemorrhage, and thickening of the membrane with whitish pock lesions on CAM. Histopathological examination of infected CAM showed vacuolar degeneration of epithelial cells, with congestion of blood vessels and haemorrhages over CAM. There was presence of eosinophilic intracytoplasmic inclusions within the epithelial cells which is the characteristic of Pox virus. The presence of virus in infected CAM was confirmed by PCR assay targeting F gene. Later, PCR products were sequenced and phylogenetic tree was constructed. Henceforth, it is inferred that this isolation of virus in ECE was simplest and cost-effective technique at hand for diagnosis but adaptation of LSDV to ECE was difficult as they were heterogenous.

**PP_4.3 : STUDIES ON ANTIBIOGRAM PATTERN OF BACTERIAL ISOLATES
FROM BOVINE AND BUBALINE SEMEN OF BREEDING BULLS IN KARNATAKA**

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Indian dairying has emerged, as the world's largest producer of milk and this was possible to a greater extent with the introduction of artificial insemination (AI) and frozen semen technology. Even under careful and strictly monitored conditions, semen often gets contaminated during collection, processing and preservation. The possibility of these organisms becoming resistant to conventional antibiotics cannot be ruled out because of indiscriminate use of Penicillin and Streptomycin which are being employed in routine dilution of semen. Keeping the above facts in mind the present study was carried out to isolate the bacterial organisms from the neat semen, extender and frozen straw and study the Antibioqram pattern for the isolates to study the efficacy of presently used antibiotics in semen stations. A total of twenty-eight HF bulls (Farm A - 08, Farm B - 12 and Farm C - 08) and fourteen



Murrah (Farm A and C), two Surthi (Farm C) buffaloes were selected for the study. A total of 205 bacterial isolates obtained from 244 samples of neat and frozen semen from three different FARMS and they were typed up to genus level by culture and staining methods. The isolates were mainly belonging to the genus *Staphylococcus*, *Klebsiella*, *Proteus* and *Bacillus*. Of the 205 isolates, the number of isolates from different FARMS were 49 from FARM A, 63 from FARM B and 93 from FARM C. Many isolates have shown the higher sensitivity to the newer generation antibiotics like Imipenem, Piperacillin Tazobactam and least sensitivity to resistance to the commonly used antibiotics like Penicillin and Streptomycin. The details will be presented during the presentation.

PP_4.4 : MOLECULAR CONFIRMATION OF PNEUMONIC PASTEURELLOSIS AND TACHYGONETRIA SP., IN A STAR SHELLED TORTOISE

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A star-shelled tortoise (*Geochelone elegans*) was presented to the Small Animal Medicine Referral Clinic of Veterinary College and Research Institute, Orathanadu, Thanjavur by the Forest Department, Pattukottai division with a history of sero-nasal discharge and diarrhoea. On clinical examination, the tortoise was dull, bubbling of nasal discharge from the nostril, whistling, open mouth breathing was noticed with pale oral mucosa. Blood from the sub-carapacial vein was collected for hematological and biochemical analysis. Faecal and cloacal swab with worms were collected for parasitological examination whereas nasal swab was collected for microbiological examination. Morphological examination of the worms present in the fecal sample was identified as *Tachygonetria* sp. To ensure precision in identification, molecular confirmation using polymerase chain reaction (PCR) was employed. In parallel, nasal and tracheal swabs were subjected to bacterial culture, revealing the colonies of *Pasteurella* sp. on blood agar. To fortify these results, PCR was employed for confirmation, providing molecular evidence, supporting the presence of *Pasteurella* sp. This dual-method approach ensured robust identification of the bacteria and parasite. The tortoise was diagnosed with the concurrent infection of *Pasteurella* sp., and *Tachygonetria* sp., which were the culprit for pneumonia and diarrhoea. Importantly, the study highlights the importance of employing a multidisciplinary diagnostic strategy, combining traditional methods with advanced molecular techniques, to comprehensively unravel the complex etiology of infections in reptiles. As per the literature, this is the first report on molecular confirmation of *Pasteurella* sp., and *Tachygonetria* sp., from star-shelled tortoise in India.



TECHNICAL SESSION V

**NANOTECHNOLOGY &
BIOINFORMATICS**





IMMUNOINFORMATIC APPROACHES IN VACCINE DESIGN

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Immunoinformatics is an emerging field that combines bioinformatics, immunology, and computational biology to develop computational tools and technologies to study the immune system. Immunoinformatics helps in identification of potential antigens, understanding immune responses, and designing novel immunotherapeutics. In recent years, immunoinformatics has opened the doors for vaccine development at a lightning-fast speed as compared to the traditional *in vitro* and *in vivo* approaches.

The worldwide spread of COVID-19, an infectious disease caused by SARS-CoV-2 has led to over millions of deaths around the world. The emergence of SARS-CoV-2 and the subsequent COVID-19 pandemic underscored the critical importance of having efficient and rapid platform technologies for developing vaccines against emerging viral diseases. The traditional vaccine development process can be time consuming and may not be well suited to respond quickly to novel and rapidly spreading viruses. Platform technologies offer a more adaptable and streamlined approach to vaccine development. Vaccines are urgently needed to prevent the spread of the diseases caused by emerging of new viruses which lack an existing effective medical therapy. Despite the inherent propensity of viruses to mutate and evolve over time, constant efforts are required to produce better vaccine candidates to counteract emerging viral strains. Structurally, SARS-CoV-2 contains four structural proteins; spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The spike protein is an attractive target for vaccine design because it facilitates viral entry into the host cell during the process of infection.

The spike (S) protein of SARS-CoV-2, which plays a key role in the receptor recognition and cell membrane fusion process, is composed of two subunits, S1 and S2. The S1 subunit contains signal peptide (SP), N-terminal domain (NTD), receptor-binding domain (RBD); The S2 subunit contains fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2); transmembrane domain (TM), cytoplasmic (CP) domains. Receptor-binding domain recognizes and binds to the host receptor, angiotensin-converting enzyme 2 (ACE2), thereby virus enters into the host while the S2 subunit mediates viral cell membrane fusion by forming a six-helical bundle *via* the two-heptad repeat domains. RBD domain of S protein was selected for the development of a pan-corona virus vaccine.

We used *in silico* methods to investigate the linear B-cell epitopes (LBL), cytotoxic T lymphocytes (CTL) epitopes in the spike protein of SARS-CoV-2 virus. We identified two B-cell epitopes and three cytotoxic T lymphocytes in the spike protein of SARS-CoV-2 virus. The human population coverage, antigenicity, allergenicity, toxicity, physicochemical properties and secondary



structure of the proposed vaccine were evaluated by bioinformatics approaches had good quality of the designed vaccine. Further conservancy analysis of the predicted cytotoxic T lymphocytes and B-cell epitopes were carried out among all the variants of SARS-CoV-2. The 3D structures of the CTL and the LBL vaccine candidates were predicted, refined and validated by *in silico* tools. Molecular docking was employed to investigate the binding affinity of the predicted CTL epitopes with TLR4 and 12 predominant HLA molecules and predicted B cell epitopes with neutralizing IgG antibody using BioLuminate (Schrödinger Release 2022-4). All the predicted epitopes had good binding affinity than the existing epitopes. Molecular dynamic simulations were performed for the predicted CTL with TLR and 12 MHC molecules; and LBLs with neutralizing antibody IgG using Desmond v7.2 module to evaluate the stability in physiological environment.

Nanotechnology benefits modern vaccine design since nanomaterials are ideal for antigen delivery as adjuvants and also mimics as viral structures. Virus-like particles (VLPs) are virus-derived structures made up of one or more different molecules with the ability to self-assemble, mimicking the form and size of a virus particle and serve as adjuvants making them efficient activators and amplifiers of antigen-specific immune response. Multi-epitope vaccine candidates with the predicted CTL and LBL epitopes were generated to enhance the immune response and were displayed on VLP which function as a combined adjuvant and epitope nano-carrier promoting antigen-presenting cells.

In silico immune simulation study was conducted for the vaccine candidates to explore the generation of adaptive immunity and also the immune interactions. Multi-epitope vaccine candidates showed elevated rates of active B-cells, plasma B-cell, helper T-cells and cytotoxic T cells; antigen-presenting cells like dendritic cell, epidermal cells and macrophage. Neutralizing effect of antigen was also observed with the production of different immunoglobulins (IgM, IgG, IgG1, IgG2 and IgM). Vaccine candidates also produced different types of cytokines which are important for cytotoxic T cells, T helper cells and B-cells production. The proposed multi-epitope vaccine candidates were observed promising against the SARS-CoV-2 viral infection and need to be evaluated in *in vitro* and *in vivo* studies.



NANO-IGY TECHNOLOGY: APPLICATIONS

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In spite of the technological advancement in the field immunology and immunodiagnostics, infectious diseases posing a problem with high morbidity and mortality. Recently “IgY technology” has attracted many researchers because of the advantages of IgY antibodies over IgG. IgY antibodies can be obtained from egg yolk non-invasively causing no pain to the animal thereby eliminating the need for blood collection. The production steps are so simple yet facilitating surplus amount of antibody production, i.e., 100-150 mg of IgY per egg. Unlike IgG, IgY is less flexible which is believed to attribute to an increased resistance against proteolytic degradation and fragmentation. Moreover, due to large phylogenetic distance between mammals and birds, the IgY antibodies developed are highly specific against mammalian proteins. Chicken IgY is able to activate mammalian complement system and not able to react with mammalian Fc receptors, rendered its use as ideal detecting agents in various immunoassays. On the other hand, administration of IgY is an attracting option with less side effects. Indeed, specific anti-venom IgY have been able to neutralize bacterial toxins. But the potential demerit of IgY in therapeutic field is its loss of activity and stability when susceptible to harsh gastric conditions in the gastrointestinal tract. Prior to the use of IgY for passive immunotherapy, it's imperative to go for an effective strategy that can overcome some of the hurdles faced. And it is in that context, these hurdles have opened up a new avenue i.e., nanotechnology-based immunotherapy.

The nanoparticles are one of the essential and promising tools of the twenty first century, having penetrated practically every industry from engineering, chemistry, physics, biology, and medicine to construction materials, military items, and electronics. It might be small of size about 1 to 100 nm but it is powerful, significant and can have an outsized effect in the modern-day medicine because of its enormous versatile nature. Additionally, the development of "nanotechnology" in the fields of diagnosis, treatment, prevention, and cure has enormous potential to transform the healthcare sector. The progress of nanoscience and nanotechnology have become most significant especially in the field of medicine i.e., medical devices, drug delivery systems, therapeutic & diagnostic applications. In essence, these small nano-scale materials exhibit good biocompatibility and biodistribution and are suitable for drug delivery applications. They have the ability to protect the loaded agents from degradation thereby increasing the stability and half-life. Hence, nanomaterials are engineered to load the suitable therapeutic agents and also enabling their release at appropriate site. These technical advantages of nanoparticles like good carrier capacity, stability and greater penetration make them one of the robust and cutting-edge tools of modern-day medicine .

Nanoparticles being the master of drug delivery platform and IgY technology being the queen of antibody therapy would undoubtedly make one of the best novel combinatorial therapies. Upon encapsulation within the nanoparticles, these nanocarriers can decrease the dosage of antibodies



thereby increasing its efficacy and potency. In addition to this, after encapsulation, these nano-based systems can be tailored with different surface functionalities for effective antibody release at intended site. Most importantly, these nanoparticles are made in such a way that they cause no harm to the encapsulated antibody i.e., the stability and bioactivity of antibody are preserved. Suresh et al.,(1) reviewed the biomedical applications of nanotechnology along with chicken egg yolk antibody (IgY).

Therapeutic Applications

Prolonged use of conventional drugs has led to the development of antibiotic resistant microbes prompting researchers to look for an alternative option and the one that is attractive is passive immunotherapy. The use of IgY antibodies as a novel passive immune therapy combined with nanoparticles can be used against multiple infectious diseases in humans and animals. IgY has proven to be effective against many enteric pathogens like *Escherichia coli*, *Salmonella* spp., and rotavirus by preventing its growth in in-vitro studies and its symptoms in in-vivo models. To maximize its therapeutic potential by preserving the bioactivity of antibodies against enzymatic and pH degradation, IgY are encapsulated with nano materials.

Bellingeri et al.,(2) reported effect nano-encapsulation of IgY using three different materials i.e., hydrogel, hydrogel-carbon, nanotube-chitosan composites with varying percentages of hydrogel on intestinal cells. Carbon nanotube (CNT) which was wrapped with chitosan and incorporated to hydrogel showed no signs of cytotoxicity on primary culture of porcine enterocytes. Addition of CNT to the nanocomposite has helped to improvise its mechanical properties and also to load IgY at higher rates without toxicity issues. Alustiza et al., further studied the application of same material to in-vivo models to check whether the nanocomposite could effectively deliver IgY without getting inactivated in the gastrointestinal tract. The IgY antibodies encapsulated within the nanocomposite were administered orally to piglets to study the effect against enterotoxigenic *Escherichia coli* (ETEC). The results were positive, as in, the hydrogel-carbon nanotube was able to protect IgY against gastric inactivation while sustaining activity in lower intestine.

Subsequently, Bhakshi et al (3) evaluated invitro safety and release of IgY loaded sodium alginate nanoparticles in intestinal cells. Studies revealed that the release of IgY in simulated gastric fluid whose pH is acidic was almost negligible (10%) during 8 hours while its release was complete in simulated intestinal fluid with a basic pH of 6.8 during 24 hours. Further, MTT assay results showed that these sodium alginate nanoparticles of any concentration held no potential in inducing cytotoxicity to vero cells. The results revealed the suitability of sodium alginate particles for oral delivery through gastrointestinal tract. Yet another study by Ma et al., used IgY conjugated chitosan nanoparticles to target Shiga toxin producing *Escherichia coli* (STEC) without disturbing the beneficial bacteria in the gastrointestinal tract. Overall, results suggested that Carbon nanotube-IgY can be a promising candidate to control STEC in the GI tract by enhancing selective killing. Results though promising need to be evaluated further using animal models for obtaining high precision and accuracy (4)



Contradicting to the usual application of IgY antibodies for oral passive immunotherapy Wu et al., used it to treat Allergic rhinitis, an allergic reaction, induced by ovalbumin, to which currently available medications pose severe side effects allergic reaction. Poly methacrylic acid (PMAA) nanogel loaded with IgY antibodies specific against Interleukin-1beta used in the study showed sustained release of IgY and extended therapeutic effect. Guo et al., employed the use of IgY antibodies made against α -amylase to protect against glycemic variability. IgY coupled PEGcoated Fe₃O₄ nanoparticles embedded onto chitosan alginate hydrogel were used in the study. The antibodies at the small intestine as intended posing its inhibitory effect against α -amylase for an extended period of time of more than 36 hours. (5).

Diagnostic Applications

Number of distinct features of IgY compared to IgG have turned the pages towards the application of these antibodies in diagnostic assays and kits. The major point that has to be kept in mind when it comes to diagnosis is the avidity of protein towards the target antigen i.e., antigen-binding specificity. Mammalian proteins are highly conserved and when these are used as antigen in mammals, it might pose less or no antigenicity. Due to this phylogenetic evolutionary difference, IgG usage in medical diagnostics might lead to false positive results. Adding more, proteins in human serum might activate the complement system thereby interfering with the result. Thus, to avoid these hurdles, usage of IgY antibodies have been a better option (6). These IgY antibodies when conjugated with nanoparticles have a great potential in conferring increased sensitivity and accuracy for different immunoassays (7). Among the different nanoparticles that are used for diagnostic applications, gold nanoparticles are widely used owing to its good optical and electronic properties. Gold nanoparticles serve as an excellent platform in biosensing applications as they have high surface area to volume ratio and biocompatibility. Gold nanoparticle-based immunoassays have been largely benefitting biologists as they are suitable cell targeting vectors for qualitative and quantitative analysis. In order to sense the analyte, the surface of nanoparticles is fabricated using specific moieties to increase sensitivity and selectivity.

Lisa et al., developed an immune dipstick method using IgY antibodies, which is quick and accurate for detection of Dichloro diphenyl trichloro ethane (DDT) (8). In another study Zhang et al., detected Swimming crab reovirus (SCRV) a causative agent leading to 100% mortality within 10 days affecting the crab industry using IgY-gold complexes.

Egea et al., developed a diagnostic kit for the detection of indoor dust mite allergens using polyvalent IgY antibodies conjugated to colloidal gold nanoparticles. Results showed that these conjugates were able to recognize the allergens with a detection limit of 0.78 microgram. Chemiluminescence immunoassay is widely used for the detection of human IgG levels in humans. To overcome its high cost, lack of sensitivity and selectivity, Yeritsyan et al., used IgY antibodies conjugated onto gold nanoparticles for the detection of human IgG and achieved a detection limit of 30 ng/mL. (9) Selvakumar et al., developed an immunodipstick based gold nano sensor to detect



vitamin B12 in fruits and energy drinks and were able to achieve 1 ng/mL detection limit. This study has a full potential in emerging as a biosensor tool for detection of various analytes like toxins, adulterants, microbes and for monitoring purpose (10). Another similar study using gold nanoparticles to detect *E. coli* O157:H7, Yaghubi et al., showed high specificity, speed and limit of detection of about 10 CFU/mL. A different strategy i.e., rapid immunochromatographic assay using Fe₃O₄/Au composite nanoparticles were synthesised for the detection of same pathogen (*E. coli* O157:H7) and achieved detection limit of 10³ CFU/mL. Liu et al., demonstrated the detection of a food-borne pathogen - *Vibrio parahaemolyticus*, using a fluorescence quenching system along with gold nanoparticles. Similar methods with slight modification were also used for detection of other viral agents. (11).

Pang et al demonstrated the advantage of gold nanorods over gold nonospheres to absorb more biological moieties like IgY and urease enzymes. Further proved that the probes can also be tagged to specific magnetic beads which lead to the formation of sandwich-type immunocomplex, that can be separated in the presence of external magnetic field. It appears to be a novel, rapid and specific strategy to develop a test to detect at least 460 cfu/mL which has a huge potential in food safety application for screening bacteria. Yet another study by Roushani et al attached IgY on to the gold nanoparticle and further modified using glassy carbon electrode for better capture the bacteria and to amplify the electrochemical signal. This immunosensor was able to accurately detect bacteria with detection limit of 3.3 cfu/mL. (12).

The next widely used nanoparticles in the field of diagnostics are quantum dots because of their inherent optical and electronic properties. Moreover, conjugation of biomolecules with quantum dots helps achieve high specificity, sensitivity and biocompatibility. Quantum dots are easily excitable, produce strong luminescence thereby possessing high photostability. Chouhan et al., developed a diagnostic kit by conjugating IgY antibodies onto Cadmium telluride quantum dot for sensitive and specific detection of methyl parathion (pesticide). This was shown to be effective over HPLC, ELISA and other techniques. (13).

Zhang et al., developed a lateral flow immunochromatographic strip (LCS) using IgY conjugated quantum dots (CdTe/ZnS) to detect rhein, a component found in traditional Chinese medicine with detection limit of 98.2 ng/mL. (14). Very interestingly, a study by Li et al., reported the development of a detection method that uses fluorescence signal to detect a member of intracellular bacteria, *Brucella melitensis* 16M in food samples based on peptide-mediated magnetic separation (PMS) technology. One other study by Liu et al., developed a colorimetric assay using gold nanoparticles along with silver nanoclusters to rapidly detect a food-borne pathogen, *Listeria monocytogens*. (15).

A study by Kilinc et al., employed latex nanoparticles. IgY was synthesized against M2e peptide. Latex nanoparticles were conjugated to anti-M2e IgY antibody in the latex agglutination assay for diagnosing influenza A virus. This methodology was able to specifically recognize two different strains of influenza virus, that is, H3N2 and H1N1. Though this study has potential in diagnosis,



prevention and treatment of influenza A virus, the limit of detection, its specificity and selectivity are yet to be discussed (16). Xiao et al., used nano-IgY conjugates for both detection and destruction of cancer cells and proved that it is one of the most promising and suitable modalities for therapeutic and diagnostic application (17).

In conclusion nanoparticle-IgY conjugates have a great potential in the field of diagnostics and therapeutics. They possess high sensitivity, selectivity, accuracy. Nonetheless, the translation of IgY nanotechnology from laboratory settings to clinical setup still faces a significant setback due to a wide literature gap, manufacturing and commercialization problems and need to be addressed.

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ORAL PRESENTATIONS

OP_5.1 : POLYMERIC MELOXICAM NANOPARTICLES MITIGATE MONO IODOACETATE-INDUCED OSTEOARTHRITIS IN WISTAR RATS BY MODULATING NF- κ B AND *Nrf2* PATHWAYS

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The study aimed to assess the efficacy of polymeric meloxicam nanoparticles incorporated with chitosan in alleviating monosodium iodoacetate (MIA) induced osteoarthritis (OA) in male Wistar rats, focusing on pain behavior, inflammation, ankle histopathological changes, and oxidative stress. After the induction of OA, rats were divided into five groups and treated for 21 days: MIA-only, MIA with meloxicam (1 mg.kg⁻¹ b.wt), MIA with high-dose of chitosan-encapsulated meloxicam nanoparticles (CEMNPs) (1 mg.kg⁻¹ b.wt), MIA with low-dose CEMNPs (0.2 mg.kg⁻¹ b.wt), and MIA with chitosan nanoparticles (CNPs) (1 mg.kg⁻¹ b.wt). The control group received saline in the left knee joint. Treatment with CEMNPs notably improved various OA parameters such as including voluntary and involuntary movements, beam walking ability, footprint analysis, joint diameter, body weight, and total leukocyte count. High-dose CEMNPs significantly reduced lipid peroxidation and nitrosative stress while increasing levels of antioxidant enzymes (SOD, CAT, GSH, GPx, and GR) in OA rats. Additionally, high-dose CEMNPs significantly decreased CRP, NF- κ B, PGE₂ levels and improved *Nrf2* levels in joint tissue. Behavioral observations and histological analysis indicated reduced joint inflammation and cartilage destruction in CEMNPs-treated OA rats. Both low and high doses of CEMNPs demonstrated chondroprotective effects, with the low dose comparable to meloxicam. In conclusion, CEMNPs exhibit promising antiarthritic potential in MIA-induced OA, even at significantly lower doses than meloxicam, suggesting a potential avenue for clinical applications.

POSTER PRESENTATIONS

PP_5.1 : STUDY OF ANTIMICROBIAL EFFECT OF SILVER NANOPARTICLES AGAINST THE CLINICAL ISOLATES OF OVINE PNEUMONIA

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Microbial resistance to the antibiotics is a huge problem in the treatment of infections. The advancement of technological approaches like nanotechnology in medicine is coming as a novel approach to combat antibiotic resistance. The alternative strategies like nanotechnology, phytochemicals are promising well to handle this resistance problem. Phytochemical substances, symbiotics etc., are gaining importance in this aspect. Among these silver nanoparticles are reported to show good antimicrobial activity. Curcumin, the phytochemical extracted from rhizome of *Curcuma*



longa has a variety of properties including anti-inflammatory, antioxidant and anti-bacterial activity. The possible synergistic association between silver nanoparticles and curcumin in a formulation can be a good alternative to control infectious diseases. In the present study, the silver nanoparticles were synthesized phylogenically by using curcumin. 5mg of curcumin was dissolved in 10ml of distilled water containing 2mM NaOH. This solution was added drop wise to 90 ml of 2mM silver nitrate solution by stirring for 1 h at 80^oC. The change of the colour of the solution from yellow to dark brown was indicative of the synthesis of nanoparticles. Then the solution was centrifuged at 10000 RPM for 10 min and the nanoparticles separated were used for the MIC study. The pure cultures of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae* isolated from ailing Ovinos near Pellimarri, Kadapa district, Andhra Pradesh were used to derive the minimum inhibitory concentration (MIC) value for silver nano particles. The MIC of phylogenetic silver nanoparticles was evaluated against standard antibiotic enrofloxacin.

**PP_5.2 : QUERCETIN MEDIATED ZINC OXIDE NANOPARTICLES:
SYNTHESIS, CHARACTERIZATION AND *IN-VITRO* EVALUATION OF
ANTICANCER ACTIVITY AGAINST HELA CERVICAL AND MDA-MB 231
BREAST CANCER CELL LINES**

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The current research study was undertaken to synthesize and analyze zinc oxide nanoparticles (ZnO NPs) mediated by quercetin (QZnO NPs) and assess their potential in combating cancer cells, particularly HeLa cervical cancer cells and MDA-MB 231 breast cancer cell lines. The synthesized ZnO NPs measured around 75 nm, while the QZnO NPs were approximately 168 nm, exhibiting zeta potentials of -14.5 mV and -36.7 mV, respectively. Both types of nanoparticles displayed low polydispersity indices - 0.226 for ZnO NPs and 0.277 for QZnO NPs suggesting uniform dispersion characteristics. The *in-vitro* drug release studies revealed a notable sustained-release pattern (54.76% at 24 h) at pH 4.8 compared to a less sustained-release (21.07% at 24 h) at pH 7.4. This indicated a preference for sustained drug release within the acidic tumor microenvironment. The investigation also included *in-vitro* cytotoxicity assessments on HeLa cervical cancer cells and MDA-MB 231 breast cancer cell lines. Free quercetin, ZnO NPs, and QZnO NPs were tested at varying concentrations in triplicate over a 48-hour incubation period. Results from the MTT assay revealed significant differences in the IC₅₀ values between quercetin, ZnO NPs, and QZnO NPs in both cell lines ($p < 0.01$). This variance was attributed to the uptake of nanoparticles by the cancer cells and the gradual release of the drug within the cells. Notably, the findings indicated that QZnO NPs effectively impeded the progression of cancer cells *in-vitro*. Consequently, further comprehensive studies are warranted to thoroughly characterize the toxicity profile and elucidate the underlying mechanisms responsible for the anticancer efficacy of these nanoparticles.



TECHNICAL SESSION VI

INNOVATIONS IN IMMUNODIAGNOSTICS AND THERAPEUTICS





TRANSLATING INNOVATIONS IN DIAGNOSTICS: CHALLENGES AND OPPORTUNITIES

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The Oxford dictionary defines “Diagnosis” as the act of discovering or identifying by examination the exact cause of an illness or a problem. It originated from the Greek word literally meaning “to discern or to distinguish or to know thoroughly.

Diagnosis of infection / diseases is done by any one or a combination of the following methods:

- Causative pathogen isolation and identification
- Direct detection of pathogen – Electron microscopy
- Detection of the protein component of the pathogen – ELISA, Strip tests
- Detection of the nucleic acid component of the pathogen – PCR, qPCR, LAMP
- Combination of the protein and nucleic acid detection methods – PCR-ELISA
- Detection of serum antibodies to the pathogen (paired sera) – ELISA
- Differentiation of infected from vaccinated animals (DIVA) strategy

Diagnostic tests are primarily classified by purpose as

- **Diagnostic** - A diagnostic test is used to determine or confirm the presence or absence of a disease when a subject shows signs or symptoms of the disease
- **Screening** - A screening test identifies asymptomatic individuals who may have the disease. This is primarily done to monitor disease prevalence, epidemiology, facilitate prevention or in understanding disease pathogenesis

Diagnostic tool-box

Protein based immunoassays are an analytical method, quantitative or qualitative, which uses antibodies as reagents to quantitate specific analytes. Immunoassays such as ELISAs rely absolutely on the specificity and affinity of the interaction between epitope on the antigen and paratope on the antibody.

Genome based assays such as polymerase chain reaction (PCR) relies on interaction between two complementary sequences, linked to signal generation. Such assays also involve an amplification step and can be either qualitative or quantitative.



Both these technologies provide complementary information.

Ideally a diagnostic test should classify all subjects who have the disease as “diseased” and vice versa. This ability of the test under question to classify subjects is evaluated before the test is applied in diagnosis

The context of disease or infection detection and the decisions to be taken based on the diagnosis are the critical elements of choosing a diagnostic platform.

Among the OIE-approved assay formats for the fit-for-purpose tests for prevalence of infection, antibody detection by ELISA (26%), as a measure of exposure, and agent detection by PCR (22%), as a measure of infection, comprises the majority of OIE-recommended tests.

Translating Diagnostics for Field-deployability

Every academic institution is being questioned about the impact created by them. The only performance indicator for the institution’s research capability is the number of products developed that are being used widely and did they change the lives of the common man, in our case, the farmers!

Translation refers to the process of converting laboratory discoveries into clinical interventions that provide a direct benefit to animal / human health. In India, several institutions are engaged in developing diagnostic assays or diagnostic reagents but the translatability of these assays in to affordable and available kits for the stake holders seems to be lacking to the extent desired. This responsibility lies with the Academia, Government and Industry. The so called ‘rhyming phrases’ of lab to land, field to fork, bench to bedside, mind to market etc. seems to suffer from a ‘translational disconnect’.

In the Veterinary Diagnostics front, this gap is still wide, some the reasons being:

- Affordability and the clinical question whether the treatment is going to change based on diagnosis
- Lack of drive and zeal on the part of the inventor
- Technologies always keep changing
- Lack of continuity in research / personnel
- Changing priority – dictated by funding
- Only a few tests are available in the form of kits
- Mindset of the end user in terms of confirmatory or lab-based diagnosis
- Inconsistent demand and low market volumes

Some of the challenges in converting diagnostic assays in to user-friendly kits can be classified as either scientific or translational.



Scientific – Developmental challenges

- Type of assay – Genome or protein
- Determination of a cut-off value for positivity
- Diagnostic sensitivity and specificity
- Analytical sensitivity and specificity
- Validation of the test
- Availability and volume of positive control sera
- Interpretation of test results

Scientific – Application challenges

- Disease agents to be included in the test programme
- Sampling strategy
- Type of samples
- Frequency of testing

Translational challenges

- Limited market
- Inconsistent market
- Direct costs and cost benefit analysis
- Kit formulation
- Regulatory approvals
- Acceptance by international agencies

Opportunities for innovation in diagnostics

The technological explosion in making the diagnostic assays sensitive and at the same time, user-friendly and field-deployable, is the greatest opportunity available for the present-day diagnosticians. The real time PCR was the gold standard test for COVID diagnosis mainly because of the lower levels of false positives diagnosis that might have occurred had PCR was used. However, if real time PCR was coupled with a field-deployable lateral flow assay to detect the amplification of the target gene, this strategy would offer itself as a kit with all requirements of field applicability.

The isothermal amplification methods such as Loop mediated Isothermal assay (LAMP), Recombinase Polymerase Assay (RPA) etc. coupled with protein / small molecule-based detection is the way forward for the next-generation diagnostics. The fore-runner of this technology is the PCR-



ELISA method wherein a digoxigenin labeled PCR primer and a biotinylated capture probe was added to the denatured PCR product which was later captured onto a streptavidin coated ELISA plate. Detection was done using anti-digoxigenin horseradish peroxidase conjugate. Now, small molecules such as biotin or FITC are incorporated into analytes or reaction products, which are detected by gold conjugated antibodies that results in the formation of the coloured test-line on lateral flow strips.

The Point-of-Care (PoC) molecular diagnostic tool-box is headed towards detection using an isothermal nucleic acid amplification method, introducing specificity of amplification with a CRISPR detection and finally LFA-based read out platform.

The way forward for converting lab-based diagnostic assays into POC field-based kits

Of the diagnostic tool box available, nucleic acid-based assays such as (RT) PCR are now becoming the clinical diagnostic standard! However, for field-use of these assays the way forward is to initially follow these steps,

- Identification of need of kits
- Patent infringements and Technology / reagents licensing
- Technology giant-leap, favoured by collaboration with industry
- Diagnosis with relevance to humans also
- Extensive validation and trying to obtain international validation such as WOAH
- Awareness among practicing vets to confirm clinical diagnosis using laboratory tests
- Aiming for global markets

If these steps are scrupulously followed, one of our institutions would end up developing a PoC kit which is not only successful in the local market but can also obtain OIE approval, which at present is a distant dream as in India there are no veterinary kits that is approved.



IMMUNOTHERAPY IN ONCOLOGY

Dr. Nageshwar Reddy

Introduction

Immunotherapy has emerged as a revolutionary approach in the field of oncology, offering new hope to cancer patients by harnessing the power of the immune system to combat cancer. Unlike traditional treatments like chemotherapy and radiation, which directly target cancer cells, immunotherapy works by bolstering the body's natural defenses. This article explores the history, physiology, various classes of immunotherapeutic agents, mechanisms of action, applications in different cancers, adverse effects, challenges, and future directions of immunotherapy in oncology.

Brief History

The roots of immunotherapy can be traced back to the late 19th century when William Coley, a surgeon, began injecting bacterial toxins into cancer patients. His pioneering work laid the foundation for modern immunotherapy, and he observed occasional tumor regressions. However, it was not until the mid-20th century that immunotherapy gained traction as a legitimate cancer treatment. The discovery of immune checkpoint inhibitors and development of Car-T cell therapy in the late 20th and early 21st centuries marked significant milestones in the evolution of immunotherapy.

Nobel Prize in Immunotherapy

In 2018, the Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo for their groundbreaking discoveries in cancer immunotherapy. They developed immune checkpoint inhibitors, a class of immunotherapeutic agents that have revolutionized the treatment of various cancers by blocking inhibitory pathways that cancer cells exploit to evade the immune system.

Physiology: The immune system is a complex network of cells, tissues, and molecules designed to defend the body against foreign invaders, including cancer cells. T cells, a type of white blood cell, play a central role in this defense. They can recognize and destroy abnormal cells, including cancer cells, but tumors often develop mechanisms to evade this immune surveillance. T cells recognize peptide antigens, derived from proteins degraded intracellularly, that are loaded onto cell surface MHC molecules, a process called antigen presentation.

Different Classes of Immunotherapeutic Agents:

1. **Immune Checkpoint Inhibitors (ICIs):** Certain molecules that have been conserved throughout evolution play a role in dampening T cell activation. These are known as 'checkpoint molecules' and they help regulate the immune response, preventing it from becoming overly active. Two notable examples of such molecules are CTLA4 and PD1. These molecules are crucial in fine-tuning the activity of T cells to maintain a balanced immune response. Immune Checkpoint Inhibitors target molecules that inhibit T cell activity, such as CTLA-4 and PD-



- 1/PD-L1. Approved ICIs like pembrolizumab and nivolumab have demonstrated remarkable success in various cancer types.
- Cancer Vaccines:** Cancer vaccines prompt the immune system to protect the body from cancer and fall into two categories, prophylactic and therapeutic. Prophylactic vaccines against hepatitis B and human papillomavirus have been instrumental in reducing the incidence of hepatocellular carcinoma and cervical cancer, respectively. These are classic vaccines used to prevent infection by oncogenic viruses. By contrast, therapeutic vaccines aim to harness the immune system to eliminate disease-causing cells that are already neoplastic. These stimulate the immune system to recognize and attack cancer cells by presenting tumor-specific antigens. Sipuleucel-T, a vaccine for advanced prostate cancer, is one example.
 - Adoptive Cell Therapies:** These involve the infusion of genetically engineered T cells (CAR-T cells) that are designed to target specific cancer antigens. First, T cells are extracted from the patient's blood. In a laboratory, these cells undergo genetic modification to express the chimeric antigen receptor (CAR). The modified T cells are then cultured and multiplied before being infused back into the patient. This intricate production process ensures the generation of a robust population of CAR-T cells capable of effectively targeting and destroying cancer cells in the patient's body.

Applications in Different Cancers: Immunotherapy in oncology has diverse applications, leveraging the immune system's intricacies for therapeutic purposes. The deployment of immune checkpoint inhibitors, exemplified by CTLA-4 and PD-1/PD-L1 blockade, represents a pivotal intervention to modulate T cell responses. CAR-T cell therapy, involving the genetic engineering of T cells to express chimeric antigen receptors, demonstrates notable success in hematological malignancies like B-cell lymphomas and multiple myeloma.

Prophylactic and therapeutic cancer vaccines, such as those targeting hepatitis B and human papillomavirus, epitomize immunotherapy's preventive and curative dimensions. The precision of personalized medicine is harnessed through the identification and exploitation of genetic and immune profiles for tailoring immunotherapeutic interventions.

The integration of immunotherapy into standard protocols for lung cancer, melanoma, renal cell carcinoma, notably via the application of immune checkpoint inhibitors, signifies a paradigm shift. Further, the exploration of combination therapies involves intricate studies to discern optimal synergies between various immunotherapeutic agents and conventional modalities, maximizing therapeutic efficacy.

Additionally, its continuous evaluation in clinical trials for cancers such as ovarian, pancreatic, stomach and breast underscores the ongoing quest to unravel its full therapeutic scope.

Adverse Effects and Challenges: While immunotherapy has transformed cancer treatment, it is not without challenges:



1. **Autoimmune Reactions:** Immune checkpoint inhibitors can lead to immune-related adverse events, including colitis, hepatitis, and pneumonitis. Managing these side effects is crucial in ensuring patient safety.
2. **Response Heterogeneity:** Not all patients respond to immunotherapy, and the factors determining response are not fully understood. Research is ongoing to identify predictive biomarkers for treatment response.
3. **Resistance:** Some tumors develop resistance to immunotherapy over time. Investigating the mechanisms of resistance and developing strategies to overcome it is an active area of research.
4. **Cost:** Immunotherapies can be expensive, limiting access for some patients. Efforts are being made to address cost-related issues and improve affordability.
5. **Combination Therapies:** Optimizing the use of immunotherapy in combination with other treatments is an ongoing challenge. Research is focused on identifying synergistic combinations and the right sequence of therapies to maximize effectiveness.

Future Directions: The future of immunotherapy in oncology holds several promising avenues including personalize therapy based on individual's genetic and immune profile, biomarker discovery to predict response to immunotherapy, strategies to overcome resistance and exploring immunotherapy's potential to expand its indications.

In conclusion, immunotherapy in oncology represents a remarkable advancement in the fight against cancer. From its modest beginnings in the late 19th century to the recognition of Nobel laureates in 2018, immunotherapy has transformed the treatment landscape for various cancer types. With continued research, the field holds great promise for the development of more effective and personalized therapies, ultimately improving the lives of cancer patients worldwide. However, challenges such as adverse effects, response heterogeneity, and resistance must be addressed as the field moves forward, and innovative strategies are needed to maximize the potential of immunotherapy in oncology. The evolving landscape of immunotherapy offers hope for a future where more cancer patients can benefit from these innovative treatments.



ORAL PRESENTATIONS

OP_6.1 : PREGNANCY INDUCED MODULATION OF HUMORAL IMMUNE RESPONSE TO FOOT-AND-MOUTH DISEASE VACCINATION IN THE HF COW

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Pregnancy is a non-genetic factor influencing the immune response to vaccination. Except advanced pregnant cows, other cattle are covered under the biannual vaccination campaign for foot-and-mouth disease (FMD). This study evaluated the humoral immune response to FMD vaccination in the purebred HF cows that were in 80-180 days of gestation (n=10). A group of non-pregnant cows (n=10) served as control. The cows were vaccinated with commercial FMD vaccine covering serotypes O, A, and Asia1 (day 0 post-vaccination; dpv 0) as per farm health calendar. Blood was collected on dpv 0, 14, and 28 to determine neutralization antibody titer through virus neutralization (VN) test for each serotype of FMD virus (FMDV). The log₁₀ scale transformed VN antibody titer was analysed by linear mixed model by fitting the pregnancy status and dpv as fixed effects and cow as random effect. The findings showed that the log₁₀ VN titer was comparable between the pregnant and non-pregnant cows for FMDV serotype O (P=0.312) and Asia1 (P=0.09). For serotype A, pregnant group had a significantly low VN titer on dpv 14 (P<0.0001) as compared to non-pregnant group with a mean difference of 1.36. As expected, the effect of dpv on the VN titer was extremely significant for all the three serotypes of FMDV (P<0.0001). It was concluded that FMD vaccination during 80-180 days of pregnancy would not affect the VN titer on dpv 28 in the HF cows.

OP_6.2 : ORNITHOBACTERIOSIS IN LAYER CHICKENS OF NAMAKKAL REGION/TAMIL NADU – A COMPREHENSIVE REPORT

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A study was carried out to record the prevalence of ornithobacteriosis, an emerging contagious disease of poultry caused by *Ornithobacterium rhinotracheale* by isolation, identification, molecular confirmation and seroprevalence in the poultry birds with production drop and mortality. The organism was isolated in 10 per cent sheep blood agar supplemented with gentamicin from the samples such as trachea, lungs and airsacs collected during necropsy. The individual pure colonies developed on the agar was confirmed by staining, biochemical and carbohydrate fermentation test. The isolates were further confirmed by polymerase chain reaction targeting 16S rRNA gene. Additionally, sera samples were collected from the poultry flocks with production drop and mortality and no history of vaccination for *Ornithobacterium rhinotracheale* and subjected for ELISA for estimation of antibodies. The smears prepared from the colony revealed gram negative, highly pleomorphic rod shaped bacteria. No growth was observed on MacConkey agar. The isolate was positive for oxidase, Voges-Proskauer test, arginine



dehydrogenase and urease activity. Negative for catalase, citrate utilization, indole, methyl red reaction, nitrate reduction, phenylalanine deamination and gelatin liquefaction. The isolates produced the predicted size of 784 bp amplicons in the PCR. Interestingly, the seroprevalence of ornithobacteriosis was found to be 96.04 % in layers, 67.57 in growers and 43.75 in chicks. Hence, the findings of the study firmly reveal the prevalence of the contagious respiratory disease among poultry layers in large numbers irrespective of the age group and emphasize the need for adoption of appropriate preventive and control measure to avert the economic loss to the farmers due to drop in production and mortality.

OP_6.3 : DEVELOPMENT AND EVALUATION OF INDIRECT-ELISA FOR DETECTION OF *PASTEURELLA MULTOCIDA* SPECIFIC ANTIBODIES IN BOVINES

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Haemorrhagic septicemia (HS), an acute, fatal and septicemic disease of cattle and buffaloes caused by *Pasteurella multocida*. An estimation of sero-prevalence is important to know the disease burden in bovine population especially in HS endemic states. The present study reports development and standardization of an indirect-ELISA assay for HS causing *Pasteurella multocida* specific antibody detection in bovine. An indirect-ELISA was developed with heat extract antigens of *Pasteurella multocida* B:2 (strain P52), as coating antigens. A checkerboard titration was employed using HS convalescent sera and negative sera. The concentrations of heat extract antigen, sample serum and goat anti-bovine immunoglobulin G labelled with horseradish peroxidase were optimized for the assay. A positive and negative sera panel of 75 bovine serum were formed using the indirect haemagglutination (IHA) test and commercial-ELISA kit. A receiver operating characteristic (ROC) analysis was performed to estimate the percentage positive value cut-off that maximized the sensitivity (Se) and specificity (Sp) of the indirect-ELISA. An indirect-ELISA had a mean Se of 94.74% (95% CI = 87.07%- 98.55%) and a mean Sp of 90.79% (95% CI = 81.94% to 96.22%), at PP value cut-off value of 30%. Further, optimized assay was used to screen a total of 540 serum samples of buffalo origin from Haryana state for surveillance. Of which, 26.48% sero-positivity was recorded. The study warrants further screening bovine sera samples from HS endemic states of India.

OP_6.4 : EVALUATION OF THE EFFECT OF TLR3 AND TLR7 AGONISTS IN DUCK PERIPHERAL BLOOD MONONUCLEAR CELLS

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Toll-like receptors (TLR) are pathogen recognition receptors that recognize pathogens and activate both innate and adaptive immunity through secretion of cytokines and chemokines. Several TLR agonists have been used as potential vaccine adjuvants and prophylactics in mammals and



chicken. However, there have been only limited studies on the effect of TLR agonists in ducks. The present study was aimed to evaluate the individual and combined effect of TLR3 and TLR7 agonists in duck peripheral blood mononuclear cells (PBMCs). The PBMCs were isolated by density gradient centrifugation method using histopaque-1077 and were stimulated with poly I:C (TLR3 agonist) and resiquimod (R848, TLR7 agonist) at the dose rate of 5µg/ml and 2.5µg/ml, respectively. The stimulated cells were harvested at three different time intervals, 12 h, 24 h and 48 h post-stimulation and the expression of immune response genes *viz.*, IL-1β, IFN-β, IFN-γ and IL-4 were determined by real-time qPCR using the specific primers. Both individual and combination of agonists induced the expression of immune genes at all the three time intervals. The peak expression of the genes, *viz.* IL-1β at 12 h, IFN-β and IFN-γ at 24 h post stimulation were found. The combination induced higher expression of IL-1β, IFN-β and IFN-γ as compared to the individual responses in duck PBMCs with a significant difference ***P<0.001. Even though, the expression of IL-4 was upregulated by the combination treatment compared to individual stimulation, a significant synergistic effect was not evident. The combination of poly I:C and resiquimod induced the expression of various cytokine genes indicating the enhancement of proinflammatory, antiviral and Th1 mediated (IFN-γ) response in ducks. Further, *in vitro* and *in vivo* studies with different doses are necessary to explore the optimum expression of other Th1 and Th2 cytokines and immune potentiation in ducks.

**OP_6.5 : DIAGNOSTIC EVALUATION OF IMMUNODOMINANT LSDV095
RECOMBINANT CORE PROTEIN FOR DETECTION OF LUMPY SKIN DISEASE
VIRUS ANTIBODIES**

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Lumpy skin disease (LSD) is a transboundary, high impact and World Organization for Animal Health (WOAH) (formerly OIE) - notifiable pox viral disease of cattle and Asian water buffalo caused by LSD virus. The recombinant protein-based diagnostics for sero-evaluation would be a better choice in disease prevention and eradication programmes in non- endemic countries. The authors evaluated the diagnostic potential of immunogenic core protein (LSDV095) of LSDV for its use as diagnostic antigen. The predicted protein characteristics including hydrophilicity plot, antigenic index, surface probability and predicted secondary structures revealed its immunogenic potential. The protein was expressed in prokaryotic system using pRham™ N-His SUMO vector, purified under denaturing conditions using Ni-NTA superflow cartridges and eluted in 300 mM imidazole. The identity of the expressed protein was analyzed in SDS-PAGE and confirmed by western blotting. The immunoreactivity of the LSDV095 recombinant protein was evaluated by using the same in indirect ELISA and compared with the gold standard serum neutralization test (SNT). At a serum dilution of 1:160, with a percent positivity (PP) cut-off value of 37, it resulted in a diagnostic specificity (DSp) of 91.67



% (95% confidence interval (CI), 90.1 – 97.2%) and diagnostic sensitivity (DSn) of 92.17%. Of 606 random samples tested, 474 samples (78.21%) were found positive for LSDV antibodies by using the optimized i-ELISA.

OP_6.6 : TOWARDS ERADICATION OF PPR: SEROPREVALENCE OF PPR IN SHEEP AND GOATS IN NORTHERN STATES OF INDIA BEFORE IMPLEMENTATION OF PPR-EP VACCINATION

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Peste des petits ruminants (PPR) pose significant challenges to small ruminant productivity in enzootic countries, impacting the economy. In line with the global effort to eradicate PPR, this study focuses on seroprevalence of PPR in sheep and goats in Northern Indian states, as part of the PPR Global Control and Eradication Strategy. Cross-sectional studies were carried out at the epidemiological unit level (epi-unit or village) across several northern states of India, namely Jammu & Kashmir, Punjab, Uttarakhand, and Chandigarh a total of 3240 serum samples (targeted for each state/UT except Chandigarh) of sheep and goats were collected for serosurvey (to assess the immunity status of PPR before the implementation of carpet mass vaccination under PPR-EP in 2023) using a stratified random sampling methodology as per WOA/FAO guidelines, to assess the prevalence of PPRV antibodies in the population before the start of the National Strategic Plan for PPR eradication. All the sera were screened for PPR virus (PPRV) antibodies using the indigenous PPR-Competitive ELISA kit (ICAR-IVRI-Mukteswar).

The overall seroprevalence across the surveyed regions was found to be 46.1%. Chandigarh reported the highest seroprevalence at 66.9% (81 positive out of 121 samples), while Jammu & Kashmir, Punjab, and Uttarakhand demonstrated seroprevalence rates of 49.0%, 44.9%, and 36.8%, respectively with significant (<0.01) variations in PPRV antibodies in sheep and goats across different districts in various states in the studied region. Notably, a considerable proportion of epidemiological units exhibited less than 30% seroprevalence, in Punjab (40.37%, n=44) and Uttarakhand (47.13%, n=41), and Jammu & Kashmir (21.7%, n=23) before carpet mass vaccination implementation in the



year 2023, indicate extensive mass vaccination is required to achieve the desired herd immunity. To accelerate PPR eradication, a mass vaccination programme with >95% coverage of the target population aged >4 months is recommended, aiming for >85% seroconversion field-level vaccine efficacy to attain a desired herd or population immunity of more than 80%. This research provides crucial insights into PPRV antibodies in small ruminants, which plays a pivotal role in refining national zone strategies toward achieving a PPR-free India and contributing to the global eradication of the disease.

**OP_6.7 : DEVELOPMENT OF WHOLE AND RECOMBINANT ANTIGEN BASED
INDIRECT ELISAS FOR SEROLOGICAL DETECTION OF ANATID ALPHA
HERPESVIRUS -1 INFECTION IN DUCKS**

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Duck virus enteritis (DVE) is contagious virus infection of the family Anatidae that includes duck, swan and geese. The infection is caused by Anatid alpha herpesvirus-1 or DVE virus (DVEV), that belongs to Alpha herpesvirinae subfamily of the Herpesviridae, genus *Mardivirus*. The disease has a worldwide distribution and is also seen in the duck rearing regions of India. The morbidity and mortality of the birds ranges from 5 to 100 percent Recovered birds often remain latently infected and shed the virus in the faeces for a long duration of time. Serological assays can detect antibodies against this herpes virus which is very useful in large scale surveillance programmes. In this study, we have developed indirect ELISA for sensitive detection of anti-DVE antibodies in duck serum. The assay was validated by a panel of hyperimmune serum (n=8), serum from DVE infected ducks (n=40) and negative duck serum (n=31). The iELISA showed a sensitivity and specificity of 100% using this panel. However, sensitivity and specificity analysis are being done using a large panel of field serum samples. We have also expressed a truncated version of the glycoprotein gD of DVE using bacterial expression system. The recombinant protein expression was confirmed by western blotting and immunolabelling using anti DVE serum obtained from vaccinated ducks. Indirect ELISA is being developed using this recombinant protein to improve the specificity of the assay system.



POSTER PRESENTATIONS

PP_6.1 : EVALUATION OF IMMUNE RESPONSE TO LIVE ATTENUATED GOAT POX VACCINE IN GOATS

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The present study was conducted to evaluate the immune responses to live attenuated goat pox vaccine in goats. The humoral immune response was evaluated in ninety-three goats vaccinated with single dose of goat pox vaccine with 36 goats kept as control. Blood was collected before and 21 days after vaccination and serum was separated. The samples were subjected to virus neutralization test to detect the antibody level in the serum. The samples collected before vaccination showed a titer ranging from 1.5 to 2.5 log₁₀ with a mean titer of 1.57 log₁₀ ± 0.02 whereas after vaccination the titer was ranging from 3 to 10 log₁₀ with the mean titer of 4.69 log₁₀ ± 0.14. The neutralization index (mean ± SEM) in vaccinated animals was ranging from 1.5 to 8.5 with mean NI of 3.12 ± 0.14. The cell mediated immune response was evaluated by IFN-γ ELISA. A total of 13 animals at KVAFSU farm were vaccinated with single dose of goat pox vaccine. Blood samples were collected on day 0, 7, 14, 21, 28, 35, and 42nd day post vaccination and stimulated with PBS, Con A and goat pox virus and incubated at 37 °C for 24 hrs. The plasma samples were separated and subjected to IFN- γ and IL-4 ELISA. The IFN- γ started increasing from day seven and reached peak on day 35 (206.55ng/L). Similarly, IL-4 started increasing from day 21 and reached peak on day 35 (310. 49 ng/L). The study revealed production of humoral and cell mediated immune responses to goat pox vaccine in goats.

PP_6.2 : MOLECULAR AND SERO SURVEILLANCE OF RETICULOENDOTHELIOSIS IN LAYER CHICKENS

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An investigation was made to study the prevalence of reticuloendotheliosis by seroprevalence and molecular confirmation in the layer poultry birds with production drop and mortality. A total of 634 serum samples were collected from unvaccinated flocks and tested for reticuloendotheliosis by ELISA. Serosurveillance revealed 28.08 (178/634) per cent prevalence of reticuloendotheliosis in poultry with highest rate of 34.64 per cent in layers followed by 7.09 and 4.55 per cent in growers and chicks respectively. Similarly, liver, spleen and kidneys samples with tumors collected from the birds presented for necropsy from few farms were subjected to RT-PCR to detect the reticuloendotheliosis, mareks and avian leucosis complex(J) virus. The RT-PCR also confirmed the presence of reticuloendotheliosis virus in the birds by the amplification of 291 bp LTR and the absence of ALV(J) and Mareks disease infection among the birds. The findings of the study strongly disclose the



prevalence of reticuloendotheliosis infection among poultry layers in considerable population irrespective of the age group since there is no practice of vaccination against REV. The study emphasizes the need for adoption of appropriate preventive and control measure like culling the affected birds to prevent horizontal infection of the birds in the flock.

**PP_6.3 : DEVELOPMENT AND IMMUNOLOGICAL EVALUATION OF
CpG ADJUVANTED KYASANUR FOREST DISEASE VACCINE IN MICE**

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This study was taken with the objectives of formulation of Kyasanur Forest Disease vaccine adjuvanted with TLR9 agonist CpG adjuvant; Evaluation of humoral and cell mediated immune response of CpG adjuvanted KFD vaccine and its protective efficacy in mice. The KFD seed virus (P-9605) procured from NIV, Pune was used for preparation of KFD vaccine. The seed virus was confirmed by Real time Reverse Transcription-PCR. KFD vaccine prepared in Chick Embryo Fibroblast primary culture, was inactivated with 0.1 % formalin. The prepared KFD vaccine was adjuvanted with different concentrations of CpG adjuvant per dose. The highest concentrations of CpG adjuvanted KFD vaccine which passed the safety test was used for further immunological evaluation in mice. The cell mediated immune response was assessed by quantifying the IFN- γ production by T cells by flowcytometry. The evaluation of humoral immune response was done by mouse protection test. The results found that CpG adjuvanted KFD vaccine provides a significantly higher survival percentage compared to non-adjuvanted KFD vaccine. In potency test the CpG adjuvanted KFD vaccine was found more potent compared to the non-adjuvanted KFD vaccine. These findings suggest that CpG when used as an adjuvant with formalin inactivated KFD vaccine modulates the innate and the adaptive immune response increasing the immunogenicity, efficacy and potency of the vaccine in mice model.

**PP_6.4 : RESCUE AND CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE
VIRUS (FMDV) MUTANTS IN BHK-21 CELL**

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Foot and mouth disease (FMD) is a transboundary viral disease of livestock. The inactivated FMD virus (FMDV) vaccine, the mainstay tool in the control program in India, confers immunity only for 6 months demanding repeated vaccinations to maintain herd immunity. Mutating the non-structural proteins of FMDV such as L-protease (L-pro) and 3A that are essential for viral replication, virulence, and host range a potential way to generate attenuated candidates for developing vaccines with long-



term immunity. Accordingly, the wild-type FMDV serotype Asia1 cDNA clone was mutated at different regions coding for L-pro and 3A by site-directed PCR-mediated mutagenesis to generate the following six mutant clones: pAsiaΔLab, pAsiaΔLb, pAsia-sap1, pAsia-sap2, pAsiaΔ3A, and pAsia3B2. The pAsiaΔ3A was further mutated at the SAP domain of L-pro to generate the following three mutant clones: pAsiaΔ3AB1, pAsiaΔ3A-sap1, and pAsiaΔ3A-sap2. A representative clone from each mutant plasmid was sequenced to confirm the mutations. All the nine FMDV Asia1 mutant plasmid clones were transcribed *in vitro*, transfected in BHK-21 cells, and propagated for six blind passages. Out of nine, five mutants pAsia-sap1, pAsia-sap2, pAsiaΔ3A, pAsiaΔ3AB1, and pAsiaΔ3A-sap-2 were rescued. The five rescued mutant FMDV Asia1 viruses were propagated for 20 passages and the genetic stability of the mutation in each mutant virus was confirmed by nucleotide sequencing. The serotype specificity was confirmed by indirect ELISA. Virus titration by endpoint assay revealed that the titer of pAsia-sap1 and pAsia-sap2 viruses was comparable with that of the wild-type FMDV Asia1; however, the other 3 mutant viruses had a low titer. Further, mutant virus's virulence in mice and guinea pig is under study to consider its potential as an attenuated vaccine candidate.

PP_6.5 : GROWTH KINETICS OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) NONSTRUCTURAL PROTEINS L-PROTEASE AND FMDV 3A MUTANT IN THE BHK-21 CELL

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Attenuated vaccines have an advantage over inactivated vaccines due to long-term immunity. As the current foot and mouth disease (FMDV) vaccine is inactivated, developing and characterizing the attenuated vaccine virus candidates is essential. Accordingly, we developed two mutants of the FMDV serotype Asia1 by targeting the L-protease (L-pro) and one mutant by targeting 3A as these non-structural proteins are responsible for virulence, virus replication, and host range. The SAP domain of L-pro was targeted at amino acid positions 55 and 56 by PCR-mediated site-directed mutagenesis to substitute isoleucine and arginine to serine and glycine, respectively, to generate pAsiaSAP1 clone and the corresponding rescued virus. Similarly, by mutating glutamine to leucine and leucine to valine at amino acid positions 57 and 58, respectively, pAsiaSAP2 mutant virus was generated. Further, the pAisaΔ3A mutant virus was produced by deleting 114 nucleotides of the C-terminal of FMDV 3A. The growth kinetics of the three mutants of pAsia1 viruses were compared with that of parent virus by absolute quantification of virus copy number by real-time PCR, plaque assay, and end point dilution titration. The copy number of pAsiaSAP1 and pAsiaSAP2 was maximum at 16 hours post-infection (hpi) and was comparable with that of parent virus. In contrast, pAsiaΔ3A had a reduced replication rate, as well as a lag in the replication time as the maximum copy number reached 24 hpi. Similarly, pAsiaSAP1 and pAsiaSAP2 plaques' morphology was comparable with that of parent virus; however, pAsiaΔ3A mutant produced smaller plaques indicating reduced infectivity. The virus titer of the SAP



mutants was comparable with that of parent virus and ranged from 6.8 to 7.2 log₁₀TCID₅₀/mL. In contrast, the virus titer for pAsiaΔ3A mutant was 5.8 log₁₀TCID₅₀/mL and was significantly low. It is concluded that pAsiaΔ3A reduced the growth and replication *in vitro* and is a candidate for attenuation studies *in vivo*.

PP_6.6 : STUDIES ON THE ESTIMATION OF HAEMAGGLUTINATION INHIBITION TITRE VALUES IN BROILERS VACCINATED WITH NEW CASTLE LENTOGENIC STRAINS

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Studies on the estimation of the broilers vaccinated on 7th day and 28th day with lentogenic strains of Ranikhet Disease in Vencobb broiler chicks were carried out in the broiler unit of LFC ACVM. The hemagglutination inhibition (H.I) titre values were assessed as per the standard procedure and the average weight gain of the broilers also taken. Sera samples were assessed for their H.I. values on 16th, 23rd, 32nd, 38th and 46th days of vaccination. The H.I. titre values were 1:16-1:64 on 16th day, 1:32 -1:128 on day's 23rd and 32nd. The H.I. titre values on 38th and 46th days of post vaccinations were 1:164 & 1:32 respectively. The average weight gain of the broiler chicks were 161.9 gms on 16th day, 865 gms on 23rd day, 1.54 kgs on 32nd day, 2.11 kgs on 38th day and 2.5 kgs on 46th day. The study revealed immune status of the broilers with their weight gain.

PP_6.7 : IMMUNOHISTOCHEMICAL EVALUATION OF CYTOKERATIN IN VARIOUS UTERINE PATHOLOGIES OF DOMESTIC FELINES

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Immunohistochemistry plays a crucial role in diagnosis and treatment of hormonal imbalances, associated diseases and also understanding the normal biochemical pathways and physiologic responses. Cytokeratin is a family of polypeptides of intermediate filaments which in diverse epithelia are expressed in different, yet specific combinations. This study was conducted to determine the biomarker cytokeratin expression in various uterine pathologies. To evaluate the diagnostic value of keratin, immunohistochemical staining was done in formalin-fixed, paraffin-embedded uterine tissues of domestic cats. The strong immunopositivity against cytokeratin was noticed in cytoplasm of epithelial cells of endometrial glandular epithelium. A high intensity of cytokeratin expression was noticed in scirrhous adenocarcinoma, endometrial adenocarcinoma, and cystic endometrial hyperplasia. Thus, Cytokeratin plays a major role in diagnosis and differentiation of uterine disorders.



**PP_6.8 : SERO-MONITORING OF *BRUCELLA ABORTUS* S19 VACCINE
AT DIFFERENT INTERVALS OF POST-VACCINATION UNDER NADCP
BRUCELLOSIS CONTROL PROGRAM**

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Shome Rajeswari***

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Brucellosis is a communicable zoonotic bacterial disease caused mainly by *Brucella abortus* in cattle and buffaloes. Department of Animal Husbandry and Dairying (DAH&D), Government of India implemented brucellosis control through a nationwide program called National Animal Disease Control Program (NADCP) by immunizing female cattle and buffalo calves of 4-8 months old with one log reduced dose of *B. abortus* S19 vaccine (4×10^9 CFU/dose). The aim of this study is to assess vaccine induced anti-*Brucella* antibody response in vaccinated calves at various days intervals post-vaccination in different states of the country.

For the post-vaccination sero-monitoring, ICAR-NIVEDI, Bengaluru has been entrusted to design sampling plan and to undertake post-vaccination sero-monitoring. The two-stage stratified random sampling method provides recommendations for selecting villages within blocks, districts and states, as well as the number of animals to be sampled within the village based on population proportion of cattle and buffaloes to be sampled in each selected village. During the first phase of the NADCP program 2021-22, a total of 15,871 serum samples from 312 districts and 1,477 villages from 14 states and four UTs received were tested by in-house developed Protein G-based iELISA (Indian patent number No.335659).

Out of 15,871 calf serum samples (cattle-13,555 & buffalo-2134) tested by iELISA, 12,042 samples showed the presence of anti-*Brucella* vaccinal antibodies. Out of 14 states and four UTs, Karnataka state and Chandigarh (UT) were the utmost in vaccination sero-conversion. At various intervals of post-vaccination days (DPV 21-45, 46-60, 61-90 and 91-120), the elevated antibody response was observed at initial 21-45 days interval followed by 46-60 and 91-120 (76.34%, 73.06%, 72.31% and 69%), respectively. This Intensive Brucellosis Control Program in animals is envisaged for controlling brucellosis which will result in effective management of the disease, in both animals and human population.



PP_6.9 : STUDY ON P1^{A1}/P1^{A2} GLYCOPROTEIN IIIA POLYMORPHISM IN MYOCARDIAL INFARCTION BY DUAL ANTI-PLATELET THERAPY (DAPT)

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A heart attack, also called a Myocardial infarction, extremely dangerous condition that usually occurs when a blood clot blocks blood flow to the heart. This blockage can develop due to a buildup of plaque, a substance mostly made of fat, cholesterol, and cellular waste products. Patients undergoing primary Pre-Cutaneous Intervention (PCI) should receive Dual Anti-Platelet Therapy (DAPT), a combination of Aspirin and a P₂Y₁₂ inhibitor and a parental anticoagulant. In this study, we take a total of 23 case study analogs, by using Aspirin and Clopidogrel are widely used anti platelet drugs for preventing adverse thrombotic episode in patients with cardiovascular diseases. So, polymorphism was observed in males of about 23 cases, majority of males only exposed for IIIa gene polymorphism. And it is nearly about 20(86.95%). This myocardial infarction might expose for the males of age between 40-65 years of males. But females also get affected with these mild symptoms of exposure at the age between 25-55 years. Homozygous diseased type shows 0% mutational polymorphism in controlled samples. So that, odd ratios might be undefined values. However, polymorphism was observed in males of similar age groups. The Leu amino acid was changed to Proline. Of about 27 healthy samples, included 100% wild type of (Leu/Leu) platelet aggregation PLA1 with 0% (0) (pro/pro) PLA2 for that diseased case of about 18 were homozygous with 78.2% and 5 are affected with 21.73%. So that, dual non-responsiveness to aspirin and clopidogrel was 21.73 percent more than half of non-responders (10/18) showed high residual platelet reactivity by collagen that activates platelets via GPV1 receptors and involves mechanisms different from COX1- and P₂Y₁₂ – mediated platelet functions. In the present study, non-responders to aspirin and clopidogrel showed significantly enhanced plasma thromboxane B₂ levels in addition to sustained ADP, leucin and collagen induced aggregation, reflecting increased systemic levels of thromboxane A₂. The plasma thromboxane B₂ levels in patients were also found to be associated with DAPT non responsiveness. A measurement of TxB₂ in both plasma and serum of patients may provide better assessment of aspirin response in patients taking dual anti platelet drugs (Aspirin and Clopidogrel). It is not only the acute treatment of myocardial infarction (MI) but also long-term medication. Patient's response to Aspirin and Clopidogrel is critically regulated by their specific receptor function as well as by their hepatic metabolism and intestinal absorption.



TECHNICAL SESSION VII

**CURRENT TRENDS IN VACCINOLOGY,
VETERINARY PHARMACEUTICS AND
DRUG DELIVERY PMA**





LEAD PAPER-1

BIOSECURITY FROM ONE HEALTH PERSPECTIVE: VACCINES AND CRUCIAL NEED FOR IMMUNE MODULATION

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Biosecurity refers to a set of measures and practices aimed at preventing, controlling, and mitigating the spread of infectious diseases within and between populations of animals, plants, and humans. Biosecurity measures can be applied in various settings, including agriculture, healthcare, research facilities, and wildlife management. It encompasses strategies to safeguard against the introduction and dissemination of pathogens, whether they are bacteria, viruses, fungi, or other disease-causing agents. Examples of biosecurity measures include quarantine protocols, vaccination programs, strict hygiene practices, and controlled access to bio-containment laboratories.

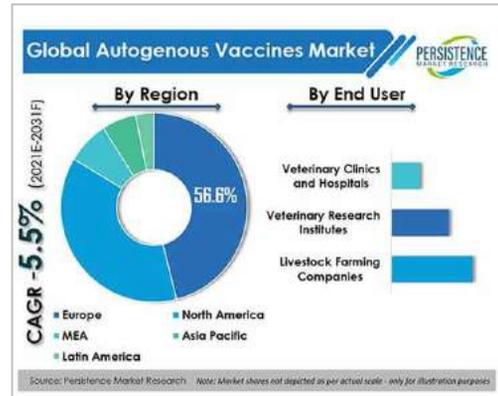
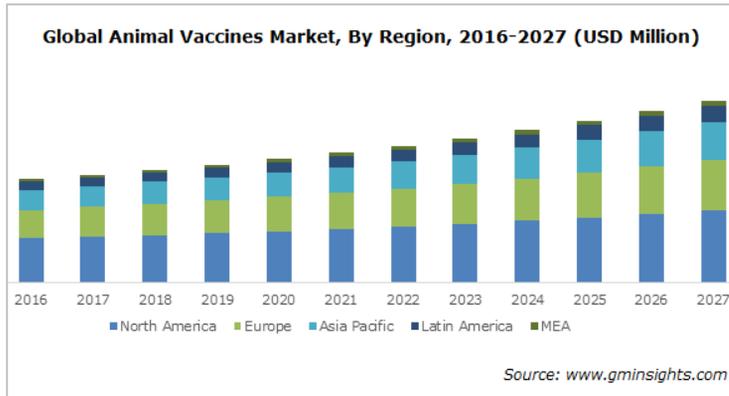
One Health is a holistic and interdisciplinary approach that recognizes the interconnectedness of human health, animal health, and the environment. It emphasizes the interdependence of these three domains in the context of health and disease. The concept of One Health acknowledges that many infectious diseases affecting humans originate in animals (zoonoses), and environmental factors can influence disease transmission and emergence. One Health promotes collaboration among medical professionals, veterinarians, ecologists, environmental scientists, and other experts to address complex health challenges. It aims to improve disease surveillance, early detection, and response to emerging infectious diseases, promote responsible antimicrobial use, and protect ecosystem health. Examples of One Health initiatives include studying the link between deforestation and the spread of vector-borne diseases, like malaria, and monitoring the emergence of zoonotic diseases like COVID-19.

Biosecurity and One Health are two interconnected essential components of the One Health approach, as they help prevent and control the spread of infectious agents at the animal-human-environment interface. Implementing biosecurity measures in animal farming, wildlife conservation, and healthcare settings can reduce the risk of zoonotic diseases spilling over from animals to humans. Additionally, One Health initiatives often involve strengthening biosecurity practices to address the shared health concerns of animals and humans which are crucial in addressing emerging infectious diseases, preventing pandemics, and ensuring the sustainability of our planet. Biosecurity measures aimed at preventing and controlling zoonotic diseases often involve strategies that modulate the innate immune system. Controlling these diseases requires a multi-faceted approach that broadly includes the following with the aim to reduce transmission at the human-animal interface.



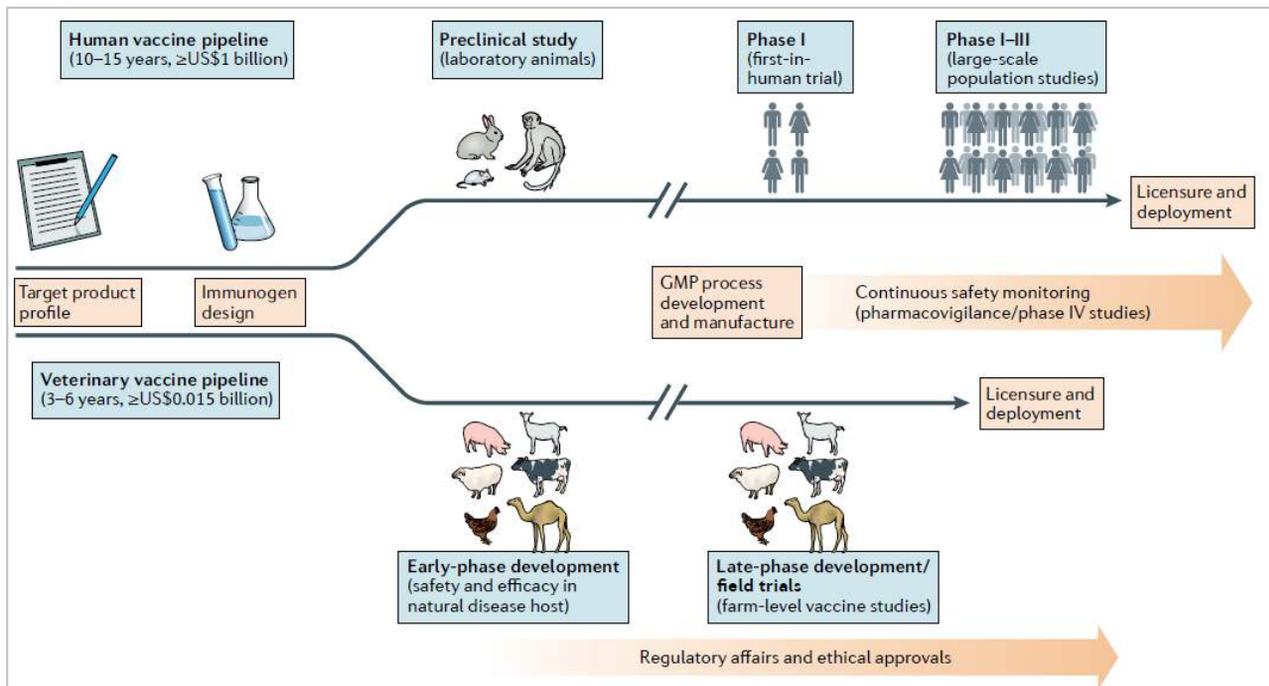
- a) **Vaccination Programs:** Vaccination is one of the most effective ways to prevent zoonotic diseases in both animals and humans. Vaccines work by stimulating the adaptive and sometimes the innate immune system to recognize and respond to specific pathogens. For example, vaccines can enhance the activity of innate immune cells like macrophages and dendritic cells, which play a role in presenting antigens to the adaptive immune system.
- b) **Antiviral and Antimicrobial Agents:** Modulating the immune response can involve the use of antiviral and antimicrobial agents. These treatments can help in the control zoonotic diseases by directly targeting the pathogens and supporting the immune system in its efforts to combat the infection.
- c) **Hygiene and Sanitation Practices:** Basic hygiene and sanitation practices in animal agriculture, such as clean water and living conditions, can help reduce the burden of zoonotic diseases. These practices indirectly support immune system by preventing the spread of pathogens in animal populations.
- d) **Surveillance and Early Detection:** Early detection of zoonotic diseases in animals is essential for preventing their transmission to humans. Surveillance systems can monitor the health of animals and detect outbreaks early, allowing for rapid response measures. This is particularly relevant in the context of emerging zoonotic diseases.
- e) **Vector Control:** Many zoonotic diseases are transmitted by vectors such as mosquitoes and ticks. Controlling these vectors through the use of insecticides or environmental modifications can reduce the risk of transmission. The immune responses in the host can also be modulated to make individuals less attractive to vectors or more resistant to their bites.
- f) **Education and Public Awareness:** Public education about zoonotic diseases and their transmission is crucial for prevention. Educating communities about practices that can reduce the risk of zoonotic disease transmission, such as proper food handling and safe animal interactions, is a form of biosecurity that relies on human behavior but can impact the immune response to infections.
- g) **Research and Innovation:** Ongoing research into the immune response to zoonotic diseases can lead to the development of new therapies, vaccines, and diagnostic tools that enhance biosecurity measures.

Rising concerns over food security and increasing animal husbandry are boosting the demand for animal vaccines for the livestock population and the driving factors include the (i) increasing demand for animal products globally, (ii) variation in food preferences due to changing lifestyles and population growth and (iii) the perpetual introduction of technologically advanced vaccines and the outbreak of livestock diseases have contributed to the shifting market dynamics. The Indian animal vaccine market is projected to reach \$ 232 million by FY 2026 as the country is the fourth largest chicken producer in the world and is among the leading exporters of high quality poultry products to various countries. The demand also had been contributed to the market growth is the increasing number of diseases in animals such as rabies, coccidiosis, swine pneumonia and many others. In addition, the government initiative on the National Animal Disease Control Programme with a total outlay of around USD1.8 billion (INR 13,343 crore) with the output targeted for 100% vaccination of 530 million animals including buffalos, sheep, pigs, etc., all over the country and eradication of livestock diseases by FY 2030.



The data presented above clearly indicates the potential market for veterinary vaccines as well the requirement of autogenous vaccines in region of Asia-Pacific and thus stressing the need of veterinary research institutions to focus on vaccine R & D but also to meet the local requirements

It is essential for the researchers to understand the typical vaccine development pipeline that is required for bringing out an effective vaccine into market starting from target product profiling to licensure and deployment. The respective stages and approximate costs for veterinary and human vaccines are shown.

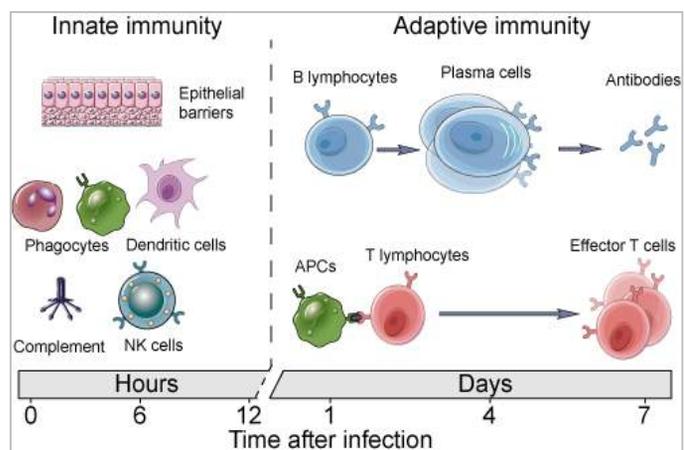


The linear chronological process, some of the different stages of the pipeline for a ‘multispecies’ vaccine can occur in parallel with basic requirement to meet the regulatory requirements of good manufacturing practice (GMP)

Before we discuss on the different types of vaccines and the recent developments it is very much essential to understand on the generated immune responses that are to be expected following the vaccination. This will provide a clear idea on the generated immune responses and its contribution to the expected protective immune responses.

Among the above factors, the implementation of appropriate vaccination programmes if of prime importance to achieve appropriate protection as well as to prevent the disease outbreak. The efficiency of the vaccination entirely depends on the immune modulation to achieve the required protection. Immunity is the body's remarkable defense system that safeguards it against harmful invaders such as bacteria, viruses, and other pathogens. This complex network of cells, tissues, and molecules works tirelessly to identify and eliminate foreign substances while distinguishing them from the body's own cells and tissues. The immune mechanisms involve various components, including white blood cells such as lymphocytes and phagocytes, as well as antibodies and cytokines. These elements collaborate in a coordinated response, featuring both innate and adaptive immunity. Infectious diseases rely on host susceptibility, tied to immune effectiveness.

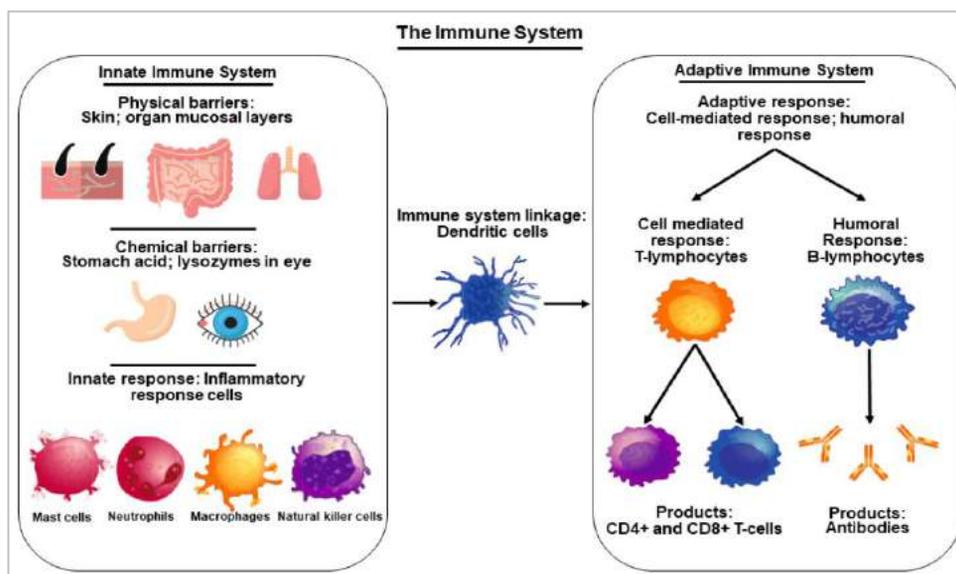
Innate immunity provides immediate but nonspecific defense, while adaptive immunity develops over time, offering a tailored and memory-based response to specific pathogens. Together, these immune mechanisms play a crucial role in maintaining the body's health and preventing illness. This lecture will focus briefly touch upon the innate and adaptive immunity and mainly describe the modulation of innate immunity and its role in biosecurity and one health. Innate immunity



plays a crucial role by serving as the first line of defence against infectious agents, including bacteria, viruses, fungi, and other pathogens. The innate immune system relies on a complex array of components to identify common pathogen patterns and respond to signals of cell stress or death. These components encompass a range of cells, including dendritic cells, monocytes, macrophages, granulocytes, and natural killer T cells, as well as the epithelial cells in the skin, lungs, and gut that form the body's interface with the environment. In addition to these cellular elements, non-cellular factors, such as the complement cascade and physical barriers, are integral in preventing pathogen entry and activating phagocytes when necessary. The Pathogen-associated molecular patterns (PAMPs) serve as recognizable cues for the innate immune system and are detected by specialized pathogen-recognition receptors (PRRs). Toll-like receptors (TLRs), a crucial subset of PRRs, are expressed on various cell types, including innate immune cells, endothelial cells, epithelial cells, and fibroblasts. When TLRs bind to their corresponding microbial ligands, they trigger a cascade of events, including the activation of phagocytes, pathogen elimination, release of pro-inflammatory cytokines, and

antimicrobial peptide production. Beyond TLRs, other factors associated with inflammation can act as danger signals, prompting the activation of phagocytes and dendritic cells. These factors encompass heat-shock proteins, lectins, cytokines, chemokines, extracellular matrix components, and specific cell-surface molecules, including lipids from necrotic cells. These danger signals facilitate phagocyte activation and pathogen destruction, as well as initiate the activation of dendritic cells, ultimately kick-starting adaptive immune responses. Anti-microbial proteins and peptides, including lysozymes, cathepsin G, defensins, cathelicidins, and others, are additional vital components of the innate immune system. These molecules possess the ability to directly eliminate pathogens through various mechanisms, such as cell lysis, while also participating in chemotaxis, attracting monocytes, dendritic cells, and T cells. A notable example is the defensin hBD-2, which not only has microbicidal properties but also serves as a signaling molecule connecting the innate and adaptive immune systems.

The adaptive immune response, in contrast to the rapid but nonspecific innate response, takes days to weeks to develop, yet it offers remarkable specificity and memory. This form of immunity arises following exposure to an antigen, whether from a pathogen or a vaccine. It comes into play when the innate immune system falls short in controlling an infection, serving as a critical complement. Indeed, the adaptive response relies on information gleaned from the innate system. There are two primary branches of adaptive immunity: the cell-mediated response, executed by T cells, and the humoral response, overseen by activated B cells and antibodies. Specific T and B cells, tailored to molecular features of the pathogen, undergo proliferation and target the invader. They can directly eliminate pathogens or release antibodies, boosting phagocytosis and disrupting the infection. Furthermore, adaptive immunity establishes a lasting memory, affording long-term protection against re-infection by the same pathogen. Upon re-exposure, this memory enables a swift and efficient response.



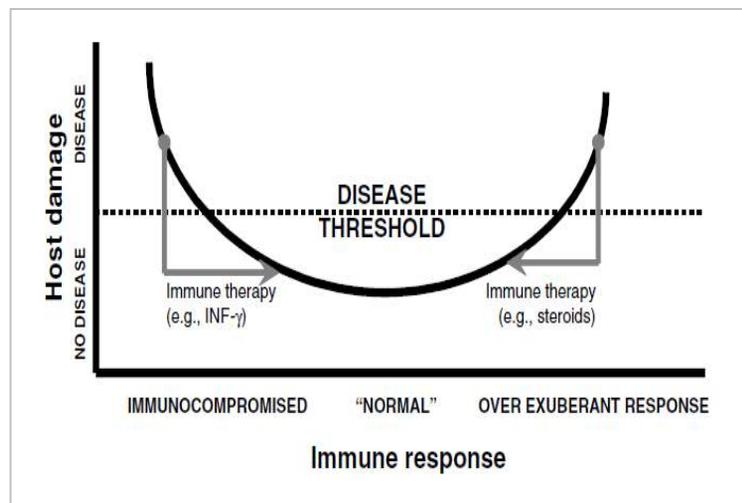
Overview of the immune system illustrating innate and adaptive immune system components ana et al., 2022)



An infectious disease can occur only in a susceptible host, with susceptibility being a function of the effectiveness of the immune response. The damage may stem from microbial or host factors, including excessive immune response or insufficiency and the host damage correlates with the immune response in a parabolic curve. Therapeutic immunomodulation aims to shift this curve, reducing host damage, instead of conventional pathogen elimination. A comprehensive immunomodulation toolkit needs agents to stimulate and suppress the immune response, along with tools to measure host damage, adapting to the host's position on the damage-immune response curve. Diverse agents for fine-tuning the immune response offer clinician's new treatment options. Developing interventions for infectious diseases through immune modulation taps into a system evolved to defend against microorganism-related diseases. Experience with vaccination, passive antibody, and cytokine therapy confirms the safety and efficacy of immune modulation. Challenges arise from the complexity of the immune system a network of interconnected subsystems maintaining immune balance while responding effectively to microorganisms. Properly stimulating and, crucially, dampening the immune response post-infection is essential to avoid uncontrolled inflammation.

Immune Response Vs Host Damage:

Damage to host stems from both the quantity and quality of the host's response to a microorganism (microbial effects on host tissues, the immune response, or both). The host damage observed (i.e., disease) occurs when the immune response is either overly vigorous or insufficient. Immunomodulation can help to mitigate host damage by either boosting or dampening the immune response



Developing immunomodulators is complex due to varying susceptibility and responses to microbial agents, as well as dynamic changes in the immune response during host-microorganism interactions. Factors like certain infections, immunosuppressive treatments, genetic background, and past microbial exposure contribute to individual variability. However, the immune response continually adapts during these interactions, making a single immunomodulator's effectiveness time-dependent. Despite the intricate nature of the immune responses, ongoing research uncovers potential immunomodulatory compounds and strategies. Notably, successful approaches like vaccination and passive antibody therapy have been developed and used effectively even without a complete understanding of their mechanisms. The topic will discuss the immunomodulation in four basic approaches which includes:



- a) Modulating the innate immunity
- b) Subdue the inflammation
- c) Modulating the acquired immunity
- d) Therapeutic vaccination

a) Modulating Innate Immunity

Innate immune responses, though once considered simplistic, are evolutionarily conserved. Recent research has unveiled the complexity of the innate immune system, involving various receptors, signaling peptides, and cytokines interconnected in feedback loops. Innate immunity acts rapidly (within minutes to hours) and lacks specificity, making it effective against various microorganisms. Unlike conventional antimicrobial therapy, it is less likely to induce resistance as it doesn't target specific microbes. Some microorganisms evade innate immunity through virulence factors, while excessive or prolonged immune responses can harm the host. Therapies modulating innate immunity need both enhancers and inhibitors, tailored to the infection stage. They can complement traditional antibiotics. Timing of intervention is critical, as pre-infection protection might worsen post-infection, and different immunomodulators may be useful at distinct disease stages. The committee examined strategies to boost and suppress innate immunity separately. In the past decade, research unveiled diverse pattern-recognition receptors (PRRs) in the human innate immune system, such as Toll-like receptors (TLRs), NOD proteins, and others. Each PRR activates unique but overlapping signal pathways, leading to cytokine production tailored to the detected microorganisms. The discovery of PRRs prompted investigations into their agonists, signaling cascades, and interactions with the immune system. Potential targets for modulating innate immunity are numerous and reports indicate that the process is highly orchestrated (regulated). The three potential boosters of innate immunity that have been shown to be promising are:

- ✓ TLR agonists and agents that modulate the TLR response pathway
- ✓ Cationic host-defense peptides and
- ✓ Direct expansion of the effector cells normally activated by the innate immune system

Compounds modulating innate immunity may yield unpredictable or conflicting host responses, depending on their interaction with natural host-microorganism responses. The complex regulatory mechanisms governing immune responses are poorly understood, making outcome prediction for innate immune modulators challenging. Examples show potential risks, like TLR agonists triggering autoimmune diseases and self-proteins sometimes causing antibody responses. Developing safe and effective innate immune modulators necessitates substantial basic research. Comprehensive understanding of the innate immune system will aid therapeutic development. Basic research in the following areas to boost the innate immune system include:



- ✓ Characterize PRR agonists, their downstream signaling, and effector mechanisms.
- ✓ Investigate elements governing the balance between proinflammatory and anti-inflammatory pathways in innate immunity.
- ✓ Study the selective immunomodulatory effects of cationic host-defense peptides.
- ✓ Identify biomarkers indicating innate immune status.

b) Subdue the inflammation

Excessive innate immune activation can generate harmful proinflammatory responses, contributing to sepsis-like syndromes with high mortality rates. Attempts to down-modulate immune responses for sepsis treatment have often failed due to the syndrome's complexity and late intervention. Many tested compounds target single steps in intricate inflammatory pathways, offering a narrow therapeutic window. To enhance outcomes, it's advisable to focus on critical checkpoints rather than individual mediators. Promising targets include HMGB1, TREM-1, MIF, and TLR4 antagonists. Strategies involve bolstering T-regulatory cell activity and controlling neutrophil responses. Neutrophil persistence at infection sites can cause extensive local and systemic damage, making the regulation of apoptosis, cytokine signaling, and mononuclear cell infiltration important areas of study. A deeper understanding of proinflammatory cytokine regulation is essential. For instance, LL-37, known for suppressing TNF- α induction by bacterial LPS, has potential to both suppress innate immune responses and protect against infections. Better comprehension of innate immunity promises more selective sepsis-suppression approaches. Prior intervention failures targeting single inflammatory pathway steps may relate to a narrow effective window. Prospective studies that categorize sepsis patients based on genomics and proteomics, using advanced gene-array and proteomics technologies, offer opportunities for early diagnosis and tailored treatment. Clinical studies should collect and analyze samples, create comprehensive databases, and identify biomarkers for patient stratification, ultimately guiding more effective treatments. The two promising approaches to the development of agents to suppress innate immunity:

- ✓ Develop therapeutics to modulate the inflammatory network, focusing on T-regulatory cell function, TLR signaling pathways, and neutrophil activity and trafficking.
- ✓ Conduct prospective studies using gene array and proteomic technology to stratify sepsis patients into distinct categories for more targeted treatment.

c) Modulating acquired Immunity

The acquired immune system's specificity, memory, and effector mechanisms have been vital in addressing infectious diseases. Interventions like smallpox eradication through vaccination and passive antibody therapy for various diseases demonstrate its power. Passive antibody therapy, involving immunoglobulin administration, is a proven and safe approach for treatment, especially beneficial for immunocompromised individuals. However, it faces limitations in specificity, the need



for microbiological diagnosis, and short antibody half-lives. Research could address these issues by developing broad-spectrum antibody reagents, identifying new antibody mechanisms, and improving diagnostics for microorganism identification and immune status. Promising applications include monoclonal antibodies to combat multidrug-resistant bacterial infections and antibodies that interact with the innate immune system, inducing damage control mediators to act as broad-spectrum immune stimulators or suppressors. Three kinds of research are recommended that would contribute to the development and effective use of passive antibody therapy:

- ✓ Understanding antibody specificity, affinity, isotype, and dose relationships can enhance the development of more effective and longer-lasting antibodies, optimizing passive antibody therapy dosage and timing.
- ✓ Investigating the mechanisms through which antibodies interact with the innate and cellular immune systems for damage control can lead to the creation of broad-spectrum antibodies effective against multiple pathogens and potential vaccine adjuvants.
- ✓ The development of rapid and specific diagnostics, capable of identifying both the infectious agent and the patient's immune status, can enable the swift identification of patients suitable for passive antibody therapy and guide precise dosing and timing.

d) Approach of Therapeutic vaccination

Active immunization, in the form of therapeutic vaccination, has successfully prevented numerous viral and bacterial diseases. Some vaccines, like rabies and varicella, are administered after infection to induce a protective immune response before the onset of disease, illustrating the potential of therapeutic vaccination for infectious diseases. Given the long history of safety and efficacy of vaccination, the committee emphasizes the importance of research to develop more effective vaccines. This includes enhancing vaccine design and exploring adjuvants that can stimulate different immune responses, particularly mucosal and Th1 responses. The use of Toll-like receptor (TLR) agonists as adjuvants for specific vaccine formulations shows promise. Therapeutic vaccination for chronic infectious diseases caused by parasites, mycobacteria, fungi, HIV, and other viruses is a promising avenue. Such diseases require more targeted antigens that elicit beneficial immune responses and control chronic infections. Current vaccines predominantly elicit IgG antibodies, but natural infections provoke a broader range of antibody isotypes and longer-lasting immunity. Research into dendritic cell vaccination and antigens targeting dendritic cells can enhance vaccine effectiveness. Additionally, understanding positive and negative T-cell signaling pathways offers the potential to regulate T-cell function during immunization, augmenting antibody production through positive pathways and enhancing immune responses by derepressing T-cell function via negative pathways. The following research was identified as most likely to contribute to improved vaccines:

- ✓ Define B- and T-cell epitopes at the molecular level for designing novel vaccines and identifying protective antibody characteristics.



- ✓ Investigate methods to elicit non-IgG protective responses and simulate mucosal immunity.
- ✓ Develop strategies targeting dendritic cells and optimize antigen delivery to enhance vaccine effectiveness.
- ✓ Characterize positive and negative signaling pathways between T cells and antigen-presenting cells for regulating immune responses during vaccination.

This lecture will provide a brief description on the crucial role of immune modulation in biosecurity and one health with case studies for modulating the innate and adaptive immune system as well as the next generation vaccine approaches



CHALLENGES IN THE BOVINE MASTITIS VACCINE DEVELOPMENT: THE WAY FORWARD

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In India there is an increase of 42% milking exotic cows and 4.34% buffaloes from 2012 to 2019 as per the recent animal husbandry statistics. The compound annual growth rate of milk in India from 2020-21 to 2022-23 is 4.79%. Though there is increase in the quantum of milk and the animal producing the milk, several factors hinder the milk producing capacity of animal. One of the major problems in dairying is mastitis which is the inflammation of udder caused by various bacterial pathogens. Mastitis is a disease of high economic importance next to FMD among various bovine diseases. It affects the profitability of animal producers/farmers, and leads to the largest production losses in dairy sector worldwide. The economic loss due to mastitis in India is about Rs. 575 million per annum and it reduces milk by 21%. In addition, the consumption of mastitis-affected milk is harmful to humans as antimicrobial resistant pathogens may be transmitted by contaminated unpasteurized milk; hence is also a major public health concern/hazard.

Challenges

The main pathogenic bacteria that cause mastitis include *Staphylococcus aureus* (*S. aureus*), *E. coli*, *Klebsiella pneumonia* and *Streptococcus*. Successful treatment of clinical mastitis depends on antimicrobial treatment, causal agent, parity, stage of lactation, history of previous SCC, clinical mastitis and other systemic diseases. Treatment protocol for mastitis involves the use of antimicrobial agents but raising problem of antimicrobial resistance is a concern. This led to treatment failure and huge economic loss to the farmers. Since there is development of resistance and under field condition, resistance against higher generation antibiotics has developed there is no treatment option left to curtail the resistance pathogens. Hence there is need for therapeutic alternatives to prevent and control mastitis in cattle.

Alternative approaches

Other than antimicrobial therapy other alternative approaches have been studied or under study. These include bacteriophage therapy, use of bacterial endolysin, antimicrobial peptides, probiotic use, nanoparticle-based therapy, stem cell therapy, herbal therapy, and use of vaccines.

Vaccination of animals can be an option for control of mastitis but commercially there are only few vaccines available. Currently, commercially available dairy cattle mastitis vaccines include the Lysigin™ vaccine against *S. aureus*, the J5 vaccine against *E. coli*, the Startvac® vaccine having *S. aureus*, and J5 mutant *E. coli*, and so on (Table 1). Furthermore, several experimental vaccines and group-specific autologous vaccines have been developed with remarkable success but there is no solid control measure available.



Table 1. Different mastitis vaccines that are commercialized or under trial

S. No.	Vaccine Antigens	Efficacy	Limitations
1.	<i>E. coli</i> J5 bacterins	Decreased severity of coliform mastitis in field experiments, little effect in experimental infections	Little effect on incidence of cases, variable among herds and experiments. Unknown mechanism of action
2.	<i>E. coli</i> J5 bacterin with killed <i>S. aureus</i> (StartVac®, Hipra)	Decreased mastitis severity in field studies	No effect on incidence of cases. Unknown mechanism of action
3.	<i>E. coli</i> enterobactin FepA or siderophore receptor FecA	Reduction of bacterial growth in vitro	Not tested in vivo (FepA) or not effective in challenge experiment (FecA)
4.	<i>Klebsiella</i> siderophore receptors and porin proteins (KlebVax™)	Little reduction in risk of coliform mastitis, some increase in milk yield	Administration with a J5 vaccine confounding the interpretation. Effect variable depending on experiments
5.	<i>S. aureus</i> bacterins and toxoid or bacterial lysate	Some reduction in severity and incidence of mastitis	Variable results, little prevention of chronic infections
6.	<i>S. aureus</i> protein A	Increased spontaneous cure after experimental challenge	Not tested in field conditions Mechanism not identified
7.	<i>S. uberis</i> live bacteria and surface extract	Reduction in bacterial shedding in milk and local inflammation	Not tested in field conditions Mechanism not identified
8.	<i>S. uberis</i> slime preparation (UBAC®, Hipra)	Reduction in milk production losses and incidence of clinical mastitis cases	Few published field experiments. Little confirmed effect on the prevalence of infections. Mechanisms not identified

Though several reports document the effect of vaccine in controlling mastitis, yet there is no effective vaccine that can prevent mastitis. Major hurdles of development of a successful mastitis vaccine are mentioned below.

Challenges of bovine mastitis vaccine development

- 1) multiple pathogens can cause mastitis, hence a single vaccine cannot be cure for mastitis
- 2) new infections/ new pathogens can cause mastitis
- 3) protection against severe clinical attacks requires an autogenous vaccine and repeated injections
- 4) cost of vaccines is high
- 5) delivery of vaccine



The way forward

A single vaccine that can effectively control the pathogens from causing mastitis is the need of the hour. A combined or cocktail vaccine containing mixture of subunits from Gram positive and Gram-negative pathogens and their whole bacterins that can cause mastitis has to be developed. Other options will be development of a bacterial ghost of Gram-negative bacteria expressing proteins of Gram-positive bacteria so that it can provide immunity against both pathogens. Immunogenic targets are another important feature for developing subunit vaccine. Outer membrane proteins (omp) of Gram-negative bacteria is a good immunogenic target that can be exploited for vaccine development. Coliform organisms are the common Gram-negative pathogens isolated from mastitis cases, hence 'omp' from these organisms can be tried as a vaccine that can cross protect the major mastitis causing pathogens. Similarly, for *Staphylococcus* and *Streptococcus* sp. immunogenic targets has to be identified which can be used in the cocktail vaccine. This identification of targets can be achieved by systems biology approach. Aims to consider the complex interactions between all parts of a biological system, with a view to extracting from data information used to understand and predict the behaviour of the biological system. This is made possible by the extraordinary progress in molecular biology, particularly in genome sequencing, and the high-throughput measurements of the "omic" technologies.

Similarly, vaccine delivery and adjuvants to be used has to be studied to achieve better results. To achieve this, data on the mastitis pathogen prevalent in the geographical area is essential. Hence as a preliminary work, mastitis samples were collected from Tamil Nadu and were screened for pathogens and AMR. A total of 1539 milk samples were collected in a study conducted at Vaccine Research Centre- Bacterial vaccine, CAHS, Madhavaram Milk Colony, TANUVAS, Chennai from six different districts of the North eastern regions of Tamil Nadu namely Tiruvallur, Kanchipuram, Chengalpat, Krishnagiri, Vellore and Dharmapuri. 252 *Staphylococcus* spp. was isolated, of which 61 was coagulase +ve (CoPS) and 191 was coa-ve (CoNS). The Gram-negative bacteria, *E. coli* which predominantly causes clinical mastitis was found to be 180 in CM milk and 114 in SCM milk. Thus, *Staphylococcus* spp. and *E. coli* are the predominant pathogens in mastitis in various districts of Tamil Nadu. The success of any vaccine rests on the selection of suitable candidates for vaccine preparation and hence, candidate selection is in progress.

Conclusion

Several solutions can be proposed to get out of the current mastitis vaccine deadlock. The root causes of vaccine shortcomings have not been considered in a conscious way. All the factors starting from the choice of antigen to the delivery of vaccine needs to be studied thoroughly so that a better preventive vaccine can be formulated. A cocktail vaccine that can be used as a single shot solution for mastitis control will be a huge boon to the dairy industry in this country.



ORAL PRESENTATIONS

OP_7.1 : EVALUATION OF AN AUTOGENOUS SNUFFLES VACCINE FOR RABBITS UNDER FIELD CONDITION

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Rabbits are important domestic pets and livestock animals contributing for meat and fur production. However, rabbits are highly susceptible to many infections which impacts the rabbit industry (Soriano-Vargas et al., 2012). One of the most common health problems in rabbits is pasteurellosis. Pasteurellosis is a bacterial disease which causes severe epidemics and adverse economic losses in rabbit production worldwide (Nasser et al., 2013). It is caused by *Pasteurella multocida* (*P. multocida*) and is a highly contagious disease affecting at 4–8 weeks old domestic rabbits. Rabbits older than 8 months to 1 year of age show lower incidences. In this study, an autogenous vaccine for Snuffles was prepared using the local isolate with capsular type A and a field trial was conducted in a private rabbit farm, in Tiruvannamalai dt. Of Tamil Nadu between December, 2020 and January, 2021. A formalin inactivated vaccine was prepared using *P. multocida* type A isolate's overnight culture having 1.2×10^9 cfu/ml concentration after sterility testing blended with alum gel at 3:1 ratio (3 parts of bacterin + 1 part of alum gel). A private rabbitry (Sathyaraj rabbit farm, Tiruvannamalaiddt) having 200 Nos. of rabbits of White Giant, Grey Giant, Soviet Chinchilla breeds of different age group were used for the trial. All the rabbits above 4 weeks' old were vaccinated with 0.5 ml dose per animal and blood samples were collected randomly at 0 and 21 days' post vaccination. Ten rabbits were used as control. The antibody response was assessed by indirect haemagglutination or passive haemagglutination test. The results were presented in the table showing all the vaccinated rabbits had good antibody levels (1:32 to 1:1024) but few animals had antibody levels (0 to 1:16) on 0th day itself indicating that prior infection with *P. multocida*. and there were no or very less antibody level in the control rabbits. Generally, in rabbit farming practices, vaccines are not used for any diseases. Snuffles is the only disease occurs in rabbits lading to sudden death. So, through this study, we advocate the use of snuffles vaccine in rabbits lading to sudden death. So, through this study, we advocate the use of snuffles vaccine in rabbits at the age of 4-6 weeks, a booster after 2 weeks' interval and annual revaccination.



OP_7.2 : DESIGN OF MULTI-EPITOPE SUBUNIT VACCINE AGAINST INFECTIOUS LARYNGOTRACHEITIS VIRUS USING IMMUNOINFORMATICS APPROACH

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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 design a multi-epitope based subunit vaccine using envelope glycoprotein B and glycoprotein D of the ILT virus using an immunoinformatics approach. The glycoproteins B and D play an essential role in the attachment and entry of ILTV into the cell which makes a potential vaccine candidate. The cytotoxic T lymphocyte, helper T lymphocyte, and interferon-gamma inducing epitopes were predicted and high-scoring predicted epitopes were joined in an organized manner using suitable linkers to design the final vaccine candidate. The avian beta-defensin 1 was added into the N terminal of the vaccine as an adjuvant that possesses antimicrobial activity and histidine residues at the C terminal for purification purposes. The final vaccine candidate was evaluated for its physicochemical characters, solubility, antigenicity, stability, and allergenicity and validated for its modeling. Molecular docking, binding affinity, and interacting residues between vaccine candidate and immune receptors like TLR 3, Class I, and II MHC were analyzed. Further, the immune response profile generated by the vaccine construct was evaluated by immune simulation. The results of the study showed the final vaccine candidate was antigenic, nonallergenic, stable, interacts with immune receptors, and able to generate humoral and cell-mediated immune responses against ILTV infection.

OP_7.3 : EXPLORING THE PROTEOMES OF *LEPTOSPIRA INTERROGANS CANICOLA* TO IDENTIFY POTENTIAL VACCINE CANDIDATES AGAINST CANINE LEPTOSPIROSIS

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Leptospirosis is the most neglected zoonotic disease of global public and animal health. It is a wide spread disease among pet animals and stray dogs and poses major threat to public health. Vaccination is the prime strategy to control the disease. However, the current vaccines are ineffective due to serovar specific protection, lack of cross reactivity and require booster doses. Therefore, it is



highly essential to identify conserved protein candidates that can provide immunity against the majority of leptospiral serovars using the immunoinformatic tools. In the present study, the proteome database of *L.interrogans canicola* and other causative serovars were analysed to develop a recombinant multi-epitope based vaccine against canine leptospirosis. Comparative analysis of complete proteomes of 20 *Leptospira* serovars with *L.canicola* yielded 664 gene products. Further non-homology search against the canine genome reduced the protein candidates to 346 numbers. Of these, 159 proteins were identified based on more than fivefold expression. Sub cellular localization of the selected proteins lead to 54 potential outer membrane and extra cellular proteins. Finally 18 promiscuous proteins were identified based on signal peptides. Both B-cell and T-cell (MHC Class-II) and cytotoxic T lymphocyte (MHC Class-I) epitopes were predicted for the conserved and most immunogenic outer membrane lipoproteins. All these epitopes were analysed for allergenicity, toxigenicity, IL-2 inducer, IL-10 non-inducer, water solubility and half life period. Based on the above criteria, 10 proteins were selected for predicting B cell, MHC class I and Transporter Associated Protein binding affinity predictions. Following sequence based predictions, structure based analysis of epitopes and DLA alleles were done by PEPstr, HADDock, and validation with MD simulation, PROCHEK and ProSA tools. Overall, this study provides four novel vaccine candidates namely stearyl-CoA, Beta-propeller repeat, TonB dependent and Sphingomyelin proteins which may prospect for further development of vaccines against leptospirosis.

OP_7.4 : EVALUATION OF IMMUNOGENIC POTENTIAL OF *STREPTOCOCCUS SUIIS* PHAGE LYSATE AS VACCINE CANDIDATE

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Streptococcus suis serotype 2 infection is considered as a major pathogenic disease worldwide both in humans and swine. In the present study, *S. suis* vaccine development was aimed with main criteria is to induce heterologous protection by both humoral and cell mediated immune response and to reduce the mortality of piglets by reducing the bacterial load and prevent the carrier animals in herd by immunotherapeutic approach of using phage lysate vaccine preparations. In the present study, the objectives were to isolate and characterize bacteriophage against *Streptococcus suis* serotype 2, to standardise preparation of phage lysate bacterin against *Streptococcus suis* serotype 2 and to evaluate the immunogenic potential of lysate against the direct challenge with *Streptococcus suis* serotype 2. Four different lytic phages SS1, SS2, SS3 and SS4 were isolated belong to the family myoviridae under order caudovirales. Phages SS1, SS2 and SS3 were having broad host range lytic activity to other serotypes of *S. suis*, to other streptococcal species and MRSA. Upon chemical characterisation, increased phage titre was observed on incubation with 10% Phenol and 1% SDS. 0.1 MOI of phage bacteria ratio shown to have high phage titre and is selected for Phage lysate vaccine preparation. Upon Lymphocyte transformation test, heightened Cell mediated immune response was observed for adjuvanted phage lysate vaccine group on 28th day post immunisation.



POSTER PRESENTATIONS

PP_7.1 : DEVELOPMENT OF RECOMBINANT FIMBRIAL VACCINE AGAINST OVINE FOOTROT

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Footrot is a contagious disease affecting the interdigital epidermis and living tissues of digits of sheep and goats. The causative agent is the anaerobic, gram-negative bacteria, *Dichelobacter nodosus*. Fim A of *D. nodosus* serotype B1 which was found to be more prevalent serotype in the study was cloned and expressed in *Pichia pastoris*. The gene coding for fimbriae was amplified and subsequently cloned in pPICZ α and transformed into DH5 α cells and then to *Pichia pastoris*. The expression of 17KDa *fimA* protein by *Pichia pastoris* was analyzed SDS- PAGE and confirmed by Western blotting. Immunogenicity studies of the recombinant *fimA* proteins were carried out in mice and rabbits. The titres recorded in mice/rabbits on day of vaccination were ranged from 1:2 to 1:4. The vaccination titers of 512 were recorded on 30th day of post vaccination and 1024 on 60th day of post vaccination in rabbits with whole cell antigen. Rabbits vaccinated with recombinant *fim A* vaccine shown the titre of 128 on 30th day and 256 on 60th day of post vaccination. The geometric mean titer of mice immunized with whole cell vaccine was 724 (512-1028) on 30th day and 1290 (1028-2056) on 60th day of post vaccination. Whereas mice immunized with r-*fimA* vaccine the geometric mean titer was 203 (128-256) on 30th day and 256 (128-512) on 60th day.

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