XXVII Annual Convention of ISVIB and National Conference

"LEVERAGING EMERGING BIOTECHNOLOGIES FOR ONE HEALTH"

VIBCON - 2022

27th to 29th July, 2023

BOOK OF ABSTRACTS

Under the aegis of

Indian Society for Veterinary Immunology & Biotechnology (ISVIB)

Organised by:

Divisions of Veterinary Microbiology
& Immunology and Animal Biotechnology,
Faculty of Veterinary Sciences & Animal Husbandry
SKUAST-Kashmir, Shalimar, Srinagar-190025

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Chief Editors: 1. Prof. Mohd Altaf Bhat
2. Prof. Riaz Ahmad Shah

Editors: Dr. Sabia Qureshi, Dr.Md Ishfaqul Hussain, Dr. Zahid Amin Kashoo, Dr. Pervaiz A. Dar, Dr. Shaheen Farooq

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Acknowledgement

The Indian society for veterinary immunology and biotechnology, popularly called as "ISVIB" is a conglomerate organization of primarily veterinary scientists from microbiology, biotechnology, preventive medicine, parasitology, animal reproduction and fisheries disciplines with the objective of fostering the growth of veterinary immunology and biotechnology in the era of biological technology. The organization was started in the year 1990 by collective visionary zeal of Dr. P. Richard Masillamony, B. B. Mallick, and B. S. Keshavamoorthy, during an interactive session at Tirupathi. XXVII Annual Convention of ISVIB and National Conference on "Leveraging Emerging Bbiotechnologies for One Health" was organized by Divisions of Veterinary Microbiology & Immunology and Animal Biotechnology, FVSc & AH, Shuhama & Indian Society for Veterinary Immunology & Biotechnology at SKUAST-Kashmir, Shalimar, Srinagar.

On behalf of ISVIB, the Organizing Secretary, Prof. Mohd Altaf Bhat and Divisions of Veterinary Microbiology & Immunology and Animal Biotechnology, FVSc & AH, Shuhama would like to acknowledge tremendous support and generous contribution made by Sponsors like NAHEP-SKUAST-Kashmir, SERB India, Indian Immunologicals Limited, Aviagen, Biovet and Hester to foster incredible conference like VIBCON-2022. Your support goes a long way in helping us to achieve these goals. The VIBCON-2022 was only able to fulfil its mission by receiving support from generous sponsors.

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I am delighted to know that SKUAST-Kashmir is organizing VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) with focal theme of "**Leveraging Emerging Biotechnologies for One Health**."

I have been told that the focal theme of VIBCON 2022, underscores the crucial role of cutting-edge biotechnological advancements in addressing the challenges related to human and animal health including antimicrobial drug resistance, emerging and re-emerging diseases, newer diagnostic tools, vaccines, immunoprophylaxis and other biotechnological interventions.

I trust that the conference will serve as a vital platform for the convergence of knowledge in the fields of veterinary immunology, biotechnology, and animal health and role of technological interventions to address emerging challenges as well as evolve a broader policy framework towards fulfilling our national commitments to implementation of UN SDG's for better human health.

Use of Technology should be at the core of our planning towards a sustainable public healthcare system that encompasses healthy soils, plants, animals, humans and an overall healthy ecosystem.

I believethat the conference will provide a platform for fruitful discussions, contribute to the collective understanding of the critical issues, and evolve a collaborative engagement among stakeholders towards better animal health with some tangible policy and action frameworks coming out of this conference.

I compliment the organizers and extend my best wishes to all participants for a successful and productive VIBCON 2022.

(**Manoj Sinha**)

July 19, 2023
Srinagar

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
www.skuastkashmir.ac.in

Prof. Nazir A. Ganai
Vice-Chancellor

Message

It gives me pleasure to extend my heartfelt greetings and support to the organizers and participants of VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Leveraging Emerging Biotechnologies for One Health."

I am happy to witness the convergence of distinguished professionals, academicians, scientists, and researchers from various disciplines related to life science, medicine, veterinary science and biotechnology. This conference provides a remarkable platform for sharing knowledge, exchanging ideas, and fostering collaborations that are crucial for the advancement of veterinary immunology and biotechnology.

The theme of this conference holds great significance in the current times. The exploration and utilization of emerging biotechnologies for the betterment of animal health and production is of utmost importance. By addressing antimicrobial drug resistance, emerging and re-emerging diseases, diagnostics, vaccines, and immunoprophylaxis, we can collectively work towards achieving the goals of One Health, which emphasizes the convergence of human, animal, and environmental health.

I applaud the organizing committee for their tireless efforts and I urge all participants to actively engage in the conference, share their research findings, and contribute to generation of new insights during this event. Collaborative efforts are crucial for bringing about significant positive changes in the field of veterinary immunology and biotechnology and I hope event will set a platform for initiating inter-national collaboration.

I extend my best wishes for a successful and enlightening conference. May VIBCON 2022 serve as a catalyst for transformative advancements, fostering stronger collaborations, and enabling the dissemination of innovative research that will shape the future of veterinary science.

(Nazir Ahmad Ganai)

Place: Shalimar, Srinagar
Dated: 24.07.2023

Shalimar,Srinagar-190025, J&K India
Phone: (Office) 0194-2464028; Fax: 0194-246160 (Resid.) 0194-2463655, 2461543
e-mail: vc@skuastkashmir.ac.in

Message by ISVIB President

I am delighted to extend my warmest greetings and support for VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Leveraging Emerging Biotechnologies for One Health."

In my capacity as the President of ISVIB, I commend the dedicated efforts of the organizing committee in convening this significant event, which brings together a diverse gathering of veterinary and animal professionals. This conference serves as an exceptional platform for robust discussions, analysis, and the formulation of strategies aimed at safeguarding animal health, enhancing animal production, and fostering advancements in veterinary immunology and biotechnology.

The chosen theme, "Leveraging Emerging Biotechnologies for One Health," underscores the vital role of biotechnological advancements in addressing contemporary challenges. From combating antimicrobial drug resistance to tackling emerging and re-emerging diseases, diagnostics, vaccines, and immunoprophylaxis, these topics are of paramount importance in ensuring the well-being of animals and humans alike.

To all participants, I earnestly encourage active engagement in this conference, sharing your research findings, and fostering knowledge exchange among fellow scientists, professionals, and stakeholders. Your expertise and insights are integral to shaping the future of veterinary immunology and biotechnology, propelling the field to new horizons.

My sincere appreciation goes to the organizing committee for their unwavering dedication and meticulous planning in orchestrating VIBCON 2022. Their commitment to nurturing collaboration and facilitating knowledge sharing is truly commendable. I am confident that this conference will foster meaningful interactions, stimulate innovative ideas, and lead to the development of transformative solutions that contribute to improved animal health and sustainable agriculture.

I wish you all a successful and enriching conference experience. May your collective efforts substantially advance veterinary immunology and biotechnology, ultimately benefiting animal well-being and promoting the principles of One Health.

Warm regards,

Raj Kumar Singh
President,
Indian Society for Veterinary Immunology & Biotechnology (ISVIB)

Sher-e-Kashmir
University of Agricultural Sciences and
Technology of Kashmir
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Prof. M.T. Banday
Dean

It is my great pleasure to extend my warmest greetings and support to the organizers and participants of VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Leveraging Emerging Biotechnologies for One Health."

As the Dean of the Faculty of Veterinary Science & Animal Husbandry at SKUAST-K, I am thrilled to witness the convergence of distinguished experts, scientists, academicians, and researchers from various disciplines related to animal health and biotechnology. This conference will provide a valuable platform for sharing knowledge, exchanging ideas, and fostering collaborations that are pivotal for the advancement of veterinary science.

The theme of this conference holds great significance in the current era. The exploration and utilization of emerging biotechnologies in addressing the challenges related to animal health, production, and the interconnectedness of human, animal, and environmental health are of utmost importance. By focusing on topics such as antimicrobial drug resistance, emerging and re-emerging diseases, diagnostics, vaccines, and biotechnological interventions, we can collectively work towards achieving the goals of One Health.

I express my heartfelt appreciation to the organizing committee in their tireless efforts in organizing this prestigious event, their dedication and commitment in promoting the growth of veterinary immunology and biotechnology is truly commendable. I encourage the participants to actively engage in the conference by presenting their research findings, and contribute to the collective knowledge in this field for making significant positive changes in veterinary Science.

I extend my best wishes for a successful and fruitful conference. May VIBCON 2022 serve as a platform for transformative discussions, foster meaningful collaborations, and contribute to advancements in veterinary immunology and biotechnology, ultimately benefiting animal health and welfare.

Warm regards,
Prof. Mohammad Tufail Banday
Dean, Faculty of Veterinary Science & Animal Husbandry, SKUAST-K

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

Message

Our country has made significant progress in milk production to become the largest producer of milk in the world. At this juncture we need to focus on novel technologies for increasing animal production and disease prevention. Rapid diagnostic kits and safe vaccines inducing long lasting immunity are the ideal inputs required to further enhance the productivity of livestock and poultry. Research outputs from different laboratories need to be taken up for further development to transfer technology for commercialization. Big data analytics and artificial intelligence are the new tools which can be used for animal health surveillance, disease diagnosis and farm monitoring. Presently, more research studies are required focusing on zoonotic diseases, one health approach and mitigation of antimicrobial resistance. In this context it is timely and appropriate to organize a national conference on "Leveraging emerging biotechnologies for one health".

I hope the deliberations of the ISVIB convention and National conference will help the scientists and research scholars to identify thematic areas, plan research and establish linkages.

I appreciate the efforts taken by organizers to conduct this National conference in the Faculty of Veterinary Sciences & Animal Husbandry, SKUAST-Kashmir, Srinagar, J&K. I offer my warm felicitations to the organizers and participants of the National conference and wish the event a grand success.

Date: 20.07.2023
Place: Namakkal

(A.THANGAVELU)

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir

Prof. M.A. Bhat
Organizing Secretary
VICON 2022

Dear Esteemed Participants,

Warm greetings and a heartfelt welcome to VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Leveraging Emerging Biotechnologies for One Health.

As the Organizing Secretary, it is my honor and privilege to extend my sincere appreciation to all of you for joining us in this prestigious event. VIBCON 2022 serves as a prominent platform for experts, scientists, researchers, and professionals to share their knowledge, experiences, and insights in the fields of veterinary immunology, biotechnology, and animal health.

The central theme of this conference, "Leveraging Emerging Biotechnologies for One Health," highlights the critical need to harness the potential of emerging biotechnologies in tackling the pressing challenges we face today. We will explore topics such as antimicrobial drug resistance, emerging and re-emerging diseases, diagnostics, vaccines, immunoprophylaxis, and biotechnological interventions, all of which play vital roles in enhancing animal health, production, and public health.

The significance of this conference cannot be overstated. By engaging in fruitful discussions, exchanging ideas, and fostering collaborations, we can collectively work towards a healthier future for animals, humans, and the environment. Your participation and contributions hold immense value in shaping the discourse and driving meaningful change in veterinary science and biotechnology.

I express my sincere gratitude to the organizing committee for their unwavering dedication and tireless efforts in bringing this conference to fruition. Their commitment to delivering an exceptional scientific program is truly commendable. To the participants, I encourage you to actively participate, present your research findings, engage in thought-provoking discussions, and seize the opportunity to forge new collaborations.

Once again, I extend my warmest welcome to all participants. May your presence and active involvement in VIBCON 2022 lead to fruitful interactions, shared insights, and groundbreaking advancements. Together, let us chart a path towards a healthier and more sustainable future for animals, humans, and our shared environment.

Best regards,

Prof.Mohd Altaf Bhat

Message from Co-organizing Secretary-ISVIB-2022, Prof. Riaz A Shah:

Esteemed Colleagues and Participants,

I extend my warmest greetings and a wholehearted welcome to VIBCON 2022, the XXVII Annual Convention of the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and the National Conference on "Leveraging Emerging Biotechnologies for One Health."

As the Co-organizing Secretary, it is an honor for me to be part of this remarkable event that unites experts, scientists, researchers, and professionals from diverse backgrounds in the pursuit of advancing veterinary immunology, biotechnology, and animal health. Your presence at this conference amplifies the significance of collaboration and knowledge exchange in driving meaningful change.

The theme of this conference encapsulates the essence of our collective goals: leveraging emerging biotechnologies for the betterment of animal health, production, and the overall well-being of our ecosystems. Throughout the conference, we will delve into crucial topics such as antimicrobial drug resistance, emerging and re-emerging diseases, diagnostics, vaccines, immunoprophylaxis, and biotechnological interventions, exploring innovative strategies to address these challenges.

The importance of this conference cannot be overstated. By converging our expertise, experiences, and ideas, we have the power to shape the future of veterinary science and biotechnology, making substantial contributions to One Health. I commend the organizing committee for their tireless efforts in curating an outstanding scientific program and creating a platform for fruitful discussions, scientific exchange, and networking opportunities.

To all the participants, I encourage you to actively engage in the conference, seize this opportunity to share your research, insights, and experiences, and foster collaborations with like-minded professionals. Together, we can bridge gaps, spark innovation, and drive positive change for the benefit of animals, humans, and the environment.

Once again, I extend my warmest welcome to VIBCON 2022. May your participation be fulfilling, enlightening, and transformative. Let us embark on this collective journey to unlock the immense potential of emerging biotechnologies and pave the way for a healthier and more sustainable future.

Best regards,

Prof. Riaz A Shah
Co-organizing Secretary, VIBCON-2022

Inaugural Session

[VIB-IAS-KNL-01]

In search for faster, easier avenues for generation of transgenic animal bioreactor

Subeer S. Majumdar[1], Abhishek Das, Gautam Ulgekar and Nirmalya Ganguli
National Institute of Animal Biotechnology, Hyderabad and [1]Gujarat Biotechnology University, Gujarat

ABSTRACT

Different proteins are required to be supplemented as therapeutic agents to certain patients lacking them, naturally. Such proteins are produced exclusively in mammalian expression systems to maintain their bioactivity during clinical applications. Similarly animal proteins like bovine LH and FSH is also needed in large quantities. To tackle high production costs, various attempts have been made to create transgenic animal bioreactor for expressing various therapeutic proteins in the milk. Though various techniques exist to produce transgenic farm animals, their efficiency is very low. The testicular transgenesis method, being a simple and less cumbersome procedure provide a better option for transgenesis in farm animals. The large animals mostly deliver one or two offspring after many months of gestation as compared to ~10 pups/litter in 21 days in mice. Therefore, it is essential to sort the transgene-bearing sperm in the ejaculate of farm animal used for testicular transgenesis, for there use in IVF or artificial insemination to guarantee production of transgenic animals at end of long gestation. For this, we engineered a fusion protein using EGFP and different signal peptides of the sperm surface glycoprotein Basigin (BSG) under CMV promoter, with an objective to localize GFP flagged fraction of BSG on the sperm surface. Such membrane-bound EGFP signal will help in easy and large-scale sorting of transgene-bearing sperm from semen through fluorescence (FACS) or affinity-based (MACS) methods. We characterized the expression of engineered protein in-vitro using HEK293 cells and in-vivo, by detection of GFP on the tail and midpiece of sperm collected from the epididymis of mice electroporated with a transgene (in the testis). We have sorted the transgene-bearing sperm using GFP antibody-coated MACS beads and successfully performed IVF. GFP was expressed in mice embryos fertilized with sorted transgenic sperm. The same strategy will now be extrapolated in farm animals using sperm specific promoters for the large-scale sorting of transgenic sperm followed by generation of transgenic farm animals.

Regulations to use transgenic farm animals may limit this avenue in future. Therefore, strategies involving direct transfection of mammary gland using transfecting solution carrying transgene through udder teat could be an alternate option. Since transfection agents are costly and not effective always, the synthesis of novel biocompatible and cost-effective, efficient chemical agents offering robust transfection efficiency was undertaken. To this end, we have modified the existing transfection agent i.e., branched-polyethyleneimine (bPEI) with hexanoyl group using anhydride chemistry. Our in vitro studies indicated that hexanoylated-bPEI (FA6-bPEI) tagged with a transgene can be used for enhanced expression of a therapeutic protein (human-interferon-γ) in cell culture-based systems. Furthermore, we have validated the capabilities of FA6-bPEI as in vivo transfection agent for the expression of human interferon-gamma (hIFN-γ) in the mammary gland of the mouse model. To reproduce this in livestock, we have hormonally induced lactation in goats and transfected the mammary gland by injecting FA6-bPEI tagged transgene through teat canal. This system provided hope for the cost-effective large-scale production of therapeutics, in the milk.

WE thank DBT for the financial support

Day 1
Technical Sessions

Session I
Molecular and Microbial Biotechnology
(Oral)

[VIB-MMB-IVL-01]

To be *Mycobacterium orygis* and not be *Mycobacterium bovis*–that is the question

G. Dhinakar Raj, K. Karthik and A. Jawahar

Department of Animal Biotechnology
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai

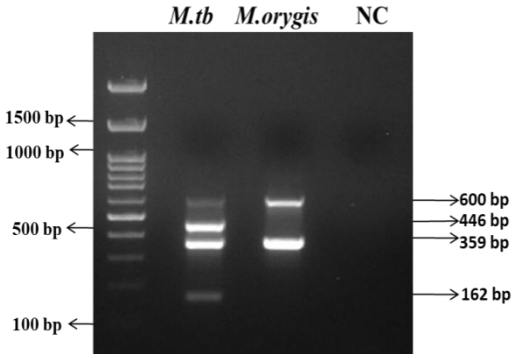
The *Mycobacterium tuberculosis* complex (MTBC) includes, but not limited to, organisms of *M. tuberculosis, M. bovis* and the more recently identified *M. orygis*. Following the first report of the 'Oryx bacillus' in a captive oryx in the Netherland Zoo in 1987, this organism along with other genetically similar bacteria were named *M. orygis* in 2012. In India, *M. orygis* has been isolated from cattle, spotted deer, and bison. Of the 940 MTBC isolates recovered from India, there was no report of *M. bovis* while there was 0.7% prevalence of *M. orygis* in human. Similarly, out of the 715 MTBC whole genome sequences from South Asia in the NCBI database there was no wild type *M. bovis* identified (Duffy *et al*., 2020). Further, presently there is lack of clarity regarding considering *M. orygis* as a pathogen either of zoonotic or zooanthroponotic importance or both. However, one certain finding from the recent literature is the total absence of *M. bovis* isolations or its detection from humans or bovines.

Genomic sequencing of *M. orygis*

Under the DBT funded project of MyDAN, there were no isolations of *M. bovis* while 10 *M. orygis* were isolated. These isolates from cattle (n=4), deer (n=4) and buffalo (n= 1) from Tamil Nadu and 1 buffalo isolate from Haryana were whole genome sequenced. In whole genome phylogeny analysis, all the *M. orygis* genome from Tamil Nadu clustered together but separately from the Haryana strain which clustered with the already reported strains from India. Comparative pangenomics of all *M. orygis* strains worldwide (n= 54) showed a closed pangenome structure. Pairwise SNP between TANUVAS_2, TANUVAS_4, TANUVAS_5, TANUVAS_7 and NIRTAH144 was less than 15 indicating that same *M. orygis* strain may be the cause for infection. Region of difference (RD) prediction showed absence of RD7, RD8, RD9, RD10, RD12, RD236a, RD301, RD315 in all the *M. orygis* (n= 54) analyzed. RD301 and RD315 was found to be unique regions that are absent in *M. orygis* and that can be exploited as a diagnostic marker for *M. orygis*.

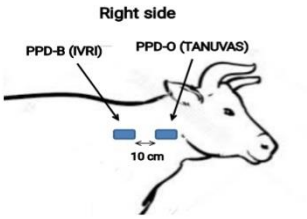
Multiplex PCR for differentiating *M. tuberculosis* and *M. orygis*

TB Multiplex PCR kit (MycoID) is a diagnostic test kit that utilizes the polymerase chain reaction (PCR) for the amplification of MTBC genome region, *IS* 6110 (OIE 2018), followed by a multiplex PCR that allows the simultaneous amplification of multiple targets of MTBC species-*M. tuberculosis, M. bovis and M. orygis* in one reaction. Pathogen diagnosis by PCR is based on both the presence and absence of specific regions of the pathogen genome detected by the amplification or non-amplification of these regions.



The primers for multiplex PCR were designed based on the RDs that targets regions of absence in MTBC complex for species identification. If the strain is *M. orygis*,2 bands of 359 bp and 600 bp would be present but if the strain is *M. tuberculcosis,* bands of 600 bp, 446 bp, 359 bp and 162 bp would be present. If *M. bovis* is present 2 bands of 600 bp and 162 bp would be present

Purified Protein Derivative prepared from *Mycobacterium orygis* strain (PPD-O)



In India, presently Purified protein derivative (PPD) is prepared from the *M. bovis* strain AN5 only by IVRI, Izatnagar. Since all isolations from bovine in our laboratory belonged to the *M. orygis* **s**train, it was felt that tuberculin skin testing with PPD prepared from a homologous strain would be a better option since *M. bovis* was almost non-existent in animals in India.

Fifty-one cattle and nine buffaloes were inoculated with PPD-O (TANUVAS) and PPD-B (IVRI) and skin thickness were measured at 72 h post inoculation. The mean ± SD skin thickness produced by PPD-O and PPD-B were 4.5 ± 3.53 mm and 3.12 ± 1.98 mm respectively. When, increase in skin thickness of more than 4 mm is taken as criteria, PPD-O and PPD-B (IVRI) gave positivity for 32 (53%) and 20 (33%) animals respectively. When skin thickness produced by PPD-A (Prionics) was subtracted from skin thickness produced by respective PPDs, the positivity was dramatically reduced to 9 (15%) and 3 (5%) animals respectively for PPD-O and PPD-B. The increased positivity rate may be attributed to the use of homologous *M. orygis* strain as PPD since *M. orygis* strain was the only strain isolated from that farm.

M. orygis* or *M. bovis

Recently, all reports of isolations from animals in India have shown to be *M. orygis* with no *M. bovis*. Though reports have shown the presence of *M. bovis* earlier, it is likely that *M. orygis* might have been misidentified as *M. bovis* due to the lack of differentiating diagnostic assays or molecular markers. Further there are no reports of the complete genome of *M. bovis* available in NCBI database thereby confirming this possibility. Sub-speciation of MTBC is not routinely followed in many clinical settings and hence it is very important to speciate the MTBC organisms from humans or animals for better understanding of the epidemiology of bovine tuberculosis. Presently there are molecular markers and next generation sequencing and real time PCR methods that can differentiate *M. orygis* and *M. bovis* which needs to be routinely incorporated during diagnosis, based on which the prevalence of *M. orygis* can be determined unequivocally.

*Salmonella enterica*subsp. *enterica*is a pathogen of global concernfor both human and animals.Itcauses infection ranging from a mild, self-limiting diarrhea to severe gastrointestinal, septicemic disease and enteric fever. The genus *Salmonella* comprises a large and related population of zoonotic pathogens that can infect most mammals, including humans and domestic animals, birds, reptiles and amphibians. It continues to be one of the most important foodborne pathogens worldwide.The organism is commonly transmitted via food chain;however, outbreaks have been reported recently in which it has been transmitted through direct or indirect contact with animals. An estimated 11% of all *Salmonella* infections are attributed to animal exposures, with the highest rates of illness and death occurring among children. Since 2007, numerous outbreaks of human *Salmonella* infections linked to contact with animals and their environments have been reported. Poultry and pigs can be the persistent subclinical shedders and can appear healthy while continuously shedding the bacteria in faeces. As zoonotic salmonellosis outbreaks occur at the intersection of human and animal health, One Health approach may be more appropriate for their investigation.

Although serotyping using the Kauffman-White scheme remains the standard method for identification of *Salmonella*, it has certain significant deficiencies. Besides being labor-intensive and expensive, it is also time-consuming, often requiring three or more days for a highly trained laboratory technician to produce a result. A total of 2579 serovars of *Salmonella* have so far been described, majority of which are non-host-specific and can cause infections across species. Pathogenesis of *Salmonella* infections is a complex process, in which numerous virulence genes clustered within *Salmonella* pathogenicity islands (SPIs) are involved and so far as many as 21 such islands (SPI-1 to SPI-21) have been reported. However, occurrence of SPIs and individual virulence genes varies among serovars.

Pathogens of a single species generally comprise of diverse strains showing wide variations in their epidemiological association with disease in respect of spatial and temporal distribution as well as host specificity.Several different methods may be applied for determining the molecular diversity among the *Salmonella* isolates.We developed a novel multiplex PCR-based rapid method supported by an online server based on differential distribution of 16 genes in various serovars of *Salmonella* for detection of common clinical serovars. Comparative efficacy of four different methods, *viz.* plasmid profiling, PFGE, rep-PCR and automated DiversiLab System®was also evaluatedfor determining molecular diversity among the*Salmonella* isolates from various sources belonging to different serovars.A multiplex PCR protocol was also developed for simultaneous detection of seven major virulence genes of *S. enterica*subsp. *enterica*.

Outer membrane proteins (OMPs)are integral membrane proteins and lipoproteins that are anchored to the outer membrane of gram-negative bacteria.Thesehelp in maintenance of the integrity and osmotic permeability of the bacterial membranes. They are highly immunogenic and considered aspromising candidates for development of vaccines and diagnostics. Some of the OMPs also act as bacterial adhesins and important virulence factors. The outer membrane proteins of *Salmonella* are also known to have a significant role in eliciting immune responses. Among the outer membrane lipoproteins, InvHacts as an adhesin that helps in the entry of the bacteria into the epithelial cells of the host. It is an integral component of SPI-2 of the Type III Secretion System and has been proposed as a potential target for vaccine development. This adhesin is almost universally present in all *Salmonella* strainsexcept for*S. enterica* subsp.*arizonae*.

We successfully expressed the InvH protein of *Salmonella* Typhimurium in *E. coli* host and evaluatedthe 15 kDa recombinant protein for its potential as a vaccine candidate by testing its immunogenicity in mice. For this, the complete sequence of the*invH* gene was cloned and expressed in *E. coli*host and the expressed recombinant protein was purified under denaturing condition. On experimentally inoculated into mice, the purified recombinant InvH protein showed significant IgG response and induced protective immunity against both homologous and heterologous challenges.

[VIB-MMB-IVL-02]

Anthrax Diagnosis and Control

K.Manimaran,T.M.A.Senthilkumar and C.Soundararajan
Zoonoses Research laboratory,
Centre for Animal Health Studies, TANUVAS
Madhavaram Milk Colony,Chennai-600 051.

1. Introduction

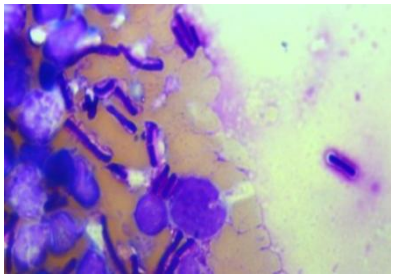
Anthrax is a potentially fatal disease for humans, domestic and wild animals, and is a bioterrorism agent. Anthrax is an enzootic disease of livestock in many parts of India including Tamilnadu. An estimate of global annual human anthrax cases is around 2000. Haemorrhage from the nose, mouth, vagina and/or anus with poorly clotted blood at death is clinical feature of disease. Proper disposal of carcass as per biosafety measures is required to avoid environmental contamination.

2. Aetiological agent:

- *Bacillus anthracis*
- Gram-positive, Rod-shaped
- Capsulated, spore forming
- Formation of endospore formers (1–2 μm in size), results in resistance of the organism to heat treatment and disinfection procedures
- The production of toxins and capsule act as virulence factors

3. Anthrax in humans

Of the three forms of the disease, cutaneous anthrax is the most frequent, followed by the inhalational and GI or oropharyngeal form. All three forms of anthrax can be fatal if not treated in time, but the cutaneous form is often self-limiting. A typical anthrax skin lesion corresponds to an ulcer covered by a characteristic black eschar and only few case reports have been published regarding anthrax meningitis.



4. Indian scenario

Anthrax is widespread in India. As per NADRES data ((from 1991 to 2010) Anthrax has been reported in eighteen states viz., Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamil Nadu, and West Bengal. The occurrence of anthrax showed a gradual increase in the number of outbreaks since 1992 with a peak seen between 2000 and 2002. Afterwards there was a gradual decline, could be attributed to awareness amongst the farming community and the control measures.

5. Anthrax scenario in Tamil Nadu

Anthrax is an endemic disease particularly in the northern part of Tamil Nadu. A total of 619 anthrax outbreaks in cattle were documented over 15 years (from April 1991 to March 2006) with peaks between 1993 and 1995 and between 2001 and 2005. The larger extent of outbreaks in districts like Vellore and Tiruvannamalai are due to factors such as alkalinity of soil, the presence of leather tanneries, and free range system of rearing, relatively poor awareness of the disease.

6. Epidemiology

Anthrax bacilli are also destroyed by normal post mortem changes. However, on exposure to air, it forms highly resistant spores. Spores remain viable for many years in animal products,

soil and industrial environments. Alkaline soil (pH 9) containing adequate nitrogen, calcium, and organic matter favour the growth. It occurs in conjunction with extreme weather changes such as a drought followed by heavy rains. When these conditions are met, the organisms are thought to undergo a vegetative cycle in soil and then re-sporulate leading to high soil concentration of anthrax spores to cause disease in grazing animals' leading to outbreaks. Anthrax spores are transmitted to animals through ingestion of contaminated water, hay or grazing in areas which have previously affected with anthrax. The incidence of infection may increase as a result of drought or overgrazing when there is a greater likelihood of animals breathing or ingesting spore contaminated dust. The disease will be most prevalent in summer when pastures are poor and the animals during grazing have closer contact with soil. Heavy rain fall leads to flushing spores from one area to other and contaminate that area. The deep ploughing of pastures previously contaminated with effluent from or tanneries, or the unearthing of old graves (for example, by flooding).

7. Mode of transmission

- It is not contagious — that is, spread from animal to animal is insignificant.
- It is spread by release of bacterial spores from blood discharges and exudates from the carcass of an animal that has died from the disease and the subsequent ingestion of these spores by other animals.
- Pigs and carnivores have acquired infection from inadvertent feeding of anthrax-affected carcasses and offal, feeding of contaminated product, such as inadequately processed blood-and-bone fertiliser.
- There are no evidence of transmission through milk from infected animals
- Mechanically by insect vectors, including flies, mosquitoes and ticks (e.g.Tabanid flies, blow flies)
- Scavenging animals, including hyenas and vultures

8. Clinical signs

Herbivores exposed to infection develop clinical signs within 4–10 days. The bacteria continuously filtered out of the bloodstream by the spleen and lymph nodes until the last few hours of life, when the bacteria rapidly build up in the bloodstream to cause a terminal septicaemia only hours before death. Dairy cattle may show a change in temperament and a drop in milk production. Blood-stained discharges at external orifices are characteristic of the disease, but not all anthrax cases show these signs. A reliable sign is the failure of blood to clot. The clinical forms of anthrax in animals are traditionally described as:

• Peracute - death occurs suddenly (within a few hours at most) of the onset of clinical signs
• Acute — death occurs from 24 hours to a few days after onset
• Subacute or localised — disease lasts for several days and might end in recovery
• Chronic — recovered animals might show localised swelling and fever, but the only sign might be enlarged lymph glands

09. Laboratory Diagnosis

The success of laboratory diagnosis is dependent on collection of right choice, quality and method of transportation of specimens.

- A veterinarian or microbiologist trained in handling disease causing agents should do the sample collection
- Safety precautions to be followed which includes use of protective clothing protective eye shields and N95 face masks and wear an apron
- Needs disposable double covers for your hands and feet and strong bleach solution (10 000 ppm)
- After specimen collection, discard disposable items into disposal bags for subsequent sterilization (boiled for 30 min or pressure cooked for 15-20 min or incineration)

- After specimen collection, rinse or wipe down gloved hands with 10% hypochlorite solution and discard outer gloves. Wash hands thoroughly with soap and water

10. Samples to be collected

Type	Sample	Container
Fresh carcass	Smears of from blood, lymph or oedematous fluid. Blood from ear vein (0.1 ml) or other vein and fluid from body cavity.	Glass slide Vacuum tube, Small vial, or leave in syringe
	Piece of highly vascularised tissue (ear clipping).	Small vial
Putrefied carcass	Piece of highly vascularised tissue	Swab tubes
	Swabs of vascularised regions (nostrils, eye socket any bloody material).	Swab tubes
	Bloody soil from under head or tail	sealable specimen container or double plastic bags
Very old carcass,	swabs of nostrils, eye sockets	Swab tube
Hides, bones		Sealable specimen container
Industrial sites (tanneries)	Soil samples up to 0.25 m from the top	Sealable specimen container
Carcass sites	Samples up to about 2 m below surface	Sealable specimen container

11. Anthrax: Different laboratory diagnostic tests

Test	Specimen required	Test detects	Time taken to obtain result
Agent detection			
Blood smears	Blood, oedema fluid	Organism	15 minutes
Bacterial culture	Blood, fluids, tissue, in swabs	Organism	1–2 days
ICT (point-of-care test)	Blood	Bacterial antigen	15 minutes
PCR (multiplex)	Blood, blood smears, tissue, cultured bacteria	Bacterial DNA	2-3 days
Real-time PCR	Blood, tissue, cultured bacteria	Bacterial DNA	1 day

12. Strategy for control and eradication of anthrax

i. Vaccination

- All other animals in the affected herd are to be vaccinated with coverage of 85% of population.
- Affected premises are to be quarantined for at least 20 days after the last case.
- All cattle on neighbouring premises should also be vaccinated.
- A buffer zone, 20-30 Km wide, is to be established around the infected area with in which all cattle and exposed sheep are vaccinated and quarantined.

ii. Vaccines

- In the early 1890s, a double-dose Pasteur vaccine was used
- Sterne developed an attenuated live animal vaccine in 1935 that is still used, worldwide. It contains spores of the non-capsulated, naturally avirulent (live) Sterne $34F_2$ strain of *B. anthracis*.

- The animal vaccines that use strain $34F_2$ are essentially as originally formulated (Sterne, 1939) with approximately 10^7 spores per ml suspended in 0.5% saponin in 50% glycerine saline.
- The protective effect of a single dose of strain $34F_2$ vaccine is said to last about 1 year and annual boosters are recommended for livestock in endemic areas.
- The most significant problem with the Sterne vaccine is an elevated temperature 12–36 hours after vaccination, causing reduced milk yield and possibly abortion in dairy cows as that it retains some virulence. Goats, horse it causes severe reaction occasionally may die following vaccination.
- Sheep and goats should be vaccinated in the caudal fold only
- All vaccinated stock are withheld from slaughter for at least 42 days after vaccination but the vaccinated cows do not shed *B. anthracis* in their milk

iii. Available vaccines

Vaccine	**Manufacturer**	**Content**	**Dose**	**Primary vaccination**	**Booster vaccination**
Raksha-Anthrax (Prophylactic only)	Indian Immunologicals	Suspension of live spores of attenuated non-capsulated strain of *B. anthracis* in 50% glycerinated saline, each dose $\geq$ $1x10^8$ viable spores	1 ml, i/m or s/c (Vial: 50 ml)	one month before grazing season or prior to the time the disease usually occurs	Annual revaccination
Note: Protect animals from overexertion 3 days following vaccination. Do not vaccinate the animal 60 days before slaughter					
Sterne Vaccine	Institute of Veterinary Preventive Medicine, Tamil Nadu	Live spores of highly antigenic non-encapsulated avirulent Sterne strain (34F2) of *B. Anthracis* suspended in glycerol with saponin added as an adjuvant.	Cattle, pigs - 1 ml S/c on neck region Sheep, goats: 1 ml injected S/c. in tail-fold	Approx. 4 weeks prior to the time the disease usually appears	Revaccinate after 2-3 weeks in heavily contaminated areas annual vaccination in endemic areas

[VIB-MMB-OP-01]

First report of Duck Hepatitis A virus genotype 2 in India

Ramya R , Jaisree S , Jayanthi N, Kalaiselvi G, Karthik K , Madhanmohan M , Manoharan S, Madhuri S, Revathi S,Lakshmana Rao P , Sridhar R, Balakrishnan G, and Soundararajan C

Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai-600051, India

Disease investigation was carried out to determine the etiological agent causing sudden deaths in a duck farm located at Tiruvallur district in Tamil Nadu, India. Enlarged pale-pink / pale-yellow liver with multifocal petechiae and ecchymosis were noted upon necropsy of dead birds.A positive amplification with duck hepatitis A virus specific primers by reverse transcription-polymerase chain reaction (RT-PCR) on the tissue samples collected from dead birds indicated infection by duck hepatitis A virus (DHAV), an avian picornavirus, known to cause acute and high-mortality in ducklings. The virus isolation was successful in 9-days old embryonated chicken eggs, in primary chicken embryo fibroblast (CEF) cells and from experimentally infected ducklings. The embryonic death on day 5 to 7 post inoculation in chicken embryos with signs of cutaneous hemorrhage, edema and greenish yellow liver together with histopathology of embryonic liver and kidney further confirmed DHAV infection. TEM analysis of the infected allantoic fluid and infected CEF cell culture supernatant showed the presence of spherical shaped, non-enveloped virion particles of ~20-38 nm diameter, typical for DHAV. Experimental infection of ducklings with RT-PCR positive tissue supernatant caused 40% to 50% mortality with typical petechial hemorrhages on the surface of liver. Further, histopathological analysis and RT-PCR of the inoculated duckling's tissues confirmed the presence of DHAV. Nucleotide sequencing of the 5′UTRregion and VP1 regionconfirmed duck hepatitis A virus genotype 2 (DHAV-2). To the best of our knowledge, this is the first report of laboratory confirmation of DHAV-2 in India. This study warrants the need for the extensive epidemiological surveillance to understand the prevalence of DHAV-2 in India and to take appropriate control measures to curtail the disease spread.

[VIB-MMB-OP-02]

Molecular characterization of *Canine parvovirus* and Phylogenetic analysis of VP2 gene from clinical cases of dogs in Nagpur

Warke S and Nair S.

Department of Veterinary Microbiology and Animal Biotechnology T & R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra
E-mail: shubhangi _warke@rediffmail.com

Canine parvovirus (CPV) infection is an infectious and contagious viral disease of canine especially puppies of age under 6 months. CPV-2 infected dogs are characterized by a gastroenteritis disorder with clinical signs of anorexia, lethargy, vomiting, fever, and diarrhoea. Conventional PCR is time consuming; it takes time for diagnosis. keeping this in mind, the current study was designed to detect *Canine parvovirus* using SYBR green dye based Real-time PCR, which provide accurate and specific results for CPV-2 and sequencing and phylogenetic analysis of VP2 gene.

A total of 200 faecal samples were collected from diarrheic dogsin and around Nagpurin this study. The faecal samples were screen for the HA test, Antigen detection kit by Ubioquick VET kit, Conventional PCR, Multiplex PCR and SYBR green dye based Real-time PCR., followed by VP2 gene sequencing and phylogenetic analysis.

Overall incidence of *Canine parvovirus* in and around Nagpur is 10% by HA test, 18% by antigen kit test, 32% by conventional PCR, Multiplex PCR reveled 18% positive samples had all the four antigenic types [CPV-2c (81.25%) ; CPV-2a (75%) ; CPV-2 (50%) ; CPV-2b (31%)].All 200 samples were screened by SYBR green dye based Real-time PCR, where 96 samples (48%) were found positive. Further Six PCR products were sequenced commercially and obtained sequence data which was analysed using bioinformatics toolsSequences revealed that two each sample belonged to CPV-2a, CPV 2b and CPV 2c antigenic type.

The conventional method shows low sensitivity as compared to qPCR. The sensitivity and specificity of qPCR was 100%. SYBR green dye-based qPCR was found to be useful for detecting CPV directly from the faecal samples over the conventional PCR method and could be employed in surveillance strategies.

[VIB-MMB-OP-03]

Molecular epidemiology of kobuvirus in pig population of Punjab, India

Aakriti Pathania* and Yashpal Singh Malik#

College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, 145001

The livestock sector contributes significantly to India's social and economic growth, accounting for 25.6% of GDP. An annual growth of 7% was observed in the livestock industry over the past three decades. Viral gastroenteritis is one severe illness that mainly infects new-born piglets which is transmitted by faeco-oral route. Diarrhoea, vomiting, dehydration, nausea, a mild to severe fever, and faeces with an unpleasant smell are few of the clinical signs of gastroenteritis. Fecal samples (N=40) were collected from pigs, from different regions of Punjab from diarrhoeic/healthy animals; animals of organized/unorganized farms; animals from different age groups (below 3 months of age/ 3–6 months of age/ and above 6 months); different seasons winters (December-February)/ monsoon (August-November) /summers (March-July). Detection of porcine kobuvirus was carried out by using reverse transcriptase PCR. Universal primers of Porcine Kobuvirus were used to check the prevalence of viruses. In pigs, out of 40 samples screened for porcine kobuvirus, 24 samples were found positive (60%) from different regions of Punjab. From the positive 24 samples, 5 samples selected from different regions of Punjab respectively were sent for sanger sequencing and it was observed to show 98-99% of similarities with strains of kobuvirus from china. Various risk factors *viz*., type of farming system, healthy or diseased status, age group as well as seasonal prevalence were also found crucial. The current study first time reported the circulation of Kobuvirus viruses in farm animals in Punjab. The finding of the study warrants strict vigilance of livestock population for emergence of various emerging and re-emerging as well as novel viral pathogens in the region. Further, appropriate screening strategies are to be adapted for effective control of the ailments.

Keywords: Kobuvirus, Farm animal, gastroenteritis, diarrhoeic

[VIB-MMB-OP-04]

Molecular detection & phylogenetic analysis of VP6 gene from Rotavirus strains infecting neonates, porcine & canines in Nagpur

Kaul V, Warke S and Tikoo M
Department of Veterinary Microbiology and Animal Biotechnology T & R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

Background:
Group A rotavirus (RVA) is one of the major pathogens causing gastroenteritis in humans and animals worldwide. The virus contains 11 dsRNA segments encoding six viral structural proteins (VP) and six non-structural proteins (NSP). It causes moderate to severe gastroenteritis in neonates of all species.

Methodology:
In the present study, 50 faecal samples each were collected from pups, piglets and human infants showing clinical signs of diarrhoea from Nagpur region. The samples were processed for Antigen detection kit. The positive samples detected by antigen detection kit will further subjected for PCR & Sequencing & phylogenetic analysis.

Results:
Antigen detection kit revealed the overall prevalence of rotavirus infection in canines as 6% (3/50), piglets 12% (6/50) and human infants 8% (4/50), respectively. The positive samples were tested for rotavirus group A, using one-step RT-PCR specific to the VP6, All 13 samples were found positive for VP6 gene
One representative amplicon of VP6 gene, each from canine, piglets and human infants were sequenced and subjected to phylogenetic analysis. VP6 gene from human infant (H19) showcased high diversity amongst the compared sequences and may be showing defined diversity among tested sequences. Sequence from piglet (P-16) was 93.80 % homologous with other sequence from pig of Maharashtra region (Accession No. LC379954.1). The sequence from canine (C-8) was 100% identical with a sequence from China (Accession No. OL388440.1).

Conclusion:
Upon nucleotide sequencing and phylogenetic analysis of VP6 RT-PCR positive samples recovered from human infants and piglets in the present research with one of the Indian human rotavirus sequences it indicative of likelihood of threat stood in the form of inter-species transmission of rota viral strain circulating in the area, raising the zoonotic significance of the organism

Key words: Rota virus , VP6 gene and Phylogenetic analysis.

[VIB-MMB-OP-05]

Molecular detection of a new lineage of Dengue virus serotype - 2 in naturally infected Aedes mosquitoes in Bhopal, Central India.

Sarma D K[1*], Rathod L[1], Mishra S[1], Das D[1], Agarwal A[2], Sharma G[1], Singh T A[3], Kumawat M[1], Singh S[1], Kumar M[1], Shubham S[1] and Tiwari R R[1]

[1] *ICMR-National Institute for Research in Environmental Health, Bhopal, Madhya Pradesh, India.*

[2] *State Virology Laboratory, Department of Microbiology, Gandhi Medical College, Bhopal, Madhya Pradesh, India.*

3 Maharaja Ranjit Singh College of Professional Sciences, Indore, Madhya Pradesh, India.

Dengue is an emerging public health burden in India and molecular surveillance of vector mosquitoes can not only aid in understanding the dengue disease dynamics, hotspot identification but also helps in efficient management and control of dengue in an area. This study aimed to detect and characterize the circulating dengue virus (DENV) in field-collected *Aedes* mosquitoes in Bhopal, Central India. Adult *Aedes* mosquitoes were collected from 29 different areas of Bhopal city using mechanical aspirator from October 2020 to September 2022. DENV infection was assessed in the individual head and thorax regions collected *Aedes* mosquitoes using reverse transcriptase PCR. Positive samples were sequenced, and the circulating serotypes and genotypes were determined using phylogenetic analysis. A total of 2,371 adult female *Aedes* mosquitoes (1,498 *Ae. aegypti* and 873 *Ae. albopictus*) were collected. Of these, 1,890 mosquitoes (1,186 *Ae. aegypti* and 704 *Ae. albopictus*) were tested for DENV infection. DENV RNA was detected in 7 *Aedes aegypti* and 1 *Ae. albopictus*, with infection rates of 0.59 and 0.14%, respectively. Phylogenetic analysis revealed all the isolates belonged to DENV serotype 2 and distinctly clustered with the non-Indian lineage (cosmopolitan genotype 4a), which was not recorded from the study area earlier. The most common recent ancestor (TMRCA) of these sequences was 7.4 years old, with the highest posterior density (HPD) of 3.5–12.2 years, indicating that this new lineage emerged during the year 2014. This is the first report on the emergence of a non-Indian lineage of DENV-2 in Bhopal, which coincides with the gradual increase in DENV cases in the area since 2014. This study emphasizes the importance of DENV surveillance and risk assessment in this strategically important part of the country to decipher its outbreak and severe disease-causing potential.

Keywords: Dengue virus, *Aedes* mosquito, surveillance, Epidemiology, India.

[VIB-MMB-OP-06]

Molecular Characterization of *Haemonchus contortus* in sheep from Palani Hills, Tamil Nadu, India, based on the genes encoding COI and Cysteine Proteinase

G. Nagarajan, K. Pachaiyappan, S.M.K. Thirumaran, S. Jegaveera Pandian, P. Thirumurugan, S. Rajapandi and K. Kanakarajadurai

Southern Regional Research Centre
ICAR- Central Sheep and Wool Research Institute
Mannnavanur, Kodaikanal, Tamil Nadu -624103, India

During the first week of December 2021, an Avikalin sheep at SRRC, Mannavanur, died due to severe anaemia. Upon Post mortem examination, the abomasum of the dead sheep was having plenty of round worms. The worms were washed in PBS and preserved in 70 % alcohol. A few worms were stored in in Trizol™ reagent and brought to the laboratory in ice. The worms taken out of 70 % alcohol and Trizol™ reagent were thoroughly ground to make a fine powder using liquid Nitrogen in a sterile mortar & Pestle for the isolation of the worm's total genomic DNA and cellular RNA respectively using the commercially available kits. Using the worm's genomic DNA as a template, the cytochrome c oxidase subunit I (COI) was amplified by PCR using the forward primer, HCCOX-F (23 mer): 5' CCTACTATAATTGGTGGGTTTGG 3' and reverse primer, HCCOX-R (24 mer): 5' TAGCCGCAGTAAAATAAGCACG 3'. The PCR amplified DNA fragments were then cloned into pGEM®-T Vector and the positive recombinant plasmids were subjected to Sequecing experiments at M/s. Eurofins Genomics India Pvt. Ltd., Bengaluru-560048, Karnataka, India. The GenBank accession No. obtained for the COI gene sequences of *H.contortus* from Palani Hills, Tamil Nadu, India, is ON005159.

The total cellular RNA isolated from the worm was reverse-transcribed using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara) in a 20µl volume reaction mixture according to the manufacturer's instructions. Using the worm's cDNA sample, the Cysteine Proteinase of *H. contortus* was amplified by conventional PCR using the forward primer, HCCP6F (24 mer): 5' ATGAGGTACAACGTAGTTGCACTC 3' and reverse primer, HCCP6R (24 mer): 5' TCACAGTAGGACATGTCCGGCGAC 3'. Resultant PCR products were then cloned into pTZ57R/T vector (Fermentas) and the confirmed recombinant plasmids were eventually subjected to sequencing experiments using Sanger sequencing method by M/s. Eurofins Genomics India Pvt. Ltd., Bengaluru-560048, Karnataka, India. The determined nucleotide sequences of Cysteine Proteinase gene of *H. contortus* from Palani Hills, Tamil Nadu, India, were then submitted to GenBank and the accession No. ON007367 was obtained. Both the nucleotide sequences encoding COI and Cysteine Proteinase of *H. contortus* in Sheep from Palani Hills, Tamil Nadu, India, were analysed using the standard Bioinformatics tools.

Key words: Sheep; Palani Hills, India; *Haemonchus contortus;* COI; Cysteine Proteinase; Bioinformatics analysis

[VIB-MMB-OP-07]

Investigation of Concurrent Infections of African Swine Fever Virus and Porcine Parvovirus 2 in a Swine Herd in Punjab, India

Adarsh Mishra, Manu M and Yashpal Singh Malik
College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

The piggery sector in India has been largely overlooked and faces significant challenges. Currently, there are over 9 million pigs in the country, and the sector is plagued by various infectious viral pathogens that pose a threat to the health of the swine population. Among these, African swine fever (ASF), caused by the African swine fever virus (ASFV), is particularly devastating and has resulted in high mortality rates in affected pigs. This disease is a major concern in many South and South-East Asian countries, and its cross-border transmissibility has a severe impact on the porcine industry. Recently, ASF has emerged in the Indian subcontinent, spreading from the North-eastern to the Northern and Southern states of the country. The lack of an effective surveillance strategy has further exacerbated the situation, leading to significant mortality in swine herds. Additionally, concurrent infections with other viral pathogens, such as porcine parvoviruses (PPVs), have been reported, causing stillbirth, mummification, embryonic death, and infertility in pigs. These concurrent infections with ASFV and PPVs have been observed in Punjab, India, where a semi-organized farm in the Amritsar district reported mortality among piglets and pregnant sows. The affected pigs, which were of the Large White Yorkshire breed, exhibited acute symptoms, including respiratory distress, shivering, and elevated body temperatures. Notably, reddened patches were observed on the skin over the ear extremities, resembling symptoms of classical swine fever. PCR-based detection platforms confirmed the presence of ASFV and porcine parvovirus 2 (PPV2), while differential diagnoses for classical swine fever virus, PPV1, and porcine circovirus 2 (PCV2) were negative. These findings highlight the need for strict vigilance and accurate detection of suspected cases in the swine population to effectively identify ASFV and other concurrent infectious pathogens and safeguard the swine herds. It is crucial to strengthen surveillance systems, enhance collaboration among veterinary authorities, researchers, and industry stakeholders, and raise awareness about biosecurity measures and vaccination programs to mitigate the impact of these diseases on the piggery sector in India and ensure its long-term sustainability.

Keywords: Porcine, Concurrent infections, African swine fever (ASF), Porcine parvoviruses, India

Session I
Molecular and Microbial Biotechnology
(Poster)

[VIB-MMB-PP-01]

Incidence of SARS-COV-2 infection among asymptomatic patients undergoing elective surgeries - experience from an apex tertiary care institute in north india

Qayoom T, Ahmed T, Fomda B A
Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, Soura-190011, J&K, India

The COVID-19 pandemic has significantly affected healthcare delivery and delayed routine and preventive services with many elective surgeries being postponed or cancelled. Studies have documented a significantly higher risk in patients undergoing surgeries who were affected by SARS-CoV-2 than in the general population. The rationale behind knowing the COVID-19 status of any surgical candidate is to ensure patient safety, reduction of postoperative complications, and potential transmission of the virus. Recently published data have suggested a 27.5% postoperative mortality rate in COVID-19-positive patients undergoing surgeries which is significantly higher to the expected rate. Given the increased vulnerability of patients in resource-limited settings, we aim to report the incidence of asymptomatic SARS-CoV-2 infection in screening tests done perioperatively.

The study was conducted in a 1200-bedded, apex tertiary care hospital in North India which served as a referral centre for COVID-19 during the initial phases of the pandemic. Besides functioning as a referral centre, the hospital serves as a super specialty hospital that carried out routine surgeries for the most part of the pandemic barring the initial period. Patients scheduled to undergo surgeries were screened for symptoms at a dedicated "COVID Clinic" in the Infectious Diseases Block. Only those who were asymptomatic were planned for surgery and underwent a mandatory preoperative COVID-19 test. Nasopharyngeal and oropharyngeal swabs collected were subjected to RNA extraction and subsequent one-step RT-PCR test with primers for at least two target viral genes (S/N/E/ORF genes).

A total of 1435 patients were screened during the study period. The mean age of the studied population was 41.1 years. Male: Female ratio was 1.36:1. The overall prevalence of COVID-19 was found to be 4.04%. Positivity varied during different intervals and the increase in positivity coincided with the peak of the waves of COVID-19 in the country.

Knowing the preoperative COVID-19 status will be of great clinical importance and will lead healthcare providers to use the right protocols aiming to reduce postoperative complications and fatal morbidity. It will further help to guide institutional decisions to continue preoperative testing and establish protocols for infected patients requiring surgery.

[VIB-MMB-PP-02]

Urinary tract infections in kidney transplant recipients: a single-centre experience

Zahra F, Fomda B A, Ahmed T,
Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, Soura-190011, J&K, India,

Background: Kidneys are the most frequently transplanted organs and renal transplantation is the preferred method for treating patients with end-stage renal disease. Post-transplantation urinary tract infection (UTI) is the most common infection in renal transplant recipients ranging from 6-86% and accounting for approximately 40–50% of all infectious complications leading to morbidity and graft failure. Additionally, renal transplant recipients develop UTIs more frequently than the general population. The frequency of UTIs depends on many factors such as age and gender, kidney function and co-morbidities, immunosuppressive protocol or the follow-up period.

Objectives: There is a paucity of data on post-transplant UTIs. With this in mind, a retrospective analysis of records was done to elaborate on the pathogens involved and document their susceptibility profile and other demographic factors.

Methods: Urine samples of renal transplant patients that were received by the department from 2020 to 2022 were included. All the cases were transplant patients and there were no specific inclusion and exclusion criteria.

Results: Of the 98samples received during the study period, 43 showed microbiological evidence of UTI. The patients included 38 males and 60 females, with ages ranging from 18-54 years. UTI developed 3-45 days (mean 19.5 days) after transplantation. Recurrent infection was observed in 11/98 patients. Female patients were more susceptible than males. The most common isolated bacteria were *Escherichia coli* followed by *Klebsiella pneumoniae*. In general, the susceptibility profile is better in this subset as opposed to other admitted patients. All episodes showed a favourable course.

Conclusions: Infection is still one of the most important problems in renal transplantation and can acutely compromise graft function and if left uncontrolled may lead to patient death. If suspected, prompt initial empiric antibiotic therapy is recommended, and further thorough investigations are required to identify potential underlying causes. One of the current challenges of the treatment of post-transplant UTI is the careful and selective use of antibiotics since current data indicate a rising incidence of multi-resistant uropathogenic strains. Active surveillance of UTI for the first 3 months is a good option for improving the quality of life of renal transplantation patients and curbing the rise in antibiotic resistance.

[VIB-MMB-PP-03]

Exploring the Clinical and Molecular Characteristics of Orf Virus in Small Ruminants of Kashmir

Rabiya Bashir1, Abdul Qayoom Mir2, Zubair Shah1, Gulzar Badroo1, Junaid lone1, Sabia Qureshi1, Altaf Bhat1, Ishfaqul Hussain1, Zahid Kashoo1, Pervaiz Dar1

1Division of Veterinary Microbiology and Immunology, FVSc & AH, SKUAST-Kashmir
2 Mountain Research Centre for Sheep and Goat, Shuhama, SKUAST-Kashmir

Orf virus, a zoonotic pathogen primarily affecting small ruminants such as sheep and goats, poses a significant threat to both animals and humans. This study aimed to investigate the clinical and molecular characteristics of Orf virus in small ruminants in the region of Kashmir, focusing on the impact, clinical manifestations, and genetic diversity of the virus.
A comprehensive assessment was conducted, involving meticulous clinical observations and documentation of the various manifestations associated with Orf virus infection. Papular, vesicular, and pustular lesions were carefully recorded, along with an analysis of their distribution and progression. The severity and duration of lesions were evaluated, taking into consideration important factors such as the age, breed, and management practices of the affected animals.

Furthermore, molecular analyses were performed to investigate the genetic diversity and identify specific strains of Orf virus circulating among the small ruminant population. Viral DNA was extracted from the lesion tissues, and PCR assays targeting the B2L gene, a conserved region of the Orf virus genome, were carried out. Subsequent sequencing and phylogenetic analysis allowed for the determination of genetic relationships among different isolates and reference strains.

The findings of this study will provide valuable insights into the clinical and molecular characteristics of Orf virus in small ruminants in the Kashmir region. Understanding the epidemiology, pathogenesis, and genetic diversity of the virus will significantly contribute to the development of effective control strategies, improved diagnostic tools, and the potential for vaccine development specifically tailored to the region. Ultimately, this research will enhance our knowledge of Orf virus and its implications for small ruminant health, thereby assisting in the protection of livestock and the advancement of veterinary practices in Kashmir.

Keywords: *Contagious ecthyma, Orf virus, molecular characterization, Small Ruminants, Kashmir,*

[VIB-MMB-PP-04]

Investigating clinico-pathology and molecular features of fowl adenovirus in Kashmir, India

Zubair Shah[1], Abrar Hassan[2], Gulzar Badroo[1], Rabiya Bashir[1], Junaid lone[1], Majid Shafi[2], Showkat Shah[2], Basharat Wani[2], Shoaib Kamil[2], Sabia Qureshi[1], Altaf Bhat[1], Ishfaqul Hussain[1], Zahid Kashoo[1], Akeel Bashir[2], Pervaiz Dar[1]

[1]*Division of Veterinary Microbiology and Immunology, FVSc & AH, SKUAST-Kashmir*

[1]*Divison of Veterinary Pathology, FVSc & AH, SKUAST-Kashmir*

Fowl adenovirus (FAdV) infections pose significant challenges to the poultry industry worldwide, including in the Kashmir valley, India. This study aimed to investigate the clinico-pathological and molecular features of FAdV in Kashmir to enhance our understanding of the disease dynamics and facilitate effective control measures.

A comprehensive investigation was conducted utilizing a combination of clinical observations, pathological examinations, and molecular analysis. A total of 18 dead birds presenting with suspected FAdV infection were analyzed in a poultry farm. Clinical signs including respiratory distress, depression, anorexia, and increased mortality rates were observed in the affected birds. Necropsy examinations revealed characteristic lesions such as hepatomegaly, spleenomegaly, enlarged kidneys and Hydropericardium.

Histopathological analysis demonstrated distinct hepatic necrosis, lymphoid depletion, and intranuclear inclusion bodies in the affected organs. The molecular characterization of FAdV was performed through polymerase chain reaction (PCR) targeting the hexon gene. The obtained PCR products were sequenced and analyzed for genotyping and phylogenetic relationships.

The results revealed the presence of FAdV group D species belonging to serotype 11 in the investigated samples. Phylogenetic analysis indicated the circulation of FAdV strain in the Kashmir region similar to the strains recently reported from other parts of India. Furthermore, sequence analysis provided insights into the genetic diversity and evolution of FAdV strains prevalent in the area.

This study highlights the significant impact of FAdV infections on the poultry population in Kashmir, India. The comprehensive approach combining clinico-pathological observations and molecular analysis provides valuable insights into disease manifestation, viral diversity, and epidemiology. These findings contribute to the development of effective control strategies, including vaccination programs and biosecurity measures, aimed at minimizing the economic losses associated with FAdV infections in the region.

Keywords: *Fowl adenovirus, clinico-pathology, molecular characterization, Kashmir, India.*

[VIB-MMB-PP-05]

Molecular Characterisation and seroprevalence of *Listeria monocytogenes* from reproductive disorders in ruminants.

Warke S and Bhatkar S
Department of Veterinary Microbiology and Animal Biotechnology T & R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

Background: Listeriosis is one of the most important bacterial zoonotic infections categorized under List C of OIE diseases. The symptoms in the affected animals include encephalitis, depression, anorexia, turning head on one side, unilateral facial paralysis, and abortion in late pregnancy. It is estimated that about 90% of the economic loss due to reproductive disorders in ruminants is due to infectious agents. *L. monocytogenes* have been reported from a wide variety of food types in various countries of the world, but the work done on the role of *L. monocytogenes* in reproductive disorders in livestock is very scanty.

Methodology: A total of 350 samples comprising of 175 serum samples and 175 vaginal swabs from the cases of abortion, retention of placenta, endometritis, pyometra and repeat breeding in farm animals (Cattle, Buffalo, and Goat) in and around Nagpur were processed for isolation and identification of *Listeria monocytogenes*. Samples were processed for isolation of *Listeriae* by two-step enrichment in UVM- 1 and UVM- 2 enrichment broth. The isolates were further confirmed based on biochemical tests as well as *in vitro* pathogenicity studies viz; haemolysis on sheep blood agar, CAMP test and PI-PLC assay. Virulence marker genes of *L. monocytogenes* were detected by employing polymerase chain reaction. All *L. monocytogenes* isolates were characterized by serotyping PCR assay. Further all the serum samples were analysis for the presence of antibodies against Listeriolysin -O (ALLO) with indirect ELISA.

Result: On cultural examination of 350 samples, 16 (4.57%) were found to be positive for *Listeria* spp. On further biochemical characterization of these samples, 9 (2.57%) were found to be *L. monocytogenes.* All the isolates of *L. monocytogenes* were haemolytic, CAMP test and PI-PLC positive indicating all are pathogenic nature. PCR conditions for detection of virulence marker genes of *L. monocytogenes*, were optimized for three genes (*hlyA, iap* and *plcA*) in combination, and individually for five genes (*actA, inlA, inlB, prfA* and *mpl).* Among all the strains of *L. monocytogenes* virulence marker genes i.e. *hlyA, actA and inlB* were detected, while, *plcA* was not detected in any of the isolates. Genes like *prfA* and *mpl* were detected in a isolate of *L. monocytogenes*. Serotyping PCR revealed two isolates of *L. monocytogenes* as 4b serotype. Analysis of all serum samples with indirect ELISA has recorded only 04 positive sera samples.

Conclusion: It is concluded that *Listeria monocytogenes* is associated with reproductive disorders in ruminants. Prevalence of *L. monocytogenes* in cattle is higher in contrast to other animals.

Keywords: *Listeria monocytogenes*, abortion cases. ELISA and PCR.

[VIB-MMB-PP-06]

Epidemiology of Lumpy Skin Disease outbreak across the UT of Jammu and Kashmir, India and GPCR gene-based characterization of the outbreak virus of 2022

Gazal S[1], Badroo G[2], Shikha D[1], Bhat MA[2], Farooq S[2], Akhter S[1], Gazal S[1], De S[3], Brahma B[1], Sharma N[1]

[1]*Faculty of Veterinary Science & Animal Husbandry, SKUAST-Jammu*
[2]*Faculty of Veterinary Science & Animal Husbandry, SKUAST-Kashmir*
[3]*Department of Animal Biotechnology, NDRI*

Lumpy skin disease (LSD) is a highly contagious and fatal transboundary viral disease of cattle and water buffaloes caused by Lumpy Skin Disease virus (LSDV) which belongs to genus capripox virus of *Poxviridae* family. Here, we present the epidemiology and characterization of Lumpy Skin Disease outbreak in J&K, the first ever outbreak of LSD in the UT of J&K, India. The outbreak of LSD started in the month of June in Jammu and rapidly spread across all the 20 districts of J&K. The affected animals exhibited the clinical symptoms including high fever, anorexia, nasal and ocular discharge, drop in milk production and generalized skin nodules all over the body. Out of the total cattle population of J&K which is 24,64,086 a total of 56,477 animals exhibited clinical manifestations of LSD thus accounting to an overall morbidity rate of 2.29%. The morbidity rate in Jammu Division was 3.1% while in Kashmir Division was 1.32%. The case fatality rate in the UT of J&K was calculated as 2.8%. During the outbreak, 1746 samples were collected from affected cattle as well as in-contact animals in Jammu Division and screened for LSDV by qPCR. A total of 704 samples were tested positive for LSDV. Three of the positive samples were randomly selected and the LSDV GPCR gene was amplified, cloned and sequenced. 12 nucleotide deletions were observed in the GPCR gene when compared to 2019 Indian LSDV isolate. In this article, we present a detailed report on the epidemiology of Lumpy Skin Disease in J&K, India and the detection of LSDV by qPCR as well as GPCR gene based molecular characterization of the virus.

[VIB-MMB-PP-07]

Prevalence and species distribution of candida bloodstream infections in neonates in a tertiary care hospital in North India

Dr Mir Saba Muzaffar, Prof (Dr Anjum Farhana)

Government Medical College, Srinagar
Department: Department of Microbiology, Government Medical College, Srinagar

Background: The occurrence of candidemia is on rise worldwide. Non albicans candida is emerging as a major cause of candidemia. The aim of the study was to determine the changing distribution of candida species and emergence of non albicans candida species.

Materials and methods:

Study design: Hospital based cross sectional study

Study setting: Department of Microbiology, Government Medical College, Srinagar

Data Collection: 1 January 2023 to 30 June 2023

Procedure: Total 636 blood culture samples were received during the study period. Candida and isolates were identified by chromogenic agar and Vitek 2 identification system

Results: Out of 636 samples obtained, 275(43.2%) samples were positive blood cultures. Candida species were responsible for 37(5.81%) bloodstream infections. C. krusei 11(29.7%) was the most common isolate followed by C. tropicalis 8(21.6%), C. albicans 5 (13.51%) and C. glabrata 3(8.10%).

Conclusions: Candidemia is emerging as a significant problem in hospitalised patients. The increased prevalence of uncommon Candida species is alarming and necessitates a prompt stewardship program

[VIB-MMB-PP-08]

Molecular detection of 'Mycobacterium tuberculosis complex' in lymphadenitis cases in dogs

Tikute P1*, Narang D1, Chandra M1, Turkar S2 and Gupta K3.
PG research scholar and Presenting author, Department of Veterinary Microbiology, 1 Department of Veterinary Microbiology, 2 Department of Veterinary Medicine, 3 Department of Veterinary Pathology, GADVASU, LUDHIANA-141004

The Genus *Mycobacterium* is classified into two main types, namely Nontuberculous Mycobacteria (NTM) and Mycobacterium tuberculosis Complex (MTC). Mycobacterium tuberculosis complex is involved in causing disseminated and systemic infections such as lymphadenopathies, alimentary tract infection, respiratory tract infection, granulomatous or pyogranulomatous inflammation of the spleen and liver particularly in immunosuppressed and immunocompromised dogs. Due to the increasing interaction between dogs and humans, the likelihood of transmission of these organisms between the two species is also increasing. For this reason, to get more knowledge about the association of Mtb complex with lymphadenopathies, a total of 123 samples (100 lymph node aspirates, 15 lymph node tissues and 8 blood samples) from 83 dogs suspected for lymphadenitis accompanied with gastroenteritis, chronic skin infections, immunosuppression, chronic pulmonary diseases and other chronic undiagnosed diseases were studied. The samples were collected from these suspected dogs and further processed for cytological (Leishman stain) and microscopic examination by Ziehl-Neelsen staining for the presence of target organisms. A cytological study revealed pyogranulomatous inflammation of the lymph node tissue. Impression smear from lymph node tissues displayed the presence of acid-fast organisms. Following the decontamination procedure, the lymph node aspirates and lymph node tissue samples were inoculated into Middlebrook 7H11 media for up to 8 weeks. The aspirated material was also directly used for molecular detection by triplex Nested Polymerase Chain Reaction (nPCR) assay. Out of 83 cases of dogs, 5 dog cases were found to be positive for *Mycobacterium* spp and they belonged to *Mycobacterium tuberculosis* complex (MTB complex).

[VIB-MMB-PP-09]

Identification and in vitro validation of potential inhibitors of RNA-Dependent RNA Polymerase of Infectious Bursal Disease Virus

Khan M, Shabir N, Chikan N, Akram T, Shah R A, Andrabi S M, Hussain I, Raja T, Ganai N A, Bhat H F

University: Department of Animal Biotechnology, FVSc and AH, SKUAST-K

Infectious Bursal Disease (IBD) is an acute, host-specific, highly pathogenic, immunosuppressive viral disease of poultry that is reported to cause huge economic impact on the poultry industry worldwide. Currently, live attenuated vaccines are used against this virus. However, there is a safety concern involved with the use of these vaccines which primarily includes reversion of this live vaccine to wild-type virus that can lead to immune-suppression in vaccinated birds, emergence of new serotypes and disease transmission. Thus, alternate strategies to combat the disease need to be explored.

Previous studies using computer-aided drug designing (CADD) have explored potential natural and synthetic compounds that can block the active sites of viral proteins involved in viral synthesis or its replication. In the current study computer –aided drug designing was employed to explore inhibitor compounds against RNA-dependent RNA polymerase of IBD virus from two compound libraries, one consisting of 66,609 natural compounds and the other consisting of 120 bioactive compounds of western Himalayan region. Four conserved active sites of RNA-dependent RNA polymerase of infectious bursal disease virus viz. ADN-403, ASP-402, ASP-416 (involved in polymerization) and SER-166 (involved in self-guanylation) were used for the in-silico drug designing. This in silico part of the drug designing was conducted in three steps viz., a) Virtual screening of each compound for free binding energy against each site using ArgusLab v 4.0.1. b) Top compounds with least free binding energy were evaluated for toxicity and mutagenecity using PreADMET software. c) Those compounds with least levels of toxicity were subjected to more stringent docking using AutoDock 4.0 software. Further, the best docked top two lead compounds with best docked conformation and least binding energies were selected for in vitro assays. Cytotoxicity assay of these compounds was performed in Chicken Embryo Fibroblasts (CEFs) using MTT assay following which the multi-step growth curve analysis of infectious bursal disease virus culture (0.1 Multiplicity of Infection) treated with the lead compound at four different sub-cytotoxic concentrations was evaluated at 0, 24, 48-, 72-, 96- and 144-Hours Post-Compound Treatment.

After virtual screening and stringent molecular docking of compounds from two libraries used in the present study, Azulene was found to be the lead compound for all the selected target sites. The concentration at which Azulene was found to be significantly cytotoxic in chicken embryo fibroblasts was ≥300µM. Following multi-step growth curve analysis, it was found that Azulene significantly reduced the overall replication of infectious bursal disease virus at 200µM, 250µM as compared to the infectious bursal disease virus control. Thus, it was concluded that Azulene can significantly inhibit the replication of infectious bursal disease virus in vitro at concentrations more than 200µM.

Key words: Infectious Bursal Disease Virus (IBDV), RNA-Dependent RNA Polymerase (RdRp), Azulene.

Session II
Microbiology Spectrum Human-Animal Interface (Oral)

[VIB-MS-IVL-01]

Zoonotic Dermatophytosis: Trends in epidemiology and insights into changing Spectrum in India

Abhishek1*, Sonu S. Nair1, Athira V1, Prasad Thomas1, V. K. Chaturvedi1, Bablu Kumar2 P Dandapat1

1Division of Bacteriology and Mycology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.

2Division of Biological Products, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India

Animals and humans are prone to infectious diseases caused by a variety of organisms including bacterial, viral, fungal, and parasitic agents. Many studies have been conducted regarding the etiological agents, risk factors, pathogenesis, clinical signs, therapeutics, and preventive measures of bacterial, viral, and parasitic diseases. But the fungal diseases are underrated and neglected till the last few decades. Fungal-borne diseases are gaining importance due to their impact on social, economic, and well being of the humans. Even though mortality due to the fungal diseases is low in animals, it may result in declined productivity among milch and meat animals. Fungal agents, especially dermatophytes are important in pet animals from an aesthetic and a welfare point of view.

Dermatophytosis is a superficial fungal disease common in both animals and humans. It comprises of 7 genera including *Microsporum*, *Trichophyton*, *Epidermophyton*, *Paraphyton*, *Lophophyton*, *Nannizzia* and *Arthroderma*.Dermatophytosisis among the most commonly diagnosed skin diseases in India. The climatic condition of India is predominantly hot and humid with severe monsoons, promoting its fast spread. Dermatophytosis is considered as a zoonotic disease and may transmit to the humans from both pet and farm animals and vice versa. Zoophilic dermatophytes like *M. canis*, *T. mentagrophytes* and *T. verrucosum* may also transmit to the humans. The transmission among animals, cost of treatment, difficulty in implementing control measures, and public health significance underlines the importance of animal Dermatophytosis. The commonly isolated dermatophytes from animals are *M. canis*, *T. verrucosum*, *N. gypsea* and *T. mentagrophyte*. The information on the exact role of animals in transmitting dermatophytosis to the humans is lacking. Even though most of the zoophiles and anthropophiles cannot propagate in the soil, it can act as a reservoir for the infective spores and serves as a medium for contracting the disease to the susceptible host. Geophiles, in contrast to the zoophiles and anthropophiles can propagate in the soil. Thus a triangular transmission cycle exists between humans, animals and the environment and many times cross species infections are reported. The species infecting animals and humans may change with time, region and country. Therefore real-time study on the isolation, incidence and prevalance of animal dermatophytes is need of an hour and to get a clear picture of the most common species and to understand the transmission cycle of dermatophytes between animals and humans. Humans can be infected with anthropophilic, zoophilic or geophilic strains of dermatophytes. In India, the data on the carriage of dermatophytes among animals is severely lacking. The real-time carriage data of dermatophytes in animals which may help to identify the role of animals in transmitting dermatophytosis to the humans is severely lacking and estimation of the real zoonotic potential of the dermatophytes is difficult and cumbersome. In last few years, humans dermatophytosis are exhibiting unusual clinical presentation and the number of chronic and

recurrent infections are increasing. The atypical clinical manifestations include impetigo-like, eczematous dermatitis-like, seborrheic dermatitis-like, lupus erythematous like etc. *T. rubrum* and *T. mentagrophytes* are the predominant strains of dermatophytosis in humans. At the same time, zoophilic strains such as *M. canis*, and *T. verrucosum* and the geophilic strain *N. gypsea* are among the few which are involved in the atypical dermatophytosis in humans. But the exact role of animals in transmitting the disease to humans is still under-studied. The changes in environmental conditions, mutations in the genome of dermatophytes and altered host cutaneous immune response may also contribute to abnormal clinical presentations. Dermatophytes are a highly heterogeneous group of fungi and accurate & precise identification of the species and strains may be done by the concurrent application of conventional laboratory and molecular tests. Phylogenetic analysis based on gene sequencing is helpful to understand the relationship between two genera of dermatophytes as it has been reported that a Microsporum species is closely related to *Trichophyton* species even though classified as two separate genera. However, the simultaneous application of gene sequencing & typing by multilocus sequence typing may be more helpful in identifying and differentiating closely related strains and species.

To understand the actual scenario, a real time epidemiological study on the carriage of dermatophytes in both humans and animals need to be conducted. The phenotypic and genotypic relatedness of animal and human isolates is helpful in understanding the zoonotic transmission of the dermatophytes. The identification of prevalent species and genotypes in both animals and humans, and further correlation with clinical data may be a key to assist in initiating appropriate anti-dermatophytic therapy or measures.

[VIB-MS-IVL-02]

Advancements in Point-of-Care Diagnostics for Brucellosis: Next-Generation Rapid Testing Approaches

Bablu Kumar1, Sanjana1, Moon Moon Satpathy1, Ambika Arun1, Hilari Debbarma1, Abhishek2

1Division of Biological Products, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.

2Division of Biological Products, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.

Brucellosis has been a menace to human and animal populations for centuries, with recorded instances dating back to ancient times. Today, despite medical advancements and improved awareness of infectious diseases, brucellosis remains a neglected and underreported illness in many parts of the world. Brucellosis is a bacterial zoonotic disease that has a worldwide distribution and causes heavy economic losses to the livestock industry through abortion, premature birth, retained fetal membrane, decreased milk production, delayed conception and infertility. The annual economic losses were estimated to the tune of about US$ 3.4 billion in the livestock population, of which more than 95% is attributed to the bovine industry, were reported in India. Three of the *Brucella* species are known to be endemic in most countries. They include *B. abortus* that primarily infects cattle, *B. melitensis* that infects sheep and goats, and *B. suis* which has a tropism for domestic, feral, and wild swine populations**.** Most species of *Brucella* can infect animals other than their preferred hosts when they come in close contact. *B. abortus, B. melitensis* and *B. suis* are important human pathogens. Transmission of brucellosis from animals to humans occurs in several ways with the most common route of transmission being consumption of raw milk or cheese prepared from milk of infected animals**,** undercooked traditional delicacies such as liver and spleen**.** Early diagnosis of the disease can be one of the most pragmatic methods to reduce the transmission of the disease. Among several diagnostic test Culture and isolation of organism from clinical samples is the still gold standard test. But the isolation procedure is time-consuming, gives variable results and mandates laboratory facilities (BSL-3), biosafety cabinet, proper media, expensive instruments, and skilled technicians. Thus, the disease is mainly diagnosed by serological test like RBPT, MRT, SAT & ELISA, but these tests suffer from certain disadvantages in terms of sensitivity and specificity. None of the serological tests have discriminatory power to differentiate between vaccinated and infected animals. The persistently infected animals are also not detected by serological tests. In place of isolation of causative agent, which is still considered as gold standard test, Nucleic Acid amplification tests (NAAT) are gaining popularity as preferred diagnostic test for microbial diseases. Having the ability to detect antigen these assays are rapid and help in early detection of infection and thus control the spread or transmission of the disease. PCR and multiplex PCR have been used for the detection as well as differentiation of *Brucella* species. Bruce Ladder PCR can differentiate all *Brucella* species including vaccine strain like *Brucella abortus* S19 & RB51. Real time PCR has also been developed for identification of *Brucella*. But none of these tests can be deployed for field condition due to the requirement of sophisticated equipments as well as trained personnel. These limitations confine PCR & Real time PCR diagnostics to central laboratories and limit its widespread utility, especially in low-resource settings. The use of isothermal amplification techniques, which perform at a specific temperature and do not necessitate complex thermocyclers,

could be employed for field diagnostics in locations with limited resource settings. Following assays are potential point of care test for diagnosis of Brucellosis.

Lateral Flow Immunoassays (LFAs): LFAs, also known as lateral flow tests or strip tests, are widely used rapid immunoassays. They utilize capillary flow to transport the sample and reagents across a porous membrane. LFAs typically consist of a sample pad, conjugate pad, test line(s), and control line(s). The target analytes in the sample interact with specific antibodies, or antigens immobilized on the membrane, resulting in a visible signal (e.g., colored line) at the test line if the analytes are present. LFAs provide quick results, typically within minutes. LFAs are user-friendly and do not require specialized equipment or extensive training. LFAs are often designed as portable and handheld devices, making them suitable for on-site testing and field applications. LFA for detection of antigen/antibody for *Brucella* have been developed.

Isothermal amplification techniques such as LAMP, developed by (Notomi *et al.,* 2000) is one of the most widely used rapid onsite nucleic acid detection method carried out isothermally at a temperature of 60-65°C and end point readouts can be done by naked eye. Following Isothermal assays have been developed to detect Brucella DNA from clinical specimen.

Loop-Mediated Isothermal Amplification (LAMP): LAMP is a widely used isothermal amplification technique that amplifies DNA with high specificity and sensitivity. It involves the use of four to six primers targeting multiple regions of the DNA, leading to a unique loop structure that enables rapid and robust amplification. LAMP is often used for the detection of pathogens, including viruses, bacteria, and parasites and has been attempted for *Brucella* from various researchers.

Recombinase polymerase amplification (RPA) is another isothermal nucleic acid amplification technology which can be accomplished at constant temperature in less than 20 minutes that can be operated in the resource limited field setting. Among the novel isothermal amplification technologies, recombinase polymerase amplification (RPA) shows great potential for point-of-care testing. The RPA technology uses three enzymes for rapid amplification at 37◦C and could specifically detect as few as single copy of target nucleic acid in 20 min. Real-time detection of the amplification could be carried out by using fluorescent probes.

Polymerase Spiral Reaction (PSR) is a highly efficient and rapid nucleic acid amplification technique that enables the amplification of specific target sequences of DNA or RNA. PSR combines a DNA polymerase and a set of specially designed primers to initiate the amplification process. The unique design of the primers forms a spiral structure that enables the rapid and specific amplification of the target sequence.

Rolling Circle Amplification (RCA): RCA is a technique used to amplify circular DNA molecules. It involves the rolling circle replication of circular DNA templates, generating long single-stranded DNA products that can be detected using labeled probes.

Cross-Priming Amplification (CPA): CPA is an isothermal amplification method that involves cross-priming events using two primers targeting the target DNA sequence. This technique can achieve high sensitivity and specificity for nucleic acid detection.

Nucleic Acid Lateral Flow Immunoassay (NALFIA): NALFIA is a rapid and simple diagnostic technique used for the detection of specific nucleic acid targets, such as DNA or RNA. Similar to Lateral Flow Assays (LFAs), NALFIA utilizes a test strip with immobilized capture probes or antibodies specific to the target sequence of interest. However, in NALFIA, the detection mechanism involves the hybridization of labeled nucleic acid probes with the target sequence on the test strip. When the target sequence is present in the sample, the labeled probes bind to the immobilized capture probes, forming a visible line or band on

the test strip, indicating a positive result. Following Isothermal assays have been developed to detect *Brucella* DNA from clinical specimen.

Loop-Mediated Isothermal Amplification (LAMP) and Lateral Flow Assay (LFA): LAMP-LFA, the combination of represents a powerful and user-friendly diagnostic approach for the rapid detection of specific DNA targets. LAMP-LFA's isothermal amplification process allows for quick and sensitive amplification of DNA sequences without the need for thermal cycling, while the Lateral Flow Assay enables visual result interpretation on a test strip, eliminating the need for sophisticated equipment. This integration of technologies makes LAMP-LFA highly suitable for point-of-care testing and field diagnostics, especially in resource-limited settings. Its simplicity, rapidity, and accurate detection capabilities position LAMP-LFA as a valuable tool in identifying various infectious agents, revolutionizing disease diagnosis and surveillance efforts worldwide.

Recombinase Polymerase Amplification (RPA) and Lateral Flow Assay (LFA) (**RPA-LFA)** constitutes a robust and rapid diagnostic method for the specific detection of DNA targets. RPA-LFA harnesses the isothermal amplification capability of RPA, which quickly amplifies DNA sequences with high sensitivity and specificity, without the need for complex thermal cycling. The subsequent Lateral Flow Assay facilitates easy visual result interpretation on a test strip, obviating the requirement for specialized equipment. This combination of technologies renders RPA-LFA an ideal choice for point-of-care and field diagnostics, particularly in resource-limited settings. There are several reports of RPA-LFA targeting Brucella DNA.

Paper based origami: Paper-based origami has been adapted for use in disease diagnosis, particularly in the development of diagnostic devices and tests for point-of-care and resource-limited settings. This innovative approach leverages the principles of origami folding and integrates them with microfluidics and bioassays to create low-cost, portable, and user-friendly diagnostic tools.These devices can manipulate small volumes of biological samples, such as blood, saliva, or urine, and perform various diagnostic tests, including immunoassays and nucleic acid amplification tests. Paper-based origami is applied to enhance the performance of lateral flow assays (LFAs). Combined with isothermal amplification techniques like LAMP or RCA, these paper-based devices enable rapid and sensitive detection of DNA or RNA targets associated with infectious diseases.

CRISPR-based biosensors now a days gaining popularity for chemical/biological molecules analysis coupled with different detection methods, such as fluorescence, electrochemistry, colorimetry, etc. In general, pre-amplification techniques like PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) paired with CRISPR-based sensing platforms can effectively boost analytical performance. Cas12a is one of the RNA-guided nucleases that have collateral trans-cleavage activities on fluorescent single stranded reporter DNA (ssDNA) under the guidance of a crRNA creating a desirable fluorescent signal.

Biosensors and nanotechnology

Nanoparticle-Based Assays: Nanoparticles, such as gold nanoparticles or quantum dots, can be functionalized with specific antibodies or aptamers that bind to Brucella antigens. When the target Brucella antigens are present in the sample, they bind to the nanoparticles, leading to a measurable signal, such as a color change or fluorescence. This allows for quick and visual detection of Brucella in the sample.

Surface-Enhanced Raman Spectroscopy (SERS): SERS is a powerful technique that uses metal nanoparticles to amplify the Raman signals of molecules near their surface. By functionalizing the nanoparticles with Brucella-specific ligands, such as antibodies or

aptamers, SERS can be used to detect even very low concentrations of Brucella in a sample, making it highly sensitive and suitable for early-stage diagnosis.

Lab-on-a-Chip Devices: Nanotechnology has enabled the development of miniaturized lab-on-a-chip devices that can integrate multiple diagnostic functions, including sample preparation, amplification, and detection. These devices offer portability, rapid analysis, and require only small sample volumes, making them ideal for point-of-care testing in resource-limited settings.

Magnetic Nanoparticles and Magnetic Biosensors: Magnetic nanoparticles can be functionalized with Brucella-specific ligands and used to capture Brucella antigens from a sample. Magnetic biosensors can then detect the presence of these captured antigens, providing a sensitive and quantitative measurement of Brucella.

Carbon Nanotubes and Nanowires: Carbon nanotubes and nanowires have unique electrical and mechanical properties that can be exploited for biosensing purposes. Functionalizing these nanomaterials with Brucella-specific biomolecules enables the detection of Brucella with high sensitivity and selectivity.

Electrochemical device: An electrochemical device for detection of organism refers to a diagnostic tool that utilizes electrochemical principles to detect and quantify the presence of organism in clinical sample. The device typically consists of electrodes or sensors that can measure electrical signals in response to specific interactions between the target bacterium and the sensing elements.

Rapid diagnostics of Brucellosis in animals are of utmost importance for several reasons. Brucellosis is a highly contagious zoonotic disease that can be transmitted from animals to humans, posing a significant public health risk. Early detection through rapid diagnostic tests in animals helps prevent its spread to humans, protecting both animal and human populations. Timely identification also allows for immediate isolation and treatment of infected animals, reducing the risk of disease transmission within livestock herds and preventing economic losses in agriculture. Furthermore, rapid diagnostics aid in implementing control and eradication programs, which are essential to maintaining food safety, trade, and animal welfare. By swiftly identifying and managing Brucellosis in animals, these tests play a crucial role in safeguarding human health, preserving livestock industries, and supporting overall public health efforts.

[VIB-MS-OP-01]

Therapeutic outcome of Saroglitazar, a peroxisome proliferator activated receptor (PPAR) α and γ agonist in Diabetic and Non-Diabetic NAFLD patients

Authors- Shagufta P, Shashipal, Hayat A, Irfan A, Nisar S, Showkat A K
Department of Pharmacology, Government Medical college Srinagar

Background: -Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common cause of Chronic Liver Disease and its consequences throughout world, more so in developed countries. It is more concerning in view of lack of a definite treatment. Other than life style modification and Vitamin E, we are still looking for a drug which can modify the outcome in this group of patients.
Materials and Methods: - In this prospective observational study conducted over a period of 48 weeks, we evaluated the safety and efficacy of Saroglitazar on NAFLD/NASH patients with the primary objective to evaluate the therapeutic outcome of Saroglitazar on NAFLD fibrosis score (NFS) in both diabetics and non-diabetics. After written informed consent from each patient, a total of 292 patients who met the inclusion criteria were enrolled. However, only 257 individuals completed the study. Eligible patients were put on Saroglitazar 4 mg per day for 24 weeks and followed on OPD basis for 48 weeks with special emphasis on NFS, BMI, HbA1c, lipid levels and liver biochemistry.

Observations: -We observed a male dominance (61.9%) and a significant improvement in lipid profile, liver biochemistry, HbA1c, NFS, and fibrosis and an insignificant improvement in BMI. We did not observe any significant drug related adverse events during the treatment with Saroglitazar.

Conclusion: -NAFLD/ NASH are the leading causes of CLD and its complications, hepatic and extrahepatic morbidities, and mortalities. Saroglitazar in a dose of 4 mg per day for 24 weeks resulted in marked improvement in liver biochemistry, lipid profile, HbA1c, NFS and Fibrosis. Before recommending its wider use in NAFLD/NASH in both diabetic and non-diabetic patients, more case control and randomized controlled trials are awaited to supplement our observations.

Keywords- Non-Alcoholic Fatty Liver Disease, NFS Score, Metabolic Syndrome, Fibrosis.

[VIB-MS-OP-02]

Molecular Characterisation and seroprevalence of *Listeria monocytogenes* from reproductive disorders in ruminants.

Warke S and Bhatkar S
Department of Veterinary Microbiology and Animal Biotechnology T & R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

Background: Listeriosis is one of the most important bacterial zoonotic infections categorized under List C of OIE diseases. The symptoms in the affected animals include encephalitis, depression, anorexia, turning head on one side, unilateral facial paralysis and abortion in late pregnancy. It is estimated that about 90% of the economic loss due to reproductive disorders in ruminants is due to infectious agents. *L. monocytogenes* have been reported from a wide variety of food types in various countries of the world, but the work done on the role of *L. monocytogenes* in reproductive disorders in livestock is very scanty.

Methodology: A total of 350 samples comprising of 175 serum samples and 175 vaginal swabs from the cases of abortion, retention of placenta, endometritis, pyometra and repeat breeding in farm animals (Cattle, Buffalo and Goat) in and around Nagpur were processed for isolation and identification of *Listeria monocytogenes*. Samples were processed for isolation of *Listeriae* by two-step enrichment in UVM- 1 and UVM- 2 enrichment broth. The isolates were further confirmed on the basis of biochemical tests as well as *in vitro* pathogenicity studies viz; haemolysis on sheep blood agar, CAMP test and PI-PLC assay. Virulence marker genes of *L. monocytogenes* were detected by employing polymerase chain reaction. All *L. monocytogenes* isolates were characterized by serotyping PCR assay. Further all the serum samples were analysis for the presence of antibodies against Listeriolysin -O (ALLO) with indirect ELISA.

Result: On cultural examination of 350 samples, 16 (4.57%) were found to be positive for *Listeria* spp. On further biochemical characterization of these samples, 9 (2.57%) were found to be *L. monocytogenes.* All the isolates of *L. monocytogenes* were haemolytic, CAMP test and PI-PLC positive indicating all are pathogenic nature. PCR conditions for detection of virulence marker genes of *L. monocytogenes*, were optimized for three genes (*hlyA, iap* and *plcA*) in combination, and individually for five genes (*actA, inlA, inlB, prfA* and *mpl).* Among all the strains of *L. monocytogenes* virulence marker genes i.e. *hlyA, actA and inlB* were detected, while, *plcA* was not detected in any of the isolates. Genes like *prfA* and *mpl* were detected in a isolate of *L. monocytogenes*. Serotyping PCR revealed two isolates of *L. monocytogenes* as 4b serotype. Analysis of all serum samples with indirect ELISA has recorded only 04 positive sera samples.

Conclusion: It is concluded that *Listeria monocytogenes* is associated with reproductive disorders in ruminants. Prevalence of *L. monocytogenes* in cattle is higher in contrast to other animals.

Keywords: *Listeria monocytogenes*, abortion cases. ELISA and PCR.

[VIB-MS-OP-03]

Emerging Threat of Multidrug Resistant Pathogens-An Experience from N ICU of a Tertiary Care Hospital

Dr. Aman M* Dr. Bashir
Resident, Department of Microbiology, Sheri-Kashmir Institute of Medical Sciences, Srinagar, J & K
Professor, Department of Microbiology, Sheri-Kashmir Institute of Medical Sciences, Srinagar, J & K.

Background: Multi drug resistant (MDR) pathogens are responsible for substantial morbidity and mortality in cases of neonatal septicemia (NNS) and pose a serious problem in developing countries like India.
Aims: To determine bacterial profile and antimicrobial resistance pattern in NNS.
Methods and Materials: Bacterial profile and antimicrobial susceptibility pattern of 126 isolates from blood culture positive bottles on BacT /ALERT3D system was analysed on VITEK 2 system at Department of Microbiology, SKIMS.
Results: Among clinically suspected cases of neonatal sepsis (N=1200) bacteremia was proven in 126 (10.5%) cases on blood culture during a period of eighteen months. Out of 126 isolates Gram positive isolates obtained (N=73, 57.93%) included Staphylococcus sp. (N=58) [methicillin resistant coagulase negative Staphylococcus (N=29), methicillin sensitive coagulase negative Staphylococcus (N=12); methicillin resistant Staphylococcus aureus (N=10), methicillin sensitive Staphylococcus aureus (N=7)] and Enterococcus sp. (N=5). Gram negative isolates (N=53; 42.06%) included K. pneumoniae (N=20), A. baumannii (N=14), P. aeruginosa (N=6), E. coli (N.5), E. cloacae (N=4), B. cepacia (N=2) and Serratia sp. (N=2). Overall, majority of isolates were Staphylococcus sp. with preponderance in early onset sepsis (p value <0.001 and chi square test results 33.49). All were sensitive to vancomycin with methicillin resistance of 67% (N=39). Most common Gram-negative isolates were multi drug resistant K. pneumoniae and A. baumannii with 80% A. baumannii sensitive to colistin only.

[VIB-MS-OP-04]

Secondary Bacterial Infection in Covid-19 Patients

Qadri U1 Fomda B2, Bali N3, Wani S J4

Post-Graduate1, Professor & Head2, Associate Professor 3, Department of Microbiology Senior Resident4, Department of General Medicine, Sher-i-Kashmir Institute of Medical Sciences, Soura.

Background: -Secondary bacterial infections may develop during or following COVID-19 infection. The rising number of multidrug-resistant bacteria and our limited capacity to eradicate them not only render us more vulnerable to bacterial infections but also weaken us during viral pandemics. To tackle this serious issue, we urgently need to investigate the effects of bacterial co-infections during viral infections.

Objectives: The present study was carried out to determine incidence, type of secondary bacterial infections and antibiotic susceptibility profiles in patients suffering from COVID-19.

Methods: In this prospective study, sampleswere collected from COVID positive patients and were inoculated on blood agar, Maconkey agar for identification. AST was done as per CLSI guidelines 2021.

Results: Out of total 72846 samples 1182 (34.1%) were positive for COVID-19. SBI was seen in 112(39.4%) of these patients. Incidence of SBI was more among male patients and in the age group > 60 years. Patients with SBI had longer hospital stay of more than 10 days. Hypertension was the most common co-morbidity seen among these cases. Gram negative organisms were the predominant pathogens isolated. Among Gram negative pathogens *K. Pneumonia* was isolated from respiratory samples where as *A. Baumannii* was isolated from blood samples. All gram-negative isolates were sensitive to polymyxin -B and colistin. S. aureus was common gram-positive organism isolated from both respiratory and blood samples. All gram-positive isolates were sensitive to linezolid.

Conclusions: Our study highlights the need to have a specific tailored antibiotic policy for COVID-19 patients who develop SBI.

[VIB-MS-OP-05]

Antimicrobial resistance pattern of bacterial isolates recovered from routine clinical specimen. A study from the northern state of Kashmir.

Shoaib Mohmad Khan*, Umara Amin

Department of Microbiology, Government Medical College, Srinagar J&K – 190010

Background: Antimicrobial resistance (AMR) is a global health threat that leads to significantly higher morbidity, prolonged hospital stay, higher healthcare costs and mortality. WHO and the UN estimate the global burden of AMR greater than 7,00,000 deaths/year and predicted 10 million deaths due to AMR by 2050. Accurate antimicrobial resistance data is essential for empirical treatment of common infectious diseases. Thus, early detection of bacterial resistance and selective reporting of the antibiotics can save millions of lives annually.

Objectives: This study aimed to assess the prevalence and antimicrobial resistance profiles of various bacterial species commonly encountered in clinical settings.

Materials & Methods: A specialty lab based retrospective study over a period of two years (July 2021 to June 2023). A total of 5765 individual clinical specimen (urine, blood, pus, swabs, body fluids, stools, sputum etc) were processed according to standard Microbiological techniques and then further analysed using the Biomeriux VITEK-2 compact automated system for microbial identification and antimicrobial susceptibility testing.

Results: Out of 5765 specimens, the prevalence of bacterial infections was observed in 1720 (29.8%). The highest prevalence of isolates was found in Urine (n=1016, 59.1%) followed by pus (n=158, 9.2%). A higher prevalence of infection was seen in females 69.5% (n=1195) than males. The most susceptible age group was 60-79 years (30.3%). E. coli (32.7%) was the predominant organism, followed by Enterococcus spp. (22.3%) & Pseudomonas spp. (16.9%). Among Gram-negative isolates high resistance rates were observed against Beta-lactams (66%), Beta-lactam/β-lactamase inhibitor combinations (44%), and flouroquinolones (76%). 71% isolates were ESBL positive, while 11% were carbapenemase producers. Among Gram-positive isolates high resistance rate was seen towards Beta-lactams (95%), fluoroquinolones (91%) and macrolids (75%). Among Staphylococci, 17% had modified PBPs (mecA) and 13% showed MLSB inducible resistance. Among Enterococci, 23% showed High-level Gentamicin resistance.

Conclusion: The results reveal a concerning rise in AMR among bacterial isolates recovered from routine clinical specimen. This study emphasizes the need for effective surveillance, judicious use of antibiotics, and development of novel antimicrobial agents to combat the escalating threat of AMR. Early detection of resistance and selective reporting of antibiotics can save millions of lives annually.

[VIB-MS-OP-06]

Antibiotypes and ERIC profile of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis attending a tertiary care hospital

Dr. Gulnaz Bashir1, Dr. Mohammed Sameer Chishti2, Dr. Javeed Iqbal Bhat3

1Professor, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences.
2Post-Graduate, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences.
3Additional Professor, Department of Neonatology & Paediatrics, Sher-i-Kashmir Institute of Medical Sciences.

Background: *Pseudomonas aeruginosa* (*P. aeruginosa)* is the most frequently isolated organism from respiratory samples of patients with cystic fibrosis (CF). Biofilm production is one of the main virulent mechanisms this persistent infection. These alginates producing mucoid strains have increased tolerance to antimicrobial therapy, and are also resistant to phagocytic activity of polymorphonuclear cells and host immune system. Biofilm production in CF patients is also associated with increase rate of mutations in *P. aeruginosa*. Untreated patients with *P. aeruginosa* colonization leads to permanent establishment of the pathogen in respiratory tract and invariably mutates to mucoid strains. These alginate producing strains lead to formation of protected microcolonies, as a result of which it is associated with increased morbidity and mortality in patients with cystic fibrosis.

Aims and Objective: To determine the antibiogram and ERIC profile of *P. aeruginosa* isolated from patients with cystic fibrosis cases attending the tertiary care hospital.

Material and Methods: A total of 50 *P. aeruginosa* isolates recovered from respiratory samples (throat swabs and sputum) of patients with cystic fibrosis, were subjected to 16s rDNA-PCR for confirmation and all were confirmed as *P. aeruginosa.* These 50 isolates were then subjected to antibiotic susceptibility testing by disk diffusion method and molecular typing by ERIC-PCR. Cluster analysis was done by using SPSS.

Results: Antibiogram of 50 *P. aeruginosa* isolates revealed 16 antibiotypes. Overall, 26% (n=13) of the isolates were MDR and were mostly distributed among out patients. Among the 50 isolates, 4 isolates were non-typeable by ERIC-PCR, while 46 isolates were classified into 16 ERIC profiles that could be further grouped into 3 clusters.

Conclusion: *P. aeruginosa* is a frequent colonizer in patients with cystic fibrosis. Multiple strains of *P. aeruginosa* were recovered from our patients. However, no clustering with a particular strain of *P. aeruginosa* could be detected. Genotypic heterogeneity among *P. aeruginosa* isolates in our study suggests no genetic correlation between them. There was no overlapping of antibiotypes and ERIC types.

[VIB-MS-OP-07]

Prevalence And Risk Factors of Gastroesophageal Reflux Disease (Gerd) In Adult Kashmiri Population

RAKESH KUMAR KOUL1, SHAGUFTA PARVEEN2, PADMA LAHDOL3, PROF SAMIA RASHEED4, NISAR A. SHAH 5

1Department of Medicine, 2Department of Pharmacology, 3Department of Medicine, 4Department of Medicine, 5Received: 12 Mar 2018 Revised and Accepted: 07 Jul 2018

Objective: The study was conducted with the objective of studying the prevalence of gastroesophageal reflux disease (GERD) and to study various factors associated with it in adult Kashmiri population.

Methods: It was a community based prospective cross-sectional observational study conducted by the Department of Medicine and Gastroenterology, GMC Srinagar over a period of 24 months upon native Kashmiris from urban as well as rural areas as a study group. A total of 2600 subjects above the age of 18 y were studied and the overall prevalence of disease was calculated and also the associated (risk) factors were looked for.

Results: The overall prevalence of 20.3% was seen in the study population with female gender being more prone to the development of disease ($p<0.001$). Other factors of greater significance included body mass index (BMI), smoking, physical activity, intake of spicy foods, posture after meals, dinner to sleep time, non-steroidal anti-inflammatory drug (NSAID) intake and some underlying ailments like asthma and history of abdominal surgery.

Conclusion: The overall prevalence of GERD in Kashmiri community is 20.3% with females being more prone with a definite role of factors like BMI, smoking, physical activity, posture after meals, dinner to sleep time interval, intake of spicy foods, drugs and also the co-morbidities.

Keywords:
Gastroesophageal reflux disease (GERD), Prevalence, Risk factors, Co-morbidity, Severity

[VIB-MS-OP-08]

Bacterial Vaginosis in Preterm Labour and Term Labour

Qari D, Gillani Y, Malik A
SKIMS Medical College, Bemina, Srinagar

Introduction

Bacterial vaginosis is a clinical condition in which there is a shift in flora away from lactobacillus species towards more diverse bacterial species, including facultative anaerobes which causes rise in vaginal pH. It is one of the common genital infections in pregnancy and is associated with higher risk of preterm delivery and other complications.

Aim

1. To study the prevalence of bacterial vaginosis in women presenting with preterm labour and term labour.
2. To analyse maternal and fetal complications associated with bacterial vaginosis.

Methods

This prospective descriptive observational study was conducted in department of Obstetrics and Gynaecology at SKIMS Medical College, Srinagar where antenatal patients admitted in hospital for delivery were firstly screened for bacterial vaginosis (BV) by Amsel criteria. The patients were divided into 2 groups, Group A with term labour and Group B with preterm labour for studying the effects on fetal and maternal outcome.

Results

Out of 2176 pregnant women admitted during study period 636 patients fulfilling the inclusion criteria were studied for the effects of bacterial vaginosis on maternal and fetal outcome. 463 women tested positive as per Amsel criteria making prevalence of bacterial vaginosis 21.27% at our centre. Out of 636 pregnant women 384 (60.4%) delivered via caesarean section and rest had a vaginal delivery. Bacterial Vaginosis was present in 37.2% patients who had a preterm delivery and only in 21.3% with a term delivery. In Group A patients (term delivery) with BV 23.1% babies had APGAR score <7 whereas in Group B patients (preterm delivery) with BV 34.4% babies had APGAR score<7. In Group A patients with BV 42.3% babies were small for gestational age (SGA), 19.2% babies developed sepsis and 23.1% babies were admitted in NICU. In Group B patients with BV 56.4% babies were SGA, 54.5% developed sepsis and 63.6% were admitted in NICU.

Conclusion

Bacterial Vaginosis is an independent risk factor for preterm delivery and increases the risk for adverse neonatal outcomes which can be reduced by appropriate antimicrobial therapy.

[VIB-MS-OP-09]

Knowledge, attitude, and practice of health care workers regarding needle stick injury

Dr Raiesa, Dr Asifa Nazir
Department of Microbiology, Government Medical College Srinagar

Background: NSI poses a major risk to health care workers. Despite implementation of preventive measures, they continue to occur among health care workers. Human awareness is necessary to decrease its incidence.

Materials and methods:
A descriptive survey was conducted among HCWs working in various hospitals of UT of JK utilizing a self-administered questionnaire. Link was kept active for one week. Data was analysed and is summarized below.

Results:
A total of 103 health care workers participated in the study.100% of participants identified the risk of NSI, acknowledged it as a public health issue.48.1% participants had suffered NSI but only 72.2% reported it. 53.8% had received training in prevention of NSI. Only 86.5% participants observed appropriate biomedical waste management techniques.

Conclusion:
Majority of the participants had a good level of awareness of diseases caused by NSI. However there is a need to impart continuous training to HCWs regarding prevention and treatment of NSI and biomedical waste management.

Keywords: NSI (needle stick injury), HCW (health care workers)

[VIB-MS-OP-10]

Bacteraemia In Intensive Care Unit: Clinical, Bacteriological, and Prognostic Prospective Study

Gillani Y, Qari D
Government Medical College, SMHS and Associated Hospitals, Karanagar, Srinagar

INTRODUCTION
Bacteremia is responsible for high rates of morbidity and mortality. The increasing prevalence of multidrug-resistant (MDR) bacteria in intensive care units (ICU) is a growing concern.

OBJECTIVE
To determine the epidemiological profile of bacteremia in ICU settings, as well as the place occupied by MDR bacteria in these infections.

METHODS
It was a prospective study carried out over a period of 1 year from July 2021 to July 2022 in the ICU of SMHS Hospital, Srinagar and Microbiology department of GMC Srinagar. Microorganism growth was detected using fluorescent technology, species identification was based on morphological and biochemical characteristics. Antimicrobial susceptibility testing was performed using disk diffusion method.

RESULTS
Among 600 hospitalized patients in ICU, 98 patients (16.3 presented at least one episode of bacteremia. Forty patients presented at least one episode of bacteremia due to MDR bacteria. Male gender, cardiovascular diseases, diabetes and previous hospitalization were significant risk factors for the acquisition of MDR bacteremia. Isolated bacteria were mainly Gram-negative bacilli (GNB) (*n* = 48; 48.97%) dominated by *Acinetobacter baumannii* (*n* = 19; 21.1%), *Klebsiella pneumoniae* (*n* = 17; 17.3%) and Pseudomonas aeruginosa (n=12; 12.24%). MDR bacteria were represented by multi-resistant *Acinetobacter baumannii* (*n* = 20; 20.4%), extended-spectrum beta-lactamases-producing Enterobacterales (*n* = 9; 9.18%) and carbapenem-resistant Enterobacterales (*n* = 7; 7.14%). Carbapenems (60.6%), Aminoglycosides (*n* =51.5%) and Piperacillin/Tazobactum (39.3%) were the most used antimicrobials. Mortality rates were 45.5% and 65.3% in patients with non MDR bacteremia and MDR bacteremia respectively.

CONCLUSION
Limiting the spread of MDR bacteria and improving the management of bacteremic patients require continuous monitoring of bacteremia as well as adapting the therapeutic and preventive strategy. . Early administration of antibiotics significantly reduces patients' mortality.

[VIB-MS-OP-11]

Augmenting the Isolation Efficiency of Pathogenic *Leptospira* from Environmental and Rodent Samples

Archana Pal1#, K Vinod Kumar1, Prajakta Prashant Bokade 1, Swathi M, V Bharath1, Veena R. K2, Shilpa Devaraj2, Jagadeesha K2, Chethan Kumar H. B1., B. R. Gulati1, V. Balamurugan1

1Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India
2District Surveillance Unit (DSU), Mangaluru, Dakshina Kannada 575001, Karnataka, India.

Leptospirosis, a zoonotic disease pervading multiple mammalian species globally, is engendered by *Leptospira* spp. Ascertaining the presence of these pathogenic bacteria for serotyping and genotyping is pivotal for epidemiological surveillance, facilitating the advancement of diagnostics and vaccines. Nevertheless, the process of isolating *Leptospira* from diverse specimens remains fundamentally insensitive. This study endeavours to explore the efficacy of selective agents and sample filtration in bolstering the success rate of cultivating pathogenic *Leptospira* from environmental water and rodent samples.

Methods incorporated the direct inoculation and filtration of samples, with added rabbit sera and 5 Fluorouracil (5-Fu). Experimental techniques included inoculating the sample directly into the Ellinghausen-McCullough-Johnson-Harris (EMJH) semi-solid media, with and without the addition of 3% rabbit sera. The use of a 0.22-µm pore size membrane filter to sieve samples and subsequent incubation for two days at 29°C was also applied, before inoculating the samples into EMJH with or without 3% rabbit sera. Results demonstrate that implementing a 0.22-µm pore size membrane filter augments the isolation efficiency by eliminating potential bacterial contaminants. Allowing the filtrate, a resting period of two days escalates the initial leptospires concentration threefold, thereby enhancing the isolation potential when introduced into EMJH. However, the filtration process may permit the growth of certain spirochetes alongside *Leptospira*, not impairing the leptospires proliferation. This parallel growth is mitigated in the presence of a high concentration of 5-Fu (0.25 mg/ml) and 3% rabbit sera.
PCR validation of isolates with *LipL32* gene-specific primers confirms that a strategic combination of filtration, a two-day waiting period for the filtrate, and using a high concentration of 5 Fluorouracil and 3% rabbit sera, proves efficacious in the isolation of pathogenic *Leptospira* from environmental samples and rodent urine or tissue. The outcomes of this study corroborate that the cultivation and isolation of leptospires from these sources can be substantially enhanced with refined methodologies.

[VIB-MS-OP-12]

Detection of Novel Parvovirus (Canine Bufavirus) in Faeces of Dog in Northern India

Gurpreet Kaur, Hansmeet Kour, Mudit Chandra

Bufavirus is a recently recognized member of the genus *Protoparvovirus* in the subfamily *Parvovirinae*. It was first identified in 2012 in the viral metagenomic analysis of fecal samples from diarrheic children in Burkina Faso and Tunisia (hence the name "bufavirus"). Canine Bufavirus was newly discovered and identified for the first time in Italy in respiratory and faecal samples of dogs. Later it was detected in faeces and plasma of dogs with diarrhea in China. The canine bufavirus (CBuV) is found to be distantly related to canine parvovirus type 2, sharing low amino acid identities in the nonstructural protein 1 (40.6%) and in the capsid protein 1 (33.4%). The CBuV-infected dogs have presented nasal discharge, coughing, and respiratory distress, but the overall pathogenic potential of CBuV in dogs remains unknown. Current diagnosis of CBuV is done by PCR techniques and sequencing. The present study aimed at detecting Canine Bufavirus in fecal samples of diarrheic dogs by molecular assay. This study presents the first report of CBuV in northern India.

Keywords: Canine Bufavirus, novel Parvovirus, gastroenteritis, canine, India

[VIB-MS-OP-13]

Evaluation of mpb64 PCR for rapid identification of *Mycobacterium tuberculosis* complex among clinical isolates of mycobacteria in a tertiary care hospital in Kashmir, India.

Bashir G1, Majid U2, Malik N3 Ahanger I4, Wani T5.
1Professor, Dept. of Microbiology, SKIMS. 2PG Scholar, Dept. of Microbiology, SKIMS,

Background: Mycobacterium tuberculosis (MTB), is a major public health threat that infects 8.7 million people every year and is responsible for 1.4 million deaths annually. These estimates increased due to the AIDS epidemic, the emergence of the COVID-19 pandemic, and the spread of drug-resistant strains of MTB. Infections caused by non-tuberculous mycobacteria (NTM) may mimic the clinical presentation of MTBC, however, the treatment guidelines vary. Thus, a proper diagnosis and differentiation between MTBC and NTM are paramount for the initiation of accurate treatment. This differentiation by routine laboratory methods is time-consuming and cumbersome.

Aims and objectives: To evaluate *mpb*64 PCR in identifying the MTBC and differentiate between MTBC and non-tuberculous mycobacteria using *IS*6110 PCR as the gold standard.

Method: A total of 100 mycobacterial isolates obtained from various clinical samples in broth (MP-bottles) or on LJ-medium which were AFB positive on ZN staining, were included in the study. An in-house multiplex PCR was then used for the detection of *mpb*64 and *IS*6110 genes.

Results: Sensitivity of *mpb*64 PCR was 85.92% and the specificity was 96.55%. A total of 71 isolates were positive by the *IS*6110 PCR as opposed to 62 by the MPB64 PCR.

Conclusion: Multiplex PCR using both *IS*6110 and *mpb*64 shows more sensitivity than that of each target alone as has been reported elsewhere.

[VIB-MS-OP-14]

Evidence of Adaptive selection in the evolution of Aquaglyceroporins in mammals

Rajput S1*, Gautam D1, Vats A1, Ludri A2 and De S1#
1 *Animal Biotechnology Center, National Dairy Research Institute, Karnal*
2 Department of Animal Physiology, ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana 132001, India
**Presenting author: Email: Shiveeli09@gmail.com, Mobile: 7347316233*
#Corresponding author: Email: sachinandan@gmail.com

Aquaporins (AQPs) are essential membrane proteins that facilitate the transport of water and other small solutes across the cell membranes in both eukaryotes and prokaryotes. Aquaglyceroporins (AQGPs) are a subset of AQP family that allow the transport of small solutes such as glycerol and water through the plasma membranes. These proteins are found in the epidermis are involved in various physiological conditions such as wound healing, organogenesis, and hydration. The dysfunction of AQGPs can be seen in conditions such as obesity, cancer, and diabetes, thus a better understanding of these proteins is crucial to help create new strategies to combat these conditions. Although AQGPs have been extensively studied in different species, their phylogenetic relationships, conservation patterns, and evolution in mammals remain unexplored. In this study, 119 coding sequences of AQGPs from 31 mammalian species were analysed to identify gene organisation, conserved residues, and most importantly, the nature of AQGP gene selection. The repertoire analysis revealed the absence of some AQGPs such as AQP7, 9, and 10 in certain species of Primates, Rodentia, and Diprotodontia, but not all three genes were lost in a single species. In AQP3, 9, and 10 gene, aspartic acid (D) residues, two Asparagine-Proline-Alanine (NPA) motifs at the N- and C-terminal ends, and the ar/R region were found to be conserved. It was discovered that all mammalian species have six conserved exons that code for the MIP domain, which is functional domain of AQGP genes. The evolutionary studies using Branch-site- and site-specific models revealed that AQP7, 9 and 10 genes have undergone positive selection at critical residues in primates, carnivores, and rodents, suggesting their involvement in adapting different environmental conditions. The substitution of certain amino acids located close to critical residues such as NPA and ar/R filter may alter the functionality of AQGPs, which is essential for substrate selectivity, pore formation and transport efficiency required for maintaining the homeostasis in different mammals. An understanding of these variations can be helpful in designing drugs that can target these proteins and help manage the medical conditions.

Keywords: AQGPs, AQPs, NPA, ar/R

[VIB-MS-OP-15]

Cultural and Metagenomic approach for identification of microbiome in healthy and mastitis-affected bovine milk

Tikoo M1*, Shikha D2, Rashid M3, Sharma I2, Paul A4, Badroo G5 and Mishra S2

1Department of Veterinary Microbiology and Animal Biotechnology T&R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

2 Department of Veterinary Microbiology, IIVER-Rohtak, Haryana, India

3 Department of Veterinary Public Health and Epidemiology, SKUAST-Jammu, R.s Pura, India

4Department of Veterinary Pathology, IIVER-Rohtak, Haryana, India

5Department of Veterinary Public Microbiology, SKUAST-Kashmir, Suhama, India

Mastitis is one of the most predominant bacterial infections affecting mammary glands in dairy animals. It is a polymicrobial disease with drastic economic and productive losses in dairy cattle. This study was based on cultural and metagenomic characterization of 70 milk samples (50=Mastitis milk and 20=Healthy milk) collected from cows of the Jammu region. The results of this study revealed that on cultural and biochemical identification a total of 92 bacterial isolates were obtained from mastitis milk samples, out of which the most predominant were *Staphylococcus aureus* (45;48.91%) followed by *E. coli* (26;28.26%) *Bacillus spp.* (9; 9.78%), *Staphylococcus epidermidis* (8;8.69%) and *Pseudomonas aeruginosa* (4;4.34%) respectively. In the case of healthy raw milk samples, 13 bacterial isolates were obtained out of which (10;76.92%) were identified as *Non-aureus Staphylococcus* and (3;23.07%) were identified as belonging to the genera *Micrococcus.* The samples were also molecularly characterized for *Staphylococcus aureus* and *E. coli* by targeting the *nuc* (32;71.1%) gene and *eco* (100%) gene. The metagenomic approach was able to ascertain 17 different species in the case of mastitis milk with *Lactococcus* emerging as the prime species causing mastitis followed by *Streptococcus,* and *Staphylococcus* while 22 different species were identified in the case of apparently healthy raw milk samples with predominant species being *Aeromonas, Agrobacterium, Acinetobacter, Propionibacterium, Staphylococcus, Streptococcus, Corynebacterium* and *Enterococcus*. The alpha diversity of healthy raw milk samples was found to be higher than that of mastitis milk samples. The study concluded that the use of metagenomic sequencing targeting 16S rRNA gene can be utilized as an important tool to advance our knowledge regarding the probable pathogenesis of bovine mastitis and there needs to be further assessment of results obtained from milk microbiome analysis to determine their potential role and dynamics in health and disease as well as role of different environments in causing bovine mastitis.

[VIB-MS-OP-16]

An insight towards the interaction of galectins with E and NS1 proteins of Japanese encephalitis virus: A bioinformatic approach

1Babaljeet Singh, Bani Preet Kaur2, Sachin Kumar2 and Ratan K. Choudhary1

1College of Animal Biotechnology, Guru Angad Dev Veterinary, and Animal Sciences University, Ludhiana – 141004, Punjab
2Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati – 781039, Assam

The disaccharide N-acetyllactosamine (Gal-b (1,4)-GlcNAc or LacNAc), present in both N-linked- and O-linked glycoproteins, belongs to a specific family of lectins called galectins (G). There are a total of 15 Gs known, in which 12 Gs, namely, G-1,2,3,4,7,8,9,10,12,13,14, and G-16, have been identified in humans. Japanese encephalitis caused by the Japanese encephalitis virus (JEV) affects humans and animals' central nervous systems, particularly horses and cattle. JEV, a flavivirus, has an 11 kb genome and codes for structural (capsid, pre-membrane, and envelope or E-protein) and nonstructural proteins, including NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. The flavivirus nonstructural glycoprotein NS1 has been the focus of research due to its critical role in replicating viral protein and helping the virus to evade the immune response. Likewise, E- protein neutralizes antibodies of the host immune system generated in response to JEV infection. These antibodies bind to the E-protein, preventing its interaction with cellular receptors and blocking viral entry into host cells. In our study, we performed extensive molecular docking on all human galectin proteins, with the two JEV proteins, NS1 and E-protein. We aimed to understand the interactions of host galactins with NS1 and E-proteins to evaluate the regulatory roles of galactins in viral protein production. Results of the bioinformatic analysis showed G-7,8,10,12,13, and 16 tightly bind to E-protein, while G- 2,7,9,10,13, and 16 to NS1 protein. The binding of galactins with E-protein suggests its active role in virus entry while binding with NS1 protein indicates down-regulation of viral replication. The vast and varied effects of galectins are an important and curious area of human-virus interaction research.

Keywords: Japanese encephalitis virus, galactins, E-protein, NS1, molecular docking

[VIB-MS-OP-17]

Assessment of Inflammatory Changes and Gene Expression Analysis in Mice Liver Following Fine Needle Aspiration of Canine Mammary Tumors

Ratan K. Choudhary*, Rasman Kaur, Priyanka, and Shanti Choudhary

College of Animal Biotechnology, Guru Angad Dev Veterinary, and Animal Sciences University, Ludhiana- 141004, PUNJAB

Canine mammary tumors (CMTs) pose a significant threat to dogs' health, accounting for a substantial proportion of malignancies. Fine needle aspiration (FNA) cytology is a widely employed diagnostic procedure for CMTs, providing valuable tumor-specific cells for analysis. This study aimed to assess the tumorigenic potential of CMT-derived FNA by intramammary injections into 6 CMT and 7 control mice. Histological changes and gene expression analysis of interleukin *Il10* and tumor necrosis factor alpha (*Tnfa*) were examined in mice liver using RT-qPCR to assess inflammatory changes and tumor initiation. Results showed two injections of FNA, a week apart, from both CMT and tumor-free mammary glands, tissue architecture of mice liver remained unaffected following 8 weeks post-injection. However, the CMT-derived FNA caused lymphoid aggregation, suggesting the initiation of hepatic inflammation. Gene expression analysis revealed a tendency ($P = 0.08$) towards upregulation of *Il10* transcript in CMT mice compared to control mice. In contrast, the abundance of *Tnfa* transcript did not differ significantly between the two groups. In conclusion, the administration of fine needle aspirates of canine mammary tumors induced hepatic inflammation in mice, as evidenced by histological changes and gene expression analysis. These findings underscore the potential of FNA-derived gene expression analysis in diagnosing CMTs and studying their tumorigenic properties. Further investigations are warranted to elucidate the mechanisms underlying the observed inflammatory changes and to explore their implications for CMT progression and treatment.

Keywords: canine mammary tumor, fine needle aspirates, tumorigenic, mice, RT-qPCR

Session V: Biotechnological Interventions for Enhancing Animal Health & Production

[VIB-MS-OP-18]

Drug Utilisation Pattern of Anti Depressants: A Prospective Cross-Sectional Study in Psychiatry Outpatients from A Tertiary Care Hospital.

Dr Syed Sajad H, Dr Samina F, Dr Yasir Hassan R, Dr Rehana T Dr . Mehroosh
Department of Pharmacology, Government Medical College Srinagar.

Background: Depression is a major public health problem as it can cause significant clinical distress. Major depression disorder (MDD) has been found to cause impairment of social, occupational or other areas of function. Antidepressant drugs being leading psychotropics that are prescribed worldwide, their utilization in actual clinical practice, effectiveness and safety in real life situation need continuous study.

Objective: Present study was carried out to evaluate the prescribing patterns of different antidepressants in psychiatry unit of a tertiary care hospital.

Methods: This prospective, observational and cross-sectional study was conducted in the out-patient department (OPD) of General Psychiatry Unit of Shri Maharaja Hari Singh (SMHS) hospital. A total of 600 cases were enrolled for the present study to investigate the prescribing pattern of antidepressants using a predesigned format out of which 543 subjects were taken for final results.

Results: Depression was found to be the leading cause of psychiatric morbidity among the subjects accounting for 36% of the total study population. Females suffered from depression more than their male counterparts. The most common age group suffering from MDD was found to between 20-39 years comprising almost 50% of study population Monotherapy was practiced more frequently than polytherapy with 2 or more drugs. Selective Serotonin Reuptake Inhibitors (SSRIs) like escitalopram was found out to be the most preferred antidepressant chosen by the treating psychiatrists.

Conclusion: Depression being the most common psychiatric disorder and this part of world is no exception to this. Among many other antidepressant groups, selective serotonin reuptake inhibitors are preferred over others because of their better side effect profile.

[VIB-MS-OP-19]

Detection of respiratory syncytial virus among hospitalized children with respiratory tract infection using RT PCR in a tertiary care hospital in Kashmir valley

Shah Aamina Samreen[1], *Fomda Bashir*[2], *Bhat Mushtaq*[3]
PG, Deptt. of Microbiology, SKIMS [2]Professor, Deptt. ofMicrobiology, SKIMS, [3]Additional Professor, Deptt. of Pediatrics and Neonatology, SKIMS.

OBJECTIVES: To find out the prevalence of RSV in children less than 5 years of age and also to evaluate the epidemiological and clinical patterns of RSV infection in children hospitalized for acute respiratory tract infection.

STUDY DESIGN: It is a prospective cross-sectional study
PARTICIPANTS: A total of 132 nasopharyngeal swab samples were collected from children less than 5 yr of age admitted at SKIMS Soura during the study period of 23months.

METHODS: Patient details were recorded and nasopharyngeal secretions were collected by gently rubbing the deep nasal turbinate. All the samples were tested for RSV A and B specific RNA by a customized Multiplex RT-PCR kit (RealStar® 3.0).

RESULTS: Out of 132 samples, 40 samples were positive for RSV A and 2 samples were positive for RSV B and 90 samples were negative for RSV A and B. The prevalence of RSV A among hospitalized children with respiratory tract infection was 30.3% while as prevalence of RSV B was only 1.5%. The overall prevalence of RSV among children aged less than 5 years was 31.8%. Most of the positive cases were recorded in winter season (November to January), 29 (69.05%) and the least number of positive cases were recorded in spring season 2 (4.76%).

CONCLUSION: RSV infection is an important cause of mortality and morbidity in children below 5 years of age and demands early diagnosis and treatment to avoid unnecessary use of antibiotics.

IMPLICATIONS: Our study highlights the healthcare and economic burden of RSV in children less than 5 years of age in this part of the country. It provides an insight into the extent to which RSV is responsible for causing respiratory tract infection in children.
While coming across the respiratory tract infections among children, we must test for RSV as well in suspected cases. This not only will establish the correct diagnosis in many cases and hence avoid unnecessary antibiotic therapy but also shall fasten the recovery and improve the outcome of such patients.

[VIB-MS-OP-20]

Drug Storage and Self-Medication Practices in Kashmir, India: A Cross-Sectional Study.

Dr. Rehana T, Dr. Syed Sajad H, Dr Sajad Hussain A.

Department Of Pharmacology, Government Medical College Srinagar.

Introduction: Among the various potential health risks, self-medication, and inappropriate storage of medicines at home are found to be very important. Inappropriate storage conditions and irrational use of medication without medical consultation may result in serious health problems. Worldwide there has been an increase in the self-medication rate, which can lead to
waste of resources and serious adverse reactions.

Aim: To assess the practice of self-medication and household storage of medicines amongst the study population.

Materials and Methods: This was a cross-sectional and questionnaire-based study conducted in the southern district of Kashmir, India from 1st September 2021 to 28th February 2022. A total of 471 households were included and interviewed to determine the practice of home storage of medicines and self-medication pattern. Quantitative data was presented as means and standard deviation (mean±SD) and qualitative data as frequency and 95% Confidence Interval (CI).

Results: Almost three quarters of the households were having monthly income of less than 20,000 INR whereas 219 (46.50%) of them were illiterate. Proton pump inhibitors, minerals and
vitamins, antibiotics and analgesics were the most common medicines stored at home. Drawer was the most common place used for storage of medicines whereas the refrigerator was used
in less than 1% of cases for the same. Solid dosage forms were mostly used by the householders and the injectables were least used for household storage purposes.

Conclusion: The study revealed that the studied householders stored large amount of medicines in homes, often under inappropriate storage conditions. There is a need for better public knowledge and information about the risks of reuse of prescribed medications.

Day 2
Technical Sessions

Session III
Antimicrobial Resistance
(Oral)

[VIB-AMR-IVL-01]

Artificial Intelligence-Based Discovery of Novel Antimicrobial Peptides for Addressing Antimicrobial Resistance

Sameer Shrivastava
Facility for Research & Training on Bioassays and Biosensors
Division of Veterinary Biotechnology
ICAR-Indian Veterinary Research Institute, Izatnagar 243122 [U.P]

Bacteria are one of the earliest lifeforms to appear on earth. The bacteria must have been constantly adapting and evolving to thrive even after some 2-3 billion. When humans developed antibiotics, these micro-organisms parallelly developed resistance to antibiotics due to several modifications in their genome that guarantee their survival in the presence of antibiotics. This, in turn, motivated scientists to develop even stronger antibiotics, which were effective against these pathogens but also caused serious side effects on human health. In due course of time, these antibiotics, too, started failing, and now we see multi-drug resistant (MDR) species of bacteria prevalent in the environment. Amongst the MDR bacteria, major threat is posed by seven species which are popularly represented by the acronym ESKAPEE, which stands for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, and *Escherichia coli.* These range from critical to high drug-resistant species as per the World Health Organisation (WHO) "priority pathogens list". The inefficiency of antibiotics towards such superbugs is leading us towards a "post-antibiotic future" and it is estimated that by 2050, there will be approximately 10 million fatal cases of AMR infections annually. The fast rise of drug-resistant infections has severely hampered effectiveness of antimicrobial therapy and inability of the most powerful antibiotics to kill superbugs highlights an urgent need for discovery of new antimicrobial agents. To register a decisive win against bacteria, we need some out-of-the-box solution. In this regard, developing a new generation of antibiotics based on antimicrobial peptides (AMPs) may lead the way.

Antimicrobial peptides (AMPs) are oligopeptides that exist naturally in all multicellular animals and act as first line of defence against harmful bacteria. AMPs offer various advantages over regularly used antibacterial medicines. They are naturally produced, kill the bacteria in numerous ways, have few side effects and are less damaging to the host cells. As a consequence, AMPs have lately gained a lot of attention as an alternative to presently available antibiotics and peptide-based drugs account for 7% of all drugs authorised by the Food and Drug Administration (FDA) in the previous five years. Identifying AMPs from natural sources is time-consuming and expensive. Therefore, in-silico tools are essential to obtain new AMPs in protein sequences and facilitate rapid drug discovery using machine and deep learning techniques. One application of these techniques is to construct computational models for discovering novel AMPs in proteins of various organisms to develop a new line of antibacterial drugs.

We have developed machine learning and deep learning-based models with the help of super computer, for discovering novel AMPs including antibacterial, antifungal and antiviral molecules. Further, the artificial intelligence (AI) based tools for predicting the MIC and probable haemolytic activity of therapeutic peptides are also developed. In this talk I shall cover strategies for developing the AI based models and also share some of the results of the synthetic AMPs which were designed and synthesized in our lab using these models and tested for their activity against different bacteria in the *in vitro* and *in vivo* conditions.

[VIB-AMR-OP-01]

Molecular Detection of Antimicrobial Resistance and Virulence Gene of Avian Pathogenic *Escherichia coli* (APEC) Isolated from Broiler Chickens

Patel S S, Patel A C, Sharma K K, Mohapatra S K, Shrimali M D, Pandor B R, Antiya S P and Chauhan H C.

Department of Veterinary Microbiology, College of Veterinary Science & A.H., Kamdhenu University, Sardarkrushinagar-385506, Gujarat.
E-mail: drsandipvety@kamdhenuuni.edu.in

Antimicrobial resistance (AMR) is now recognized globally as a public health challenge impacting all 'One Health' sectors. AMR is linked with high disease burden and economic consequences on people and nations. Avian pathogenic *Escherichia coli* are the pathogens which display high degree of antimicrobial resistance due to production of extended spectrum beta lactamases (ESBLs). The increasing trend of poultry meat, use of antimicrobials as growth promoters and correlation of their virulence factors with human haemorrhagic colitis and uropathogenic *E. coli* necessitate to undertake the present study. In this study, total 102 samples were collected from chickens of different flocks, died due to suspected collibacillosis and identified as *E. coli* through bacteriological and PCR methods. Phenotypic AMR was determined by disk diffusion method. ESBL detection was carried out via PCR by targeting *bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX-M group1, 2, and 9 genes. Genes of eight virulence factors and class I integrons were also detected by PCR using gene specific primers.

Results: Culture, microscopic, biochemical tests and PCR recognized 69/102 samples as *E. coli*. Phenotypic AST revealed five pan resistant isolates and higher resistance to enrofloxacin (72.46%) followed by levofloxacin (69.56%) and doxycycline (69.56%). A total of 48 (69.56%) and 7 (10.14%) isolates were positive for presence of *bla*TEM and *bla*CTX-M-G9 genes respectively; whereas 2 (2.90%) isolates each, were found positive for *bla*SHV, *bla*OXA and *bla*CTX-M-G1 genes. Among APEC associated virulence genes, *iss* (79.71%) was the most predominant, followed by *tsh* (50.72%), *ast* (30.43%), *cvaf* (26.08%), *pap* (23.18%), *vat* (8.69%) and *stx*-1 (1.44%). Thirty two isolates harboured the class I integrons, either with or without ESBL genes.

Conclusions: The isolates under study showed pan and multiple drug resistance, specifically against fluoroquinolones drugs. The ESBL production was mediated principally through blaTEM and *bla*CTX-M-G9.

[VIB-AMR-OP-02]

Non fermenting gram-negative bacilli as emerging pathogens - report from tertiary care hospital in Western India

Bushra Yousuf Peerzada
Department: Microbiology, GMC Anantnag

Background: Non fermenting gram-negative bacilli (NFGNB), which are saprophytic in nature, have emerged as important healthcare-associated pathogens. The main reason for concern about the prevalence of NFGNB is their tendency of being multidrug resistant. This study was done to evaluate the NFGNB isolates from clinical samples.

Material and methods: The nonfermenters were identified using a standard protocol that included tests for motility, oxidase production, oxidation-fermentation test, gelatin liquefaction, etc. Antibiotic susceptibility pattern (ABST) was evaluated using Kirby-Bauer disk diffusion method. Identification and ABST of some isolates was done using VITEK Automated system. The clinical significance was assessed by using various criteria.

Results: *Pseudomonas aeruginosa* and *Acinetobacter* spp were the most common isolates. Pus was the most common clinical sample.

Concluusion: NFGNB are emerging as important opportunistic pathogens. Hence, proper management of infection is necessary to avoid emergence of drug resistance.

Key words: *Acinetobacter spp.*, *Pseudomonas aeruginosa*, carbapenemases, multidrug resistance

[VIB-AMR-OP-03]

Phenotypic study, molecular identification, and antimicrobial resistance pattern of emerging pathogen *Acinetobacter junii* isolated from Zebrafish (Danio rerio L.)

Javia, BB*, Patel UD, Patel HB, Posiya PJ, Reddy S, Delvadiya RS, Tank MR
*Associate Professor
Department of Veterinary Microbiology
College of Veterinary Science and Animal Husbandry
Kamdhenu University, Junagadh-362001

The use of zebrafish (*Danio rerio L.)* as a laboratory research model has become increasingly important to scientific studies due to its multiple characteristics, which make it suitable for studying diseases, toxicity, and genetics. Among the various reasons for fish mortality, septicemic bacterial disease caused by *Acinetobacter* spp. has been reported in wetland aquaculture systems. The present investigation was carried out on zebrafish, which were kept as part of scientific studies and showed clinical signs of illness along with few mortality. The freshly dead fish, showing gross pathological lesions like ulcerated skin, was subjected to bacterial isolation. The collected samples were inoculated in brain heart infusion (BHI) broth for initial incubation and then streaked on BHI agar. The pure, smooth, white colonies upon examination showed gram-negative coccobacilli in irregular arrangement. This culture was found positive for catalase production and citrate utilization but negative for the oxidase test. For molecular study, fragments of the 16S rDNA gene was amplified by 27F and 1492R primers to confrim bacterial species. The consensus sequence of the 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The generated sequence was used to carry out BLAST with the available database. The phylogenetic tree was constructed, and based on this, the culture was identified as ***Acinetobacter junii,*** considering the highest sequence similarity. The *in vitro* antimicrobial susceptibility of the isolated bacteria was determined by Kirby-Bauer disc diffusion methods. Among the tested antimicrobial agents, ***A. junii*** was found to be resistant to oxytetracycline, cefopreazone, ceftizoxime, amoxicillin-sulbactum, and chloramphenicol while sensitive to gentamicin and levofloxacin. *A. junii* is an important emerging bacterial pathogen of zebrafish with high resistance against commonly used antimicrobials that needs to be studied further for its clinical significance.

[VIB-AMR-OP-04]

Molecular and Anti microbial profiling among *Escherichia coli* isolates from camel in north west Rajasthan

Mahender Miland Lakeshar*, Jyoti Chaudhry, Sudesh Dedar, Narasi Ram Gurjar, Parteek Khera, B. N. Shringi
Department of Veterinary Microbiology
Sri Ganganagar Veterinary College, Tantia University Rajasthan 335002

Escherichia coli is frequently associated with multiple antimicrobial resistances. A major problem in camel productivity is the high mortality rate in camel calves in the first 3 months of life. Diarrhea and other infectious diseases are considered to be the main causes of economic loss associated with poor growth, medication costs, and animal death. Various kinds of virulence factor such as adhesins, invasins, enterotoxins, hemolysin, biofilm production and antibiotic resistance gene harbored by *E. coli* is responsible for its pathogenicity. In present study a total of the 70 faecal were identified as *E. coli.* The overall prevalence of *E. coli* isolates was found to be 100% (70/70) by conventional methods. All the isolates were correctly identified through vitek2 biochemical identification system and were genotypically confirmed by 16S rRNA ribotyping. All 70 *E. coli* isolates were further subjected for hemolysis and slime production test. Overall, 52.85% *E. coli* isolates showed hemolysis on blood agar. Slime production test done by congo red agar test method and overall, 54.28% isolates were positive for slime production. The Antibiogram of 70 isolates revealed that *E. coli* isolates from camel were resistant to Penicillin (94.38%) followed by amoxicillin+sulbactam (85.71%), erythromycin (71.14%), cefixime+clavulanic acid (71.43%). Highest sensitivity to chloramphenicol (81.28%) followed by sulphadiazine (48.57%) and cotrimoxazole (48.28%). All the 70 isolates were screened for antibiotic resistance genes. Based on the molecular screening of the antibiotic resistance genes, majority of the isolates carried *Bla*TEM gene in camel (28/35; 80%) followed by *Str*A (15/35; 42.85%), *Sul*-3 (11/35; 31.42%), *Sul*-2 (09/35; 25.71%), *aad*A (14/35; 40%), *tet*(B) (11/35; 31.42%). *Bla*SHV, *Bla*CTX, *Bla*MBL, *tet*(C), *tet*(D), *tet*(E), *tet*(G) genes were not found in any of the *E. coli* isolates from faecal sample of camel.

[VIB-AMR-OP-05]

Isolation, whole genome sequencing and comparative genome sequencing of Clostridioides difficile reveals the presence of multidrug resistance toxigenic strains in Tamil Nadu

Seeralan Manoharan 1, *Kumaragurubaran Karthik 2, #, and Subbaiyan Anbazhagan 3

Vaccine Research Centre- Bacterial Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600051, India
Veterinary College and Research Institute, Udumalpet, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600051, India
ICMR-National Animal Resource Facility for Biomedical Research, Hyderabad- 500078, India3

A total of 150 samples (fecal samples, rectal swabs) were collected from dogs, cattle, sheep and goat and processed for *C. difficile* isolation. Fifteen isolates of *C. difficile* was isolated from dog samples which was confirmed by *gluD* gene PCR. Antimicrobial gene prediction PCR showed that one isolate showed positive for *erm*(B) gene (711 bp). Total of 5 isolates were whole genomes sequenced and its genomics was analysed with 107 different *C. difficile* isolated from animal species. Whole genome phylogeny showed clustering of genome based on multi locus sequence types. All the 5 genomes correspond to 5 different sequence types namely 54, 11, 35, 243, 298. Toxin gene production showed that CD15, CD26 genomes had *toxA* and *toxB* gene while D1 genome had *cdtA, cdtB* and *toxB* genes. On whole genome analysis *C. difficile* CD15 showed the presence of *aac(6')-aph(2''), erm(B)* genes that is responsible for resistance against amikacin, gentamicin, tobramycin, erythromycin, azithromycin antibiotics. Similarly CD26 genome showed the presence of *aac(6')-aph(2''), ant(6)-Ia, catP, erm(B)* which is responsible for resistance against amikacin, gentamicin, tobramycin, streptomycin, chloramphenicol, erythromycin, azithromycin antibiotics. Other 3 genome does not have any predicted antimicrobial genes. Both CD15 and CD26 genome had rep1 plasmid. The study indicated that multidrug resistant, multitoxigenic *Clostridioides difficile* strains are circulating in India.

Keywords: *Clostridioides difficile*, India, Dogs, Whole genome sequencing, AMR, MLST

[VIB-AMR-OP-06]

Status of antimicrobial resistance genes among bacterial population from faecal samples of wild animals and birds in a zoological park in Chennai, Tamil Nadu

Latha Mala Priyadharshini, M1, Manoharan, S2, Padmapriya, K3
1-Assistant Professor, 2-Professor, 3-Technician
Vaccine Research Centre-Bacterial Vaccine, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai-600051

Antimicrobial resistance occurs naturally over time through genetic changes. AMR organisms are found in people, animals, food, plants and the environment (in water, soil and air) and can spread from person to person or between people and animals. The main drivers of antimicrobial resistance include the misuse and overuse of antimicrobials. This study aimed to collect faecal samples from different species of wild animals (primates-06; herbovores-05; omnivores-04; rodents-04 and reptiles -04; snakes-06) from birds (ratites-06; parakeets-04; budgerigars-05; game birds-03; water birds-05 and others-04) that are housed in the Guindy National Park (Children's Park) at Chennai, Tamil Nadu. Totally 60 Nos. of faecal samples were processed and inoculated in suitable media and incubated aerobically at 37˚ C for 16-18 hours. Also, the same was inoculated in Robertson cooked meat medium and incubated anaerobically in a MacKintosh (anaerobic) jar at 37˚ C for 48-96 hours. The cultures were then streaked on many types of selective agars to isolate presumptive potential pathogens. Single colonies from selective plates were stained by Gram 's method and from its pure culture, DNA extraction was done by boiling method. Also the isolates were characterized by a panel of bio-chemical tests and results tabulated. Most of the isolates obtained were Gram negative bacilli or cocco- bacilli (32 Nos) and 32 Nos. of pure *Escherichia coli* isolates was obtained. Other bacterial isolates were *Bacillus* spp, *Pseudomonas* spp and *Proteus* spp etc. and few *Clostridial* spp in anaerobic conditions.

The DNA of bacterial isolates were subjected to m-PCR to screen for presence of *abr* genes for 6 classes of drugs. Each class of drug was screened by m-PCR or 3-7 target genes and the results are tabulated. It is concluded that, the antibiotic bacitracin was found to be the most resistant by amplifying for the gene *bcr* (B)-(247 bp) in 11 isolates followed by *bcr* (D)-(318 bp) in 6 isolates and only 1 isolate carried *bcr* (R) - (379 bp). The antibiotic tetracycline was resistant, wherein *tet* (A) - (764 bp) in 8 isolates and *tet* (M) (171 bp) was in only in 1 isolate. For lincomycin, gene *inu* (A)-(323 bp) got amplified in 7 isolates. For erythromycin, the genes *erm* (B)-(638bp) and *erm* (Q)-(410bp) got amplified in 6 isolates each. For gentamicin, gene *aac* (6) IB-(472 bp) and *aac* (6) II (542 bp) got amplified in only 1 isolate each.

Also, it was observed that *erm* (B) -5 and (Q)-1 was found in 6 birds; *inu* (A) was in 4 birds; *tet* (A)-6 and (M)-1 was in 7 birds; *bcr* (B)-6, *bcr* (D)-4 and bcr (R)-1 was found in 11 birds and lastly aac(6)Ib and aac (6) II was found 1 each in birds.

So among all species, birds carried many types of *abr* genes. The Alexandrian parakeet carried the maximum no. of *abr* genes (5) *i.e.,* against *erm* (B), *tet* (A) & (M), and *bcr* (B) & (D). All the other birds carried *abr* genes against inuA for lincomycin /*tet* A/ *aac* (6) Ib and *aac* (6) II for gentamicin, erythromycin *erm* (Q) & (B), lincomycin *inu* (A) and bacitracin *bcr* (B) & (D). From this study, it was concluded that the *abr* genes were present in all types bacteria irrespective of whether they are pathogenic or apathogenic, indicating the faster spread of AMR among the microbial world.

Session III
Antimicrobial Resistance
(Poster)

[VIB-AMR-PP-01]

Antimicrobial Resistance Monitored in Analysis of Blood Stream Infections in Haematological Malignancies

Rashid S1, Fomda B A, Ahmed T,

Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, Soura-190011, J&K, India,

Background: Infections are a significant contributing factor to morbidity and mortality in immunocompromised individuals especially in oncology patients. Immunity may be compromised by the cancer itself or by therapeutic interventions. Patients with haematological malignancies are at increased risk of infection among this subset due to bone marrow infiltration and suppression leading to neutropenia and lymphopenia. With advances in cancer treatment, more intensive treatment regimens have been introduced, leading to increased cure rates but also increased infection risk. Cancer patients have increased exposure to pathogens because of indwelling central vascular access devices and frequent hospitalization. This places them at risk of hospital-acquired infections, which carry high morbidity as they are frequently caused by multidrug-resistant organisms. A number of studies have described the epidemiology of BSI worldwide.

Objectives: There is a paucity of data on BSI amongst haematological malignancies in low and middle-income countries. At the research site, BSI is an important contributor to hospitalization. Therefore, this study was performed to investigate the epidemiology of BSI in haematological malignancies including the spectrum of pathogens, their antimicrobial susceptibilities, and clinical outcomes.

Methods: A retrospective cross-sectional study was conducted in the Department of Microbiology. All positive blood cultures from Haematology–Oncology Unit obtained in 2022 were retrieved to identify cases of BSI.

Results: Ninety-two positive cultures were identified during the study period among which 55.5% of the culture isolates were Gram-positive bacteria, while 35.7% were Gram-negative bacteria, and 8.8% were fungal isolates. Coagulase-negative *Staphylococcus* were the most common Gram-positive isolates and *Klebsiella* was the most common Gram-negative isolates while *Candida albicans* and *Candida tropicalis* were the most common fungal species involved. Majority the organisms were resistant to the commonly used antibiotics. Fungal infections had the highest prevalence of complications as the time taken for diagnosing and initiation of antifungal therapy was longer than other infections.

Conclusions: Patients with haematological cancer demonstrated to have a higher prevalence of BSI. The high levels of carbapenem resistance among Gram-negative isolates is a cause of concern. Better infection control practices and a proactive antimicrobial stewardship program is needed to curb the rising magnitude of antimicrobial resistance in such cases.

[VIB-AMR-PP-02]

Antimicrobial Susceptibility Pattern and Molecular Typing by ERIC-PCR of Non-Uropathogenic *Escherichia coli* in a Tertiary Care Hospital.

Dr. Gulnaz Bashir 1, Dr. Amir Sayed Malik 2

1Professor, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura.

2PG Scholar, Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Soura.

BACKGROUND: Extra-intestinal pathogenic *E. coli* (ExPEC) strains are one of the most common enteric pathogens to cause community-acquired and healthcare-associated bacterial infections. The emerging propensity of these strains to acquire new antimicrobial resistance has posed challenges in managing ExPEC infection. Typing of *E. coli* by phenotypic or genotypic methods permits timely detection of outbreaks in a healthcare setting.

OBJECTIVES: To do strain typing of ExPEC isolated from various samples using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) during the study period and to compare ERIC types with antibiotypes.

METHODS: Phenotypically identified consecutive non-urinary isolates of *E. coli* (n=60) were subjected to molecular confirmation by 16s rDNA PCR. All these 60 isolates were subjected to antibiotic susceptibility testing by disc diffusion method and molecular typing by ERIC-PCR. Statistical program IBM SPSS Statistics 20 was used.

RESULTS: Prevalence of MDR among in-patients and out-patients as 42 (89.4%) and 10 (77%) respectively, as compared to non-MDR strains, which were 5 (10.6%) and 3 (13%) respectively. A total of 23 antibiotypes were obtained. All 60 isolates were typeable by ERIC-PCR and produced multiple bands. ERIC profile of these isolates produced 3 clusters and 19 ERIC types.

CONCLUSION: Multiple, closely related MDR strains of *E. coli* are circulating in our hospital and no point source of any outbreak with a particular strain of *E. coli* could be detected during the study period.

[VIB-AMR-PP-03]

Nosocomial *Acinetobacter* meningitis: a single-centre experience

Susheem R, Fomda B A, Ahmed T,

Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, Soura-190011, J&K, India,

Background: *Acinetobacter*, a non-fermenting, Gram-negative bacillus, is responsible for various hospital-acquired infections due to its inherent Multidrug-Resistant (MDR) property. Currently, *Acinetobacter* is the second most common agent of nosocomial meningitis across hospitals. *Acinetobacter baumannii* colonisation has increased and it frequently colonises secretions and fluids in humans. Although colonisation is the rule for this organism, *A. baumannii* may cause a wide spectrum of nosocomial infections including pneumonia, meningitis, and urinary tract infections. MDR *A. baumannii* has a higher mortality rate as opposed to other species causing meningitis. The estimated mortality rate of 15% increases to 40-70% if caused by MDR *Acinetobacter.*

Objectives: To identify the incidence, clinical characteristics, drug resistance and mortality rates among the patients suffering from *Acinetobacter baumannii* meningitis.
Methods: This retrospective single-centre study was carried out in the Bacteriology division of the Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences Soura, Srinagar, Jammu and Kashmir, India from January 2020-December 2022
Results: A total of 35 *Acinetobacter* isolates were identified during the study period. The patients included 23 males and 12 females, with ages ranging from 7-74 years. The most common isolated species was *A. baumannii* (n=32) followed by *A. lwoffii* (n=3). Of the total patients enrolled, 11 patients had MDR isolates, whereas two showed XDR isolates, and one showed pan-drug-resistant (PDR) isolates. Majority of the patients were from neurosurgery and had a history of placement of foreign material. The mean hospitalization was 43.4 days. Seven patients eventually died.
Conclusions: The isolation of *Acinetobacter* MDR, XDR and even PDR strains makes treatment very difficult and seriously threatens patient survival. The rational selection of antibacterial drugs is crucial for such cases. For the first-line treatment, meropenem is the most recommended empirical treatment, but its resistance rate exceeds 40% with carbapenem-resistant strains. For such resistant *Acinetobacter*, polymyxin has been successful. However, polymyxin has poor solubility in the CNS. When administered intravenously, its CSF level can only reach approximately 25% of the serum levels. Intrathecal or intraventricular antibiotic administration is expected to be an effective choice for meningitis. Prompt identification and antibiotic susceptibility and administration of targeted antibiotics are of paramount importance in treating such patients.

[VIB-AMR-PP-01]

High prevalence of multi drug resistant *Pseudomonas* and *Klebsiella* species recovered from infected burn wounds in a tertiary care hospital of North India.

Sheikh Zainab A (MD), Nahid N (MD).

Department of Microbiology, Government medical college, Srinagar 190011

BACKGROUND:
Patients with burn injuries are at high risk for infectious complications and infections are the most common cause of death after the first 72 h of hospitalization. Hospital-acquired infections caused by multi drug resistant (MDR) Gram-negative bacteria (GNB) in this population are concerning. *Pseudomonas* and *Klebsiella* species are common cause of health care acquired infection and represents a major threat to critically ill patients particularly burn patients. The emergence of Multi drug resistant strains is up surging leading to problematic control. Thus the aim of my study was to detect and investigate the resistant profile of *Pseudomonas* and *Klebsiella* species.

MATERIAL AND METHODS:
STUDY DESIGN: Hospital based cross sectional study.
STUDY SETTINGS: Department of microbiology.
DATA COLLECTION: 1 January 2023 to 31 May 2023.
STUDY PARTICIPANTS: All Burn patients.
STUDY PROCEDURE: 186 pus samples from burn patients were received in our laboratory during the study period. Samples were processed using conventional microbiological methods and antimicrobial testing was carried out using Kirby Bauer disc diffusion method in accordance with the standards set by the Clinical and Laboratory Standards Institute.

RESULTS:
Out of the 186 pus samples received, 99(53.2%) came culture positive, 1(0.53%) sample was found to be contaminated and 86(46.2%) were culture negative. In one of the samples 2 organisms were isolated, so in total 100 organisms were isolated. From the 100 organisms, 55(55%) were *Klebsiella* species, 15(15%) *Pseudomonas* species, 9(9%) *E. coli*, 2(2%) *Citrobacter* species, 11(11%) *Acinetobacter* species, 6(6%) *Staphylococcus aureus*, 1(1%) Enterococcus species, 1(1%) *Serratia* species.

CONCLUSION: This study found a high level of multi drug-resistant Gram-negative bacteria in hospitalized patients with burn wound infection. The common MDR GNB causing burn wound infections in these patients included *Pseudomonas* and *Klebsiella* spp. Regular surveillance, in vitro antimicrobial testing and monitoring is necessary to guide empirical therapy in burn patients. The practices would in turn curb the emergence of multi drug-resistant organisms and decrease morbidity and mortality attributed to these infections.

[VIB-AMR-PP-05]

Genotypic and phenotypic characterisation of antibiotic resistance of Coagulase Negative *Staphylococci* associated with pigs and their environment

Kar I, Kaur P, Rai T S and Arora A K
Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India

The study was designed to determine the prevalence, antibiotic resistance pattern and resistance genes in Coagulase negative Staphylococci (CoNS) isolated from pigs and their environment. A total of 287 samples were collected which included nasal and skin swabs (n=125 each) from 50 apparently healthy and 75 diseased pigs and environmental samples (n=37). CoNS were isolated by conventional method and were confirmed by MALDI-TOF and genus specific PCR. A total of 108 CoNS were isolated which comprises of 23 (21.29%),79 (73.14%) and 6 (5.55%) isolates from healthy, diseased and farm environment samples, respectively. Out of the total CoNS isolates, eleven different CoNS strains *viz. S. epidermidis* (n=10 i.e., 9.25%), *S. hominis* (n=33 i.e., 30.55%), *S. xylosus* (n=4 i.e., 3.70%), *S. sciuri* (n= 22 i.e., 20.37%), *S. chromogenes* (n=18 i.e., 16,66%), *S. hemolyticus* (n=7 i.e., 6.48%), *S.simulans* (n=9 i.e., 8.33%), *S. hyicus* (n=2 i.e., 1.85%), *S. warneri*, *S. equorum* and *S. pasteuri* (n=1 i.e., 0.92%) were identified. Antibiotic sensitivity test was carried out against 26 antibiotics as per Kirby-Bauer disc diffusion method. Overall, the CoNS showed maximum resistance towards cefoxitin (78.7%), penicillin (73.14%), and tetracycline (55.55%). In case of healthy and diseased pig's isolates, highest resistance was observed towards cefoxitin i.e., 73.91% and 82.27%, respectively. While in environmental isolates the highest resistance was shown towards penicillin (83.3%). All the CoNS isolates were screened for the detection of antimicrobial resistance genes. The prevalence of *tetK* gene was found to be highest (40.74%), followed by *blaZ* (22.22%), *tetM* (19.44%), *ermB* (17.59%), *mecA* (13.88%), *aacA-aphD* (6.48%) and *ermC* (4.62%). Statistical analysis using Student T test revealed no significant difference in antibiotic resistance pattern between healthy and diseased animals. While in ANOVA test significant difference was observed between AMR patterns of CoNS, recovered from pigs and their environment.

Keywords: CoNS, Antibiotic resistance pattern, Antibiotic resistance gene, MALDI-TOF, PCR

[VIB-AMR-PP-06]

Isolation, antibiotic sensitivity, and virulence profile of *Streptococcus agalactiae* isolated from caprine mastitic milk samples from Punjab

Gazal S1, Kaur P1, Bedi J2, Singh J3, Arora A K1 and Rai T S1
1Department of Veterinary Microbiology, GADVASU, Ludhiana, India
2Centre for One Health, GADVASU, Ludhiana, India
3Department of Veterinary and Animal Husbandry Extension, GADVASU, Ludhiana, India

Streptococcus agalactiae, an important etiological agent of bovine mastitis, has been poorly studied as a causative agent of caprine mastitis in India. Therefore, the present study was aimed at studying the antibiogram and virulence profile of *S. agalactiae* isolates from cases of caprine mastitis. In the present study, a total of 100 milk samples were collected from different districts of Punjab and the samples were processed for estimating the prevalence of mastitis as well as for the isolation of *S. agalactiae*. The prevalence of caprine mastitis was estimated to be 51% by SLS test. A total of seven *S. agalactiae* isolates (7%) were obtained and confirmed by MALDI-TOF and all the isolates were obtained from mastitic milk samples. The *S. agalactiae* isolates were screened for the presence of ten virulence genes, out of which only six were detected viz., *cyl*, *gln*A, *cfb*, *hyl*B, *fnb*B and *sca*A gene. Ours is the first report on detection of *cyl*, *gln*A, *hyl*B, *fnb*B and *sca*A genes in caprine *S. agalactiae* isolates from India. Out of six antibiotic resistance genes tested, only one antibiotic resistance gene i.e., *tet*M gene could be detected by PCR. Antibiotic sensitivity results revealed highest resistance to tetracycline and ampicillin with 71.4% of isolates being multidrug resistant and all the MDR isolates exhibited a MAR index greater than 0.2 which is reflective of the wide antibiotic usage in goats across Punjab.

[VIB-AMR-PP-07]

Panorama through The One Health Spectacle: Carbapenem Resistant *Acinetobacter* Infections (CRAB), are we creeping to exhaustion?

Dr Lenah Bashir, Dr Anjum Farhana
Department of Microbiology, Government Medical College Srinagar

BACKGROUND: Carbapenem resistant Acinetobacter baumannii (CRAB) has attained intercontinental opprobrium as a critical pathogen.

OBJECTIVES:

1. To evaluate wheather carbapenem minimum inhibitory concentration (MIC) Creep occurred in Acinetobacter baumannii isolates over a 5 year period.
2. To look into the burden of environmental resistome of the state health care settings.

STUDY DESIGN: A multicenter retrospective cohort study carried out in Post graduate department of Microbiology,Government medical college,Srinagar. Shri maharaja hari singh hospital and seven other associated hospitals over the city. Infective samples from all suspected patients of infectious etiology were collected through asceptic procedures.
Hospital environmental surveillance sampling was also carried out in the intensive care facilities The microbial growths obtained were examined by grams staining and used for the preparation of suspensions for test microbes by VITEK 2 compact automated microbiology bacterial identification and antimicrobial susceptibility system. These isolates were subjected to antimicrobial susceptibility by automated microbroth dilution by VITEK 2 C. The isolates were tested for three carbapenems i.e imipenem, meropenem & doripenem The cut off values were interpreted according to CLSI (Clinical and Laboratory Standards Institute).

RESULTS: A total of 4519 samples were received over the total study period.2651 samples were excluded owing to other infectious etiologies. Out of the rest 1867 infections due to gram negative agents,501 were ascribed to *Acinetobacter baumannii* complex as identified by automated bacterial identification & susceptibility system Vitek 2C.Also periodic environmental surveillance samples were included with the intention of typing by antibiograms of 50 environmental Acinetobacter baumannii spp. 47% (237/501) of the isolates were carbapenem sensitive(CSAB);51%(256/501)were carbapenem resistant(CRAB),1% (8/501)fell under the intermediate group. The MIC of CSAB were analysed for each fiscal year to observe a creep in the resulting values.

CONCLUSIONS: One-Health strategy and the concept of the antibiotic resistome are essential to reduce the escalating pandemic of AMR between the One-Health sectors.

[VIB-AMR-PP-08]

Studies on the presence of antibiotic resistance genes in *Escherichia coli* of poultry origin

Arpit, Chandra M*, Kaur G, Narang D and Arora A K

Department of Veterinary Microbiology, College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004

The present study was aimed to know about the prevelance of antibiotic resistance and their associated genes in the *E. coli* of poultry origin. Poultry faecal samples (n=50) were collected and were subjected to isolation of *E. coli* and antimicrobial susceptibility testing was performed. Further, the DNA from all the *E. coli* isolates was extracted and the isolates were subjected to PCR to identify the presence of *blaTEM*, *blaSHV*, *sull*, *dhfrV*, *cmlA*, *aadA*, *DHAM*, *MOXM*, *tetA* and *tetB* antibiotic resistance genes using published primers. A total of 35 *E. coli* isolates were isolated from poultry samples and were subjected to antibiotic sensitivity test using 10 antibiotics revealed that they were resistant to penicillin (100%), ampicillin/sulbactum (100%), erythromycin (94.28%), streptomycin (91.4%), tetracycline (60%), chloramphenicol (60%), trimethoprim (51.4%), co- trimoxazole (48.57%), gentamicin (8.5%) and colistin (8.5%). All of these isolates subjected to PCR for the identification of the presence of antibiotic resistance genes against *blaTEM*, *blaSHV*, *DHAM*, *MOXM*, *sul1*, *dhfrV*, *aadA*, *tetA* and *tetB* revealed that only seven isolates were positive for *blaTEM*, nine for *sulI*, four for *dhfrV*, 11 for *aadA* and *cmlA* respectively and none for *blaSHV*, *DHAM*, *MOXM*, *tetA* and *tetB*. It was concluded from the present study that most of the faecal samples tested had *E. coli* that harboured antimicrobial resistance and thus we should seriously devise some strategies to reduce the prevalence of antimicrobial resistance in poultry.

[VIB-AMR-PP-09]

Antimicrobial Resistance Pattern of Methicillin Resistant *Staphylococcus aureus* from a tertiary care hospital in Jammu & Kashmir

Faheem U Din1*, Sabia Qureshi1, Anjum Farhana2, Leenah Bashir2, Nahid Nehvi2, Maliha Gulzar1, Zahid Kashoo1, Shaheen Farooq1, M Altaf Bhat1, Pervaiz Dar1, M.I Hussain1, Gulzar Badroo1, Mahrukh Hafiz1, Junaid Mehraj1

[1]*Division of Veterinary Microbiology & Immunology; SKUAST-K, J&K, India,*

[2]*Department of Microbiology, Government Medical College Srinagar*

Antimicrobial resistance (AMR), a silent pandemic, is expected to be a serious global threat to human health by 2040. An annual increase in resistance levels by 5–10% has been reported by ICMR due to an indiscriminate use of broad-spectrum antibiotics. In the present study *in vitro* resistance patterns and molecular screening for MRSA of *Staphylococcus aureus* isolates in clinical samples collected from Department of Microbiology, Government Medical College Srinagar was carried out between June 2022-June 2023. A total of 244 samples (blood, pus and other body fluids) were screened of which 84(34.4%) were identified as *S aureus* based on cultural, biochemical and molecular tests. All the isolates were screened for detection of methicillin resistant gene viz *mec*A and 62% (52) of total *S aureus* isolates were identified as *mec*A gene positive by PCR. *In vitro* antibiotic screening of *S aureus* isolates revealed 100% resistance of all the *S aureus* to penicillin, followed by levofloxicin 73% (62), tetracycline 63% (53), cotrimoxaxole 61% (51) and gentamycin 50% (42). All the *S. aureus* isolates were sensitive to linezolid and vancomycin followed by erythromycin 59.5% (50), clindamycin 53.5% (45), gentamycin 50% (42), and cotrimoxaxole 39% (33). Highest number, 78% (62) of MRSA were isolated from blood samples. The need of the hour is to to have proactive antimicrobial stewardship programs and one health approaches involving human, animal, and environmental health to lessen the effects of AMR to curb the rising magnitude of antimicrobial resistance.

[VIB-AMR-PP-10]

Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing Escherichia coli isolated from bovines in Jammu, India

Shikha D1*, Bhat MA2, Sharma I1, Paul A1, Badroo G2 and Tikoo M3

1 Department of Veterinary Microbiology, IIVER-Rohtak, Haryana, India
2 Department of Veterinary Microbiology, SKUAST-Kashmir, Suhama, India
3Department of Veterinary Microbiology and Animal Biotechnology T&R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

The emergence and spread of antimicrobial resistance genes and resistant bacteria do not recognize animal, human, or geographic boundaries. Addressing this threat requires a multidisciplinary approach involving One Health sectors. This is because antimicrobial agents used in veterinary medicine have been reported to be similar to those in human medicine. Extended-spectrum β-lactamase (ESBL) *E. coli* is a growing public health problem worldwide. The study was conducted to determine the occurrence of ESBL producing *E. coli* as well as the genetic diversity of ESBL and characterize their antimicrobial resistance and integrons in bovines. Out of 360 isolates, Resistance to cefotaxime and ceftazidime was observed in 94 (61.03%) and 60 isolates (38.96%) respectively, while 154 (42.77%) isolates showed resistance to both. Present study revealed that the total prevalence of ESBL producing *E. coli* in bovines of Jammu region is 42.77% and is reported for the first time in Jammu region. Out of 154 ESBL isolates, only 120 (77.92%) isolates carried the gene/s for $bla_{SHV,}$ bla_{TEM} and bla_{CTX-M}, four (3.33%) and 65 (54.16%) isolates carried bla_{TEM} and bla_{CTX-M} gene alone respectively and six (5.0%) isolates carried bla_{SHV}/bla_{TEM}/bla_{CTX-M}. Of the 116 bla_{CTX-M} positive isolates, 82 (70.68%) belonged to CTX-M-1 group, 09 (7.75%) belonged to CTX-M-9 group and 25 (21.55%) isolates carried both bla_{CTX-M} 1/bla_{CTX-M} 9. One representative amplicon of $bla_{SHV,}$ bla_{TEM} andbla_{CTX-M} were cloned in pGEM-T and pJET1.2 and got sequenced commercially. The sequence of bla_{CTX-M} was found to match with the database sequence of bla_{CTX-M} -15. However, bla_{SHV} gene sequence did not match 100% with the available database but showed 99.07% resemblance with $bla_{SHV-11.}$ Thus, bla_{SHV} seems to be new variant and $bla_{CTX-M-15}$ is the first report of its kind in India. Antimicrobial sensitivity test of 120 ESBL producing *E. coli* isolates showed multi drug resistance (MDR) against ampicillin (100%), cefexime (100%), neomycin (100%) followed by enrofloxacin (89.16%), amoxicillin/clavulanic acid (87.5%), aztreonam (81.60%), cefepime (81.60%), kanamycin (83.33%) and ceftriaxone (78.33%). Out of 120 MDR ESBL positive isolates, 52 (88.23%) isolates harbour *intI* 1 gene, while 2 (3.38%) isolates carried *intI* 2 gene. Five isolates, were found to harbour both *intI* 1 and *intI* 2.

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[VIB-AMR-PP-11]

Characterization of Extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from Poultry in Jammu, India

Sharma I1*, Bhat MA2, Shikha D1, Paul A1, Badroo G2 and Tikoo M3
1 *Department of Veterinary Microbiology, IIVER-Rohtak, Haryana, India*
2 Department of Veterinary Microbiology, SKUAST-Kashmir, Suhama, India
3Department of Veterinary Microbiology and Animal Biotechnology T&R cell, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra

The study was aimed at finding the prevelance of ESBL producing *E. coli* in poultry and to characterize ESBL producing *E. coli* with respect to bla_{TEM}, bla_{CTX-M} and bla_{SHV} gene. Out of total 200 faecal samples collected, 400 presumptive ESBL producing *E. coli* were isolated (2 from each sample). Of 400 isolates, 150 (37.5%) isolates were found to be resistant. Resistance to cefotaxime and ceftazidime was observed in 90 isolates (60.0%) and 60 isolates (40.0%), respectively and 70 (46.6%) isolates showed resistance to both. All the 150 isolates found to be positive in the screening test were confirmed as ESBL producers by phenotypic confirmatory test or Double disc synergy test. Present study revealed that the total prevelance of ESBL producing *E. coli* in poultry of Jammu region is 37.5% and is reported for the first time in Jammu region. All the isolates, which were declared as ESBL producers phenotypically, were tested for the presence of $bla_{TEM,}$ bla_{CTX-M} and bla_{SHV} genes by PCR. Out of 150 ESBL isolates, only 110 (73.2%) isolates carried the gene/s screened for bla_{TEM}, bla_{CTX-M} and bla_{SHV}. Out of 110 isolates, 30 (27.2%) isolates carried bla_{TEM} gene alone, 53 (48.1%) isolates carried bla_{CTX-M} gene alone, and 2 (1.81%) isolates carried bla_{SHV} only. One representative amplicon of bla_{TEM}, bla_{CTX-M} and bla_{SHV} genes were cloned in pTZ57R/T and got sequenced commercially. The sequence of bla_{TEM} matched with $bla_{TEM\text{-}1}$ and that of bla_{CTX-M} with the database of $bla_{CTX-M-15}$. However, bla_{SHV} gene sequence did not match 100% with the available database but showed 99.07% resemblance with bla_{SHV-11} with 5-point mutations and five amino acid replacements. Thus, bla_{SHV} seems to be a new variant and $bla_{CTX-M-15}$ is the first report of its kind in India.

[VIB-AMR-PP-12]

Study of Antimicrobial Sensitivity and Resistance Pattern of *Escherichia coli* Isolates from Drinking Water Sources of Jammu region

Wani Najimaana 1*, Kotwal S. K. 2 and Taku A.K. 2

**1. Division of Veterinary Public Health and Epidemiology, FVSc & A.H, SKUAST-K*
2 Division of Veterinary Public Health and Epidemiology, Khalsa College of Veterinary and Animal Sc.
3. Division of Veterinary Microbiology and Immunology, FVSc & A.H, R.S. Pura, SKUAST-Jammu-181102

The study enumerated the indicator organisms based faecal pollution of drinking water sources of Jammu region. The analysis was conducted to determine if the water fulfills the biological parameters of fitness for drinking in accordance with various regulatory bodies (WHO, 2008). To achieve the objectives 125 samples were collected from different drinking water sources of Jammu (Inputs to filtration plants, post filtrated water from these filtration plants and the households supplied by them, Tube Wells, filling stations and Water Sources of Livestock and Poultry). The *E. coli* isolates (57) recovered randomly from different drinking water sources were subjected to *in vitro* antibiotic sensitivity test against a panel of 17 antimicrobial agents carried out by Kirby Bauer disc diffusion method and the results were interpreted according to the standard procedures recommended by Clinical Laboratory Standards Institute (CLSI). The results were interpreted as sensitive, intermediate, or resistant.

Based on the CLSI interpretive standards for *E. coli* isolates, the isolates were found most sensitive to Cephalexin (92.9 per cent), Ciprofloxacin (91.22 per cent), Amikacin (91.22) while the sensitivity to Chloramphenicol, Streptomycin and Nalidixic acid was 87.09 per cent. Highest intermediate pattern (14.03 per cent) was recorded for Norfloxacin, Levofloxacin and Polymixin B. Resistance to Ampicillin was 89.4 per cent followed by Lincomycin (85.41) and Amoxycillin (77.19).

Key words: *Antibiogram*, Drinking Water, *E. coli*, Jammu

Session IV
ISVIB Award Presentation

ISVIB – IVRI Mukteswar Albert Linghard Memorial Award

[VIB-ALMA-OP-01]

Multi-antigenic recombinant Leptospira outer-membrane proteins approach for detection of anti-leptospiral antibodies in latex agglutination test for rapid serodiagnosis

Prajakta Prashant Bokade, K. Vinod Kumar, Archana Pal, M. Nagalingam, B. R. Shome, and Baldev R. Gulati. V. Balamurugan

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Post Box No. 6450, Yelahanka, Bengaluru-560 064

The study describes the expression and evaluation of recombinant proteins of *Leptospira* outer membrane in multi-antigenic approach, as coated latex bead antigens in a latex agglutination test (LAT) for the detection of anti-leptospiral antibodies in the sera of bovine. The recombinant proteins were constructed by amplifying the specific protein coding genes, which lack the signal peptide coding gene sequences, from pathogenic *L. interrogans* serovars using PCR. The amplicons were cloned separately into a pETite N-His Kan vector, and the expressed recombinant 6x histidine-tagged chimeric proteins were purified using Ni-NTA affinity chromatography under denaturation followed by renaturation methods. The purified recombinant proteins were characterized by SDS-PAGE and immunoblot, which confirmed their *Leptospira*-specificity. Furthermore, sensitized latex beads coated with the individual recombinant proteins were evaluated as a diagnostic antigen in LAT in multi-antigenic approach. The developed LAT was assessed for their ability to detect pathogenic anti-leptospiral antibodies using known Microscopic Agglutination Test (MAT) positive (n=82) and negative (n=56) sera. The multi-recombinant proteins based-LAT exhibited a relative diagnostic sensitivity of 89.02% and diagnostic specificity of 85.71%, and accuracy of 87.68%, respectively, with very good agreement of Cohen's kappa value of 0.75 against the gold standard serological test, MAT. Therefore, this rapid point-of-care test, which uses a combination of recombinant proteins, is the first of its kind. After extensive evaluation, it could be used as a preliminary screening test for the detection of anti-leptospiral antibodies or complemented by other diagnostics for the serodiagnosis of leptospirosis in humans and animals.

Keywords: Leptospira, Recombinant Proteins/ Chimeric proteins, multi-antigenic approaches, Latex Agglutination Test, Serodiagnosis

ISVIB Midcareer scientist award

[VIB-MSA-OP-01]

Chandipura virus nucleoprotein interact with heat shock cognate 71 and actin proteins in Neuro-2a cells

Vishal K Kavathekar, Susmit Sambhare, B. Anukumar

ICMR-National Institute of Virology (Kerala unit), Makki Junction, Kuravanthodu, Alappuzha, Kerala

To complete their lifecycle, viruses engage in interactions with several cellular proteins. It is conceivable that host proteins involved in the viral lifecycle will make good therapeutic targets. We reported that the cellular vimentin protein interacts with the glycoprotein (G protein) of the Chandipura virus. The cellular proteins that interact with the nucleoprotein (N) of the Chandipura virus were identified in this investigation using the recombinant nucleoprotein as bait. Protein pull-down experiments, co-immuno-precipitation, confocal microscopy, and bioinformatics techniques were used to find and confirm the interacting proteins. About 10 proteins that interact with N proteins were found by the investigation. HSC71 and actin were two potential targets were further validated. The study comes to the conclusion that the CHPV N protein interacts with HSC71 and actin. These interactions could mitigate the consequences of misfolded proteins produced by error-prone viral polymerase.

[VIB-MSA-OP-02]

Diagnostic application of the recombinant Leptospira outer-membrane chimeric protein(s) in latex agglutination test for rapid detection of anti-leptospiral antibodies

V. Balamurugan*, Prajakta Prashant Bokade, K. Vinod Kumar, S. Sowjanya Kumari, Archana Pal, Suhail Ashraf, M. Nagalingam, B. R. Shome and B.R. Gulati
ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Post Box No. 6450, Yelahanka, Bengaluru-560 064

The study describes the expression and evaluation of two recombinant chimeric proteins of Leptospira outer membrane, rLsa27-LipL32, and rLsa27-Loa22, as coated latex bead antigens in a latex agglutination test (LAT) for the detection of anti-leptospiral antibodies in the sera of humans and animals. The chimeric proteins were constructed by amplifying the Lsa27-LipL32 and Lsa27-Loa22 genes, which lack the signal peptide coding gene sequences, from pathogenic L.interrogans serovars using PCR (~1433 bp and 1191 bp, respectively). The amplicons were cloned separately into a pETite N-His Kan vector, and the expressed recombinant 6x histidine-tagged chimeric proteins were purified using Ni-NTA affinity chromatography under denaturation followed by renaturation methods. The purified chimeric proteins were characterized by SDSPAGE and immunoblot, which confirmed their Leptospira-specificity and an MW of ~55 kDa and ~45 kDa for rLsa27-LipL32 and rLsa27-Loa22, respectively. Furthermore, sensitized latex beads coated with the recombinant chimeric proteins were evaluated as a diagnostic antigen in LATs. The developed LATs were assessed for their ability to detect pathogenic anti-leptospiral antibodies using known Microscopic Agglutination Test (MAT) positive (n=150) and negative (n=122) sera. The rLsa27-LipL32 and rLsa27-Loa22-LATs exhibited a relative diagnostic sensitivity of 98.7% and 88.0%, diagnostic specificity of 94.3% and 98.4%, and accuracy of 96.7% and 92.7%, respectively, with an excellent agreement of Cohen's kappa value of 0.93 and 0.85, respectively, against the gold standard serological test, MAT. Therefore, this rapid point-of-care test, which uses a combination of recombinant chimeric proteins, is the first of its kind. After extensive evaluation, it could be used as a preliminary screening test for the detection of anti-leptospiral antibodies or complemented by other diagnostics for the serodiagnosis of leptospirosis in humans and animals.

Keywords: Leptospira, Recombinant Chimeric Proteins, Latex Agglutination Test, Serodiagnosis

[VIB-MSA-OP-03]

Evaluation of cross-protection against foot-and-mouth disease viruses of serotype A with different relative homology using monovalent or bivalent vaccines

Madhanmohan Muthukrishnan a, S. B. Nagendrakumar a, S. Yuvaraj a, David James Paton b, V. A. Srinivasana and Satya Parida b

a *Foot-and-Mouth Disease Virus Laboratory, Research and Development Centre, Indian Immunologicals Limited, Rakshapuram, Gachibowli Post, Hyderabad – 500032, India*
b Pirbright Laboratory, Institute for Animal Health, Ash Road, Woking, Surrey GU240NF, United Kingdom

Serology is used to predict vaccine induced protection against challenge with a heterologous strain of the same serotype of foot-and-mouth disease virus (FMDV). To evaluate the efficacy of the existing vaccine strains against an antigenicaly variant strain we compared the protection afforded to cattle vaccinated with the a type a Indian vaccine strain A IND 40/00 or a candidate vaccine strain A APS 66/05 against challenge with either a homologous (A IND 40/00 or A APS 66/05) or a heterologous strain (A RAJ 21/96). Serology by virus neutralization test (VNT) using antiserum raised against the two vaccine strains predicted that there would be no cross protection against such a challenge. Three sets of experiments were carried out to study the efficacy of an existing vaccine strain to protect against an emerging heterologous and variant virus. The 50% protective dose values for the monovalent A IND 40/00 vaccine against using both homologous (A IND 40/00) and heterologous challenge (A RAJ 21/96) were found to be 7.94 and 2.00 respectively and PD50 values for the monovalent A APS 66/05 vaccine against using both homologous (A APS 66/05) and heterologous challenge (A RAJ 21/96) were found to be 7.94 and 18.02 respectively. A APS 66/05 vaccine was found to be effective even at lower antigen payloads when compared to A IND 40/00 vaccine. The results showed that protection against heterologous challenge for serotype A depends not only on the high potent vaccines but also on the strains used to prepare the high potent vaccines. The results also suggested that combination of two type A vaccine strain (A/IND/7/82+A/IND/17/82, A/IND/7/82+A/APS/66/05 and A/IND/17/82+A/APS/66/05) increases the spectrum (r1 value) against the heterologous virus type A virus (A/RAJ/21/96).

[VIB-MSA-OP-04]

Studies on Canine Parvovirus; A gastroenteritis virus posing great threat to dogs

Gurpreet Kaur

Associate Professor, Department of Veterinary Microbiology, COVS, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141001

Canine Parvovirus (CPV) causing hemorrhagic gastroenteritis in dogs is much prevalent throughout India and worldwide. The antigenic types of CPV prevalent worldwide are CPV 2, CPV 2a, CPV 2b and CPV 2c. One of the objectives of the study was to study the prevalence of CPV and its antigenic types. For this, rectal swabs were collected from dogs suspected of CPV. It was observed that per cent positivity was 73.45% (202/275) in Punjab, 80.55% (29/36) in Assam, 54.54% (6/11) in M.P., 93.75% (15/16) in Haryana, 66.66% (4/6) in Chandigarh, 74.28% (26/35) in Delhi and 16.66% (1/6) in Jammu by Nested PCR. It was observed that CPV positive cases were observed in vaccinated dogs 33.09% (94/284). Further sequence analysis of positive samples revealed that samples formed similar clad with CPV 2a isolates and separate clad when compared with CPV 2b and CPV 2c. The antigenic typing by RealTime PCR also indicated more of samples positive for CPV 2a antigenic type of virus followed by CPV 2 and CPV 2b. Fourteen isolates of CPV were obtained out of which three isolates were from dogs having history of vaccination for CPV. To study the cross neutralization among CPV types, hyperimmune serum was raised in rabbits against two CPV types viz. CPV 2a and CPV 2b. This serum was used in cross neutralization assay up to the dilution of 1:16384. The titre of serum 2a at which it can neutralize virus CPV 2b was 4096 and the titre of the serum 2b at which it can neutralize virus CPV 2a was 2048. The titre of both the serum for the homologous virus type is higher i.e. 8192. When the in vitro cross neutralization assay was done for CPV types (CPV 2a and CPV 2b) and the vaccine strain (CPV 2) it was observed that the titre of the serum CPV 2 at which it could neutralize virus CPV 2a and CPV 2b is 2048. The titre of serum 2a at which it can neutralize virus CPV 2 is 4096 and the titre of the serum 2b at which it can neutralize virus CPV 2 is 4096. The titre of the serum CPV 2 for the homologous virus type is higher i.e. 8192.

[VIB-MSA-OP-05]

Exploring the Impact of Xanthosine on Stem Cells: Insights into Mechanisms and Potential Applications in Milk Production and Regenerative Medicine

Ratan K. Choudhary* and Shanti Choudhary
*College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana – 141004, Punjab, INDIA * Corresponding author's Email: vetdrrkc@gmail.com*

One potential role of xanthosine (naturally occurring nucleoside) is its ability to enhance stem cell proliferation. It has been shown to promote the growth and expansion of various types of stem cells, including mesenchymal stem cells and neural stem cells. By stimulating cell division, xanthosine may contribute to increasing the population of stem cells, which is important for their therapeutic potential. This study aimed to understand the mechanisms of xanthosine action on the mammary tissue, stem cell population, milk production potential, and regenerative medicine. We used primiparous Beetle goats (n = 15) and conducted two experiments using a half-udder design in which one gland was the treatment and the contra-later gland (Left or right) was the control. Thus, two mammary glands of a goat were the experimental unit- treatment and control glands. Five days after kidding, 20 mL of 10 mM of xanthosine (plus 1 mL injected into mammary parenchyma) was intramammary infused (2 x a day for three days) after milking in the treatment (TRT) glands while the remaining gland served as control (CON) with no infusion. Mammary tissue was collected using surgical biopsies on the 10th day for transcriptome and proteome profiling. Milking of each TRT and CON glands was done individually for 13 weeks and milk composition was evaluated. Finally, we examined the healing potential of xanthosine on mammary wounds and the functional restoration of biopsied glands in the subsequent lactation. RNA sequencing analysis revealed differentially expressed genes involved in metabolic pathways and the PPAR signaling pathway. These findings were validated using RT-qPCR. Proteomics analysis identified proteins associated with energy production, nucleic acid metabolism, and cell death and survival. The results indicated that xanthosine treatment could promote cell survival and growth in the mammary tissue. Overall, the study demonstrated that xanthosine infusion had variable effects on milk production in goats, with a modest increase observed in some cases. The study also showed that xanthosine administration promoted wound healing and functional restoration of the mammary gland after surgical tissue biopsy. The practical utility of our research was demonstrated in 1) the Impacts of xanthosine on wound healing after surgical biopsy of mammary glands, 2) the difference in milk production between 2 treated and control glands of the same animals, and 3) opening the possibility of in vivo manipulation of the expansion of stem cells and behavior that could have implications in tissue engineering and lactation physiology.

Keywords: mammary stem cell, xanthosine, transcriptomics, proteomics, in vivo expansion

[VIB-MSA-OP-06]

Emerging approaches in livestock health: From disease pathogenesis to vaccine development

Dr. Syed Mudasir Andrabi
Professor & Head, Division of Animal Biotechnology, Faculty of Veterinary Sciences, SKUAST- Kashmir

A comprehensive omics approach was used to explore the genetic potential of local cattle for milking potential and disease resistance against mammary gland infections. RNA sequencing revealed high endopeptidase and antioxidant activity in the milk of Kashmiri cattle. A comparative milk proteome study revealed the presence of a bioactive peptide/enzyme. Such properties will lead to the development of certain functional dairy products from the milk of Kashmiri cattle. Studies based on SNP analysis showed that Kashmiri cattle have resistance to mammary gland infections. In-house technology for isolating and culturing primary mammary epithelial cells (pMECs) from caprine and bovine raw milk has been developed. The technology has been patented (Patent numbered: 201911013320). Goats have been used as a model of mammary gland infections that led to the identification of scavenger receptor B1 (SCARB1), a novel molecule which acts as a main participant in host defence and its function in antibacterial advances to check mammary gland infections.

Additionally, I have been working on disease resistance in poultry for salmonellosis. Using genomic tools, genetic markers associated with salmonella resistance in local poultry germplasm (*Kashmir favorella*) has been identified. Understanding the function, regulation, and response of different immune-related genes and pathways provided new insights and a platform for the development of vaccines against salmonellosis in poultry. The data generated
led to the development of the first Poultry Infection Database in the country https://skuastk.org/pif/index.html. A recombinant vaccine for Salmonella infection in chicken has been developed. The vaccine is currently being tested in the field.

[VIB-MSA-OP-07]

Molecular Investigation of Conglutinin protein as a marker of disease resistance in Indigenous Pantja goat

Dr. Aman Kamboj
Assistant professor (Animal Biotechnology),
College of Veterinary and Animal Sciences,
G. B. Pant University of Agriculture and Technology, Pantnagar -263145 (Uttarakhand),

Innate immunity is an important arm of the immune system which helps in the recognition of various pathogen-associated molecular patterns (PAMP) through pattern recognition receptors (PRRs). The research pertaining to the elucidation of innate components of the immune system in livestock species is gaining pace. Conglutinin is a calcium-dependent collagenous C-type lectin, that acts as soluble PRR which helps in the recognition of pathogens. Initially recognized in bovines, Conglutinin is now recognized in other domestic and wild herbivores. Conglutinin is primarily synthesized in the liver. The expression level of Conglutinin can be directly correlated with the disease -resistance trait of animals. Pantja is a newly registered goat breed recognized for its similarity with deer in their morphological characteristics and is commonly found in the hot and humid climate (Tarai region) of Uttarakhand. The Pantja goats are hardy and resistant to most diseases and infections. The present study is aimed at the molecular investigation of the Conglutinin protein and its role in disease resistance in indigenous Pantja goats. The expression of Conglutinin was seen in liver tissue and blood and the full-length gene encoding Conglutinin (CGN1) was amplified. The neck and carbohydrate recognition domain (NCRD) of Conglutinin was cloned, sequenced, and analyzed for its structure. A change in single nucleotide and single amino acid at position 76 was found when compared with the sequence of another goat breed suggesting the uniqueness of Pantja in the context of disease resistance. On secondary structure prediction by Phyre2 server for protein modelling, prediction and analysis it was found that there were equal presence of alpha helix and beta sheets with only 1% disordered structure. The expression of Conglutinin was also reported in blood so the quantification in blood using qPCR can be a good indicative of immune status of the animal.

ISVIB GADVASU Woman Scientist Award

[VIB-WSA-OP-01]

Antibiotic resistance, virulence gene profiling and molecular typing of MRSA isolated from sheep, goats, and pigs in Punjab

Sundus Gazal1, Paviter Kaur1, Jasbir Bedi2, Jaswinder Singh3, A. K. Arora1 and T. S. Rai1
1Department of Veterinary Microbiology, GADVASU, Ludhiana, 2Centre for One Health, GADVASU, Ludhiana, 3Department of Veterinary and Animal Husbandry Extension, GADVASU, Ludhiana

Methicillin resistant *Staphylococcus aureus* strains cause a wide range of infections in domesticated livestock as well as in companion animals owing to the acquisition of multiple antibiotic resistance genes and virulence genes which increase the pathogenicity of these strains. The present study was aimed at determining the prevalence, antibiotic and virulence gene profile as well as molecular characterization of the MRSA isolates by SCC*mec* typing in Punjab, India. A total of 650 samples including nasal swabs from sheep, goat and pigs, caprine milk samples and human hand swab samples were collected from different districts of Punjab and were processed for isolation of *S. aureus*. 45 *S. aureus* isolates were obtained which were further subjected to MALDI-TOF for confirmation. The confirmed *S. aureus* isolates were subjected to PCR for molecular detection of methicillin resistance gene and all the MRSA isolates obtained were studied for their culture sensitivity, antibiotic resistance and virulence gene profile. A total of 15 MRSA isolates were obtained which were found to be 100% resistant to cefoxitin and penicillin, followed by ampicillin. Among the various antibiotic resistance genes tested, the *tet*K gene was found to be the most prevalent, followed by *aac-aph*D gene, *erm*C gene, *erm*B and *bla*Z gene. Among the virulence genes tested, the *eno* gene which encodes for enolase enzyme was the predominant virulence gene, followed by *luk*-PV gene, *coa* gene and tsst-1 gene. A total of five SCC*mec* types viz., SCC*mec* type IVd, Type III, Type I, Type IVc and Type V were observed with SCC*mec* Type IVd being the predominantly detected SCC*mec* type.

Keywords: MRSA, AMR, Virulence genes, SCC*mec* typing

[VIB-WSA-OP-02]

Molecular Epidemiology of *Campylobacter* species isolated from samples of sheep from Kashmir

Maliha Gulzar*, 1Mahrukh Hafiz1, Sabia Qureshi*1, Zahid Kashoo1, Shaheen Farooq1. M. Altaf Bhat1, Pervaiz Dar1, Gulzar Badroo1, Muddasir Sharief Banday2, M.I Hussain,1, Faheem u Din1, Junaid Mehraj Lone1

1Campylobacter Lab; Division of Veterinary Microbiology and Immunology SKUAST-K, FVSc& A H, Shuhama, 19006

2 Department of Clinical Pharmacology, Sheri Kashmir Institute of Medical Sciences, Soura, Srinagar

Campylobacter bacteria have long been associated with abortions in sheep, with *C. fetus* subsp. *fetus* historically being the primary causative agent. However, there has been a notable shift in recent years, with *C. jejuni* now replacing *C. fetus* subsp. *fetus* as the predominant cause of sheep abortion. Apart from its involvement in abortion cases, *C. jejuni* is also recognized as one of the leading causes of bacterial foodborne illnesses, posing a significant public health concern. In the context of Kashmir, limited information exists regarding *Campylobacter*-associated abortions and its prevalence in mutton. To address this, in the current study samples were collected and it was detected that out of 200 sheep vaginal swab samples, 7 (3.5%) were found positive for *C. fetus* subsp. *fetus*, while 3 (1.5%) tested positive for *C. jejuni*. Among 200 rectal swab samples, 4 (2.8%) were positive for *C. jejuni.* When investigating abortion cases where *Brucella* infection was ruled out, 8 out of 25 vaginal swab samples (32%) and 1 out of 8 abomasal content samples from aborted sheep fetuses (12.5%) were positive for *C. fetus* subsp. *fetus.* Additionally, *C. jejuni* was isolated from 1 out of the 25 vaginal swab samples (4%) collected from abortion cases. Finally, 3% of the 100 mutton samples were positive for *C. jejuni.*

This study sheds light on the prevalence of specific *Campylobacter* species associated with abortions in sheep and their presence in mutton in the Kashmir region. Monitoring and understanding these trends are crucial for implementing appropriate measures to prevent and control *Campylobacter*related infections and their potential impact on public health.

Keywords: *Campylobacter*, Abortion, *C. fetus subsp fetus, C. jejuni*

[VIB-WSA-OP-03]

Therapeutic efficacy of Copper Nano particles in *Staphylococcus aureus* induced rat mastitis model

Syed Taifa, AmatulMuhee
Division of Veterinary Clinical Complex, Faculty of Veterinary Sciences and Animal Husbandry, Srinagar, Jammu and Kashmir, 190006. India

A study was conducted with the objective of devising an alternative, non-antibiotic and economically viable treatment for bovine mastitis. Copper nano particles (CuNps) 25nm particle size were selected for the purpose and experimental study was conducted on 24 lactating wistar rats after proper approval from the Institutional Animal Ethics Committee (IAEC). The therapeutic efficacy of CuNps was evaluated in *Staphylococcus aureus* induced rat mastitis model. Different concentrations of CuNps in deionized water were prepared (100ug/mL, 50ug/mL, 25ug/mL, 12.5ug/mL and 6.25ug/mL). *In vitro* anti-bacterial activity was performed on pure culture of *S. aureus* and *in vitro* cytotoxicity of CuNps at these concentrations was checked in fibroblast cell line. The concentration of 6.25ug/mL was selected for use as intrmammary treatment of *S. aureus* induced mastitis in rats as this concentration showed significant zone of inhibition through *in vitro* sensitivity test and minimal cell cytotoxicity on fibriblast cell lines. The rats were divided into four groups of 6 rats each. Group 1 (Gr I) served as healthy control in which deionized water was infused intra mammary while as in Gr II, III and IV, mastitis was induced intra mammary with culture of *S. aureus.* Gr II served as mastitis control group in which no medication was given. In Gr III rats, CuNps were administered intra mammary for five days. Gr IV rats were treated with injection gentamycin intramammaty for 5 days selected on the basis of antibiotic sensitivity test. Gr I and Gr II rats were sacrificed 48 hours after infusion of deionized water and after appearance of clinical signs in Gr II. Gr III and IV were sacrificed on day 6 after the initiation of treatment. The therapeutic efficacy of CuNps and recovery of rats was evaluated on the basis of clinical signs, mammary gland weights, and bacterial load of glands, oxidative stress indices (OSI) and histopathology of mammary glands. The results confirmed that treatment with intramammary copper nanoparticles at a concentration of 6.25ug/mL showed early recovery, reduced bacterial loads, reduced oxidative stress indices and marked reduction in histopathological changes than treatment with intramammary gentamicin in *Staph aureus* induced rat mastitis model. On the basis of safety and efficacy *in vitro* as well as in rat mastitis model, copper nanoparticles may provide a potential alternative for treatment of bovine mastitis.

ISVIB Young scientist award

[VIB-YSA-OP-01]

Detection of Mycobacterium tuberculosis complex organisms in cases of lymphadenopathies in dogs

Prerna Tikute1, Deepti Narang1* Mudit Chandra1, Sujata Turkar2 and Kuldip Gupta3

1 Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India.
2 Department of Veterinary Medicine, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India
3 Department of Veterinary Pathology., College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India

Mycobacterium is connected to Human immunosuppressive diseases, including HIV-AIDS and may pose a zoonotic threat. The likelihood of these organisms spreading between canines and humans also rises as a result of the increased interaction between the two species. The organism causes lymphadenopathy in children, respiratory infection in adults, and generalized infection in individuals whose immune system is weakened (e.g. immunosuppression)**.** The study was planned to demonstrate the occurrence of Mycobacterium tuberculosis complex organisms and other Mycobacteria associated with lymphadenopathy cases. A total of 123 samples (100 lymph node aspirates, 15 lymph node tissues and 8 blood samples) from 83 dogs suspected of lymphadenitis accompanied with gastroenteritis, chronic skin infections, immunosuppression, chronic pulmonary diseases and other chronic undiagnosed diseases were studied. The samples were collected from these suspected dogs and further processed for cytological (Leishman stain) and microscopic examination by Ziehl-Neelsen staining for the presence of target organisms. Following the decontamination procedure, the lymph node aspirates and lymph node tissue samples were inoculated into Middlebrook 7H11 media for up to 8 weeks. The aspirated material was also directly used for molecular detection by triplex Nested Polymerase Chain Reaction (nPCR) assay. A cytological study revealed pyogranulomatous inflammation of the lymph node tissue. Impression smear from lymph node tissues displayed the presence of acid-fast organisms. Out of 83 cases of dogs, 5 dog cases were found to be positive for the Mycobacterium tuberculosis complex (MTB complex). Mycobacterium tuberculosis complex is the underestimated bacteria which is one of the main causative agents of lymphadenitis in animals.

Keywords: Lymph node aspirate, Lymph node tissue, Mycobacterium tuberculosis complex, Nested PCR.

ISVIB Student Travel grant award

[VIB-STGA-OP-01]

Antiviral activity of traditional medicinal plants against SARS-CoV-2 infection

Vimal K Maurya, Shailendra K Saxena*
Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a zoonotic virus that may transmit between humans and animals. As more animals are found to be infected with the COVID-19 virus, it becomes evident that a One Health approach is essential for dealing with emerging disease risks that affect both humans and animals. Approximately 60% of new infectious diseases identified globally are caused by animals, both wild and domestic. Over the previous three decades, over 30 new human infections have been discovered, 75% of which originated in animals. SARS-CoV-2 is the etiological agent of COVID-19 and is responsible for more than 768 million confirmed cases, including 6.9 million deaths globally. Therefore, this study was planned to investigate the antiviral role of the active constituents against spike glycoprotein of SARS-CoV-2 as well as its host ACE2 receptor. Structure-based drug design approach has been used to elucidate the antiviral activity of active constituents present in traditional medicinal plants. Further, parameters like drug-likeness, pharmacokinetics, and toxicity were determined to ensure the safety and efficacy of active constituents. Gene network analysis was performed to investigate the pathways altered during COVID-19. The prediction of drug-target interactions was performed to discover novel targets for active constituents. The results suggested that amarogentin, eufoliatorin, α-amyrin, caesalpinins, kutkin, β-sitosterol, and belladonnine are the top-ranked molecules that have the highest affinity towards both the spike glycoprotein and ACE2. Most active constituents have passed the criteria of drug-likeness and demonstrated a good pharmacokinetic profile with minimum predicted toxicity level. Gene network analysis confirmed that G-protein coupled receptor, protein kinase B signaling, protein secretion, peptidyl-serine phosphorylation, nuclear transport, apoptotic pathway, tumor necrosis factor, regulation of angiotensin level, positive regulation of ion transport, and membrane protein proteolysis were altered during COVID-19. The target prediction analysis revealed that most active constituents target the same pathways which are found to be altered during COVID-19. Collectively, our data encourage the use of active constituents as a potential therapy for COVID-19.

[VIB-STGA-OP-02]

One health approach to target Japanese encephalitis non-structural protein NS3 for the development of potential antiviral therapy

Swatantra Kumar, Shailendra K Saxena*

Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.

Japanese encephalitis virus (JEV) is the major cause of viral encephalitis in South-East Asia. JEV is maintained in a natural transmission cycle amongst mosquitoes and wading birds, while pigs act as an amplifying host. JEV-NS3 protein has been shown to be involved in transmission and neuropathogenesis via inducing neuronal cell death. Since, One Health concept involves interactions between people, animals, and the environment that may enhance the spread of diseases. Therefore, in the present study we have developed a hydroalcoholic formulation B200 and showed its antiviral efficacy against JEV infection. We have found that treatment with B200 increases neuronal cell survival by reducing JEV induced cytopathic effects which were evident from significant reduction in necrotic cell population by flow-cytometry analysis and caspase 3 and 8 enzymatic activities. B200 treatment was found to decrease the intracellular JEV level observed by significant reduction in JEV-FITC expression. Because microglia play a crucial role in JEV neuropathogenesis, we further investigated the anti-JEV effects of B200 on human microglia cells and elucidated the mechanism of action by performing whole-transcriptome sequencing. Gene expression analysis revealed that B200 reduces the pro-apoptotic and inflammatory gene expression observed by significant reduction in *BAD*, *BAX*, *CASP3*, *CASP8*, *IL1B*, and *CXCL10* and increase in *IL10* responsive gene expression. Interestingly, our molecular docking analysis revealed that B200 interacts with the crucial residues of NS3 protein involved in RNA unwinding and ATPase activity that was further confirmed by degradation of NS3 protein. Moreover, we have elucidated its Drug likeness, ADME (absorption, distribution, metabolism, and excretion), and toxicity analysis further suggests that molecules present in B200 crosses the blood-brain barrier, which is crucial for effective treatment of Japanese encephalitis (JE) as a One Health approach that may enhance the spread of diseases.

Session V
Zoonosis and One Health
(Oral)

[VIB-ZOH-IVL-01]

COVID-19: Learn to Live with Virus with One Health Approach

Shailendra K Saxena*

Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.

The COVID-19 pandemic serves as a persistent example of the devastation that zoonotic illness, or the transmission of viruses from animals to humans, may cause. Although it is understandable that there is a pressing need for vaccines and antivirals to treat SARS-CoV-2 infections, it is crucial to take advantage of this once-in-a-generation opportunity to comprehend how to live with this virus with one health approach and review the knowledge gaps. Hence, there is a need for a coordinated One Health approach to mitigate and address forthcoming pandemic risks for COVID-19, may be achieved by leading international policy organizations, including the Tripartite made up of the World Health Organization (WHO), the World Organization for Animal Health (OIE), and the Food and Agriculture Organization of the United Nations (FAO); the United Nations Environment Programme (UNEP); the World Bank; and others. So far, there is no specific treatment available for SARS-CoV-2 and the current treatment relies on supportive care of the infected patients. The specificity and efficacy of the variant monitoring system, as well as infectious preventive measures in each nation, are critical for efficient prevention and therapeutic management of newer variants of SARS-CoV-2.The most efficient way for COVID-19 protection and control has already been demonstrated via vaccination. Live attenuated vaccines, replicating and non-replicating viral vector vaccines, DNA/RNA vaccines, and protein-subunit vaccines are the major types of vaccines that are currently available for human use. In order to prevent SARS-CoV-2 infection, India has already achieved more than 1000 million vaccinations with at least a single dose. However, recurrent emergence of newer SARS-CoV-2 variants raises concerns that the newer variant might have increased transmissibility and infectivity, and escape immunity generated both by natural infections and vaccines in current use. Similar to flu, most of the COVID-19 infected individuals will recover on their own in a few days to two weeks. Perhaps we need to start thinking about designing vaccines against each variant of concern. Hence, greater understanding of the activities and needs of the One Health workforce during a pandemic response helps to pave the way for meaningful integration into coordinated and shared strategies for preventing, detecting, and responding to global public health emergencies like COVID-19.

[VIB-ZOH-IVL-02]

One Health approach for emerging zoonotic diseases with special reference to Kyasanur Forest disease (KFD) and Crimean Congo Hemorrhagic Fever (CCHF) in India

Md. Mudassar Chanda*, Bhimanagoud K and Sathish B Shivachandra

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, Karnataka.

One Health definitions vary, but. as per CDC, it is defined as "*One Health is an approach that recognizes that the health of people is closely connected to the health of animals and our shared environment"* (https://www.cdc.gov/onehealth).
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One Health' term was first used during emergence of severe acute respiratory disease (SARS) during the year 2003 (Monath et al., 2010). The majority of zoonotic diseases emerge from animal, especially from wildlife. The emergence of zoonotic diseases is mainly driven by increased anthropogenic changes in land use pattern, intensification in agriculture, urbanisation, international travel, and climate change. Hence, there is a need to adopt multi-disciplinary approach involving relevant stakeholders so that the effective prevention and control strategy can be developed. It is also important to have 'disease specific One Health' approach. Here, the example of two emerging tick borne zoonotic viral diseases namely Kyasanur Forest Disease (KFD) and Crimean Congo Hemorrhagic Fever (CCHF) are discussed to emphasise the importance of disease specific One Health approach.

KFD is a zoonotic viral disease caused by KFD virus belonging to the genus *Flavivirus,* and family '*Flaviviridae'*. It is about 40-65 nm in size with an icosahedral nucleocapsid. The single-stranded positive-sense RNA genome of the virus is 11Kb in length comprising three structural (C, M, and E) proteins and seven non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) proteins (Chinikar et al., 2010). The virus has been isolated in nature from man and monkeys and several species of ticks. The KFD virus is transmitted to humans by the bite of infected ticks and various *Haemaphysalis* ticks are involved in the transmission of this virus (Mourya and Yadav, 2016; Rathinam and Sidhik, 2022). Since its first report, KFD has been reported in five states of India (Fig. 1)

Congo hemorrhagic fever virus (CCHFV) (an arbovirus), a member of the family *Bunyaviridae* and genus *Nairovirus*. This family also includes genera *Orthobunyaviruses, Hantaviruses, Phlebovirus* and *Tospovirus*. In India, the first clinical case of CCHF was reported from a tertiary care hospital in Ahmadabad, Gujarat, in 2011 and sporadic reports from other states such as Rajasthan, Uttar Pradesh and Kerala (Fig. 2). CCHF virus is commonly transmitted to humans through bites of infected *Hyalomma* ticks or via direct contact with blood and tissues of viremic livestock and infected patients (Chinikar et al., 2010; Spengler et al., 2019).

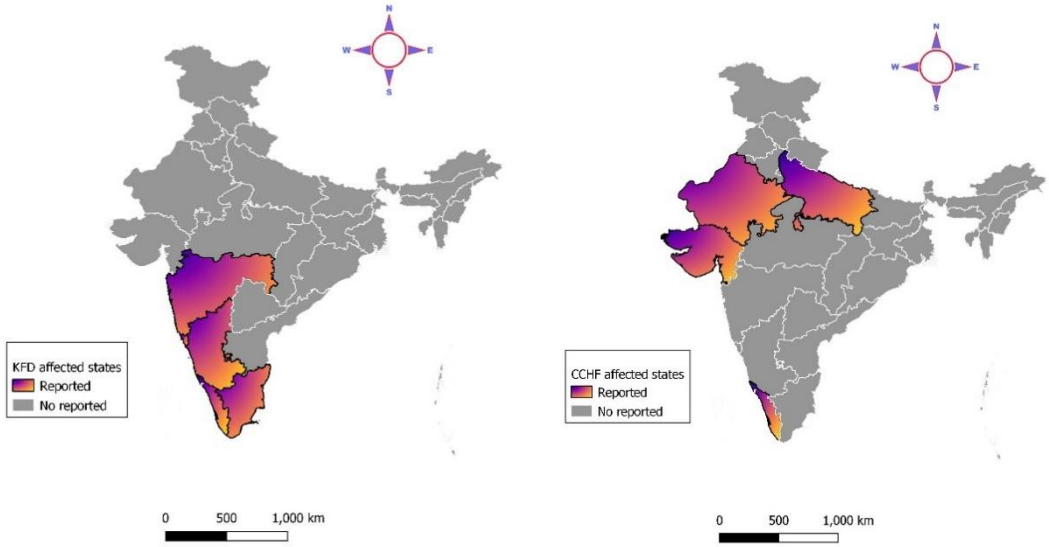

Figure 1: Maps showing KFD and CCHF affected states

One Health Approach

One Health approach can be focussed in 3 broad areas;

- Research and Surveillance priorities
- Development of policy guidelines
- Involvement of relevant stakeholders

Research and Surveillance priorities

The research priority of zoonotic disease depends on the current gaps in research and must avoid duplication of efforts being carried out. The research priority can be development of sensitive diagnostics, effective vaccines, molecular epidemiology, or host-pathogen interaction studies. The other aspect can be development of surveillance strategy for the disease with involvement of different/allied sectors.

In case of KFD, there are many research gaps such as identification of reservoirs of the disease, role of different tick species in transmission of the disease, development of serological assays for detection of antibodies against the virus in animals, efficacious vaccine etc. The surveillance can be targeted towards development of sentinel system and also to go for targeted surveillance in high-risk areas. In one of our studies, we adopted co-production approach for informing risk factors for our modelling study. We identified forest-plantation with high coverage of moist evergreen forest and plantation, indigenous cattle, low coverage of dry deciduous forest as important predictors and developed risk map for KFD in Shivamogga (Purse et al., 2020). There are several barriers to implement adaptive strategies for prevention of KFD such as lack of disease information and livelihood concerns with respect to avoiding forest visits(Asaaga et al., 2021).

In case of CCHF which is mainly reported from Gujarat with sporadic reports from Rajasthan, Uttar Pradesh and Kerala. There are many research gaps in the ecology and epidemiology of CCHF in India. There is need to work on risk assessment of the disease in other states of India. The role of reservoir hosts in maintenance and transmission of the disease is needed. Identification of ticks by both morphological and molecular methods requires huge number of trained human resources.

Development of policy guidelines

One Health approach is also required for development of policy guidelines for zoonotic diseases. The guidelines are needed on deciding on areas of vaccination and surveillance strategy based on risk mapping and development of long term prevention and control strategy. In addition, there is a need for capacity building programs on One Health.

There is need to have policy guidelines for systematic vector surveillance based on risk mapping and modelling for KFD and CCHF. These two diseases are restricted to few geographical areas and it is not known whether these two diseases can spread to other states of India. Capacity building programs on outbreak investigations, disease mapping and modelling will help to have field level Rapid Response Teams for early detection and prevention of these diseases (Fig. 2).

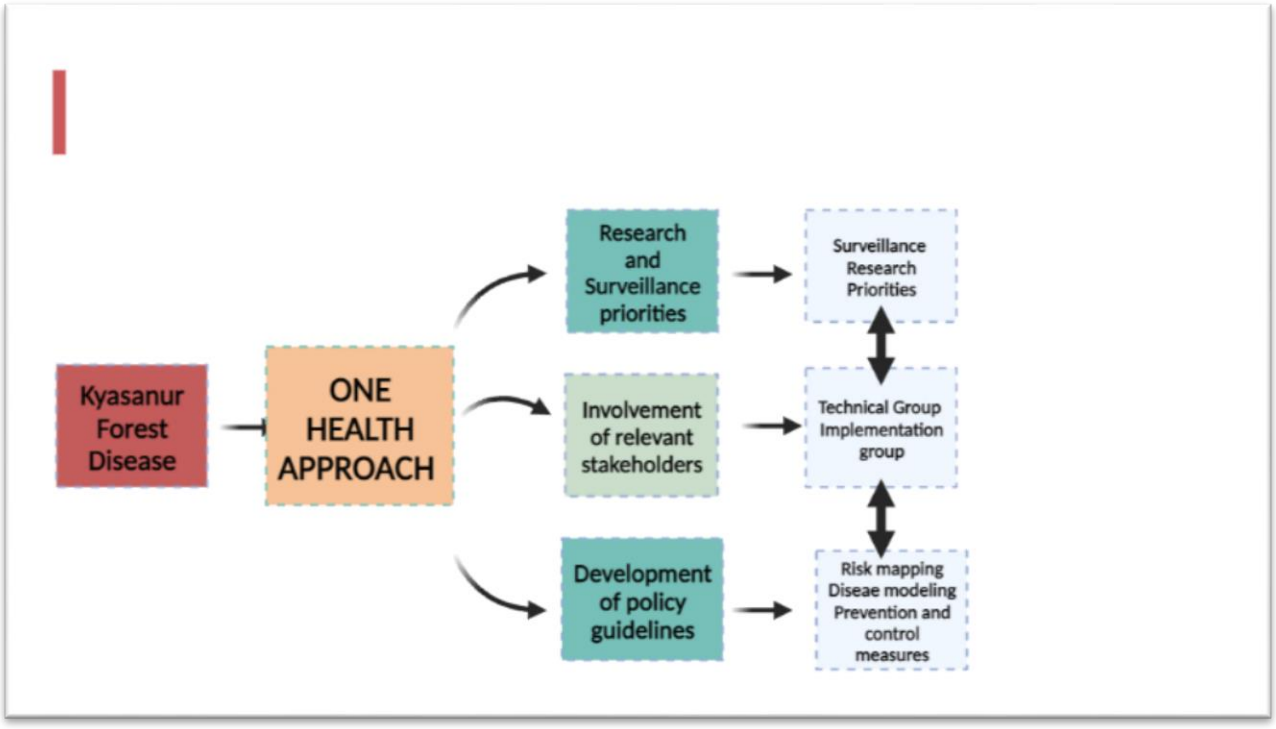

Figure 2: Components of One Health approach with KFD as an example.

Involvement of relevant stakeholders

For above two activities, we need to have relevant stakeholders. Stakeholders in One Health can be grouped into beneficiaries (animals, humans and the environment) and the organisations work to protect one health (Mazet et al., 2014). The involvement of different ministries is critical at all stage of One Health policy formulation and intervention. The formulation of One Health Policy should work both horizontally and vertically across the hierarchy. In addition, the One Health framework can have two components – technical team and implementation team

One Health team for developing of guidelines-technical team. In case of KFD for example, the technical team can comprise of representative from human health department, animal health department, environment, forest. The implementation team can comprise of other departments like municipal corporation, district commissioner, department of agriculture and any other relevant department. The department of agriculture is regularly involved in

vertebrate pest management and they can play a role of rodent control measures and other administrative departments can play a vital role in implementation of guidelines. Community engagement is important in the implementation of intervention strategies. Hence, the role of social scientist is very important at all stages of implementation of the policy guidelines.

[VIB-ZOH-IVL-03]

Zoonotic Disease in animals and One Health aspect

Minakshi Prasad[1], Kiran Bala[2], Basanti Brar[2]

[1]ICAR-NRCE Hisar; [2]Om Sterling Global University, Hisar

Zoonotic diseases impose a heavy burden on healthcare systems worldwide, particularly in underdeveloped nations. Various vertebrate animals such as birds, mammals and reptiles act as amplifiers or reservoirs for viral zoonoses. The transmission of these diseases is influenced by environmental factors, climate change, animal health and various human activities such as globalization, urbanization and travel. Infectious diseases that arise at the interface between humans, animals, environment, including zoonotic diseases, vector-borne diseases, and food/waterborne diseases, continue to pose significant risks to both animals and humans resulting in considerable mortality and morbidity. It is estimated that out of the 1,400 infectious diseases known to affect humans, 60% originate from animals. Furthermore, 75% of the emerging infectious diseases worldwide are zoonotic in nature. The term "One Health (OH)" refers to a collaborative effort among experts from various fields, including public health, healthcare, forestry, veterinary, environmental, and other related disciplines at the local, national and global levels. The goal of this collaboration is to promote optimal health for humans, animals and the environment. Although the concept of One Health is still in its early stages in India, it is gaining increasing importance. The Government of India has initiated several measures to address pressing issues such as antimicrobial resistance, zoonotic diseases and food safety using the One Health approach. However, there are significant challenges in implementing this approach effectively. These challenges include the lack of a legal framework to support One Health implementation, inadequate coordination among governmental and private agencies, insufficient surveillance of animal diseases, limited data-sharing mechanisms across sectors, and budgetary constraints. One Health approach in India requires addressing the existing challenges through the establishment of a legal framework, improved coordination among agencies, enhanced surveillance systems, better data-sharing mechanisms and increased financial resources. These efforts will help in effectively combating the rise of zoonotic diseases and ensure the optimal health of humans, animals and the environment.

[VIB-ZOH-OP-01]

Determining the load of thermophilic *Campylobacter* in surface water bodies of Uttarakhand province of India through Real-time PCR

Iram Ansari[1], Maansi[1], Ajay Kumar Upadhyay[1] and **Aman Kamboj[2]***

[1]Department of Veterinary Public Health & Epidemiology;
[2]Veterinary Clinical Complex;
College of Veterinary and Animal Sciences;
G. B. Pant University of Agriculture and Technology, Pantnagar Uttarakhand, India

Emerging water-borne pathogens constitute a significant health hazard in both developed and developing nations. According to WHO, about 6.3% of deaths happen due to unsafe water, inadequate sanitation, and poor hygiene. Thermophilic *Campylobacter* spp., is a leading food-born zoonotic bacterial pathogen regarded as one of the leading causes of gastroenteritis in humans worldwide. Animal and poultry waste are the potential sources of water contamination, and reports of isolation of thermophilic *Campylobacter* from water bodies are also available. With the advent of molecular techniques like Polymerase Chain Reaction, it is quite convenient nowadays to detect the presence of pathogens in environmental samples with utmost specificity and in the minimum possible time. In the present study, the load of thermophilic *Campylobacter* in surface water bodies of Uttarakhand province of India was determined through Real-time PCR using absolute quantification with a standard curve. It was concluded that all collected samples were positive for thermophilic *Campylobacter* with a detection range from 7.7×10^2 to 2.7×10^5 and the highest load was found from district Nainital. The work generates an important insight into the context of ensuring one-health.

[VIB-ZOH-OP-02]

Study of *Salmonella* Typhimurium isolates from hospital wastewater based on multidrugresistance profiles, multilocus sequence typing, and efflux pump activity

Manoj Kumawat[#], Bilkees Nabi, Poonam Sharma, Namrata Pal, Devojit Kumar Sarma, SwastiShubham, Rajnarayan R Tiwari, Samradhi Singh, Manoj Kumar

Department of Microbiology, ICMR- National Institute for Research inEnvironmental Health, Bhopal, Madhya Pradesh, India

Salmonella enterica serovar Typhimurium is emerging as a major cause of gastroenteritis and mortality. The widespread use of antibiotics has contributed to the development of natural resistance in *S.* Typhimurium. The objective of this research is to identify and examine multi- drug-resistant (MDR) Salmonella strains obtained from sewage samples collected from hospitals in Bhopal City, central India. The MDR isolates were subjected to molecular identification, analysis of antimicrobial resistance patterns, multi-locus sequence typing, and evaluation of efflux pump activity. Specific genes (*hilA, stn, invA, typh,* and *iroB*) were utilized to confirm the presence of *S.* Typhimurium isolates. Antimicrobial resistance profiling using the Kirby-Bauer method was conducted using 20 antibiotics. Multi-locus sequence typing, which involved the examination of seven housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA,* and *thr*), confirmed the presence of *S.* Typhimurium. Out of the five strains studied, only four were identified as *S.* Typhimurium based on MLST analysis. Efflux pump activity was assessed using the ethidium bromide (EtBr) cartwheel test. Among the 160 isolates, 38 were provisionally identified as *S.* Typhimurium through biochemical characterization, and only five MDR Salmonella strains were selected for their high resistance to multiple antibiotics. Evaluation of efflux pump activity indicated that four out of the five MDR isolates exhibited significant efflux activity by not retaining EtBr inside the cells. Furthermore, the isolated strains demonstrated a distinct correlation between their antimicrobial phenotypes and genotypes. The findings of this study enhance our understanding of the characterization of *S.* Typhimurium serotype in Bhopal City. Future research should focus on investigating changing patterns of antimicrobial resistance, pathogenicity, and the genetic background of *Salmonella* serotypes. It is crucial to prioritize further surveillance efforts to detect antimicrobial-resistant *Salmonella* in various environmental sources.

[VIB-ZOH-OP-03]

Bacteriological Quality analysis of Milk and Milk Products with special reference to coliform bacteria in Srinagar city

Innas Yousuf, **Syed Akram Hussain*,** M.A Rather, Sheikh Rafeh, Sabia Qureshi, Altaf Bhat and Shabu Shaukat

Division of Veterinary Public Health
Faculty of Veterinary Sciences and Husbandry
SKUAST-Kashmir, Shuhama, Srinagar, J&K, India

The present study was carried out to determine the hygienic quality of raw milk and certain milk products sold in Srinagar city market. In this context a total of 210 samples comprising Raw milk, pooled and packaged pasteurized milk, paneer and Ice-cream, milking can, milking pail were processed to enumerate total viable and total coliform bacteria. Presence of Enteropathogenic *E. coli* (EPEC) was also determined by detecting *eaeA* gene in the isolated *E. coli*. The mean total viable count for raw, pooled and pasteurized milk, paneer, ice-cream, milking cans and milking pails were 4.737±0.056 $\log_{10}$ cfu/ml, 4.192±0.110 $\log_{10}$ cfu/ml, 4.594±0.107 $\log_{10}$ cfu/ml, 4.939±0.039 $\log_{10}$ cfu/g, 4.564±0.088 $\log_{10}$ cfu/g, 0.621±0.016 $\log_{10}$ cfu/cm² and 0.064±0.012 $\log_{10}$ cfu/cm² respectively. The mean coliform count of raw milk, pooled milk, pasteurized milk, paneer, ice-cream, milking cans and milking pails were 4.447±0.105 $\log_{10}$ cfu/ml, 4.242±0.134 $\log_{10}$ cfu/ml, 4.217±0.232 $\log_{10}$ cfu/ml, 4.229±0.142 $\log_{10}$ cfu/g and 3.669±0.094 $\log_{10}$ cfu/g, 0.593±0.023 $\log_{10}$ cfu/cm² and 0.616±0.017 $\log_{10}$ cfu/cm² respectively. The overall occurrence of coliforms was found to be 24.28% with highest (56.6%) in raw milk. A total of three (03) isolates from raw milk were found positive for *eae*A gene. The study concluded the contamination of milk and milk products with coliform organisms indicating lack of maintenance of hygiene.

[VIB-ZOH-OP-04]

Utilization pattern of drugs among patients attending geriatric outpatient department in a tertiary care hospital in Kashmir

Rehana T, Umar H, Syed S H, Asma S, Afshan S
Presenting author: **Dr Furqaan Jan Nahum**

Department of Pharmacology, Government Medical College, Srinagar, Jammu and Kashmir, India
Department of Geriatrics, Sher-i-Kashmir Institute of Medical Sciences, Jammu and Kashmir, India

Quality and safety of prescribing in older people remains a global healthcare concern and inappropriate prescribing is a major public health issue because of its direct association with morbidity, mortality and wastage of health resources in this age group. Very limited data is available on the drug utilization pattern in geriatric population and the present study was carried out to see the prescription pattern in geriatric population in this part of the world.
Methods: The present study was conducted by the department of pharmacology in outpatient department of geriatrics in a tertiary care centre to look into the prescription pattern among geriatric age group.
Results: A total of 237 prescriptions were collected, out of which 108 (45.56%) were males and 129 (54.44%) were females. The majority of the patients were in the age group of 60-69 years (n=141, 59.5%). The most commonly found comorbidity was hypertension (63.29%) and antihypertensive agents (74.68%) were the most frequently prescribed class of drugs. Calcium (37.57%), budesonide (32.91%), thyroxine (27.84%) and pantoprazole (25.31%) were the most common individual drugs prescribed.
Conclusions: Like other studies on geriatric population polypharmacy was also observed in the present study and periodic therapeutic audit is essential to ensure rational medicine use.

[VIB-ZOH-OP-05]

Occurrence of *Streptococcus pluranimalium,* an emerging zoonotic pathogen in small ruminants

Gazal S[1], **Kaur P[1]**, Bedi J[2], Singh J[3]and Arora A K[1]

[1]Department of Veterinary Microbiology, [2]Centre for One Health, [3]Department of Veterinary and Animal Husbandry Extension
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab.

Streptococcus pluranimalium, a novel member of genus *Streptococcus* has been associated with subclinical mastitis, valvular endocarditis and septicaemia in multiple different animal hosts. It is an emerging pathogen in humans with the bacterium being isolated from cases of infective endocarditis and brain abscess. The present study was aimed at determining the prevalence, antibiotic resistance and virulence profile of *S. pluranimalium* from small ruminants, pigs and human handlers. A total of 650 samples comprising of 450 nasal swab samples from sheep, goats and pigs (150 each), 100 caprine milk samples and 100 human hand swab samples were screened and 17 *S. pluranimalium* isolates were obtained and confirmed by MALDI-TOF. All the isolates obtained were from nasal samples of sheep and goats. One of the isolate was randomly selected and its 16S rDNA region was amplified and sequenced for molecular confirmation of *S. pluranimalium*. The sequence obtained was submitted to GenBank under accession no. OR075259. The antibiogram of the isolates was also performed which revealed that the isolates were 100% resistant to vancomycin and ceftriaoxone with all the isolates exhibiting multidrug resistance with 11.7% isolates resistant to three, 41.17% isolates resistant to four, 17.6% isolates resistant to five, 11.76% isolates resistant to six, 5.8% isolates resistant to seven and 11.76% isolates resistant to eight classes of antibiotics. The MAR index of all the *S. pluranimalium* isolates was found to be greater than 0.2 which is reflective of the wide antibiotic usage in small ruminants. Out of six antibiotic resistance genes tested, the isolates were found to carry *tet*M, *erm*B and *mefE* genes. No virulence gene could be detected in any of the isolates. To our knowledge, this is the first study on isolation and determination of antibiotic resistance and virulence profile of *S. pluranimalium* from nasal cavity of small ruminants in India.

[VIB-ZOH-OP-06]

A blue print of *Acinetobacter baumannii* complex blood stream infection (ABC-BSI): Trends over seven years in Health care associated ABC-BSI in a tertiary care institute of the Kashmir valley.

Dr Lenah Bashir, Dr Anjum Farhana

Department of Microbiology, GMC, Srinagar

Long term studies to substantiate the changing prevalence, microbiological makeup and prognosis of health care associated Acinetobacter baumannii complex blood stream infections (ABC-BSI) unique to the Kashmir valley, are scarce.

OBJECTIVES: To investigate the overall trends in incidence, antibiotic resistance and clinical outcomes of ABC-BSI in Kashmir valley from 2015-2021.

STUDY DESIGN: A multicenter retrospective cohort study carried out in Post graduate department of Microbiology, Government medical college, Srinagar. Shri maharaja hari singh hospital and seven other associated hospitals over the city. Asceptic venipunctures were carried out for collection of blood samples from all suspected patients of blood stream infections. Automated blood culture bottles (bacT Alert) or conventional culture bottles were used as receptacles. These culture bottles were incubated under appropriate conditions overnight. Samples with a positive connotation by automated detection system BacT/Alert were processed further. Conventional blood culture bottles were sub cultured routinely at regular intervals till 7 days. The microbial growths obtained were examined by grams staining and used for the preparation of suspensions for test microbes by VITEK 2 compact automated microbiology bacterial identification and antimicrobial susceptibility system. These isolates were subjected to antimicrobial susceptibility by automated microbroth dilution by VITEK 2 C. These isolates were tested for 9 groups of drugs. The cut off values were interpreted according to CLSI (Clinical and Laboratory Standards Institute).

RESULTS: A total of 15962 samples were received over the total study period.11442 were excluded due to negative connotations by the detection system. Over the years from 2015-2021 the incidence of ABC-BSI has significantly increased. The proportion of drug resistant to drug sensitive isolates has also increased ($p<0.05$). Increase in the degree of resistant isolates is associated with notably lower survival days in our study.

CONCLUSIONS: According to the WHO's 2017 publication, Acinetobacter baumannii complex is among the critical group of priority pathogens. Priority should be given to the urgent need for novel antibiotics as well as the sensible use of those already available. Antimicrobial stewardship is an exigent reality.

Session V
Zoonosis and One Health
(Poster)

[VIB-ZOH-PP-01]

Emphysematous osteomyelitis: a case report and review of literature

WanI S A[1], Khan U H[2]

Senior Resident, Department of General Medicine SKIMS[1]
Associate Professor, Department of Geriatric Medicine SKIMS[2]

Emphysematous osteomyelitis is a rare form of osteomyelitis characterized by the presence of intraosseous gas. It is caused by gas-forming organisms, most common being members of the Enterobacteriaceae family (particularly E coli) or anaerobes. Comorbidities like diabetes mellitus, malignancy, alcohol abuse, Crohn's disease, and other etiologies causing immunosuppression, predispose to this condition. A prompt diagnosis of this potentially fatal condition is required to expedite early management. We present a patient diagnosed with multifocal emphysematous osteomyelitis (involving pelvic bones, left proximal femur, vertebral bodies, ribs and clavicle) caused by *E coli* to highlight the unusual diffuse involvement by this rare pathology. In addition, we reviewed 58 reported cases of emphysematous osteomyelitis including our case.

[VIB-ZOH-PP-02]

A rare case of Strongyloides Hyper infection Syndrome in a CKD Patient in temperate region

Sheikh Zaid Amin, Khandi Muzafar Ahmad, Bashir Ahmad Fomda

Department of Microbiology, Sher-i-Kashmir Institute of medical Sciences. Srinagar 190011.

Strongyloides stercoralis is infrequent in temperate areas but endemic in tropical and subtropical regions. Even one adult female can proliferate quickly in immunocompromised host, which can accelerate autoinfection and/or dissemination. The term "hyperinfection syndrome" refers to accelerated autoinfection, which is typically the outcome of a compromised immune system, it implies the existence of signs and symptoms linked to enhanced larval migration. Presence of larva in stool and sputum is the hallmark of hyper infection associated with worsening of gastro-intestinal and pulmonary symptoms. Disseminated infection happens when larvae travel beyond the organs of the autoinfective cycle. To the best of our knowledge this is the first case of strongyloidiasis in CKD patients triggered by intake of steroids in Kashmir, a temperate region in India.

CASE REPORT

We reported a case of hyper infection with *Strongyloides stercoralis* in a chronic kidney disease patient who was on steroids for his pre-existing ailment. The patient presented with complaints of persistent epigastric pain, multiple episodes of vomiting and fever. Upper Gastrointestinal endoscopic biopsy and bronchoalveolar lavage for cytology were suggestive of strongyloidiasis, stool microscopical examination was carried out which revealed presence of rhabidiform larva and patient was managed with ivermectin. Later on he developed hyper infection syndrome with *Escherichia coli* septicemia with meningitis and was subsequently managed with intravenous meropenem.

CONCLUSION

Hyperinfection with Strongyloides although quite uncommon, this syndrome's potent severity indicates that even in non-endemic areas, clinicians and microbiologists must be aware of the disease. In patients with chronic kidney disease who are on immunosuppressive therapy having coexisting abdominal and respiratory symptoms, this infection should be ruled out as a potential etiology.

[VIB-ZOH-PP-03]

Determining the load of thermophilic *Campylobacter* in surface water bodies of Uttarakhand province of India through Real-time PCR

Iram Ansari[1], Maansi[1], Ajay Kumar Upadhyay[1] and **Aman Kamboj[2]***

[1]Department of Veterinary Public Health & Epidemiology;
[2]Veterinary Clinical Complex;
College of Veterinary and Animal Sciences;
G. B. Pant University of Agriculture and Technology, Pantnagar Uttarakhand, India

Emerging water-borne pathogens constitute a significant health hazard in both developed and developing nations. According to WHO, about 6.3% of deaths happen due to unsafe water, inadequate sanitation, and poor hygiene. Thermophilic *Campylobacter* spp., is a leading food-born zoonotic bacterial pathogen regarded as one of the leading causes of gastroenteritis in humans worldwide. Animal and poultry waste are the potential sources of water contamination, and reports of isolation of thermophilic *Campylobacter* from water bodies are also available. With the advent of molecular techniques like Polymerase Chain Reaction, it is quite convenient nowadays to detect the presence of pathogens in environmental samples with utmost specificity and in the minimum possible time. In the present study, the load of thermophilic *Campylobacter* in surface water bodies of Uttarakhand province of India was determined through Real-time PCR using absolute quantification with a standard curve. It was concluded that all collected samples were positive for thermophilic *Campylobacter* with a detection range from 7.7×10^2 to 2.7×10^5 and the highest load was found from district Nainital. The work generates an important insight into the context of ensuring one-health.

[VIB-ZOH-PP-04]

One health approach to target Japanese encephalitis non-structural protein NS3 for the development of potential antiviral therapy

Swatantra Kumar, Shailendra K Saxena*

Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.

Japanese encephalitis virus (JEV) is the major cause of viral encephalitis in South-East Asia. JEV is maintained in a natural transmission cycle amongst mosquitoes and wading birds, while pigs act as an amplifying host. JEV-NS3 protein has been shown to be involved in transmission and neuropathogenesis via inducing neuronal cell death. Since, One Health concept involves interactions between people, animals, and the environment that may enhance the spread of diseases. Therefore, in the present study we have developed a hydroalcoholic formulation B200 and showed its antiviral efficacy against JEV infection. We have found that treatment with B200 increases neuronal cell survival by reducing JEV induced cytopathic effects which were evident from significant reduction in necrotic cell population by flow-cytometry analysis and caspase 3 and 8 enzymatic activities. B200 treatment was found to decrease the intracellular JEV level observed by significant reduction in JEV-FITC expression. Because microglia play a crucial role in JEV neuropathogenesis, we further investigated the anti-JEV effects of B200 on human microglia cells and elucidated the mechanism of action by performing whole-transcriptome sequencing. Gene expression analysis revealed that B200 reduces the pro-apoptotic and inflammatory gene expression observed by significant reduction in *BAD*, *BAX*, *CASP3*, *CASP8*, *IL1B*, and *CXCL10* and increase in *IL10* responsive gene expression. Interestingly, our molecular docking analysis revealed that B200 interacts with the crucial residues of NS3 protein involved in RNA unwinding and ATPase activity that was further confirmed by degradation of NS3 protein. Moreover, we have elucidated its Drug likeness, ADME (absorption, distribution, metabolism, and excretion), and toxicity analysis further suggests that molecules present in B200 crosses the blood-brain barrier, which is crucial for effective treatment of Japanese encephalitis (JE) as a One Health approach that may enhance the spread of diseases.

[VIB-ZOH-PP-05]

Rising trend of *Burkholderia* septicaemia among neonates in NICU of a major tertiary care centre of Kashmir valley.

Fazila M, Anjum F

Department of Microbiology, Government medical college, Srinagar 190011.

BACKGROUND:
Neonatal sepsis is a major cause of morbidity and mortality worldwide. Burkholderia cepacia is a rare cause of sepsis in newborns and its transmission involves contact with heavily contaminated medical devices and disinfectants. Now a days, neonatal sepsis due to Burkholderia cepacia is on rise. The aim of this study was to evaluate the clinical presentation and antibiotic sensitivity pattern from blood culture proven Burkholderia septicaemia in neonates.

MATERIAL AND METHODS:
STUDY DESIGN: Hospital based cross sectional study.
STUDY SETTING: Department of Microbiology.
DATA COLLECTION: 1 January 2023 to 31 may 2023
STUDY PARTICIPANTS**:** Neonates with clinically suspected septicaemias.
STUDY PROCEDURE:
273 paediatric blood culture bottles were received in our laboratory during the study period. Blood culture was processed by BacT/Alert automated blood culture system. Identification and antimicrobial susceptibility test were done by VITEK 2 system according to the CLSI guidelines.

RESULTS:
Out of 273 samples that were received, 152 (55.6%) came out to be positive. Among the positive cultures, Burkholderia cepacia was responsible for septicaemia in 31 (20.39%) cases. All neonates were inborn. 19 (61.29%) were male and 12 (38.7%) were female (Male to female ratio was 1.58:1). 5 (16.2%) were term and 26 (83.8%) were preterm. 23 (74.2%) had low birth weight and 8 (25.8%) had very low birth weight. 13 (41.9%) had early onset sepsis and 18 (50.06%) had late onset sepsis. Respiratory distress was the most common clinicalpresentation.

CONCLUSION:
The study contributes to the knowledge of changing epidemiological trends of Burkholderia septicaemia in neonates. The drugs for treating Burkholderia cepacia are already limited due to its intrinsic resistance against a large number of antimicrobials. High degree of suspicion, proper and timely identification can provide an early diagnosis and help to confine morbidity due to such infections.

[VIB-ZOH-PP-06]

Detection of *Klebsiella pneumoniae* in Mastitis in dairy animals In Jaipur region

Patel P*, Ingle V C and Khichar K

Post-Graduation Institute of Veterinary Education and Research
Jaipur, Rajasthan

Klebsiella spp. are important opportunistic pathogens commonly defined as environmental clinical mastitis agents. Mastitis caused by Klebsiella species is an emerging issue associated with contaminated environments, often with poor response to therapy. The aim of this study was to characterize Klebsiella in clinical and sub-clinical mastitis in terms of microbiological identification and AMR. A total of eighty five milk samples from mastitis (clinical and sub clinical) cases of mastitis from cows (57) and buffaloes (28) were collected from different area of Jaipur and screened by using California mastitis test. The milk samples found positives were further processed for isolation and identification of the causative agent by inoculating on selective medium and then further biochemical test. Out of total samples screened, 60 samples yielded bacterial growth and rest of the 25 samples could not yield any growth. On further identification out of these 60 samples, *Klebsiella pneumoniae* were isolated from 34 samples on the basis of their cultural, morphological, biochemical characters. Whereas rest of the twenty six samples had mixed growth. The incidence of *Klebsiella pneumoniae* in mastitis was 56.66%. The *in vitro* antibiotic sensitivity test revealed highest sensitivity to ceftriaxone (82.35%) followed by ciprofloxacin (73.53%), levofloxacin (67.65%), and gentamicin (58.82%) and highest resistance to ampicillin (91.18%) followed by tetracycline (70.59%) and kanamycin (50.00 %). This study helps to treat clinical and sub- clinical mastitis with effective antibiotics and helps in an epidemiological study in Jaipur Region as well as helps to create public health awareness.

[VIB-ZOH-PP-07]

Detection of Norovirus GII in Wastewater Samples of Bhopal Region Using Droplet Digital PCR

Nema R K[1], Singh S[2], **Singh A K[1]**, Tandekar A[1], Nagar J[1], Sarma D K[1], Tiwari R R[3] Mishra P K[1].

[1]Division of Environmental Biotechnology, Genetics, and Molecular Biology, ICMR-National Institute for Research in Environmental Health, Bhopal - 462030 (MP), India
[2]Division of Environmental Monitoring and Exposure Assessment (Water & Soil), ICMR – National Institute for Research in Environmental Health, Bhopal – 462 030 (MP), India
[3]ICMR – National Institute for Research in Environmental Health, Bhopal – 462 030 (MP), India

Noroviruses are a significant cause of global gastroenteritis outbreaks, underscoring the importance of effective surveillance. Wastewater-based epidemiology (WBE) has emerged as a valuable method for identifying viral pathogens within communities. Recent advancements in wastewater-based molecular surveillance have revealed early virus excretion in faeces and urine, making sewage monitoring a crucial indicator of viral presence. We aimed to design, develop and validate a novel method for the detection and real-time monitoring of Noroviruses GII in Bhopal's wastewater using Automated Droplet Digital PCR (ddPCR) technology.. A ddPCR assay targeting the NSP-3 region of Norovirus GII was developed, allowing viral nucleic acid quantification without a standard curve. A total of 27 samples from five Sewage Treatment Plants (STPs) located in Bhopal city were collected during the summer season (April and May 2023) at fortnightly intervals and analyzed for the presence of norovirus using the novel ddPCR assay. These STPs are situated in Bansal Hospital (9.5 MLD), Charimli (4.5 MLD), Shirin River (5 MLD), Professor Colony (2 MLD), and Jamuniya Cheer (3.5 MLD) areas of Bhopal city and each have a well-defined catchment area. Among the samples tested, 33% tested positive for norovirus, with the highest detection rate observed in the Bansal Hospital STP (72.72%), followed by Charimli STP (25%). Other STPs did not show any presence of noroviruses. The concentrations of Noroviruses GII in positive samples ranged from 0.06 to 6.60 copies/µl. These findings indicate a potential higher patient population within the catchment area of Bansal Hospital compared to the other STPs in the Bhopal region. The increased patient population consequently leads to a higher likelihood of norovirus shedding in faeces and urine, resulting in an elevated virus load in the sewage. The study underscores the varying prevalence and distribution of norovirus in wastewater across different STPs in Bhopal. Moreover, it demonstrates the utility of wastewater surveillance and digital PCR in accurately and specifically detecting norovirus in wastewater. The practical application of this wastewater surveillance strategy could serve as an early warning system for communities, enabling timely preparedness for impending viral outbreaks, implementation of effective administrative containment measures, and intensified vaccination campaigns.

[VIB-ZOH-PP-08]

Elderly Multigravida with Guillain Barre Syndrome in a SARS- COV 2 ; A Case Presentation

Qari D; Gillani Y; Malik A

SKIMS Medical College Hospital, Bemina, Srinagar

Guillain- Barre syndrome (GBS) is an autoimmune disorder and occurrence in COVID-19 pregnancy is rare. Herein, we report a case of 36 years old pregnant female, G3P2L2(LSCS) with 7 months of amenorrhea (28 weeks and 3 days of gestational age) with live singleton pregnancy hospitalized with ascending muscle weakness of lower limbs , oxygen saturation of 90 % on NRM on 15 litres per minute , tachypnea (RR 25 bpm) and Rapid antigen test for SARS COV2 positive. She had type 1 respiratory failure, so was put on invasive ventilation. Treatment as per guidelines was started. Given her deteriorating condition, a decision of terminating this pregnancy was taken and caesarean section was done. Her condition improved gradually and there was improvement in muscle power too. This case highlights the importance of individualised decision-making in cases with COVID-19 infection in pregnancy and that prompt treatment of the complications are lifesaving.

[VIB-ZOH-PP-09]

Pattern of Fosfomycin Sensitivity Among Uropathogens Isolated from Hospitalised Patients in A Tertiary Care Hospital of North India

Dr Unairah Naqash, Dr Asifa Nazir, Dr Humaira, Dr Farhat
Department: Govt Medical College Srinagar

Urinary tract infections (UTIs) are the most common infections in community and hospitalized patients. Worldwide, about 150 million people are diagnosed with UTI each year. The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide. It is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits. Fosfomycin exhibits excellent antimicrobial activity even against the isolates with relatively high levels of antimicrobial resistance and hence has a promising role in UTI as such may be considered as a highly effective empirical oral treatment for UTI along with nitrofurantoin until a urine culture report is available

Objectives:

1. To find out the prevalence of uropathogens isolated
2. To determine the Antibiotic susceptibility pattern of the uropathogens with emphasis on fosfomycin

Material and methods:

A total of 6883 urine samples were obtained from the hospitalized patients. Data of 1 year from July 2022 to June 2023 was retrospectively viewed from the departmental record system The significant growth of pathogenic bacteria recovered from the urine using microbial culture on Hi Chrome media were tested for antibiotic susceptibility testing by the standard Kirby Bauer's disc diffusion method The MIC was confirmed for the current standards for clinical breakpoints using VITEK-2 COMPACT SYSTEM.

Results:

1708 out of total 6883 urine samples were culture positive *E. coli* (35.1%) was the leading isolate followed by *Enterococcus* (26%). Fosfomycin was recorded as the most active antibiotic against all the bacterial pathogen The MIC of fosfomycin was recorded ≤ 32 against most of the isolates.

Conclusion:

Identifying the characteristic of uropathogens and antimicrobial sensitivity and resistance patterns play a crucial role to successfully treat and decide empiric treatment for the patients of UTI..The resistance pattern is increasing due to uncontrolled abuse of the available antibiotics. A strong decision regarding the antibiotic policies for UTI and stringent measures need to be taken to ensure the effectiveness of the same. Failing to do so, the time is not far where we would have to stand helplessly against these organisms**.**

Day 3
Technical Sessions

Session VI
Immunoprophylaxis, Immunodiagnostics & Advances in Vaccine Research
(Oral)

[VIB-IAVR-IVL-01]

Virulence Markers and Molecular Detection of *Salmonella* serovars and the Prospect of Developing a Novel OMP-based Recombinant Vaccine

Probodh Borah
Professor & Head, Department of Animal Biotechnology
College of Veterinary Science, Assam Agricultural University
Khanapara, Guwahati-781 022

Salmonella enterica subsp. *enterica* is a pathogen of global concern for both human and animals. It causes infection ranging from a mild, self-limiting diarrhea to severe gastrointestinal, septicemic disease and enteric fever. The genus *Salmonella* comprises a large and related population of zoonotic pathogens that can infect most mammals, including humans and domestic animals, birds, reptiles and amphibians. It continues to be one of the most important foodborne pathogens worldwide. The organism is commonly transmitted via food chain; however, outbreaks have been reported recently in which it has been transmitted through direct or indirect contact with animals. An estimated 11% of all *Salmonella* infections are attributed to animal exposures, with the highest rates of illness and death occurring among children. Since 2007, numerous outbreaks of human *Salmonella* infections linked to contact with animals and their environments have been reported. Poultry and pigs can be the persistent subclinical shedders and can appear healthy while continuously shedding the bacteria in faeces. As zoonotic salmonellosis outbreaks occur at the intersection of human and animal health, One Health approach may be more appropriate for their investigation.

Although serotyping using the Kauffman-White scheme remains the standard method for identification of *Salmonella*, it has certain significant deficiencies. Besides being labor-intensive and expensive, it is also time-consuming, often requiring three or more days for a highly trained laboratory technician to produce a result. A total of 2579 serovars of *Salmonella* have so far been described, majority of which are non-host-specific and can cause infections across species. Pathogenesis of *Salmonella* infections is a complex process, in which numerous virulence genes clustered within *Salmonella* pathogenicity islands (SPIs) are involved and so far as many as 21 such islands (SPI-1 to SPI-21) have been reported. However, occurrence of SPIs and individual virulence genes varies among serovars.

Pathogens of a single species generally comprise of diverse strains showing wide variations in their epidemiological association with disease in respect of spatial and temporal distribution as well as host specificity. Several different methods may be applied for determining the molecular diversity among the *Salmonella* isolates. We developed a novel multiplex PCR-based rapid method supported by an online server based on differential distribution of 16 genes in various serovars of *Salmonella* for detection of common clinical serovars. Comparative efficacy of four different methods, *viz.* plasmid profiling, PFGE, rep-PCR and automated DiversiLab System® was also evaluated for determining molecular diversity among the *Salmonella* isolates from various sources belonging to different serovars. A multiplex PCR protocol was also developed for simultaneous detection of seven major virulence genes of *S. enterica* subsp. *enterica*.

Outer membrane proteins (OMPs) are integral membrane proteins and lipoproteins that are anchored to the outer membrane of gram-negative bacteria. These help in maintenance of the integrity and osmotic permeability of the bacterial membranes. They are

highly immunogenic and considered as promising candidates for development of vaccines and diagnostics. Some of the OMPs also act as bacterial adhesins and important virulence factors. The outer membrane proteins of *Salmonella* are also known to have a significant role in eliciting immune responses. Among the outer membrane lipoproteins, InvH acts as an adhesin that helps in the entry of the bacteria into the epithelial cells of the host. It is an integral component of SPI-2 of the Type III Secretion System and has been proposed as a potential target for vaccine development. This adhesin is almost universally present in all *Salmonella* strains except for *S. enterica* subsp. *arizonae*.

We successfully expressed the InvH protein of *Salmonella* Typhimurium in *E. coli* host and evaluated the 15 kDa recombinant protein for its potential as a vaccine candidate by testing its immunogenicity in mice. For this, the complete sequence of the *invH* gene was cloned and expressed in *E. coli* host and the expressed recombinant protein was purified under denaturing condition. On experimentally inoculated into mice, the purified recombinant InvH protein showed significant IgG response and induced protective immunity against both homologous and heterologous challenges.

[VIB-IAVR-IVL-02]

Development of recombinant virus-based vaccines against respiratory viral diseases of equines

Nitin Virmani*, B.C. Bera and Taruna Anand
ICAR-National Research Centre on Equines, Sirsa Road, Hisar, Haryana 125 001, India

Amongst the respiratory diseases affecting equines, Herpes and Influenza viruses are the most important viruses. Equine Herpes virus-1 (EHV1) is particularly common and ubiquitous in horse population while equine influenza (EI) caused by H3N8 influenza A virus is considered as one of the most important re-emerging respiratory pathogens of horses that leads to substantial economic losses. Both equine rhinopneumonitis (caused by EHV1 and EHV4) and equine influenza are OIE listed diseases of horses. EHV1 is characterized by respiratory symptoms, late-term abortion, neurological disorders and neonatal mortality. Equine influenza is an extremely contagious acute respiratory disease of horses, mules, donkeys and zebras. India has suffered 2 major epizootics, the first of which was reported in 1987 and involved more than 83,000 equines, whereas 2008-09 outbreak involved equines in more than thirteen states.

Vaccination is an efficient mean to prevent or limit the spread of both equine diseases alongside managerial strategies. Different types of commercial vaccines available for the control of EIV are whole inactivated virus, subunit, live attenuated and viral vector-based vaccines. Whole inactivated virus used along with aluminium hydroxide as adjuvant. These vaccines stimulate strong antibody response but lack cellular immune response. Another study revealed specific antibody response and protection against H3N8 challenge infection, immunized by inactivated H3N8 whole virus vaccine adjuvanted with aluminium hydroxide. Subunit vaccine on the other hand contains only purified virus proteins instead of whole virus. These are used with adjuvants to stimulate proper immune responses. Stimulation of humoral as well as CMI (EIV specific IFN-g) has been demonstrated in ponies vaccinated with ISCOM-Matrix. With advancement of vaccine development attempts were made to create live attenuated vaccine which stimulates long lasting immune response. But it holds less effectiveness in adults and have a risk of reassortment. Subsequently, viral vector-based vaccines were made, which were non-pathogenic recombinant viruses containing foreign DNA expressing as antigens after immunization. Classic examples of this category is canarypox vectored vaccine. While DNA vaccine refers to injection of DNA plasmid expressing gene of pathogen encoding whole antigen protein or epitope eg. Influenza DNA vaccines expressing viral HA, M2e, NA, and NP has been found efficient. DNA vaccines have certain limitations like host autoimmunity, possible risk of malignancies due to integration of exogenous DNA into host genome and less effectiveness in elder and new-born. Hence, there is a need for development of a vaccine bearing very less limitations to combat the disease in future. For EHV1, traditionally inactivated vaccines were in use but they were known to be weak inducers of cell mediated immunity and provided only partial clinical and virological protection with no prevention against cell associated viraemia. Despite widespread vaccinations, outbreaks still continue and remain a severe problem for the horse industry. Thus, vaccination program has to be aimed at strengthening both humoral and CMI responses for preventing EHV1 infection.
Strategies that result in the stimulation of both humoral and cell mediated immune responses in a manner similar to natural infection need to be explored. With the advent of recombinant

technology, novel vaccination strategies entail the use of second-generation vaccines that stimulates both humoral and cell mediated immune responses in a way analogous to naturally occurring disease. Recombinant technology has been employed to introduce some clear-cut modifications in the genome of viruses for proper and stable attenuation in order to develop recombinant live vaccines. Further, the genetically engineered vaccines have set up a foundation for vaccination strategies based on DIVA. NRCE has developed different mutants of EHV1 through deletion of specific genes (gE, IR6, gE +IR6, gE+IR6+pUL43 +pUL56) employing bacterial artificial chromosome technology. These mutants have been tested for their attenuation employing *in vitro* and *in vivo* studies followed by challenge studies in murine model for their further use as modified live vaccine candidate. Mice immunized with the mutant viruses and challenged with pathogenic wild virus showed less pathology in terms of clinical signs, body weight loss, gross and histopathological lesions accompanied with early shedding of virus from the respiratory tract. This could be attributed to the higher protective efficacy brought about by strong cellular immunity as evidenced by high CD8 response and high neutralizing antibody titres. As such these mutants qualify to be good MLV vaccine candidates for vaccinating against EHV1.

EHV1 holds its importance as a delivery vector for immunization, as it has a high packaging capacity due to numbers of non-essential genes, broad host range in cultured cells and absence of anti-vector immunity. Hence, it can be exploited as a vector for expressing another protein encoded genome. Same as Canarypox vectored vaccine, a herpes virus vectored vaccine can be produced incorporating the HA gene of equine influenza virus creating a new combination vaccine candidate against both the diseases. As such NRCE ventured into the development of a combined vaccine against EHV1 and EIV employing the genetically constructed EHV1 BAC. The HA gene from Clade 1 and Clade 2 viruses of EIV (recommended to be used by WOAH) have been cloned into the backbone of EHV1. The strategies for the development of combined vaccine against EHV1 and EI are discussed.

[VIB-IAVR-OP-01]

Effect of polyherbal immunomodulator on immune response in foot-and-mouth disease and haemorrhagic septicaemia vaccinated dairy cattle

Mishra N, Singh R, Sirohi R, Ganguly B, Gupta A K, Singh A P and Yadav S K
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura – 281001 (UP)

Control of foot-and-mouth disease (FMD) and haemorrhagic septicaemia (HS) is mainly dependent on inactivated vaccines though, outbreaks are reported. Plant extracts such as phytogenic feed additives (PFA) have been used to enhance livestock production by improvement in nutrient utilisation, immunity and overall health. The present study was undertaken to study the effect of a polyherbal immunomodulator (restobal) at improving immune response to FMD and HS vaccination in dairy cattle. Animals were orally fed restobal from day 0 to day 10 and vaccinated on day 5 of treatment with commercially available oil adjuvanted FMD vaccine and oil adjuvanted FMD + HS + BQ combined vaccine. Antibody response and cytokine expression was studied in blood samples collected at different intervals. In relative mRNA expression studies, mean fold change increase was observed in *IL-2, IFN-ɣ, IL-4, IL-10* and *IL-12* genes after vaccination with FMD vaccine in control animals on day 7 and day 12 of vaccination and then declined on day 21 of vaccination. No such change was observed for *IL-6*. However, in FMD vaccinated treatment animals there was no mean fold change in gene expression and it declined after vaccination. In FMD + HS vaccinated animals in control and treatment animals mean fold change increase in gene expression of *IL-2, IFN-ɣ, IL-4* was observed on day 21 of vaccination. FMDV titre for all three serotypes increased to 2.4 $\log_{10}$ by day 21 of vaccination and were not different significantly in both control and treatment group. In estimation of antibody titre for HS, animals were partially protected on day of vaccination (titre < 1.8 $\log_{10}$) and were fully protected on 90 day of vaccination (> 1.8 $\log_{10}$) in both control and treatment group. Antibody titres were not significantly different in FMD vaccinated and FMD + HS vaccinated animals for FMDV serotype O, A, and Asia 1. Restobal treated group was no different than control group in improving antibody response in FMD and HS vaccinated dairy cattle. No difference in antibody response for FMDV was observed for Monovac and Triovac vaccinated cattle, supporting use of combined vaccine as a cost-effective measure to control FMD and HS.

[VIB-IAVR-OP-02]

Towards eradication of peste des petits ruminants: Serosurveillance and post-vaccination seromonitoring of PPR in sheep and goats in Karnataka state, India

V. Balamurugan*1, Rakshit Ojha1, K Vinod Kumar1, Asha A1, Suhail Ashraf1, Annett Helcita Dsouza1, Archana Pal1, Prajakta Prashant Bokade1, Harshitha, S. K.1, Ramachandra Deshpande1, K. P. Suresh1., G. Govindaraj1, Subramanya Prasad H.,2 Chandra Sekar3, D. Hemadri1, Anirban Guha4 and B. R. Gulati1

1Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India

2Commissionerate of Animal Husbandry and Veterinary Services, Pashupalana Bhavana, Hebbal, Bangalore-24

3ICAR-Indian Veterinary Research Institute (IVRI), Mukteswar 263138, Nainital (Distt.), Uttarakhand

4Department of Animal Husbandry & Dairying, GoI, Animal Husbandry and Dairying Krishi Bhawan, New Delhi - 110001

Peste des petits ruminants (PPR) primarily affects sheep and goats, causing major constraints in augmenting the productivity of small ruminants in enzootic countries. Due to the vast economic impacts of PPR, following the eradication of rinderpest, a global consensus was extended on the need to eradicate PPR with the adoption of a PPR Global Control and Eradication Strategy under the GEP 2030. This study addresses the surveillance and seromonitoring of PPR in sheep and goats in Karnataka, India, with the goal of contributing to global PPR eradication efforts by 2030. The cross-sectional serosurveillance and post-vaccination seromonitoring at an epidemiological unit level (village) across various regions in the state were carried out to know the status of PPRV antibodies prevalence in the population before the start of the National Strategic plan for PPR eradication and assess the efficacy of PPR vaccination at the field level. Using a stratified random sampling methodology, a total of 3417 serum samples were collected for serosurveillance, while 1052 samples were planned for post-vaccination seromonitoring, as per the WOAH guidelines all of which were screened for PPR virus (PPRV) antibodies using an indigenous IVRI-PPR-C-ELISA kit.

Results indicate a seroprevalence of 61.1% among small ruminants in the studied state, with significant associations of PPRV antibodies across different districts (sheep $\chi 2 = 234.86$, $p < 0.01$; goats- $\chi 2 = 266.89$, $p < 0.01$). The serosurveillance identified 61 epidemiological units with a >70% seroconversion rate, verifying the population's immune status due to the earlier implementation of regular vaccination and the vaccine's field-level efficacy. However, 47 villages in the designated district with less than 30% seroprevalence call for additional, comprehensive vaccination efforts. The post-vaccination seromonitoring study displayed an encouraging 72.3% seroconversion for the 2023 year with IAH&VB PPR vaccine in the age group 6-12 months. To maintain momentum towards PPR eradication, strategic implementation of mass vaccination programmes in scheduled periods is recommended, ensuring a >70-80% prevalence of PPRV antibodies or immunity status. Future interventions could limit vaccination to bordering districts, animal markets, and check posts if no PPR outbreaks are detected in all the epidemiological units of the State. This

research provides critical insights into PPRV antibodies in small ruminants, instrumental for refining strategies towards a PPR-free India and the global eradication of the disease.

[VIB-IAVR-OP-03]

Prevalence of anti-leptospiral antibodies and frequency distribution of *Leptospira* serovars in buffaloes in enzootic states of India

Prajakta Prashant Bokade, K Vinod Kumar, Bharath V, Swathi M, Neha P, D. Hemadri, B. R. Gulati, V., Balamurugan*

Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India

Leptospirosis, an often overlooked, emergent, re-emergent, and neglected anthropozoonosis, has a global distribution. It is attributed to various serovars of pathogenic spirochetes from the *Leptospira* genus and finds hosts in a multitude of animal species. Buffaloes, in the livestock sector, are significant contributors to the maintenance and transmission of this infection. The disease in water buffaloes frequently goes unnoticed due to limited awareness about its prevalence. It is essential to identify the prevalence of specific serovars in certain geographical areas for effective leptospirosis diagnosis in both animals and humans. This study was initiated to determine the seroprevalence of leptospirosis and the frequency distribution of *Leptospira* serovars in buffaloes from enzootic states of India. Between July and May 2023, 973 serum samples were collected from 23 districts spanning three Indian states: Kerala (n=115), Tamil Nadu (n=197), and Gujarat (n=661). These samples were subjected to a microscopic agglutination test (MAT) using five to seven-day-old *Leptospira* reference serovars (n=20) at a concentration of 1-2 $x10^8$ organisms/ml. An overall seroprevalence of 10.59% (103/973) was observed, with the highest in Kerala at 60.87% (70/115), followed by Gujarat at 3.78% (25/661), and Tamil Nadu at 4.06% (8/197). Among the 103 reactive sera, 40 samples demonstrated reactivity with multiple serovars, indicating a 45% prevalence of multiple serovars. The serovars with the most reactivity included Pomona (41.75%), Hardjo type Prajitno (37.86%), Autumnalis (29.13%), Grippotyphosa type Moskva (25.24%), and Kaup (22.33%). This study's findings highlight a concerning high seroprevalence of leptospirosis in buffaloes in Kerala. The determined prevalent serovars could be beneficial as reference panels for *Leptospira* antigens in MAT for diagnosing leptospirosis in humans and animals, thereby increasing diagnostic precision. Moving forward, measures such as promoting disease awareness, enhancing vaccination programs, and conducting routine serosurveillance in buffaloes and other livestock should be emphasized to curb the rising incidence of leptospirosis in enzootic regions.

[VIB-IAVR-OP-04]

Development of latex agglutination test (LAT) using recombinant ErpY-like lipoprotein for detection of anti-leptospiral antibodies for serodiagnosis of leptospirosis

K Vinod Kumar#1, Prajakta Prashant Bokade 1, Archana Pal1, S. Sowjanyakumari1, V Bharath1, B. R. Shome, B. R. Gulati1, V. Balamurugan1*

1 Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India
#National Post-Doctoral Fellowship, Science and Engineering Research Board, Department of Science and Technology, Government of India.

Leptospirosis is a significant global concern affecting both public and animal health, and accurate diagnoses are imperative for preventing severe complications in humans and animals. Existing diagnostic challenges highlight the need for novel, easily accessible field screening tests. The ErpY-like lipoprotein (LIC11966) is exclusively pathogenic *Leptospira* specific, eliciting antibody response in humans and animals during infection. This study explores the diagnostic potential of recombinant ErpY-like protein in the Latex Agglutination Test (LAT) to detect anti-leptospiral antibodies in the sera of humans and animals. The ErpY-like lipoprotein-coding gene sequences were amplified from pathogenic *Leptospira interrogans* serovar Hardjo type Prajitno strain Hardjoprajitno and cloned into the pETite vector and expressed in the *Escherichia coli* host system. The expressed recombinant ErpY-like protein (rErpY) with a molecular weight of ~16 kDa was characterized by SDS-PAGE and Western blot technique using *Leptospira*-specific standard sera. The NiNTA-purified rErpY was assessed for its suitable diagnostic potential as an antigen in the form of sensitized coated with rErpY latex beads in LAT. Further, on evaluation, the rErpY-LAT against microscopic agglutination test (MAT) revealed a relative diagnostic sensitivity and specificity of 92% and 94.44 %, respectively, with an accuracy of 93.20% for the detection of the anti-leptospiral antibodies in the sera of animals (Bovine, Canine and swine). This extremely simple and rapid standardized rErpY-LAT, after an exhaustive evaluation, could be used as a preliminary screening diagnostic tool at the field level.

[VIB-IAVR-OP-05]

Development and evaluation of swine brucellosis vaccine in mice model

S. Manoharan1, K. Viswanathan2 and K. Padmapriya3
1 Professor, 3 Technician, Vaccine Research Centre-Bacterial Vaccine, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai-51
2 Scientist, Translational Research Platform for Veterinary Biologicals, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai-51

Brucellosis is a worldwide popular zoonosis caused by gram-negative bacteria of the genus *Brucella.* Many studies pointed that brucellosis vaccines based in *B. abortus S19* and *B. melitensis* Rev1 in swine does not provide complete protection. There is an acute need for the development of *B. suis* specific vaccine based on circulating biovar in a region. An attempt was made in this study to develop and evaluate the efficacy of the vaccine in mice model. *B. suis* isolate was obtained from the aborted fetal tissue samples collected froma a private farm which had abortion problem during October, 2020 in Nagapattinam district. A simple inactivated vaccine was developed using biovar 1 of *B. suis* local isolate by incorporating novel agent - imiquimod to enhance cell mediated immune response. Three groups (12 mice per group) were made comprising Group I - bacterin+alum gel, Group II - bacterin+alum gel+imiquimod and Group III = unvaccinated control and were given the optimal dose of the respective vaccine (0.2 ml/ mouse, s/c route). After 21 days of immunization, the mice were challenged with LD_{50} of *B. suis* virulent bacteria with 0.2 ml inoculum by intra-peritoneal route (1.0×10^5 cfu/ml) in 6 Nos. of mice from each group. The remaining mice in the groups were given booster dose and again challenged with virulent *B. suis* after 21 days post immunization. The blood samples were also collected prior to challenging study, to assess the serum antibody levels by using BruAlert kit available at TRPVB, TANUVAS. The results showed that in the first challenge study, 20%, 50% and 0 survival in the Groups I, II, III respectively whereas 80, 100 and 0% survival in the Groups I, II, III respectively were noticed in the second challenge after booster immunization, indicating that the booster dose was required to give complete protection to the mice. The ELISA results showed presence of antibody levels which was correlating with the challenge study. The role of adjuvants and imiquimod agents in conferring required immune responses will be discussed in the presentation.

[VIB-IAVR-OP-06]

Molecular Detection of Bacteria Causing Respiratory Tract Infection in Chickens

Bhanderi B B and Modi A R
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Himmatnagar,
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand
Kamdhenu University, Gujarat

Chickens are domesticated birds that are primarily raised for their eggs, meat, and feathers. Compared to illnesses that affect other organs, respiratory tract illnesses constitute a major part of diseases that affect poultry and lead to significant economic losses for the industry globally. In the present study, 52 poultry comprised of chicken (46), colored birds (04), and RIR (02) showing respiratory tract infections were selected for the molecular detection of bacteria during the period from December 2022 to March 2023 in and around Anand district of Gujarat. From the all 52 birds, lung and trachea were collected and pooled in a sterilized vial and processed for the extraction of DNA. PCR based detection of the major bacteria was carried out using specific primers. The result Out of 52 chicken, 98.07% (51/52) found to be positive for *E. coli*, 59.61% (31/52) for *Staphylococcus* spp., 9.61% (5/52) for *Mycoplasma gallisepticum*, 7.69% (4/52) for *Ornithobacterium rhinotracheale*, 3.84% (2/52) for *Avibacterium paragallinarum* and 1.92% (1/52) for *Mycoplasma synoviae* while negative in *Bordetella avium*, *Pseudomonas aeruginosa*, and *Pasteurella multocida*. The birds with etiology observed as 33.33%, 52.94%, 9.80% and 3.92% have one, two, three and four bacterial infections respectively. It is to be concluded that respiratory tract infection in chicken is caused by multiple etiological agents.

Keywords: Molecular detection, Bacteria, Respiratory tract, Chicken

Session VI
Immunoprophylaxis, Immunodiagnostics & Advances in Vaccine Research
(Poster)

[VIB-IAVR-PP-01]

Evaluation of Probiotic Potential of Traditional Kashmiri Fermented Rice Water (Sader Kaa'nz)

P. G Programme in Microbiology, Centre of Research for Development, University of Kashmir Srinagar, 190006 Hazratbal Srinagar
Abrar P, Ruqaya N and Uzma C

The scientific data supporting probiotic bacteria's positive impacts on human health has been steadily growing over the past 20 years, which has led to an increase in their popularity. Probiotics boost the mucosal defence against infections and the equilibrium of the gut microbiota, which both benefit human health. The aim of this work was to isolate, characterize, and identify certain lactic acid bacterial strains from traditional Kashmiri fermented rice water known as "Sader Kaa'nz" that may have probiotic properties. Seven isolates in all were found, and all of them were cocci-shaped and gram positive under the microscope. Primary screening was performed on them, and the colony morphological traits were examined. Four of the seven isolates that tested positive for catalase were further characterised in vitro for their probiotic traits, antibacterial activity against certain common bacterial pathogens of humans, and antibiotic susceptibility. The findings demonstrated that all four isolates could safely withstand acidic pH for three hours, 0.3-0.5% bile salts for four hours, and 0.2-0.4% phenol for twenty-four hours. They demonstrated potent antibacterial activity against the studied human pathogenic strains and were sensitive to the majority of widely used antibiotics. All four isolates displayed favourable percentages of co-aggregation, auto-aggregation, and cell surface hydrophobicity. In light of our findings, all the four isolates met the requirements for probiotic selection, making Sader Kaa'nz a beverage with strong probiotic potential. The results of this study could have important implications for the food industry, particularly as there is growing interest in traditional fermented foods that are believed to have health benefits and it may be possible to develop new probiotic products that are capable of combating common pathogens and withstanding the challenging conditions of the human gastrointestinal tract. Consequently, this study represents a valuable contribution to the field of probiotic research, offering a promising avenue for addressing microbial infections in humans. However, more thorough and in depth research for clinical usage is required to fully assess its benefits for human health.

[VIB-IAVR-PP-02]

Comparative Evaluation of Rapid Antigen Detection with Reverse Transcriptase Polymerase Chain Reaction for Detection of Novel SARS-CoV-2

Shah F Z1 , Fomda B2, Bali N3
Post-Graduate1, Professor & Head2, Associate Professor 3
Department of Microbiology Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar.

Background: The rapid and accurate detection of SARS CoV-2 is crucial to control outbreaks in the community and in hospitals. rRT–PCR is considered as the "gold-standard" test to diagnose SARS CoV-2. However, requirement of specialized instruments and technical expertise to perform the rRT-PCR assays coupled with the need for a sophisticated laboratory preclude the use of rRT-PCR. Rapid antigen tests have emerged as point of care diagnostic assays for testing. It is important to compare the diagnostic accuracy of these methods viz a viz RT-PCR. Objectives: The present study was designed to find out the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall diagnostic accuracy of rapid antigen test taking RT-PCR as the gold standard. Methods: A total of 300 nasopharyngeal/ororpharyngeal swabs were collected from patients suspected of having COVID-19 . Rapid antigen test was performed from the tube using STANDARD Q COVID-19 Ag test. RT-PCR of the sample was done after RNA extraction. Results: The sensitivity and specificity of rRT-PCR in our study was 95% and 92% respectively. The sensitivity and specificity of RADT was 86% and 90% respectively. The PPV and NPV of rRT-PCR was found to be 95% and 90% respectively whereas the PPV and NPV of RADT was 91% and 88% respectively. Conclusion: rRT-PCR was the most sensitive and effective method to diagnose SARS CoV-2 infection, however RADT showed a high.

[VIB-IAVR-PP-03]

Evaluation Of Vitek 2 Susceptibility Test Against the Clsi Agar Proportion Reference Method for Nitrofurantoin in A Tertiary Care Hospital

Bashir G, Tariq S, Fomda B A.
Department of Microbiology; Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, India.

BACKGROUND: Amongst the Enterobacteriaceae, Escherichia coli accounts for 75 to 95% of cases of urinary tract infection (UTI), the remaining being Klebsiella and Proteus mirabilis. The upsurge of drug resistant uropathogens in recent years has perplexed the treatment of UTIs, causing an escalation in morbidity and mortality. In terms of candidate antibiotics for UTI, especially for cystitis which is very common infection in community, selective pressure by antibiotics as well as treatment efficiency should be carefully considered. With this perspective nitrofurantoin would be a good candidate antibiotic for uncomplicated cystitis from the community.

OBJECTIVES: To evaluate VITEK 2 susceptibility test against the CLSI Agar Proportion Reference Method for Nitrofurantoin in urinary isolates of MDR Enterobacteriaceae.

METHODS: Of the 7971 routine urinary samples received in the Department of Microbiology from both inpatient and outpatient departments during the study period, 130 samples with isolation of MDR Enterobacteriaceae were recruited considering the inclusion and exclusion criteria. On the day of testing, the MDR strains of Enterobacteriaceae freshly sub-cultured on Hi-Chrome agar were subjected to MIC determination by Agar Dilution Method and Vitek 2 Compact system and the methods were compared statistically.

RESULTS: Overall, most common Enterobacteriaceae isolated from urine was E. coli (77.22%) followed by K. pneumoniae (20.87%) and Proteus spp. (1.92%). In patients of MDR UTI, E. coli was most common (73.1%) followed by K. pneumoniae in 26.9%. Of the 130 MDR Enterobacteriaceae 61.5% were sensitive to Nitrofurantoin by Agar Dilution, and 66.2% by VITEK 2. There was a substantial agreement between the two methods (Kappa=0.73).
CONCLUSION: MDR Enterobacteriaceae isolated from urine have a higher susceptibility to Nitrofurantoin as compared to other drugs and can be used as a dependable treatment option for UTIs empirically in Kashmiri population

[VIB-IAVR-PP-04]

Augmenting the Isolation Efficiency of Pathogenic *Leptospira* from Environmental and Rodent Samples

Archana Pal1#, K Vinod Kumar1, Prajakta Prashant Bokade 1, Swathi M, V Bharath1, Veena R. K2, Shilpa Devaraj2, Jagadeesha K2, Chethan Kumar H. B1., B. R. Gulati1, V. Balamurugan1

1Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, India
2District Surveillance Unit (DSU), Mangaluru, Dakshina Kannada 575001, Karnataka, India.

Leptospirosis, a zoonotic disease pervading multiple mammalian species globally, is engendered by *Leptospira* spp. Ascertaining the presence of these pathogenic bacteria for serotyping and genotyping is pivotal for epidemiological surveillance, facilitating the advancement of diagnostics and vaccines. Nevertheless, the process of isolating *Leptospira* from diverse specimens remains fundamentally insensitive. This study endeavours to explore the efficacy of selective agents and sample filtration in bolstering the success rate of cultivating pathogenic *Leptospira* from environmental water and rodent samples.

Methods incorporated the direct inoculation and filtration of samples, with added rabbit sera and 5 Fluorouracil (5-Fu). Experimental techniques included inoculating the sample directly into the Ellinghausen-McCullough-Johnson-Harris (EMJH) semi-solid media, with and without the addition of 3% rabbit sera. The use of a 0.22-µm pore size membrane filter to sieve samples and subsequent incubation for two days at 29°C was also applied, before inoculating the samples into EMJH with or without 3% rabbit sera. Results demonstrate that implementing a 0.22-µm pore size membrane filter augments the isolation efficiency by eliminating potential bacterial contaminants. Allowing the filtrate, a resting period of two days escalates the initial leptospires concentration threefold, thereby enhancing the isolation potential when introduced into EMJH. However, the filtration process may permit the growth of certain spirochetes alongside *Leptospira*, not impairing the leptospires proliferation. This parallel growth is mitigated in the presence of a high concentration of 5-Fu (0.25 mg/ml) and 3% rabbit sera.

PCR validation of isolates with *LipL32* gene-specific primers confirms that a strategic combination of filtration, a two-day waiting period for the filtrate, and using a high concentration of 5 Fluorouracil and 3% rabbit sera, proves efficacious in the isolation of pathogenic *Leptospira* from environmental samples and rodent urine or tissue. The outcomes of this study corroborate that the cultivation and isolation of leptospires from these sources can be substantially enhanced with refined methodologies.

[VIB-IAVR-PP-05]

Antiviral activity of traditional medicinal plants against SARS-CoV-2 infection

Vimal K Maurya, Shailendra K Saxena*
Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a zoonotic virus that may transmit between humans and animals. As more animals are found to be infected with the COVID-19 virus, it becomes evident that a One Health approach is essential for dealing with emerging disease risks that affect both humans and animals. Approximately 60% of new infectious diseases identified globally are caused by animals, both wild and domestic. Over the previous three decades, over 30 new human infections have been discovered, 75% of which originated in animals. SARS-CoV-2 is the etiological agent of COVID-19 and is responsible for more than 768 million confirmed cases, including 6.9 million deaths globally. Therefore, this study was planned to investigate the antiviral role of the active constituents against spike glycoprotein of SARS-CoV-2 as well as its host ACE2 receptor. Structure-based drug design approach has been used to elucidate the antiviral activity of active constituents present in traditional medicinal plants. Further, parameters like drug-likeness, pharmacokinetics, and toxicity were determined to ensure the safety and efficacy of active constituents. Gene network analysis was performed to investigate the pathways altered during COVID-19. The prediction of drug-target interactions was performed to discover novel targets for active constituents. The results suggested that amarogentin, eufoliatorin, α-amyrin, caesalpinins, kutkin, β-sitosterol, and belladonnine are the top-ranked molecules that have the highest affinity towards both the spike glycoprotein and ACE2. Most active constituents have passed the criteria of drug-likeness and demonstrated a good pharmacokinetic profile with minimum predicted toxicity level. Gene network analysis confirmed that G-protein coupled receptor, protein kinase B signaling, protein secretion, peptidyl-serine phosphorylation, nuclear transport, apoptotic pathway, tumor necrosis factor, regulation of angiotensin level, positive regulation of ion transport, and membrane protein proteolysis were altered during COVID-19. The target prediction analysis revealed that most active constituents target the same pathways which are found to be altered during COVID-19. Collectively, our data encourage the use of active constituents as a potential therapy for COVID-19.

[VIB-IAVR-PP-06]

Comparison of Immunological and Molecular detection tests for diagnosis of Canine Parvovirus from dogs of Himachal Pradesh

Deepika Thakur, Himani Ravi*, Prasenjit Dhar, Subhash Verma, Rajesh Chahota and Monika Bharadwaj
Department of Veterinary Microbiology
DGCN COVAS CSKHPKV, Palampur, H.P (1760612)

Diarrhoea is one of the most common clinical conditions seen in dogs caused by a variety of different etiological agents. Among common causative agents, Canine Parvovirus remains the most common cause of hemorrhagic diarrhea along with vomiting and which is often accompanied by high mortality among susceptible dogs. CPV can infect dogs of any age with the most severe infection commonly seen in the age group of 2-4 months old pups. The CPV is a rapidly changing virus leading to emergence of newer antigen strains and thus leading to its rapid spread among the canine population. This has led to appearance of clinical disease even in previously vaccinated animals thus leading to difficulty in accurately diagnosing the disease. A combination of molecular and immunological techniques thus offers the best hope of correctly diagnosing the disease. With this objective, the study was done to detect different antigenic variants of CPV in dogs of Himachal Pradesh and to find out the relative comparison of the molecular and immunological tests for the detection of CPV from clinical samples. A total of 238 clinical samples from affected dogs like faecal samples, urine samples and necropsied tissue samples were collected from different parts of Himachal Pradesh and processed in the laboratory. For molecular detection, the CPV DNA of all the 238 samples were initially extracted and subjected to PCR amplification using primer pairs CPV-2, CPV-2ab, CPV-2b and CPV-2c targeting *VP2* gene while CPV DNA of Megavac-P vaccine strain was used as a positive control. For immunological detection, all the samples were initially screened for HA activity using 1% washed pig erythrocytes. Samples showing positive HA activity were then serologically tested by HI and Dot ELISA using hyper- immune sera raised in rabbits.

A total of 109 samples out of 238 (45.8 %) were found positive for CPV DNA on PCR. No sample was found positive for original CPV-2 strain, CPV-2a and CPV-2c strains out of the 238 samples processed. All the 109 positive samples were found to be CPV-2b antigenic variant. In HA test, 89 out of 238 (37.39 %) samples showed positive HA titres ranging from 1:2 to 1:2048. that were then confirmed using HI test with similar results. However, 91 out of 238 (38.23 %) samples showed positive results in Dot ELISA with formation of distinct brown coloured spots on nitrocellulose membrane.

In conclusion, molecular tests like CPV specific PCR appears to be the more sensitive test for detection of CPV in clinical samples while Dot ELISA was found to be the more sensitive immunological test. The study also revealed that CPV-2b antigenic variant was the most prevalent strain in dogs in the state of Himachal Pradesh and so future vaccines should incorporate this strain for better protection in field conditions.

[VIB-IAVR-PP-07]

Development of a recombinant *Lactobacillus*-based vaccine for salmonellosis in poultry

Junaid Nazir1,2, Tasaduq Manzoor, Afnan Saleem1, Zulfikar ul Haq3, Ishfaq ul Hussain4, Priyanka Jha2, Syed Mudasir Ahmad1*

1 Division of Animal Biotechnology, FVSc & AH, Shuhama, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, 190006, India

2 Department of Clinical Biochemistry, Lovely Professional University, Phagwara, 144411, Punjab, India

3 Division of Livestock Production and Management, FVSc & AH, Shuhama, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, 190006, India

4 Department of Veterinary Microbiology and Immunology, FVSc & AH, Shuhama, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, 190006, India

Salmonellosis poses a serious threat to the poultry industry, causing huge economic losses and posing risks to human health. Vaccination is essential for controlling *Salmonella* infection in poultry, and there is growing interest in alternative vaccine delivery platforms, such as recombinant vaccines. This study aims to present the development of a recombinant *Lactobacillus*-based vaccine for salmonellosis in poultry. The vaccine involves genetically modifying *Lactobacillus* to express antigens that protect against *Salmonella*. The recombinant *Lactobacillus* strains serve as antigen-presenting cells and mucosal adjuvant, stimulating immune responses at the site of infection. The vaccine's immunogenicity and protective efficacy were evaluated in a poultry model, which revealed robust immune responses and significant protection against *Salmonella* challenge. This study contributes to the development of a safe and effective *Salmonella* vaccine strategy in poultry, with advantages in administration, mucosal immune responses, and cost-effective mass production. Future research will concentrate on vaccine optimization, large-scale production and application.

Keywords: Salmonellosis, poultry, recombinant vaccine, *Lactobacillus*, immunogenicity, protective efficacy.

[VIB-IAVR-PP-08]

Evaluation of Vitek 2 Compact system in identifying *C. auris* in comparison to MALDI-TOF, which is the gold standard in identifying *C. auris*

Bashir G1, Majid U2,
Professor, Dept. of Microbiology, SKIMS. 2PG Scholar, Dept. of Microbiology, SKIMS
Department of Microbiology
Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar

Background: *C. auris*, a novel *Candida* species first reported in Japan in 2009, is an emerging pathogen that has been isolated on five mainlands. *Candida auris* is an emerging fungus that presents a serious global health threat. it is resistant to multiple antifungal drugs. It is difficult to identify with standard laboratory methods, and it can be misidentified. It is difficult to identify with standard laboratory methods, and it can be misidentified. Patients who are colonized with *C. auris* can develop an invasive bloodstream infection day to months after becoming colonized.

Aims and objectives: Evaluation of VITEK 2 Compact system in identifying *C. auris* in comparison to MALDI-TOF, which is the gold standard for identification.

Method: a total of 21 isolates were subjected to two different diagnostic modalities for identification of *C. auris*, one being a gold standard i.e; MALDI TOF-MS and the other being VITEK 2 Compact

Results: 71.50% were identified by MALDI TOF-MS against 62.00% by VITEK 2 Compact. P value = 0.326, therefore the difference between identification by both methods compared to each other was statistically non-significant

[VIB-IAVR-PP-09]

Prevalence of Hepatitis A and E positive cases among clinically suspected patients at SKIMS

Ayoub M1, Fomda B2. Bali N3, Wani S4
Post-Graduate1 , Professor & Head2 , Assistant Professor3, Associate Professor4
Department of Microbiology Sher-i-Kashmir Institute of Medical Sciences,
Soura, Srinagar.

Introduction:
The hepatitis A virus (HAV) is a positive-sense, single-stranded RNA virus that belongs to the family Picornaviridae. The hepatitis A virus (HAV) is a common infectious etiology of acute hepatitis worldwide. HAV is most commonly transmitted through the oral-fecal route via exposure to contaminated food, water, or close physical contact with an infectious person.

Hepatitis E virus (HEV) is the aetiological agent of non-HAV enterically transmitted hepatitis. It is the major cause of sporadic as well as epidemic hepatitis, which is no longer confined to Asia and developing countries but has also become a concern of the developed nations. In the Indian subcontinent, it accounts for 30–60% of sporadic hepatitis.

Aim and objectives:
To study the prevalence of Hepatitis A and E positive cases among clinically suspected patients admitted in SKIMS, Soura hospital.

Methodology: A total of 453 and 504 blood samples were collected from clinically suspected Hepatitis A and Hepatitis E patients respectively. Diagnosis was made based on serologic testing i.e., IgM anti HAV and IgM anti HEV to detect Hepatitis A and E respectively.

Results:
Out of 453 clinically suspected cases, 73 patients were positive for hepatitis A virus and out of 504 cases, only 3 were positive for hepatitis E virus.

Conclusion:
The positive cases for both Hepatitis A and E belonged to the age group of 6-35 years.

High-risk groups for hepatitis A patients include, people traveling to endemic areas, and isolated communities. HAV does not cause chronic liver disease unlike hepatitis B or C.

It is generally accepted that hepatitis E is mostly self-limited and never progresses to chronicity. It has a higher mortality in pregnant women where the disease condition is accentuated with the development of fulminant liver disease.

Session VII
Biotechnological Interventions for Enhancing Animal Health & Production (Oral)

[VIB-BI-IVL-01]

Application of MaLDI TOF in the disease diagnosis

Mudit Chandra, Gurpreet Kaur, Deepti Narang and A K Arora
Department of Veterinary Microbiology, College of Veterinary Science, GADVASU, Ludhiana

Age old standard method of identification of bacteria from a clinical case or cases relies on culturing of the bacteria on a suitable medium to allow its growth. Once the growth has been achieved on a medium the next task is to identify the bacteria that have grown on the medium. The standard method is to grow the bacteria on some selective medium (which helps in the growth of those bacteria) and further it being subjected to various biochemical tests for its accurate identification. This standard method is time tested and is a being followed in most of the microbiological laboratories. Though, these methods of identification are good but they have some inherent problems or pitfalls; one being very cumbersome (requires preparation of different media), requiring lot of time in delivering results and the other being that it requires technical expertise. Also species level identification of microorganisms is difficult and requires need of other techniques for it.

Since the clinicians are partially dependent on the report of microbiologist so as to start treatment thus time required to deliver identification results becomes one major issue which needs our focus.

Ever since, scientist have tried to reduce the time for diagnosis by using different strategies such as roping in of some immunological or molecular based tests which again require greater technical expertise and cannot be adopted in a routine diagnostic laboratory. Recently with the introduction of Matrix assisted laser desorption ionization- Time of Flight (MALDI-TOF) bacterial identification system, a lot has changed. Using this technology we could identify bacteria very quickly. One major advantage of MALDI-TOF is that besides being quick, it is highly specific and doesn't require much technical knowledge and expertise.

MALDI is based on mass spectrometry where mass-to-charge ratio (m/z) of an analyte, providing spectra within minutes is detected. This provides a unique mass spectral fingerprint of the microorganisms which upon comparison from the database provides us the identity of the bacteria isolated in the laboratory. In mass spectrometry biopolymer molecules present in the condensed phase are converted into intact, isolated ionized molecules in the gaseous phase. Then, ions are separated according to their molecular weight after migration in an electric field. Each molecule detected is characterized by: the molecular mass (m), the charge (z), the ratio mass/charge (m/z), and the relative intensity of the signal. This leads to accurate analysis of peptides and its determination.

Initially only molecules of low molecular masses were analyzed and the limit size of these varied from 1 kilodalton (KDa) for biopolymers to 9.0 KDa. Later, soft ionization techniques such as MALDI-TOF and electrospray ionization (ESI), which were introduced in the late 1980, have largely overcome the problem of harsh ionization. Of these two, MALDI-TOF proved to be most effective for bacterial identification as it allows the detection of macromolecules in complex mixtures without prior purification of samples.

The procedure of MALDI-TOF includes several steps:

1. Sample is spotted onto a MALDI-TOF sample target plate.
2. It is loaded with an appropriate matrix and allowed to air dry at room temperature.
3. Then, the plate is inserted into the machine.

4. The dried matrix-sample mixture is bombarded with a laser to create gas phase ions that are then pulsed into a flight tube.
5. Generally, only a singly ionized species having a single charge is produced.
6. The species of interest are identified by their mass/charge ratio; the m/z value is obtained from the centric of the peak.

The detection of mass spectral fingerprint has become a convenient tool for the rapid analysis of bacteria. The method analyzes the profiles of bacterial components that are extracted from intact bacteria.

Databases

The identification by MALDI-TOF is based on the following findings:
1. Spectral fingerprints vary between microorganisms.
2. The molecules detected in the spectrum, some peaks (molecular masses) are specific to genus, species, and sometime to subspecies,
3. Spectra obtained are reproducible as long as the bacteria are grown under the same conditions.

One of the major components used in MALDI-ToF, besides equipment is the matrix. Matrix serves two major functions viz., absorption of energy from the laser and isolation of the biopolymer molecules from each other. Many matrices are available commercially but they all require almost same physical and chemical properties:
1. An efficient absorbance at the laser wavelength,
2. An efficient ionization,
3. An important stability not to interfere with the mass spectrum of the sample.

Examples of matrix include:

1. 2, 5-dihydroxybenzoic acid (gentisic acid), (for studying oligosaccharides, glycopeptides, and glycoproteins.
2. 3, 5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid),
3. α-cyano-4-hydroxycinnamic acid (α-CHCA).
4. Ferulic acid

Thus, as we find that MALDI-TOF is simple and easy to use thus I feel that it will soon be adopted in routine clinical laboratories for bacterial identification.

[VIB-BI-OP-01]

Effect of different dietary sources of betaine on production performance and immune response in commercial broilers

Dr. N. R. Senthil, *Central Clinical Laboratory, Madras Veterinary College, Vepery, Chennai – 600 007*
Dr. G. Srinivasan, *Pulikulam Cattle Research Centre, Manamadurai – 630 606*
Dr. C. Pandian, *Veterinary University Training and Research Centre, District Collectorate Campus, Sathuvachari, Vellore – 632 009*
Dr. Richard Churchil. R, *Department of Poultry Science, Madras Veterinary College, Vepery, Chennai – 600 007*

Poultry has become an indispensable part of the nutritional requirement of the current dietary plants across the world due to the nourishment it provides. Such that the Indian poultry industry has amassed a revenue of over Rs.2,500 billion in FY 2022-23, due to an increase in the demand. At this juncture, a demand of proper diet to improve the production performance of commercial broiler chicken have also risen, with a subsequent increase of the price of these diets. Hence, we developed different dietary sources of betaine that could significantly impact the production performance and the immune response in commercial broilers. Having understood the importance, we studied the impact of different dietary sources of betaine on the genes involved in the immune response pathways. A total of 250 broilers was considered for the study that spanned across 28 days, and were divided into 5 groups of 50 broilers in each group. Group 1 served as control, Group 2 received OptiBetaine at 750 g/ton orally, Group 3 received Natural Betaine at 500 g/ton, orally. Group 4 received OptiBetaine at 1000 g/ton orally, and Group 5 received Synthetic Betaine at 1000 g/ton orally. At the end of study, intestine, liver and spleen were extracted from the chickens and gene expression was studied using real time PCR. The genes studied were HSP70, IL2, IL4, IL6, IL12, IL17D, TGF-beta, TNFsf15, IFN-gamma and CXCL4. Significant upregulation of IL12 expression was seen in group 3 when compared to Group 2. Significant regulation of HSP70 gene was seen in Group 2 intestine, liver and spleen of Group 2 broilers when compared to other treatment groups. Similar trends were also observed in Group 2 broilers when compared to other treatment groups. The genetic response significantly correlates with the haematology and biochemistry parameters in Group 2 fed with betaine highlighting its ability against heat stress and a significant increase in the production performance.

[VIB-BI-OP-02]

Development of Tumour Specific vectors for therapy of canine cancers

Saxena Sonal *, Subas Jena, Sameer Shrivastava, Pradeep Kumar, Bishnu Prasad Mishra and Raj Kumar Singh

*Corresponding author: Dr Sonal, Senior Scientist, *ICAR-IVRI, Izatnagar, sonalvet@gmail.com*

ICAR-Indian Veterinary Research Institute, ICAR-IVRI, Izatnagar

Cancer is still the most common cancer type and the leading cause of cancer-induced mortality both in humans and animals, especially pet animals. In case of pet animals, canine mammary tumours (CMTs) have the highest incidence in female dogs, with thrice higher mortality rates as compared to human breast cancer. Majority of canine mammary tumors have poor clinical outcomes and frequently result in death due to metastatic disease. Many chemotherapies used in dog fail to produce appropriate response, besides being associated with high cost and side effects. Considering the poor prognosis associated with CMTs, there is a need for newer diagnostic and therapeutic strategies for disease management. In this study tumour specific promoters associated with CMTs were identified and therapeutic constructs were designed utilizing tumour specific promoters for delivery of target genes to tumour cells. The gene constructs showed specific expression only in cancer cells and no expression in healthy canine cells. Therapeutic constructs were developed incorporating apoptotic genes such as bax, bid and tumour necrosis factor alpha which were cloned under tumour specific promoters. The therapeutic constructs showed specific apoptosis in canine cancer cells *in vitro*. One of the constructs when tested in clinical cases of canine mammary tumour, showed selective killing of tumour cells, as shown by decrease in tumour volumes, along with decrease in CMT associated biomarkers. These constructs are safe, cost effective and more effective, and can be used for treatment of CMT cases.

[VIB-BI-OP-03]

Production of β-lactoglobulin (BLG) gene knockout blastocyst stage embryos of Indian water buffalo using CRISPR and SCNT technology

Tara A*, Singh P, Gautam P, Tripathi G, Malhotra S, De S, Singh M K, Selokar N L
Presenting author: Tara A
Corresponding author: Selokar N L (naresh.selokar@icar.gov.in)
Animal Biotechnology Division (ABTD), ICAR-National Dairy Research Institute, Karnal, Haryana, 132001

In several tropical countries, buffalo milk has a high-value demand than cow milk due to its nutritional and economic value. In India, buffalo is the main dairy animal and contributes 45% of the total milk produced in the country. Besides the nutritive value of milk, several allergen proteins such as casein, α-lactalbumin, β-lactoglobulin (BLG), and immunoglobulins have been reported. The breeding strategies, nutritional management, and quantitative genetics have improved milk yield, but these approaches could not lead to significant changes in milk composition. With the development of biotechnology, especially genome editing tools (CRISPRs), it is possible to generate new value-added products such as designer hypoallergenic milk for human health benefits. Keeping this in mind, we planned to utilize the CRISPR tools to disrupt the buffalo β-lactoglobulin (BLG) gene for production of hypoallergenic milk in long run. To achieve our aims, 3 sgRNAs against the BLG locus of buffalo were designed, and their editing efficiency was determined using Sanger sequencing followed by TIDE and ICE analysis. Among 3 sgRNAs, best efficient sgRNA was used to generate the clonal population of edited cells. Several single-cell clones were established and screened using the TA cloning and Sanger sequencing methods. Of 14 single-cell clones screened, 8 were found to have BLG gene disruption events (57% editing rates). We successfully produced cloned blastocyst stage embryos from 4 BLG-gene disrupted clonal cells using the SCNT. The cloned blastocyst production rates (25 to 30%) were similar to non-edited control cells. Efforts are ongoing to establish pregnancies from BLG-KO cloned embryos. This work can lead to generation of the designer buffaloes for the production of hypoallergenic milk for human benefits.

Keywords: Buffalo, Milk allergy, β-lactoglobulin, CRISPR, SCNT, and Hypoallergenic milk.

[VIB-BI-OP-04]

Effect of lab-made transfection buffer on delivery of genome modification components into primary cells of buffalo, cattle, goats, and sheep

Malhotra S*, Singh P, Tara A, Gautam D, Parsad R, Tripathi G, De S, Singh M K and Selokar N L
Presenting author: Shreya Malhotra
Corresponding author: Naresh L. Selokar (naresh.selokar@icar.gov.in)
Animal Biotechnology Division (ABTD), ICAR-National Dairy Research Institute, Karnal, Haryana, 132001

The transfer of genome-modification components into farm animal cells is indispensable for the production of genome-modified and transgenic farm animals. Electroporation is a physical method of transfection when appropriately used; this technique is safe, simple to use, affordable, and efficient in transfecting cells from a number of lineages. Electroporation efficiency depends on various physical parameters, of which cell type is considered to be a major factor for transfection efficiency. Primary cells are generally less susceptible to transfection than other cell types due to their finite lifespan and limited expansion capacity. Previously we developed an indigenous transfection buffer for the delivery of exogenous genetic components into mammalian cells. In the present study, we examined the effect of developed buffer on transfection rates and cell viability of primary somatic cells from buffalo, cattle, goats, and sheep. To achieve the aims of this study, the primary somatic cells from skin biopsies were established and were transfected with Venus-expression vector (pCAGGs- Venus). We noticed that transfection rates of pCAGGs-Venus were 22.51%, 17.56%, 22.81%, and 16.16% for buffalo, cattle, goats, and sheep cells, respectively. We also noticed that cell viability and proliferation rates were better in the case of goats, sheep, and cattle cells; also, these cells have less vacuolation than that buffalo cells. In addition, we also generated MSTN KO cell clones from these cell populations, in which the efficiency of single-cell clone generation was high for goats and sheep cells. In conclusion, our lab-made transfection buffer can be efficiently used to generate genome-edited or transgenic farm animals for agriculture, biomedical, and veterinary applications.

Keywords: Transfection buffer, genome modification, and CRISPR

[VIB-BI-OP-05]

Poly I:C Stimulation Unveils Cell-Type-Specific Antiviral Responses in Water Buffalo (Bubalus bubalis): Insights into RLR Pathway Activation and Interferon Expression.

Devika Gautam1,2, Anil Sindhu2, Ashutosh Vats1, Shiveeli Rajput1, Sachinandan De1*

Animal Biotechnology Centre, ICAR-National Dairy Research Institute, Karnal-132001(HR), India. Tel: + 91 0184-225-503

Indian cattle (Bos indicus) and buffalo (Bubalus bubalis) have adapted to India's diverse climate, developing resistance to infectious diseases unlike exotic cattle. Reports indicate lower susceptibility to bacterial, viral, and parasitic diseases in Indian zebu cattle and buffaloes compared to Bos taurus breeds. Factors such as T-cell proportions, interferon response, cellular receptors, apoptosis regulation, and cytokine interplay contribute to their non-specific disease resistance. Upon viral invasion, the antiviral innate immune system activates by recognizing viral components, such as dsRNA, through specific receptors. This triggers the production of type-I interferons (IFNs), which confer an antiviral state in both the invaded cells and surrounding cells. The fibroblast, monocyte, and macrophage cells derived from water buffalo (*Bubalus bubalis*) were exposed to a synthetic dsRNA analogue, poly I:C to mimic viral invasion. Recognition of poly I:C through cytosolic helicase receptors, namely RIG-I and MDA5, led to the activation of the RLR pathway, subsequently triggering the MAVS-IRF3/7 cascade and the production of antiviral effector molecules like IFNβ and ISGs. Differential expression pattern of RLR receptor and IFNβ expression by different cell types was observed following poly I:C treatment. Fibroblasts displayed a rapid and robust IFNβ response, followed by macrophages and monocytes. Despite their absolute expression differences across three cell types, the expression trend of RLR pathway genes remained similar. The length of the poly I:C molecule also influenced IFNβ expression through the RLR pathway. Short poly I:C molecules induced stronger IFNβ expression in myeloid cells (macrophages and monocytes), while long poly I:C molecules preferentially elicited higher IFNβ expression in non-myeloid cells (fibroblasts). Therefore, MDA5 and RIG-1 play essential roles in triggering the antiviral response in non-immune host cells, particularly fibroblasts. Stimulation of the RLR pathway using suitable and potentially cell-type-specific agonist molecules effectively induces an antiviral state in the host animal, with fibroblasts conferring a stronger antiviral state compared to monocytes and macrophages.

[VIB-BI-OP-06]

Enhanced developmental efficiency of in vivo produced Myostatin Gene edited embryos derived through CRISPR Cas9 microinjection.

Abrar A Malik1, Suhail N Magray1, Nida Handoo1, Younis Farooq1, Syed Hilal Yaqoob1, Muneer A Dar1, Mujeeb Fazili1, Syed Mudasir Ahmad1, Abdul Majeed Ganaie2, Riaz A Shah1*

1Division of Animal Biotechnology, Faculty of Veterinary Sciences, SKUAST-Kashmir, J & K, India

2Professor Incharge, MRCS&G, Shuhama, Faculty of Veterinary Sciences, SKUAST-Kashmir

**Presenting author: - Email: drriazshah@gmail.com, Mob. No. 9797794864*

The CRISPR Cas system has advanced the field of genome engineering in an unprecedented way. This research is focused to increase productivity of Sheep by enhancing body weight gain through generation of double muscled Sheep employing plasmid-based CRISPR/Cas9 gene editing technology to target MSTN exon-1 coupled with zygote microinjection.

Briefly Guide RNA's spanning 135 bp apart of exon 1 of MSTN gene were designed using online CRISPOR tool. Each single guide RNA coding DNA sequence was cloned into linearized PX459 vector backbone to generate two separate constructs. The dual guide construct harboring two guides was assembled by Gibson cloning method. Validation of construct was done by sequencing. The editing activity of the construct was evaluated in primary dermis derived fibroblasts of Sheep after electroporating the said construct and evaluating the cells by in house PCR and sequencing.

Ten non pregnant healthy ewes were selected and estrus synchronization was done using intravaginal progesterone sponge. An 18 day superovulation protocol was followed wherein sponges were removed on day 15/16. PGF2α shot (125mcg/animal) was given on day 8 followed by a constant 12 hrly dose of pFSH from day 13 to day 17. 24 hours after the last shot of pFSH, single shot of GnRH (10mcg/animal) was administered. Heat detection and breeding was done using a trained merino ram from day 16 evening till day 17 evening. On day 18, presumed zygotes were collected via laparoscope-assisted flushing in TCM 199 based collection media. The CRISPR Cas construct was purified using endotoxin free kit-based purification method. 2-4 picolitres of 100mg/ml solution of the plasmid construct was injected into each presumed zygote. mSOF was used as IVC medium in all the experiments afterwards. The 18-day superovulation protocol coupled with natural mating yielded an average of Eight (08) presumed zygotes per animal. A total of 43 zygotes were selected after quality evaluation for microinjection. 10 blastocysts were analyzed for editing efficiency of MSTN exon 1 using on site PCR based detection & genotyping. 7 out of 10 analyzed blastocysts revealed 135 base pair monoallelic deletion in MSTN exon 1. Selected blastocysts were transferred to synchronized recipient ewes. The sequencing results showed that editing at specific sites was achieved in sizeable proportions, demonstrating that the delivery of CRISPR Cas9 system utilizing plasmid-based approach for microinjection of zygotes has the potential to become a method of choice for generation of gene edited animals.

[VIB-BI-OP-07]

Plant based nanobiotics as novel feed additive improved the gut microbiology and immune status of broiler chicken

Adil S[1], Banday M T[1*], Wani M A[1], Kashoo Z[2], Muzamil S[3] and Sheikh I U[1]*

[1]Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir, India, [2]Division of Veterinary Microbiology and Immunology, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir, India, [3]Division of Veterinary Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, SKUAST-Kashmir, India

***Correspondence:** Banday MT, drtbandayade@gmail.com; aadilsheikh5@gmail.com*

This study aimed to investigate the effect of supplementation of nanoencapsulated Lavender essential oil (LEO) as a novel feed additive against antibiotic growth promoters on gut microbiology and immune status of broiler chicken. 420, one week old broiler chicks (Cobb 400) were randomly allocated to 7 treatments of 4 replicates (floor pens) each having 15 chicks. The treatment groups included: CON (Control) fed basal diet only, AB (Antibiotic) fed basal diet+ 10 mg/kg enramycin, CS fed basal diet+ 300 mg/kg Chitosan nanoparticles, LEO_{F200} and LEO_{F400} fed basal diet+200 mg/kg and 400 mg/kg free LEO respectively, LEO_{N200} and LEO_{N400} fed basal diet +200 mg/kg and 400 mg/kg nanoencapsulated LEO respectively. The results revealed that the cecal microbial population was remarkably influenced ($p < 0.05$) in all the dietary treatments compared to CON. Cecal coliforms decreased ($p < 0.05$) and Lactic acid bacteria increased ($p < 0.05$) notably in LEO supplemented groups with better values in nanoencapsulated groups (LEO_{N200} and LEO_{N400}). Total bacterial count decreased in the cecal contents of all treated birds compared to CON. Cell mediated immunity tended to improve ($p < 0.05$) in a dose dependent manner in LEO groups with significant value in LEO_{N400} as against CON. Humoral immunity was also found to increase significantly ($p < 0.05$) in LEO groups than CON particularly in nanoencapsulated groups. The expression of TNF-α upregulated and IL-10 downregulated in LEO groups with highly significant ($p < 0.05$) values in nanoencapsulated LEO groups when compared to CON. In conclusion, the nanoencapsulation of LEO proved to be a viable strategy in improving the gut microbiology and immune status of broiler chicken.

Key words: Broiler chicken, essential oil, nanoencapsulation, immunity, microbiology

Biotechnological Interventions for Enhancing Animal Health & Production (Poster)

[VIB-BI-PP-01]

Isolation and Characterization of Exosomes Derived from Buffalo Oviductal Epithelial Cells

Naheef K*, Brindha K, Senthil Kumar TMA, Sesh PSL, Rangasamy S, Dhinakar Raj G
Affiliation: Dept. of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-07 Correspondence Author: Naheef K, Ph.D. Scholar (Animal Biotechnology)
Email: nahifkhan04vet@gmail.com

Exosomes are the nano-sized vesicles secreted by cells and they function as bioactive cargo reflecting particular cells' kind and physiological state. Just as every cell does, oviductal epithelial cells also release their molecular cargo as exosomes to their luminal microenvironment to establish the earliest maternal signal between the oviduct and the developing embryo. In the present study, exosomes were successfully isolated by culturing the oviductal epithelial cells (OECs) of buffaloes *in vitro*. Nanoparticle analysis revealed that the size of the oviductal exosomes ranged between 40-150nm. Imaging by Transmission Electron Microscopy showed that these exosomes exhibited circular or cup morphology. The identity of isolated exosomes was further confirmed by analysing the expression of surface markers- tetraspanins (CD9 and CD63) by flow cytometry. Such characterization studies along with further profiling of molecular cargo of oviductal exosomes would help in better understanding of pre-implantation embryo-maternal communication within the oviduct which is missing in *in vitro* embryo production systems.

[VIB-BI-PP-02]

Successful establishment of CRISPR-based genome-edited clonal cell populations from primary cells of buffalo, goats, and sheep

Singh P*, Jose B, Gautam D, Tara A, Malhotra S, De S, Singh M K, Selokar NL
Animal Biotechnology Division (ABTD)
ICAR-National Dairy Research Institute
Karnal, Haryana, 132001

Genome editing technology has great potential for precise modification of DNA in any mammalian cells. The ability to precisely generate the clonal population of CRISPR-edited genotype is of great importance in gene function/pathway analysis, drug discovery, and production of genome-edited animals. In the present study, we demonstrated an efficient method to generate CRISPR-edited single-cell clonal populations of farm animals including, buffalo, goats, and sheep. To generate clonal cell populations, the primary fibroblasts were established through explant culture, and then, electroporated with CRISPR/Cas RNPs targeted for the disrupted MSTN gene. We used a single-cell pickup method in which one cell was picked up using ultra fined glass capillary, and transferred into each well of a 96-well plate. For promoting the growth of single cells, we used growth factor supplemented media. After seeding of single cell to each well, the plate was kept undisturbed for 5-7 days, and then cell attachment rates were noted. We reported that the cell attachment rates for buffalo, goat, and sheep cells were 40%, 77.08%, and 83.67%, respectively. The proliferation rates were 70.83%, 75.67%, and 78.05% for buffalo, goat, and sheep cells, respectively. We noticed that cell attachment as well as proliferation rates were better in the case of goat and sheep cells; also, these cells exhibited less vacuolation compared to buffalo cells. In the present study, we generated 11, 20, and 20 single-cell clones of MSTN-gene edited buffalo, goat, and sheep cells. In conclusion, our method can be efficiently used to generate genome-edited single-cell clones to harness the potential of CRISPR technologies in farm animals.

Keywords: CRISPR, single cell, clonal population, genome-editing, and farm animals

[VIB-BI-PP-03]

Isolation and growth kinetics of canine distemper virus in Vero cells

Manu M* and Yashpal Singh Malik#

College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

**Presenting author: manumohankrishna123@gmail.com;*

#Corresponding author: malikyps@gmail.com

Abstract

Canine distemper virus (CDV) infection results in high morbidity and mortality in dogs. CDV is pantropic and infects a broad range of animal families such as *Canidae, Mustelidae, Procyonidae, Ursidae, Viverridae, Hyaenidae, Felidae and Ailuridae*. The disease is enzootic in the Indian dog population and free-ranging dogs pose a threat of CDV transmission to wildlife. In the present study, CDV was successfully isolated using clinical sample from naturally infected dog in Vero cells. Cytopathic effects (CPE) were observed from the third passage onwards. At the fifth passage level, N gene targeted in house developed reverse transcription polymerase chain reaction was used to confirm CDV. Virus infectivity testing done and titre was calculated based on the observation of CPE. The growth kinetics of the isolated virus was studied using one-step growth curve with three (1.0, 0.1 and 0.01) different multiplicities of infection (MOI) using CDV of the fifth passage. The growth curve study revealed that MOI of 0.1 is the most suitable for obtaining high titres of virus ($10^{6.5}$ $TCID_{50}$/ml) and the optimum harvest time is 96 hours post infection. It has been observed that higher and lower MOIs result in the reduction of CDV titres ($10^{5.6}$ and $10^{5.9}$$TCID_{50}$/ml). The isolated virus can be used as an antigen for development of diagnostics. The growth curve data can be useful for the cultivation of CDV for different research purposes.

Keyword: Canine distemper virus (CDV), cell culture, isolation, one step growth curve.

Session VI

[VIB-BI-PP-04]

Development of Collagen based polymeric scaffolds from chicken feet incorporated with anti-bacterial compounds for wound healing applications.

Tasaduq Manzoor1†, Syed Mudasir Ahmad1†, Sahar Saleem1*
1 Division of Animal Biotechnology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Kashmir
**Corresponding Author*
**Sahar Saleem, SERB Scientist, Division of Animal Biotechnology*
Faculty of Veterinary Sciences and AH, Shuhama, SKUAST-Kashmir, 190006
saleem.sehar@gmail.com

Abstract: The poultry sector generates a large volume of chicken feet as a by-product. An effective method has been utilised to extract the collagen from it. The objectives of this study were to isolate the collagen from chicken feet and use it as source of biomaterial incorporating antimicrobial compounds. Collagen was isolated from chicken feet by chemical method with a %age yield of 24.26 % by dry weight. Collagen extraction consisted of three stages of pre-treatment, hydrolysis, and hydro-extraction. Chemical characteristics of chicken feet collagen were evaluated using SDS-PAGE, FTIR spectra and NMR. The SDS-PAGE profiles of the extracted collagen comprised of α_1 and α_2 chain protein units indicating the presence of type I collagen. The FTIR results showed the characteristic peaks corresponding to amide A, amide I, amide II, and amide III bands. The NMR indicated the presence of peaks characteristic of collagen. The scaffolds based on collagen and gelatin scaffolds were incorporated with beberine as an antibacterial compound. The surface morphology of scaffold was determined by SEM. The *in vitro* anti-bacterial efficiency of the developed scaffold was tested against *S. aureus.*

[VIB-BI-PP-05]

Innovative technique of keyhole ovariohysterectomy in female dogs for post-operative pain management

Taziyun Imtiyaz, Dil M Makhdoomi and Hakim Athar
SKUAST-K, Shuhama, Alusteing, Jammu and Kashmir, India
Taziyun Imtiyaz *Division of Veterinary Surgery and Radiology, F. V. Sc. &A. H., SKUAST-K, Shuhama, Alusteing, Jammu and Kashmir, India* taziyunimtiyaz99@gmail.com
Dil M Makhdoomi *Director Extension SKUAST-Kashmi*r
Hakim Athar Asstt. Professor *Division of Veterinary Surgery and Radiology, F. V. Sc. &A. H., SKUAST-K, Shuhama, Alusteing, Jammu and Kashmir*

Present study was undertaken to determine the intraperitoneal analgesic effect of bupivacaine and tramadol in female dogs undergoing keyhole ovariohysterectomy. The pain assessment was done before surgery and at different time intervals up to 18th hour after surgery using two pain scales Glasgow composite pain scale (GCPS) and University of Melbourne pain scale (UMPS)}. To undertake the study animals were divided into three groups, group A, group B and group C (each containing 12 animals). The animals of group A were given bupivacaine and tramadol intraperitoneally, group B were given bupivacaine intraperitoneally and in group C animal's normal saline was used intraperitoneally. Significant number of animals in Group A showed earlier recovery after 6^{th} hours post-operatively of the study and in group B the recovery was seen in 8^{th} postoperative hour and group C showed recovery at the end of the study that is at the 18^{th} post-operative hour of the study.

Keywords: Intraperitoneal, keyhole, bupivacaine, tramadol, normal saline solution

[VIB-BI-PP-06]

RNA-Seq Analysis of *E. coli* Induced Apoptosis in Bovine Mammary Epithelial Cells: Unraveling Gene Expression Dynamics

Afnan Saleem*1, Syed Mudasir Ahmed1, Tasaduq Manzoor1, Junaid Nazir1
1Division of Animal Biotechnology, FVSC & AH, SKUAST-Kashmir

Bovine mastitis, a prevalent and economically significant disease in dairy cattle, is often caused by bacterial infections, with *Escherichia coli* (*E. coli*) being a major pathogen. *E. coli*-induced apoptosis of bovine mammary epithelial cells (bMECs) is a critical aspect of the host-pathogen interaction, leading to tissue damage and impaired milk production. To comprehensively explore the molecular mechanisms underlying this apoptotic response, we performed RNA sequencing (RNA-Seq) analysis on bMECs exposed to E. coli. The apoptotic response was assessed through multiple techniques, including cell morphology evaluation, Annexin V-FITC/propidium iodide staining, caspase-3 activity and gene expression analysis by qPCR analysis. RNA-sequencing was conducted to identify changes in key apoptotic signaling pathways and inflammatory mediators. The results revealed a substantial number of DEGs in *E. coli* infected bMECs compared to uninfected controls. Several key apoptotic genes, such as Bcl-2 family members, caspases, and death receptors, showed significant dysregulation, consistent with the induction of extrinsic pathway of apoptosis. Additionally, genes associated with inflammatory and immune responses were prominently upregulated, reflecting the interplay between apoptosis and immune activation. Moreover, pathway enrichment analysis unveiled the involvement of various signaling pathways in highlighting the complex network of molecular events associated with the apoptotic response in bMECs. This comprehensive RNA-Seq analysis provides valuable insights into the gene expression dynamics underlying *E. coli* induced apoptosis in bMECs. The identification of dysregulated genes and enriched pathways will enhance our understanding of the intricate interplay between bacterial infection, apoptosis signaling pathways, and inflammation in the context of bovine mastitis. These findings will contribute to the development of targeted therapeutic strategies to mitigate the impact of bacterial infections on mammary epithelial cell health and milk production in dairy cattle.

[VIB-BI-PP-07]

Fabrication of gelatin, and chitosan-based scaffolds loaded with oxygen and nitric oxide for wound healing and management

Dar L A1,2, Syed M A1*, Shafi S2*
Division of Animal Biotechnology, Faculty of Veterinary Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India, Srinagar, India
Department of Pharmaceutical Sciences, University of Kashmir, India, Srinagar, India

Chronic wounds are characterized by their inability to heal within expected time due to persistent inflammation with inadequate oxygen and nutrient supply. Insufficient oxygen supply arising due to the biological events like impaired angiogenesis in the wound itself or from a physiological condition, results in oxidative stress due to increase in the production of reactive species (ROS). It is very crucial for optimal healing and to prevent the infection that a continuous supply of molecular oxygen is to be provided. Nitric oxide (NO), the endogenous signaling molecule, plays an important regulator of many wound healing processes, like inflammation phase, antibacterial, proliferation, and angiogenesis. The NO serve as a potential wound therapeutic agent alone or in combination of other wound therapy. To address these concerns, here, we report the development of gelatin-based oxygen-producing adhesive hydrogel for sustained delivery oxygen and NO. We have incorporated the calcium peroxide (CPO) and NO In the scaffolds to provide sustained release of oxygen over a period of 7 days. The gel showed good adhesiveness towards the various tissues and metals. The adhesive hydrogel being porous, high-water uptake capacity (~94%-98%), swelling ratio (14–17) and more than 90% of degradation within a period of 10 days. The release kinetics reveals the release of ~72% (within 10 days) and ~94% (within 10 days) oxygen and NO, respectively. An in vitro analysis of the scaffolds showed great potential in proliferation and migration of NIH-3T3 cells, with cell viability being significantly better than the normal scaffolds. The developed hydrogel may be used as cell and cell free therapeutics for the regeneration of damaged tissue, particularly with ischemic conditions such as myocardial infarction and chronic wound healing.

[VIB-BI-PP-08]

Single Step Ribonucleoprotein based CRISPR-CAS gene editing of MSTN exon 1 in ovine, caprine and bovine dermal fibroblasts using dual sgRNA and single sgRNA based strategies

Muneer Ahmad Dar*, Abrar Ahad Malik, Suhail Nabi Magray, Younis Farooq, Syed Hilal Yaqoob, Nida Handoo, Saba Nazir, Towseef Akram and Riaz Ahmad Shah
Division of Animal Biotechnology, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Alusteng, SKUAST-K

In this study gene editing of MSTN exon 1 in ovine, caprine and bovine dermal fibroblasts employing same pair of sgRNAs was targeted. The Ribonucleoprotein (RNP) method of CRISPR-Cas editing using preformed Ribonucleoprotein complexes from CAS enzyme and sgRNAs was utilized. Following the invitro validation of guides in the three species dual sgRNA RNP CRISPR-Cas gene editing strategy was adopted for ovine cells and single sgRNA RNP CRISPR-Cas gene editing strategy was followed in caprine and bovine cells. Electroporation was carried out for the delivery of the RNP gene editing components to the target cells. Both poring and transfer pulses at voltages of 87.5V/mm and 10.0V/mm and decay rates of 10% and 40% respectively were used. The gene editing in ovine cells was confirmed by PCR and sequencing whereas gene editing in caprine and bovine cells was confirmed by surveyor assay. The agarose gel electrophoresis of PCR product obtained from upscaled electroporated ovine cells clearly showed an additional band of about 360bp obtained by deletion of about 135bp from original length from 497bp. Sequencing of the edited ovine MSTN exon 1the confirmed the deletion of 135bp. Surveyor assay of upscaled electroporated caprine and bovine cells confirmed the gene editing by depicting two extra bands of about 330bp and 160bp length in addition to the original product length of 497bp.

Keywords: CRISPR-Cas, Gene editing, MSTN, exon, RNP

[VIB-BI-PP-09]

Aquaporin gene expression pattern in buffalo cumulus cells

Shavi, S. Saini, P. Singh, N.L. Selokar and M.K. Singh
Embryo Biotechnology Lab, Animal Biotechnology Division
ICAR- National Dairy Research Institute, Karnal, Haryana, India

The family of major intrinsic membrane proteins includes aquaporins (AQPs), which are necessary membrane proteins that serve as carriers for the movement of water and small molecules in cells. It also facilitates the passage of volatile substances such as ammonia (NH_3) and carbon dioxide (CO_2) etc. through membranes. Both unicellular and multicellular species contain numerous aquaporin isoforms that are variably generated and altered by post-translational processes, enabling precise tissue-specific osmoregulation. In the present study buffalo fetal fibroblast and cumulus cells were cultured in vitro and cDNA was prepared. The mRNA abundance of AQP3, AQP4, AQP7 and AQP9 was (128.21±1.11) (35.40±0.36), (35.18±0.35) and (13.63±0.12) times respectively, significantly higher ($P<0.01$) in cumulus cells compared to fibroblast cells. These aquaporins are crucial for regulating water transport in cells and results show that AQPs are differentially expressed in buffalo cumulus cells and fibroblast cells. This may have implications for cumulus cell function and oocyte development. Increased water input and elevated AQPs in cumulus cells are related to the growth and structural stability of cumulus cell layers and participate in the development of ovarian cells.

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