Compendium-cum-Souvenir

VIBCON 2025

XXX Annual Convention of ISVIB
and

INTERNATIONAL CONFERENCE

on

Envisioning Livestock Production and Protection under the One Health Landscape

November 06-08, 2025

ICAR-INDIAN VETERINARY RESEARCH INSTITUTE

Mukteswar Campus, Nainiital-263 138 (Uttarakhand) INDIA

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XXX Annual Convention of ISVIB

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Envisioning Livestock Production and Protection under the One Health Landscape

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अजय भट्ट
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भारत सरकार
Minister of State for Defence and Tourism
Government of India

पत्रांक...../संसद/लो.सभा/2025
दिनांक...02/11/2025

Message

It gives me immense pleasure to learn that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is organizing its XXX Annual Convention and International Conference (VIBCON 2025) at the ICAR-Indian Veterinary Research Institute (IVRI), Mukteswar Campus on the theme "Envisioning Livestock Production and Protection under the One Health Landscape."

As a representative of Uttarakhand, a state where farming and animal husbandry form the soul of rural life, I have witnessed firsthand the dedication, resilience, and hard work of our farmers and livestock keepers. The hills and valleys of our region depend on livestock not only as a source of food and income but also as a cornerstone of traditional ecological balance. Strengthening this sector through innovation, training, and scientific support is vital to sustaining rural livelihoods and empowering our farming families.

The Government of India has been deeply committed to promoting farmers' prosperity and livestock health through transformative programs such as the National Livestock Mission, Animal Husbandry Infrastructure Development Fund, and the Rashtriya Gokul Mission. These initiatives are creating new opportunities for value addition, entrepreneurship, and women's participation in the livestock economy - particularly in hilly and remote regions like Uttarakhand.

I firmly believe that advances in veterinary immunology and biotechnology will further strengthen disease surveillance, improve animal productivity, and contribute to food and nutritional security. The One Health concept, which recognizes the interconnection of humans, animals, and the environment, is especially relevant in our Himalayan context where biodiversity and community well-being are closely linked.

I appreciate the efforts of ISVIB and ICAR-IVRI Mukteswar for providing a global platform through VIBCON 2025 where scientists, academicians, and policymakers can exchange ideas and collaborate for the collective good. I am confident that the deliberations during this conference will inspire new initiatives that benefit farmers across India, including those in Uttarakhand, and help realize our vision of a self-reliant, prosperous, and sustainable rural India.

I extend my warm congratulations to the organizers and my best wishes for the grand success of VIBCON 2025.

Ajay Bhatt

(Ajay Bhatt)

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Dr. Raghavendra Bhatta
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No. AS/PS/DDG(AS)/Message/2025
Dated 31st October, 2025

Message

It is with great pleasure that I extend a warm welcome to all of you gathered for the XXX Annual Convention of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the International Conference VIBCON 2025, hosted at the ICAR-Indian Veterinary Research Institute (IVRI), Mukteswar Campus. The theme of this year's conference, 'Envisioning Livestock Production and Protection under the One Health Landscape,' profoundly highlights the interconnections between animal health, human well-being, and environmental sustainability.

In India, livestock is not merely an economic asset—it is the backbone of rural livelihoods, contributing to nutrition, employment, and financial stability for millions of smallholders and farming communities. In this era of emerging infectious diseases, climate change, and a globalized food system, the health of our livestock cannot be viewed in isolation. The One Health approach, which acknowledges the inextricable links between animals, humans, and the environment, offers a comprehensive framework to tackle the multifaceted challenges facing both health and productivity.

To strengthen livestock systems, we must adopt a holistic strategy that integrates cutting-edge research, technological innovations, and informed policy interventions, Advances in veterinary immunology, diagnostics, biosecurity measures, and disease surveillance are critical to controlling zoonoses, improving productivity, and ensuring sustainable animal agriculture. By translating scientific knowledge into actionable solutions, we empower farmers, enhance food security, and foster resilient rural communities.

VIBCON 2025 provides an invaluable platform for interdisciplinary collaboration, bringing together scientists, policymakers, and industry leaders to exchange ideas and develop innovative strategies for advancing livestock development under the One Health framework. Such collective efforts will allow us to anticipate emerging threats, mitigate risks, and build sustainable livestock production systems that benefit not only farmers but also society at large.

I am confident that the discussions and collaborations at this conference will lead to actionable insights, inspire meaningful partnerships, and stimulate research that will propel India's livestock sector forward—while simultaneously safeguarding the health of animals, humans, and the environment in harmony with the One Health approach.

I extend my best wishes to the organizers and all delegates for a productive, intellectually enriching, and impactful VIBCON 2025.

(Raghavendra Bhatta)

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डॉ. प्रवीण मलिक
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पशुपालन आयुक्त
Animal Husbandry Commissioner
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Message

It is a matter of profound pleasure to extend my greetings to all participants, distinguished delegates, and experts attending the XXX Annual Convention of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the International Conference (VIBCON 2025) at the ICAR—Indian Veterinary Research Institute (IVRI), Mukteswar Campus. The theme, "Envisioning Livestock Production and Protection under the One Health Landscape," is both timely and visionary, highlighting the inseparable links between animal health, human well-being, and ecosystem sustainability.

In today's interconnected world, livestock health cannot be viewed in isolation. Diseases affecting animals often reverberate across human populations and ecosystems, reminding us that the health of all living beings is deeply intertwined. The One Health paradigm provides a powerful framework to address these challenges - integrating veterinary science, public health, environmental stewardship, and socio-economic considerations to achieve holistic and sustainable outcomes.

From my perspective, the future of India's livestock sector depends not only on scientific breakthroughs but also on the translation of knowledge into action at the grassroots level. This entails the development of innovative vaccines, rapid diagnostics, molecular surveillance, and biosecurity frameworks that are accessible, effective, and sustainable. By empowering farmers with evidence-based strategies, we not only protect animal health but also safeguard human health, secure food systems, and strengthen rural livelihoods.

VIBCON 2025 is a critical intellectual platform that catalyzes cross-disciplinary dialogue, inspires cutting-edge research, and fosters collaboration among scientists, policymakers, and industry stakeholders. Through such synergistic engagements, we can anticipate and address emerging zoonotic threats, combat antimicrobial resistance, and mitigate climate-linked challenges in a proactive, integrated, and evidence-driven manner.

I am confident that the deliberations at this conference will generate actionable insights and foster networks that advance our shared vision: a resilient livestock sector, a safer food ecosystem, and empowered farming communities, all under the unifying principle of One Health. I extend my best wishes to the organizers, delegates, and participants for a highly productive and intellectually enriching VIBCON 2025.

(Praveen Malik)

भाकृअनुप- भारतीय पशु-चिकित्सा अनुसंधान संस्थान
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डॉ त्रिवेणी दत्त
निदेशक
Dr. Triveni Dutt
Director

MESSAGE

In today's interconnected world, the health of animals, people, and the environment are inseparable. Livestock Production and Protection under the One Health Landscape calls upon us to rethink how we produce, manage, and safeguard livestock in ways that promote sustainable livelihoods, protect ecosystems, and ensure global health security. Livestock systems are at the heart of food security and economic growth, yet they also sit at the crossroads of emerging disease threats, antimicrobial resistance, and climate change. Embracing the One Health approach means recognizing that animal health cannot be viewed in isolation it must align with human health goals and environmental stewardship. Through collaboration among veterinarians, public health professionals, ecologists, and farmers, we can foster innovation in disease prevention, biosecurity, animal welfare, and sustainable production. Together, we can build resilient livestock systems that not only feed communities but also safeguard the well-being of future generations.

The ICAR-Indian Veterinary Research Institute (IVRI), since its establishment in 1889, has been a cornerstone of veterinary science in India. The Mukteswar Campus, in particular, holds a special place in the institute's illustrious history. It has been the cradle of pioneering research in veterinary microbiology and vaccine development. From the early breakthroughs in Rinderpest to contemporary work in molecular diagnostics and vaccine innovations, IVRI Mukteswar has consistently contributed to safeguarding India's livestock health. Over the decades, the campus has evolved into a center of excellence for vaccine development, viral diagnostics, and zoonotic disease research, embodying the very essence of the One Health approach. As we move into an era of rapid technological advancement and global health challenges, the legacy of Mukteswar campus continues to inspire innovation and scientific excellence.

It is a matter of great pride and pleasure to convey my greetings to all delegates, scientists, academicians, and participants attending the XXX Annual Convention of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the International Conference (VIBCON 2025) being organized by ICAR–IVRI, Mukteswar. This event provides an excellent opportunity for scientists, policymakers, and industry partners to deliberate on innovative approaches for sustainable livestock development under the One Health paradigm.

I am confident that VIBCON 2025 will provide a vibrant platform for sharing ideas, strengthening collaborations, and translating scientific knowledge into practical outcomes which will benefit farmers, animals, and society at large. I extend my sincere wishes to the organizers, participants, and the entire scientific community for the grand success of this prestigious scientific event.

(Triveni Dutt)

भा.कृ.अनु.प.-राष्ट्रीय पशुरोग जानपदिक एवं सूचना विज्ञान संस्थान
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Dr. Baldev Raj Gulati
Director and
President, ISVIB

Message

It gives me immense pleasure to extend warm greetings to all members of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) on the occasion of the XXX Annual Convention of ISVIB and International Conference being organized at the ICAR–IVRI, Mukteshwar Campus from 6–8 November 2025.

The Society was established with the vision to advance, promote, and integrate scientific knowledge in the vital fields of veterinary immunology and biotechnology. These disciplines form the cornerstone of modern animal health research—driving innovations in vaccines, diagnostics, and therapeutics that are essential for effective disease control, improved livestock productivity, and the realization of the national One Health goals. Through sustained efforts in research, training, and networking, ISVIB continues to provide a platform for meaningful exchange among scientists, policymakers, and industry partners.

The theme of this year's conference, "Envisioning Livestock Production and Protection under the One Health Landscape," is highly relevant in the present context of emerging and re-emerging infectious diseases. It emphasizes the need for integrated approaches that consider the interconnections between animal, human, and environmental health. The deliberations during the conference are expected to foster innovative thinking, new collaborations, and practical strategies for achieving sustainable livestock development and biosecurity in India.

The ICAR–IVRI Mukteshwar Campus, with its illustrious legacy in infectious disease research and vaccine development, offers a historic and inspiring venue for this scientific congregation. Nestled amidst the serene hills of Uttarakhand, Mukteshwar has long been synonymous with pioneering veterinary science and continues to motivate generations of researchers.

I sincerely congratulate the Organizing Committee for their tireless efforts in hosting this important event and extend my best wishes to all delegates, scientists, and students for fruitful deliberations and enriching interactions. Together, let us continue to strengthen the scientific foundations of veterinary immunology and biotechnology for the benefit of animal and public health.

(Baldev R. Gulati)

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Dr. S. Manoharan, Ph.D.
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Secretary, Indian Society for Veterinary Immunology
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MESSAGE

The Indian Society for Veterinary Immunology and Biotechnology is marching on its 35 years of journey, has been associated with its XXX annual convention and national/international conferences including VIBCON 2025. The very purpose of this society is to bring together all innovative scientists, scholars, students and stakeholders across the country and abroad to deliberate in the fields of Animal Biotechnology, Immunology towards the animal production and protection every year. Amidst rising one health issues like AMR, Rabies etc, VIBCON 2025 has been conceptualized.

In an era defined by global interconnection and ecological uncertainty, livestock production stands at the crossroads of human health, animal welfare, and environmental sustainability. The One Health approach which recognizes the intricate linkages between people, animals, and their shared ecosystems that offers a holistic framework for transforming how we produce and protect livestock.

Under the One Health landscape, envisioning the future of livestock production means going beyond traditional agricultural goals of productivity and profit. It calls for systems that prioritize disease prevention, responsible antimicrobial use, biodiversity conservation, climate resilience, and equitable livelihoods for farming communities. Integrating science, policy, and innovation, this approach seeks to balance the growing demand for animal-based food with the imperative to safeguard public health and the environment.

As we look ahead, collaboration across disciplines—veterinary science, public health, ecology, and socioeconomics—will be key to building resilient livestock systems. By embracing the One Health vision, we can ensure that livestock production not only feeds the world but also sustains it.

My heartfelt wishes to the organizers for a grand success of VIBCON 2025.

Welcoming you all for the VIBCON 2025!

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Chennai - 600 007

Organizers, VIBCON 2025
ICAR-Indian Veterinary Research Institute
Mukteswar-263138, Uttarakhand, India



Amit Kumar
Org. Secretary

Karam Chand
Org. Secretary

Y.P.S. Malik
Chairman

MESSAGE

We take immense pride and pleasure in extending a warm welcome to all distinguished delegates, scientists, academicians, entrepreneurs, industry partners, and students participating in the XXX Annual Convention of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the International Conference (VIBCON 2025). This prestigious event, hosted by the ICAR-Indian Veterinary Research Institute, Mukteswar Campus, from 6th to 8th November 2025, represents a convergence of intellect, innovation, and interdisciplinary collaboration. It is a celebration of scientific spirit and collective resolve to advance animal health and productivity through the powerful lens of biotechnology and immunology.

The theme of this year's conference - “Envisioning Livestock Production and Protection under the One Health Landscape”- reflects our collective commitment to addressing global challenges at the interface of animal, human, and environmental health. In an era when zoonotic diseases, antimicrobial resistance, and climate variability are reshaping the contours of animal health management, VIBCON 2025 provides a vital platform to foster dialogue, share innovations, and develop strategies that align with the One Health approach.

This conference brings together leading experts, researchers, policymakers, entrepreneurs, and young scientists from India and abroad to deliberate on cutting-edge developments in immunology, biotechnology, diagnostics, vaccine design, and sustainable livestock production. The technical sessions, plenary talks, and poster presentations are designed to encourage meaningful exchange of ideas and promote translational research that benefits both science and society.

We gratefully acknowledge the guidance and support of the Indian Council of Agricultural Research (ICAR), the Indian Society for Veterinary Immunology and Biotechnology (ISVIB), and the Director, ICAR-IVRI, in organizing this event. We also express our heartfelt thanks to all sponsors, collaborators, and participants whose contributions have made this conference possible.

We are confident that VIBCON 2025 will serve as a catalyst for scientific advancement, policy integration, and innovation in veterinary and allied sciences, ultimately strengthening our shared mission toward animal health, food security, and sustainable livelihoods.

We warmly welcome you all to IVRI Mukteswar, and wish you an intellectually stimulating and memorable experience at VIBCON 2025.

Amit Kumar

Karam Chand

Y.P.S. Malik

ACKNOWLEDGMENTS

The Organizing Committee of VIBCON 2025 gratefully acknowledges the enthusiastic participation of scientists, faculty members, delegates, students, and researchers from various institutions across India and abroad. Their valuable contributions through oral and poster presentations have enriched the scientific content and deliberations of this conference. Our heartfelt thanks are due to the distinguished speakers, chairpersons, judges, and rapporteurs for sharing their expertise and ensuring the smooth conduct of the scientific sessions.

We also express our sincere appreciation to our industry partners and sponsors for their generous support, collaboration, and active engagement in making this conference a success. Their valuable association not only provided essential financial assistance but also strengthened the bridge between research, academia, and industry. The presence and participation of representatives from leading organizations added great value to the scientific deliberations, exhibitions, and discussions. Their commitment towards promoting innovation, research, and sustainable livestock development under the One Health framework is deeply acknowledged and appreciated.

We also gratefully acknowledge all the Chairmen and members of various committees associated with VIBCON 2025. Their meticulous planning, dedicated supervision, and tireless efforts, often extending late into the night, ensured the smooth and timely execution of every component of this multifaceted event. The synergy, commitment, and sense of shared purpose demonstrated by all committee members have been instrumental in transforming the collective vision of this conference into a resounding success.

Special appreciation is extended to the staff of ICAR-IVRI, Mukteswar, whose unwavering commitment, hard work, and exemplary teamwork made the successful organization of this conference possible. Their dedication in managing every aspect, from logistics, accommodation, transport, and catering to technical arrangements, venue preparation, and on-site coordination, was truly commendable. Despite the challenges posed by the remote and difficult terrain of Mukteswar, the entire team worked tirelessly with enthusiasm and a spirit of cooperation that ensured the smooth conduct of all events. Their collective efforts and sense of responsibility have been the backbone of the seamless execution and overall success of VIBCON 2025.

We also gratefully acknowledge the constant guidance, encouragement, and support received from Indian Council of Agricultural Research (ICAR), Indian Society for Veterinary Immunology and Biotechnology (ISVIB), DDG (Animal Sciences) and Director, ICAR-IVRI.

Finally, the Organizing Committee expresses heartfelt thanks to all individuals, organizations, and well-wishers who, in one way or another, have contributed to making VIBCON 2025 a resounding success.

We warmly welcome you all to IVRI Mukteswar, and wish you an intellectually stimulating and memorable experience at VIBCON 2025.

About ISVIB

The Indian Society for Veterinary Immunology and Biotechnology (ISVIB), popularly known as ISVIB, is a conglomerate organization comprising primarily veterinary scientists from disciplines such as microbiology, biotechnology, preventive medicine, parasitology, animal reproduction, and fisheries. Established in 1990 through the collective visionary zeal of Drs. P. Richard Masillamony, B. B. Mallick, and B. S. Keshavamoorthy during an interactive session at Tirupati, the society was formed with the objective of fostering the growth of veterinary immunology and biotechnology in the era of advancing biological sciences. As a registered professional body, ISVIB motivates its members to discuss, analyze, and formulate effective strategies for safeguarding animal health, thereby ensuring improved animal production and sustainable livestock development. To achieve these goals, the society organizes annual national conventions and symposiums, such as the Veterinary Immunology and Biotechnology Conference (VIBCON), to critically analyze and update knowledge on emerging biotechnological and gene-based technologies in animal health and production. Since its inception, ISVIB has grown in strength, now boasting a membership of over 1,000 scientists from India and abroad.

The society also publishes the peer-reviewed journal, which features high-quality research papers, reviews, and scientific communications on key topics such as immunogenetics, vaccine development, genomics, diagnostics, and reproductive biotechnology. Through its research-oriented and collaborative approach, ISVIB provides a vital platform for scientists, academicians, and students to exchange ideas, share innovations, and advance the frontiers of veterinary biotechnology.

ISVIB continues to play a pivotal role in strengthening the link between research institutions, universities, and industries, fostering innovations aimed at improving animal health, productivity, and disease resistance. The society envisions maintaining the scientific momentum initiated by the first green revolution-driven by genetics and microbiology—by leading the second revolution through biotechnology. Upholding its commitment to creating a hunger-free world with balanced nutrition and protection from zoonotic diseases, ISVIB remains dedicated to applying biotechnology for the benefit of animal health, human welfare, and sustainable agricultural development.

About ICAR-IVRI Izatnagar

The Imperial Bacteriological Laboratory (IBL) established at Pune in 1889, was first led by Dr Alfred Lingard, as In-charge of the laboratory in 1890. Two years later, it was felt that handling highly infectious micro-organisms and pathological materials in a densely populated city like Pune is hazardous. Consequently, the laboratory was shifted in 1893 to an isolated site amidst the dense conifer forest of Mukteswar in the Kumaon hills of United Province located at about 1500 m above the mean sea level. Cattle plague or rinderpest was the most devastating disease in those days and the institute gave top priority for controlling this deadly disease. In fact, the first batch of anti-rinderpest serum was produced in 1899. By 1906, the institute started production of antisera against anthrax, haemorrhagic septicaemia and tetanus, a vaccine against black quarter and a diagnostic against equine glanders. To produce more of these biological products, a sub-centre was established at Kargaina, in the outskirts of Bareilly city. But limitation of space at this site led to acquisition of 306 ha. of land in Izatnagar in 1913, where the institute stands today. The Imperial Bacteriological Laboratory was renamed several times. It was known as Imperial Institute of Veterinary Research in 1925, Imperial Veterinary Serum Institute in 1930 and Imperial Veterinary Research Institute in 1936. Finally when India gained independence it was renamed as Indian Veterinary Research Institute. It came under administrative control of Indian Council of Agricultural Research in 1966.

Over the years, the institute expanded by establishing Regional Stations at Palampur and Kolkata and Campuses at Bengaluru, and Pune. The scientific strength of the institute also contributed to establishment of some reputed institutes such as the Central Avian Research Institute (CARI) at Izatnagar in 1979 and the High Security Animal Diseases Laboratory at Bhopal in 1998 (known as National Institute of High Security Animal Diseases, since 2014). In 2015, the institute established the Training and Education Center at Pune, Maharashtra.

The institute has contributed immensely for enhancement of livestock production through control of economically important diseases and eradication of some of them, most notably eradication of Rinderpest, CBPP, African horse sickness and Dourine.

About ICAR-IVRI, Mukteswar Campus

The history of foundation of the mother campus of ICAR-Indian Veterinary Research Institute at Mukteswar campus can be traced from the recommendations of Col. J.H.B. Hallen, Chairman, Indian Cattle Plague Commission to focus on livestock conservation since the animal diseases were rampant and breeding operations were generally unsuccessful. This commission recommended the establishment of Veterinary Research Laboratory in India in 1885 with a view to undertake a geographical survey of the major contagions with which field workers in this country have to contend and to discover prophylactic measures for the various diseases disclosed by the survey. The foundation stone of research laboratory was laid by the Governor of Bombay on 9th December, 1889 at Pune on 5.5 acres of land adjoining the College of Science.

The original Imperial Bacteriological Laboratory was housed in the College of Science at Pune and Dr. Alfred Lingard, a distinguished medical bacteriologist was appointed in 1891 as Imperial Bacteriologist. In the beginning, the laboratory devoted its attention to the preparation and distribution of Pasteur's anthrax vaccine. Later, when it was realized that the magnitude of the cattle disease,anthrax, in India, was only next to rinderpest, the Govt. of British India took upon the task of investigation, research and manufacture of a serum for the protection of bovines against rinderpest. For this purpose, the densely populated city of Pune was regarded as unsuitable. In August, 1893 it was decided to shift the laboratory to the Himalayas. Mukteswar was selected as the new site and about 3,000 acres of estate was purchased in the Kumaon hills.

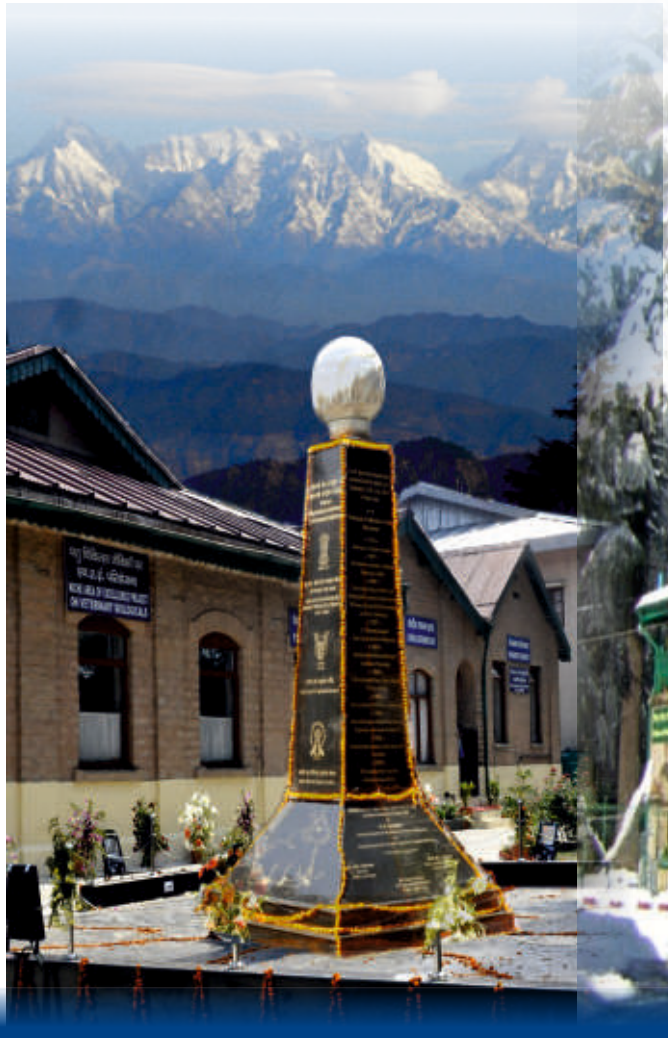
The salubrious climate, unpolluted natural environment and cold climate of Mukteswar was found to be congenial for the preservation of vaccines and sera and suitable for work on infectious microorganisms. In addition to large reserve forest area surrounding the experimental locations, it was sufficiently remote from populous areas for any fear of disease spreading from its experimental herds to the neighbouring cattle herds. In addition, hill cattle of Kumaon and Garhwal, a breed known till today for its high susceptibility to rinderpest were available in abundance.

Mukteswar is situated at a height of 7520 feet on the peak at junction of Gagar and Lohaghat ranges of the Kumaon hills, an area of exquisite natural scenic beauty, with its thick pine and oak forests, fast running springs and rivulets, rich fauna and flora, simple and hospitable people with rich cultural heritage. Overlooking the Institute Campus and adoring the highest point is the historic temple of Lord Shiva known as "Mahadeo" and from which the name of Mukteswar is derived for this place. The towering peaks of Nanda Devi (25,645 ft.), Trisul (23,360 ft.) and Panchuli (22,650 ft.) ever standing so high and kissing the sky with their thick cloak of perpetual snow, feeding the rivers emanating from this region, add to the grand panorama

In 1925, the name of the Imperial Bacteriological Laboratory was changed as Imperial Institute of Veterinary Research and the name of the Imperial Bacteriologist as the Director. Dr.

J.D.E. Holmes expanded the dimension of research paving the way for the commercial manufacture of sera, to be carried out in the sub-station on the plains with the advantage of cheaper costs and more readily available transport to the field. Accordingly, a sub-station was established at Izatnagar in 1913 for large scale manufacture of sera and vaccines, and also for undertaking investigations which could best be conducted in the plains. With the passage of time and resultant expansion of activities, the daughter (Izatnagar) outgrew the mother (Mukteswsar) in 1931. Dr. Ware divided the work at Mukteswar into three sections, viz, Pathology, Serology and Protozoology, each headed by a Veterinary Research Officer. In 1936, the Imperial Veterinary Serum Institute, Izatnagar, became the Biological Products Section and the Institute was renamed as Imperial Veterinary Research Institute. Dr. Frank Ware planned the addition of new Sections at Izatnagar, namely, Animal Nutrition (1936), Poultry Research (1938) and Animal Genetics (1945). Soon after independence, the Institute was redesignated as the Indian Veterinary Research Institute.

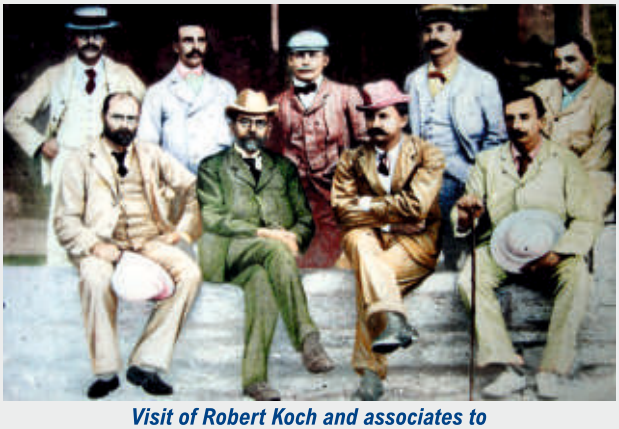
Mukteswar spread over 3450 acres of land, now houses the Division of Virology and the Division of Temperate Animal Husbandry. The Virology Division conducts basic, adaptive and applied research on viral diseases of animals. The Central Typing Laboratory under development as International Centre of FMD virus typing also functions at this Campus. However, the Coordinating Unit of All-India Co-ordinated Research Project for Epidemiological Studies on FMD, is located at the main campus of IVRI, Izatnagar. The Division of Temperate Animal Husbandry conducts research on livestock production under temperate conditions and improvement of goats for pashmina production. There is a proposal to develop National Veterinary Museum at Mukteshwar



IVRI Milestones

Major Events/ Research Milestones of ICAR-IVRI, Mukteswar Campus

1889	: Foundation of Imperial Bacteriological Laboratory (IBL) at Poona, Maharashtra
1890	: Appointment of Dr Alfred Lingard, a noted medical scientist as the founder Director
1893	: Shifting of the IBL to Mukteswar, Kumaon Hills of Uttar Pradesh (now in Uttarakhand)
1897	: Historical visit of renowned bacteriologists, Robert Koch, R. Pfeiffer and G. Gaffky to Mukteswar
1899	: Production of first batch of anti-rinderpest serum
1920-21	: Eradication of Dourine disease of horses
1927	: Developed goat tissue vaccine (GTV) against rinderpest
1940	: Development of vaccine against Ranikhet disease of poultry
1947	: Shifting of Headquarters of the Institute from Mukteswar to Izatnagar
1947	: Renaming as Indian Veterinary Research Institute (IVRI) under Govt. of India
1958	: Establishment of a Postgraduate College of Animal Sciences at Mukteswar, affiliated to Agra University
1960-65	: Control and eradication of African horse sickness
1966	: Transfer of administrative control to Indian Council of Agricultural Research and recognition as a National Institute
1986	: Division of Temperate Animal Husbandry was established (from LPR-CG & LA)
2001	: Development of competitive-ELISA diagnostic kit for rinderpest, approved by OIE and validated by IAH, Pirbright, UK
2002	: Development of live modified PPR vaccine
2012	: Erection of Commemorative pillar on Global Rinderpest Eradication at Mukteswar
2014	: Development and commercialization of Sheep pox vaccine using indigenous strain
2018	: Developed the IVRI-M PPRV Antigen capture ELISA kit
2019	: Developed Sandwich ELISA (sELISA) kit for the detection of bluetongue virus (BTV) antigen
2022	: Developed a homologous live-attenuated LSD vaccine in collaboration with ICAR-National Research Centre on Equines (ICAR-NRCE), Hisar (Haryana)
2023	: Live Attenuated PPR- Goatpox Combined Vaccine
2024	: Live Attenuated PPR-Sheeppox Combined Vaccine
2024	: IVRI-M Recombinant PPR Marker Vaccine
2024	: IVRI-M Thermoadapted PPR Vaccine
2025	: Goat breed from Kumaun region registered as Chougarkha name with Accession number of INDIA_GOAT_2400_ CHAUGARKHA_06040
2025	: Modified Live Attenuated Canine Parvovirus Cell Culture Vaccine



Visit of Robert Koch and associates to Mukteswar, 1897

Dr J.T. Edwards: Discoverer of GTV, 1927

Dr S.G. Iyer: Discoverer of R2B Vaccine, 1940

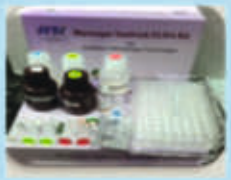
Diagnostics and Vaccines

Major Vaccines Developed

Major Diagnostics Developed

Indirect ELISA for Bluetongue

- Diagnostic specificity : 91%
- Diagnostic sensitivity: 96%
- In use for sero-surveillance and sero-monitoring
- Commercialized to industrial house



Polyclonal antibody based sandwich ELISA for Bluetongue

- Analytical sensitivity: $10^{3.4}$ $TCID^{50}$ / ml.
- Analytical specificity : do not cross react with viruses of small ruminants
- Used for detection of Bluetongue virus antigen in cell culture and blood samples.

Monoclonal antibody based blocking ELISA for Bluetongue

- Diagnostic sensitivity (dsn): 98.8%
- Diagnostic specificity (dsp): 97.1%
- Used for screening of serum from multiple susceptible species and is suitable for sero-surveillance and sero-monitoring

Modified Live Attenuated Canine Parvovirus Cell Culture Vaccine

Area specific mineral mixture for high altitude of Kumoaun hills

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VIBCON-2025

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and

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on

Envisioning Livestock Production and Protection under the One Health Landscape

November 06-08, 2025

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Envisioning Livestock Production and Protection under the One Health Landscape

November 06-08, 2025

Organised by:

ICAR-Indian Veterinary Research Institute, Mukteswar-263138, Uttarakhand, INDIA

&

Technical Programme Schedule

DAY 1 - 06.11.2025

Time	**Programme**
08.30 AM – 10:00 AM	Registration
10:00 AM – 11:30 AM	Inauguration
11:30 AM – 12:15 PM	**High Tea**
12:15 PM – 1:00 PM	**ISVIB Dr Richard P. Masillamony Oration Award Lecture** **Dr R. K. Singh** FAO-IN and Former Director, ICAR-IVRI, Izatnagar, India
Lunch Break 01:00 PM to 02:00 PM	
Session 1 - One Health Frontiers: Combating Emerging Zoonoses (Conference Hall - 1)	
Keynote address	
14:00 PM – 14:20 PM	**1. Strengthening National Preparedness for Emerging Zoonoses through One Health Collaboration** Dr Ashok Kumar, Ex-ADG (Animal Health), ICAR, New Delhi
14:20 PM – 14:40 PM	**2. One Health Dimension in Animal Health: Nepal and India's Perspective** Dr Shital Kaji Shrestha, Former President, Nepal Veterinary Association
Lead address	
14:40 PM – 14:55 PM	**1. The Changing Landscape of Pandemic Influenza A(H1N1) pdm09 virus (pH1N1): Implications for One Health Professionals** Prof. (Dr) Shailendra Saxena, Vice-Dean, KGMU Lucknow
14:55 PM – 15:10 PM	**2. Rabies in One Health, as a model** Dr Shrikrishna Isloor, Professor & Head, WOAH Reference Lab for Rabies, KVAFSU, Bengaluru
15:10 PM – 15:25 PM	**3. Triage Model: Early signals of increased zoonotic risk** Dr Harmanmeet Kaur, Associate Professor, Amity University, Noida
15:25 PM – 15:40 PM	**4. Prevalence of Zoonotic and Transboundary Animal Diseases in West Bengal, India: A One Health Initiative** Dr T. K. Dutta, CAU, Aizawl
15:40 PM – 16:00 PM	**Discussion over Tea**
16:00 PM – 16:50 PM	Oral Presentations
16:50 PM – 17:00 PM	Session wrap-up
Poster presentations for Sessions 1 and 2 will be conducted as parallel sessions	

Session 2 - One Health Approach to Antimicrobial resistance, Microbiome, Food safety and biosecurity (Conference Hall - 2)	
Keynote address	
14:00 PM – 14:20 PM	**1.Predict, Prevent, Protect: Harnessing Epidemiology and Informatics for Health Security** Dr B. R. Gulati, Director, ICAR-NIVEDI, Bengaluru
14:20 PM – 14:40 PM	**2. A Cross-Sectoral Framework for Food Safety: The One Health Connection** Dr Bimlesh Mann, ADG (EP&HS), ICAR, New Delhi
Lead address	
14:40 PM – 14:55 PM	**1. Reinventing Food Safety: Natural Solutions for the Antimicrobial Resistance Era** Prof. (Dr) Vikas Pathak, Dean, CoVSc, DUVASU, Mathura
14:55 PM – 15:10 PM	**2. Integrons: Genetic Architects of Antimicrobial Resistance** Dr Amit Kumar, Professor & Head, SPVUAT, Meerut
15:10 PM – 15:25 PM	**3. Genomic Insights and One-Health Approaches for Controlling Zoonotic Tuberculosis in India** Dr P. Dandapat, Head (B&M), ICAR-IVRI, Izatnagar
15:25 PM – 15:40 PM	**4. Transforming Brucellosis Diagnosis through Next-Generation Molecular Tools** Dr Babloo Kumar, BP Division, ICAR-IVRI Izatnagar
15:40 PM – 16:00 PM	**Discussion over Tea**
16:00 PM – 16:50 PM	Oral Presentations
16:50 PM – 17:00 PM	Session wrap-up
Poster presentations for Sessions 1 and 2 will be conducted as parallel sessions	

Gala Dinner (5:30 pm onwards)

DAY – 2 (07.11.2025)

Session 3 - Omics and Innovation in Vaccines, Diagnostics and Therapeutics	
10:00 AM – 10:20 AM	**Plenary lecture: Food systems as Driver for Species Spill-over of Animal Viruses** Dr Gaya Prasad, Former ADG (AH), ICAR/Former Vice-Chancellor, SVPUAT, Meerut
Key-note address	
10:25 AM – 10:40 AM	**1. A Futuristic Vision of Status of Transboundary diseases of Livestock in India: Linking it with "Viksit Bharat" @2047** Dr R. P. Singh, Director, ICAR-NIFMD, Bhubaneshwar
10:40 AM – 10:55 AM	**2. Unraveling Respiratory Pathogenesis in Buffalo: Cellular Perspectives for Livestock Health and Protection** Dr R. S. Sethi, Dean, CoABT, GADVASU, Ludhiana
10:55 AM – 11:15 AM	**Discussion over Tea**
Lead talks	
11:15 AM – 11:30 AM	**1. Omics and Innovations in Viral Disease Diagnostics** Dr Minakshi Prasad, Emeritus Sci., ICAR-NRCE, Hisar
11:30 AM – 11:45 PM	**2. Diagnostic Methods for Microbial Pathogens: Current Trends and Future Prospects** Dr Ravi Kant Agrawal, Head (BP Division), ICAR-IVRI Izatnagar
11:45 PM – 12:00 PM	**3. Assessing the efficacy of inactivated, adjuvanted *Brucella suis* vaccine in piglets** Dr S. Manoharan, VRC-BV, CAHS, TANUVAS, Chennai
12:00 PM – 12:30 PM	**Oral Presentations**
Poster presentations for Sessions 3 and 4 will be conducted as parallel sessions	
12:30 PM – 13:00 PM	**General Body meeting (ISVIB)**
13:30 PM – 14:00 PM	**Lunch Break**

Session 4 - Genomics, Disease surveillance and epidemiology (Conference Hall - 2)	
Key-note address	
10:25 AM – 10:40 AM	**1. An overview of the One Health Needs and Priorities** Dr Gyanendra Gongal, Senior Public Health Officer, WHO Regional Office, New Delhi
10:40 AM – 10:55 AM	**2. Leptospirosis at the Human-Animal-Environment Interface: Advancing One Health Strategies** Dr V. Balamurugan, ICAR-NIVEDI, Bengaluru
10:55 AM – 11:15AM	**Discussion over Tea**
Lead talk	
11:15 AM – 11:30 AM	**1. Molecular Profile of Antimicrobial Resistance in Methicillin-Resistant *Staphylococcus aureus* isolates from various sources** Dr P. Borah, Director of Res. (Vet.), AAU, Guwahati
11:30 AM – 11:45 PM	**2. Antimicrobial-Resistant, Virulent and Biofilm-forming *Salmonella*: Molecular and Phenotypic Evidence from Duck at the Human-Animal-Environment Interface** Dr S. N. Joardar, WBUAFS, Kolkata
11:45 PM – 12:00 PM	**2. Initiatives and Strategies for the Eradication Plan for *Peste des Petits Ruminants* (PPR-EP) in India by 2030: A Comprehensive Approach to Combatting Small Ruminants Plague** Dr Ashwini K. Singh, DAHD-CCSNIAH, Baghpat
12:00 PM – 12:30 PM	**Oral Presentations**
Poster presentations for Sessions 3 and 4 will be conducted as parallel sessions	
12:30 PM – 13:00 PM	**General Body meeting (ISVIB)**
13:30 PM – 14:00 PM	**Lunch Break**

Session 5 - Future Poultry in Vikshit Bharat@ 2024 and Industry-Academia Interface (Venue: Ojaswi Resort, Shitla, Muketswar)	
(Chair: Dr R S Chauhan **Co-chair:** Dr Babloo Kumar**)**	
10:00 AM – 10:25 AM	**Plenary lecture: Envisioning Poultry Production and Protection under the One Health Landscape in India** Prof. (Dr.) Pankaj Kumar Shukla, Head, DUVASU, Mathura
10:25 AM – 10:45 AM	**Key-note address** Dr. Shirish Nigam, MD (EW Nutrition-SA), Former President (INFAH)
Lead talks	
10:45 AM – 11:00 AM	**1. Studies on characterization of Fowl pox virus (FPV) from outbreaks in backyard poultry clusters of West Bengal** Dr Arnab Sen, SIC, ICAR- IVRI, ERS Kolkata
11:00 AM – 11:15 AM	**2. Isolation and molecular characterization of Fowl Adenovirus serotype 11 associated with inclusion body hepatitis from India** Dr V. Gopinath, Indovax Pvt. Ltd., Gurgaon
11:15 AM – 11:30 AM	**Discussion over Tea**
11:30 AM – 12:00 PM	**Oral Presentations**
12:00 PM – 12:30 PM	**Industry-Academia Interface**
12:30 PM – 13:00 PM	**Orientation of IVRI Campus for Industry partners**
13:00 PM – 14:00 PM	**Lunch Break**
14:00 PM – 15:00 PM	**Poster presentations for Poultry Session will be conducted at IVRI Campus**

Session 6 - Genome Editing and Data-Driven Smart Livestock Farming and production **(Conference Hall - 1)**	
Key-note address	
14:00 PM – 14:20 PM	**1. Genomic Insight of Mastitis-causing ESBL (Extended Spectrum Beta Lactamase) producing *E coli*** Dr S. De, National Professor, ICAR-NDRI, Karnal
14:20 PM – 14:40 PM	**1. Identifying Universal Principles of Host-Pathogen Interactions: Lessons from Morbilliviruses** Dr Rajeev Kaul, Dean, Delhi University South Campus
Lead talks	
14:40 PM – 14:55 PM	**1. Overcoming Insecticide Resistance: A Critical Step Towards Eradication of Vector-Borne Diseases** Dr Kailash C. Pandey, ICMR-NIMR, New Delhi
15:10 PM – 15:25 PM	**2. Addressing Anti-microbial Resistance (AMR) using Indigenous Means** Dr Anu Rahal, Principal Scientist, DKMA, ICAR, New Delhi
14:55 PM – 15:10 PM	**3. Author's Responsibility in Scientific Communication** Dr Aruna T Kumar, Ex-Editor, ICAR, New Delhi
15:25 PM – 15:50 PM	**Discussion over Tea**
15:50 PM – 16:50 PM	Oral Presentations
16:50 PM – 17:00 PM	Session wrap-up
14:00 PM – 17:00 PM	**Poster session of sessions 5 and 6**
Session 8 - ISVIB Award Session (Conference Hall - 2)	
14:00 PM – 15:25 PM	ISVIB Award session presentations
15:25 PM – 15:50 PM	**Discussion over Tea**
15:50 PM – 17:00 PM	ISVIB Award session presentations

Cultural Programme- Spirit of Hills (5:30 pm onwards)

DAY-3 (08.11.2025)

Session 7 - Frontiers in Translational Biotechnology (Conference Hall – 1)	
10:00 AM – 10:25 AM	**Plenary lecture:** Dr Praveen Malik CEO, AgrInnovate India: Animal Husbandry Commissioner, GOI
10:25 AM – 10:40 AM	**Key-note address: Enhancing Large Viral DNA Genome Editing Using Stable Cell Line Co-Expressing CRISPR/Cas9 and gRNAs** Dr P.K. Gupta, PC (NP-GET), ICAR-IVRI, Izatnagar
10:40 AM – 11:00 AM	**Discussion over Tea**
Lead talks	
11:00 AM – 11:15 AM	**1. Designing Multi Epitope Vaccines Against Emerging and Re-Emerging Human Viruses** Dr Manoj Kumar, NIPER, Mohali
11:15 AM – 11:30 AM	**2. Regional Disease Management: Challenges and Prospects of Immuno-Molecular interventions** Dr Anish Yadav, ICAR- National Fellow, SKUAST-J
11:30 AM – 11:45 AM	**3. Development of Multi-Epitope DIVA Vaccine Constructs Against Brucellosis: An Immuno-Informatics and Recombinant Approach** Dr J.S. Sohal, Chandigarh University, Mohali
11:45 AM – 13:00 PM	**Oral presentations**
13:00 PM – 14:00 PM	**Lunch Break**
14:00 PM – 15:00 PM	**Award and Valedictory session**

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Oration Award Lecture

From Mukteswar to Modernity: India's Pursuit of Excellence in Animal Vaccinology

Raj Kumar Singh
Former Director/Vice Chancellor
ICAR-Indian Veterinary Research Institute (ICAR-IVRI), Izatnagar-243122, Bareilly, UP, India

The journey of animal vaccination in India is both historic and inspiring. In the early twentieth century, the ICAR–Indian Veterinary Research Institute (IVRI) at Mukteswar pioneered path-breaking work on immunotherapy for cattle using serum from rinderpest-infected animals and developed the world's first rinderpest vaccine, famously known as the Goat Tissue Vaccine (GTV). This seminal achievement marked the beginning of organized vaccinology in India. With subsequent expansion at Izatnagar, IVRI established advanced bacterial and viral vaccine production units (Division of Biological Products), transforming itself into the epicentre of immunological innovation. The institute's contributions were instrumental in the eradication of diseases such as Dourine and African Horse Sickness (AHS) in equines; Rinderpest (RP), and Contagious Bovine Pleuropneumonia (CBPP) in cattle and buffalo, and in bringing major control over economically devastating infections like Foot-and-Mouth Disease (FMD), Hemorrhagic Septicemia (HS), and Brucellosis in bovines; Sheeppox and Goatpox in small ruminants; Classical Swine Fever (CSF) in pigs; and Newcastle Disease (ND) and fowlpox, and Infectious Bursal disease (IBD) in poultry. These sustained scientific endeavors have transformed animal husbandry in India from a traditional practice into a scientifically grounded, disease-resilient enterprise.

The IVRI has also played a pivotal role in the standardization and quality assurance of veterinary biologicals, including vaccines, antigens, and diagnostic reagents. Through its Division of Biological Standardization, the institute has established robust protocols for potency, safety, and purity testing, thereby ensuring the reliability and efficacy of the biological products used across the country. By developing reference standards, validating testing methodologies, and contributing to the formulation of regulatory guidelines, the IVRI has laid a scientific foundation for India's veterinary biological control system. These efforts have not only enhanced vaccine credibility and public confidence but also supported national regulatory authorities in aligning with international norms and advancing biosafety and biosecurity frameworks for animal health management.

Immunotherapy and modern vaccinology have evolved remarkably, integrating molecular biology, recombinant technology, and adjuvant optimization. However, challenges remain substantial, including ensuring antigenic stability, maintaining cold-chain logistics, upgrading biosafety infrastructure, and translating laboratory breakthroughs into affordable field products. Farmer access, particularly for smallholders, continues to depend on equitable distribution systems, local manufacturing capacity, and last-mile delivery mechanisms.

As we move into the era of genomic surveillance and "One Health," the future of animal vaccines must embrace innovation, including mRNA and vector-based platforms, thermostable formulations, and DIVA (Differentiating Infected from Vaccinated Animals) strategies. Strengthening public-private partnerships, fostering indigenous R&D ecosystems, and harmonizing regulatory frameworks are essential to ensure timely vaccine availability and pandemic preparedness.

India's vision must be bold yet inclusive–vaccines for all species, accessible to every farmer, produced with excellence and scientific integrity. Guided by the legacy of the IVRI and national institutes, let us pledge to make our livestock not only disease-free but also future-ready–safe, productive, and resilient for generations to come.

Session 1:

One Health Frontiers: Combating Emerging Zoonoses

Strengthening National Preparedness for Emerging Zoonoses through One Health Approach

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Zoonotic diseases are a global health priority, as they account for over 60% of all human infectious diseases and nearly 75% of emerging infections, highlighting their immense impact on human health, economy, and biosecurity. Their emergence is driven by rapid urbanization, agricultural intensification, deforestation, wildlife trade, biodiversity loss, climate variability, and growing interconnections between humans, domestic animals, and wildlife. India may face a major threat of zoonotic spillovers due to its unique combination of high human population density, extensive livestock population of over 536 million animals, more than 800 million poultry birds, widespread backyard farming, poorly regulated wet markets, rich biodiversity including known wildlife reservoirs such as bats and rodents, expanding human encroachment into forests, and changing peri-urban ecosystems. Climate-sensitive vectors such as mosquitoes and ticks have expanded geographically, increasing the transmission of diseases like Nipah virus infection, Japanese encephalitis, Kyasanur Forest Disease, scrub typhus, and Zika, etc.

To address this challenge and other related issues, India has proactively adopted the One Health approach by integrating the human, animal, and environmental health sectors. Key national mechanisms include the National One Health Mission, National Standing Committee on Zoonoses, ICMR–ICAR collaborations, the National Centre for Disease Control's multisectoral coordination initiatives, the Animal Pandemic Preparedness Initiative (APPI), Animal Health System Support for One Health (AHSSOH), and national disease control programs such as the control of brucellosis and the Rabies Elimination Plan. India is also strengthening its Integrated Disease Surveillance Programme (IDSP) to incorporate animal health data and aims to establish 100 zoonotic sentinel surveillance sites by 2027, of which 75 are already functional, including 11 dedicated sites for animal health. Support from the World Bank Public Health System for Pandemic Preparedness Programme is enhancing the institutional capacities of the ICMR, NCDC, and state public health laboratories. Surveillance capabilities are advancing through the adoption of genomic epidemiology, next-generation sequencing, wastewater-based monitoring, wildlife and farm-level pathogen detection, and AI-driven predictive modelling to identify high-risk spillover hotspots. Despite these efforts, the country continues to face challenges, such as fragmented data-sharing systems, limited interoperability between the veterinary and public health sectors, and shortages of trained multidisciplinary personnel. To build a resilient zoonotic preparedness framework, India must operationalize legally empowered One Health institutions, establish federated data platforms with global standards, expand genomic and environmental surveillance networks, strengthen workforce capacity across veterinary, epidemiological, and ecological disciplines, and promote public–private partnerships for vaccines, diagnostics, and modelling technologies. A proactive, predictive, and collaborative approach is essential for mitigating future threats, preventing spillovers at their source, and ensuring national and global health security.

Bridging Boundaries: Integrated one Health Approach to the Control and Management of Leptospirosis

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Leptospirosis, caused by pathogenic *Leptospira* species, is an emerging zoonotic disease and a growing public health challenge in tropical regions such as India. Transmission occurs through contact with water or soil contaminated by urine from infected animals, disproportionately affecting agricultural laborers, sewage workers, and urban slum populations. Its complex epidemiology, spanning human, animal, and environmental reservoirs, requires coordinated multisectoral responses. A One Health framework, integrating medical, veterinary, and environmental sectors, is essential for sustainable control. Human leptospirosis is frequently underdiagnosed due to nonspecific symptoms, while rodents, livestock, and dogs act as major reservoirs and carriers. Environmental factors including flooding and poor sanitation further intensify transmission. Veterinary contributions in surveillance, diagnostics, vaccine development, and vector control are vital components of a sustainable control strategy. A case study from Dakshina Kannada, Karnataka, demonstrates the effectiveness of an integrated One Health model. Initiated in 2019, collaboration between ICAR-NIVEDI, NCDC, and state health authorities strengthened diagnostics, surveillance, and community engagement. Intensive training, stakeholder coordination, and field interventions led to a marked reduction in prevalence, with human seropositivity declining from 26.7% in 2020 to 12.7% in 2023. Livestock surveillance confirmed bovines as key reservoirs (26.7%), rodent screening revealed highly virulent strains, and environmental sampling showed 55% positivity. These findings informed targeted awareness programs and region-specific interventions. A PPCL-funded regional laboratory established in 2023 enabled rapid diagnostics, quality assurance, and real-time reporting, ensuring program sustainability. Despite this progress, challenges remain, including fragmented surveillance, limited diagnostics, low animal vaccine uptake, and weak intersectoral coordination. The study emphasizes the need for a national One Health surveillance platform, expanded animal vaccination, rapid field diagnostics, eco-friendly rodent control, and policy integration. Leptospirosis highlights the urgent need for integrated health governance to reduce zoonotic threats in interconnected ecosystems.

An overview of the One Health Needs and Priorities

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One Health (OH) is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, plants, and ecosystems. The unprecedented, transboundary, and massive outbreaks of highly pathogenic avian influenza in Asia from 2003 to 2006 triggered the One Health conscience, as humans became victims of poultry diseases. The One Health approach has evolved over time with other cross-cutting concepts contributing to its development, such as "One Medicine," "Eco Health," "One World One Health," and "Planetary Health." Although One Health initially focused on zoonotic spillover, prevention, and control, its scope has expanded beyond zoonoses in subsequent years, which have been transformed into six Action Tracks under the One Health Joint Plan of Action. Integrating the environment and agriculture into OH has gained momentum in recent years owing to biodiversity loss, pollution, and climate change. However, it retains a strong biomedical dimension while neglecting socioeconomic dimensions.

One Health is gaining momentum and is now an integral part of international health-related policies, strategies, and agreements, particularly in the wake of the devastating impact of the COVID-19 pandemic on all aspects of our lives. The entire government and societal approach that emerged during the COVID-19 pandemic can be considered an expansion of the socioeconomic dimension of the One Health principle. The adoption of the Pandemic Agreement by the World Health Assembly on May 20, 2025, was a historic step toward strengthening global pandemic preparedness, cooperation, and equitable benefit sharing. The Pandemic Agreement incorporates the One Health approach in Article 5. It requires coordinated action across the human health, animal health, and environmental sectors to prevent zoonotic spillovers, recognizing that future pandemics may emerge at the human-animal interface based on past events.

The world is witnessing an alarming increase in the incidence of emerging infectious diseases with epidemic and pandemic potential. It is impossible to predict precisely when another pandemic will occur, but it could happen at any time in the future. We must bolster our collective efforts toward future pandemic preparedness and response. Governments, international organizations, civil society, academic institutions, and private sector partners must work together to build robust systems for prevention, early detection, and response. The Pandemic Fund is the first multilateral financing mechanism dedicated exclusively to strengthening critical pandemic prevention, preparedness, and response using the One Health approach.

One Health agenda setting should be need-based and country-driven, rather than donor-driven. Many donors and partners have generously invested in developing the One Health workforce and supported research activities over the past decades. However, an enabling environment is lacking for the rational use of trained manpower at the country level, as One Health research is not a priority, and the government is hesitant to invest in it. More emphasis should be given to One Health policy research and the added economic value of OH investment, considering the country's context, needs, and priorities, that is, strengthening the evidence base and translating research output to inform policy decisions.

The Changing Landscape of Pandemic Influenza A(H1N1) pdm09 virus (pH1N1): Implications for One Health Professionals

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Since its emergence in 2009, the Pandemic Influenza A(H1N1) pdm09 virus (pH1N1) has undergone continuous genetic evolution, maintaining its prominence in seasonal influenza activity. As of early 2025, pH1N1 remains the predominant circulating strain in the Northern Hemisphere, including Europe, North America, and Asia, with the co-circulation of A(H3N2) and B viruses in some regions (World Health Organization). This persistence underscores the necessity for One Health professionals to adopt an integrated approach to surveillance, prevention, and control of this disease. Recent developments have highlighted the interconnectedness of human, animal, and environmental health. Notably, the 2024–2025 influenza season coincided with the heightened activity of highly pathogenic avian influenza A(H5N1), leading to human infections in the United States and raising concerns about potential reassortment events. These occurrences emphasize the critical role of One Health strategies in monitoring and mitigating zoonotic threats. Advancements in genomic surveillance, such as the Emergenet platform, enable the rapid assessment of viral evolution and emergence risks, facilitating preemptive interventions. Additionally, the integration of artificial intelligence in epidemiological modeling enhances predictive capabilities, informing timely responses to influenza outbreaks. This study reveals the current landscape of pH1N1, examining its molecular evolution and transmission dynamics and their implications for One Health professionals. Emphasizing the importance of interdisciplinary collaboration, it advocates for strengthened surveillance systems, enhanced cross-sector communication, and the application of innovative technologies to address the evolving challenges posed by the influenza virus.

'Rabies in One Health' as a Model

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Rabies is a fatal disease that is primarily transmitted through rabid dog bites. It takes tens of thousands of lives each year, particularly in low- and middle-income countries. Although rabies can be prevented, it remains a serious public health issue. This is primarily due to the lack of animal rabies control, especially in free-roaming dog populations, and limited access to post-exposure prophylaxis (PEP). The One Health approach, which interlinks human, animal, and environmental health, provides a robust strategy for controlling rabies. By focusing on the main source, the dog population, through mass dog vaccination (MDV), monitoring, and community involvement, this model can help reduce human rabies cases and manage the disease in animals. Studies indicate that vaccinating 70% of dogs can create herd immunity and stop the spread of the virus, making it possible to eliminate rabies from the dog population. Regions such as Latin America, Asia, and Africa have seen a decrease in human rabies cases by applying One Health principles. This involves not only coordinated vaccination campaigns but also strong collaboration between the health, veterinary, and local sectors. Real challenges remain, such as weak disease monitoring, limited access to life-saving post-exposure treatment (PEP), and a lack of coordination among key agencies. To overcome these issues, steady funding, reliable tracking systems, and meaningful involvement from local communities are required. The global "Zero by 30" initiative sets a strong but achievable goal of ending dog-mediated rabies deaths by 2030. Achieving this milestone will depend on collaboration across sectors, maintaining political commitment, ensuring that resources are used wisely, and empowering communities to take an active role. With the One Health approach leading the way, a rabies-free future is possible in Brazil.

Triage Model: Early Signals of Increased Zoonotic Risk

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Zoonotic infections are majorly due to viruses like Avian influenza, Nipah virus, SARS, SARS-CoV-2, Kyasanur Forest Disease, Crimean Congo Haemorrhagic Fever, etc are transmitted between animals and humans, accounts for nearly 75% of emerging infectious diseases worldwide[1]. It is essential to mitigate epidemics, outbreaks, pandemics to prevent socio-economic loss to the population. The fragmented delays across different sectors-human, animal and environment plays a key role in recognizing early warning signals. The early signs like anomalies, patterns that precede the emergence of any outbreak become utmost important to understand. There is an essential requirement to keep a watch on genomic mutations in the virus, unusual mortality and morbidity of animals/birds, and community level alerts. The pathogen survival depends upon various environmental and meteorological factors like humidity, rainfall, temperature. These environmental patterns must be integrated with other patterns to predict the early warning signs.

- Identify environmental changes that favour pathogen emergence or spread.
- Monitor vectors or reservoirs that can bridge the infections from animals to humans.
- Data Collection: Real-time collection of information from human, animal, and environmental sources.
- Signal Verification: Not all signals are real threats; verification ensures accuracy and prevents false alarms.
- Risk Assessment: The multidisciplinary teams assess the probability, impact, and propagation.
- Response Activation: Rapid communication triggers public health measures, veterinary interventions, or environmental management actions.

Complementing the prediction, community level alerts, event-based surveillance, and syndromic surveillance that align with genomic mutations play a paramount role. This triage will help in implementing appropriate strategy by monitoring unusual events reported by healthcare workers and media that link to a rise in symptoms of confirmed diagnosis. To have a successful model, technical, semantic, and organizational interoperability across all sectors should be made. Once this system is developed, more coordinated and timely response can be generated to combat future outbreaks and epidemics.

Passive and active surveillance of zoonotic diseases through sentinel surveillance laboratory in the hills of Kumaon, Uttarakhand

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Zoonoses pose a significant public health challenge, especially in hilly regions where close human-animal interactions & ecological factors favor disease transmission. The Sentinel Surveillance Laboratory (SSL) serves as a critical component in monitoring & controlling priority zoonotic threats by integrating laboratory-based public health actions and supports the "One Health" approach. The study was done to monitor, detect and assess the burden of major zoonotic diseases in Kumaon through integrated active and passive surveillance, enabling early timely response and evidence based public health interventions. The current study conducted in the SSL, SSJGIMS&R, Almora.

1. Passive Surveillance: The patients visiting OPD or admitted, upon using standardized clinical case definitions or with PUO origin were included.
2. Active Surveillance: The region with abrupt increase in cases of undiagnosed fevers were visited. The suspected cases as per the case definition or screened febrile/compatible patients and high-risk contacts were included.

Samples were tested for ELISA, serological testing, culture, Western blot, etc. The laboratory data and clinic-socio-demographic details were uploaded in IHIP-IDSP and IHIP-NOHPCZ portals. The results were shared with competent authority and for source detection veterinary department and entomologist were roped in. Samples from livestock, ticks and mites sample pool were collected. A total of 1529 samples were tested for zoonotic diseases, out of which 360 (23.5%) were tested positive. 250/1017 (24.6%) were positive for IgM scrub typhus, 71/354 (20.1%) for IgM *Brucella*, 16/96 IgM (16.7%) for *Leptospira* and 23/61 (37.1%) for IgM *Borrelia burgorferi* (Lyme Disease). Among 61 sample of Lyme Disease, 48 were collected from Active surveillance where 20 samples (41.7%) were positive. A notable number of zoonotic infections were identified, especially Scrub Typhus in Passive and Lyme Disease in Active surveillance, indicates a significant disease burden in Kumaon. The SSL provided vital for early detection & response, emphasizing the need to strengthen integrated "One Health" surveillance system.

Sequencing, Phylogenetic, and *in-silico* Molecular Docking Analysis of the Nucleocapsid Protein of Crimean Congo Hemorrhagic Fever Virus (CCHFV) to find its Potential Inhibitor

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Crimean Congo Hemorrhagic Fever (CCHF) is a potentially fatal tick-borne viral zoonotic disease affecting humans in a wide geographical area including India. The current study is focused on the sequencing and structural analysis of the nucleocapsid gene of CCHF virus (CCHFV), followed by in-silico molecular docking studies to elucidate its phylogenetic origin and possible inhibitor. The nucleocapsid gene was amplified and cloned, followed by its sequencing. The sequence was then aligned with the other reported sequences from different countries and phylogenetic analysis was done by MEGA. The structure of nucleocapsid protein was then predicted using swiss model. The protein

structure was analysed and subjected to in-silico molecular docking with different ligands to elucidate the possible therapeutic agent for inhibition of virus. On sequence and phylogenetic relationship based on the genomic sequence of the nucleocapsid segment of various strains of CCHFV circulated in India over the past 15 years, it was found that there were considerable differences in the nucleotide sequences, and the Indian strains were found to be related to two different phylogenetic groups, i.e., Asia-1 and Asia-2. It suggests the transboundary nature of the disease, which may be well coordinated with the travel history of the patients affected. The structure of the nucleocapsid protein that is essential for viral infection, replication, and assembly, was predicted by Swiss Model and docked with the Withanolide E using different docking packages like Pymol, UCSF Chimera, PyRx, and MzDock. On docking analysis, a strong binding was found between protein and ligand, suggesting the possible role of Withanolide E as an antiviral agent against CCHF. The phylogenetic study gives the possible origin of the virus and its transboundary nature. The molecular docking studies revealed the strong binding with Withanolide E, suggesting its possible role as an antiviral agent against CCHFV.

Genomic insights into Indian Buffalopox virus strains: Evolution, host adaptation, and phylogenetic relationships

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Buffalopox is a contagious, emerging, and re-emerging viral zoonotic disease, caused by buffalopox virus (BPXV), affecting buffaloes, cows, and humans. Apart from India, it is also prevalent in many buffalo-rearing countries, affecting buffaloes, cows, and humans. Limited genomic data from Indian BPXV strains warrant comprehensive genome analysis. This study aimed to characterize the complete genomes of Indian BPXV -BP4 strain (reference strain) and Vijayawada/96 (Vij/96) strain using next-generation sequencing to elucidate their genetic diversity and evolutionary relationships. The BPXV strains were revived in Vero cells, and genomic DNA was extracted and confirmed by species-specific PCR. Whole-genome sequencing was performed using the Illumina HiSeq platform with 150 bp paired-end reads. Quality-trimmed reads were reference-mapped and the consensus genomes were annotated. Comparative genomic and phylogenetic analyses were conducted with vaccinia virus (VACV) and other orthopox viruses to determine percentage identity, evolutionary relationships, and genetic mutations. The genome sizes of BPXV-BP4 and BPXV-Vij96 were ~194 kbp, with 66% A+T content and 177 complete ORFs. Nucleotide identity among the BPXV strains was 99.82%-99.9%. Phylogenetic analysis of BPXV strains based on whole genome and eight host-range genes from Indian BPXV isolates revealed the presence of two distinct subclades, both closely related to the VACV-CVA strain. In comparison with VACV, several genes encoding ankyrin proteins, A-type inclusion protein, kelch-like protein, and semaphorin-like protein were found to be truncated. A deletion of approximately 2 kbp affecting the CrmC (TNF receptor-like protein) gene resulted in a frameshift mutation and the loss of an adjacent kelch-like protein. These deletions and truncations may potentially influence host range and adaptation mechanisms in BPXV. This study enhances understanding of the genetic origin, evolutionary divergence, and host adaptation mechanisms of Indian BPXV strains, offering valuable insights into their emergence and zoonotic potential.

Seroprevalence of Brucellosis in Cattle and Yak/Chauries of Nepal

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Brucellosis is one of the most important contagious bacterial zoonotic disease of livestock reported throughout the world resulting a serious economic threat in animal production sector and downturn of public health. The study was done to determine the prevalence of *Brucella abortus* antibody in raw milk of cows in Tanahu district, serum of Yak/Nak in Mustang district and serum of cattle in Chitwan, Rupandehi, Kanchanpur and Gorkha district. Different cross-sectional studies were conducted from July 2021 to September 2021 (Tanahu), April 2021 to May 2021 (Mustang), November 2022 to January 2023 (Chitwan), May to June 2023 (Rupandehi) and June to August 2023 (Kanchanpur) and August to September 2025 in Gorkha (180). A total of 130 milk samples and 888 serum samples were collected using a purposive sampling technique from different household and farms. Thus, collected samples were transported to National Cattle Research Program laboratory, Chitwan for indirect ELISA. Statistical analysis was done by using SPSS version 19 and Fisher exact test was used. These samples showed an overall prevalence of brucellosis to be 0.59% (6/1018) in the study area. Considering district wise prevalence of brucellosis, 3% (4/130) in Tanahu, 0% (0/92) in Mustang, 0.29% (1/348) in Chitwan, 0% (0/141) in Rupandehi, 0.78% (1/127) in Kanchanpur and 0% (0/180) in Gorkha was found. The result showed no statistical significance (P>0.05) to any of the factors including age, breed, breeding method, location, history of abortion/repeat breeding/ROP etc. With no history of vaccination, positive reactors were asymptomatic animals that harbored natural infection. Thus, further studies need to be done in different areas of the study sites to detect animals in the carrier state with molecular testing facility like PCR. Large scale vaccination programs to the animals along with awareness programs among people may aid in the prevention of disease transmission and public health problems.

Characterization of a Virulent Leptospira interrogans Strain: Advancing Challenge Models for Vaccine Development in India

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Leptospirosis severity varies by region and host species, requiring region-specific vaccines for effective control. However, vaccine efficacy studies are hindered by the lack of virulent, well-characterized challenge strains and standardized protocols. High-passage reference strains often lose virulence, emphasizing the need for low-passage, regionally adapted strains. To address this, ICAR-NIVEDI isolated a pathogenic *Leptospira* strain from a Greater Bandicoot Rat (*Bandicota indica*) from a household, following a severe human case in Dakshina Kannada, Karnataka (November 2024). The isolate, maintained in EMJH medium, was passaged in Golden Syrian hamsters to restore virulence. By the third passage, animals exhibited severe clinical signs and mortality, confirming virulence restoration. The strain was maintained at low passage for use in challenge studies. Whole genome sequencing (Oxford Nanopore+Illumina) was performed at Gujarat Biotechnology Research Centre. Both the original and hamster-passaged isolates were identified as *Leptospira interrogans* (Genome

GenBank Accession Numbers: JBRBPH000000000 *Leptospira interrogans* Lepto/12-24/R(BH)-NIVEDI and JBRBPI000000000 *Leptospira interrogans* Lepto/11-24/R(B)-NIVEDI), with further analysis of virulence genes underway. For model development, hamsters (3–4 weeks old) were inoculated intraperitoneally with 10-fold serial dilutions (10□–10¹ cells/ml). Animals were monitored and euthanized upon clinical signs or study endpoint. Blood and tissue samples confirmed systemic infection with multi-organ damage. The strain belonged to the Autumnalis serogroup, and LD□□ was calculated using the Reed–Muench method, confirming dose-dependent virulence. This study successfully isolated, restored, and characterized a virulent *L. interrogans* strain fulfilling Koch's postulates. It provides a critical tool for standardized challenge models and supports the development of region-specific leptospirosis vaccines in India.

Poster Presentations

A novel isothermal CRISPR-Cas12a–based diagnostic platform for the detection of Rabies virus

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Rabies remains a highly fatal zoonotic disease, posing a major challenge to veterinary and public health due to the limited accessibility of rapid and field-based diagnostics. This study aimed to develop a rapid, sensitive, and field-deployable CRISPR-Cas12a–based molecular assay integrated with an isothermal assay for efficient detection of the rabies virus. A total of 110 suspected animal specimens from different Indian states were screened for the rabies nucleoprotein (N) gene using WOAH-recommended primers. Isothermal amplification methods, including RPA, PSR, and LAMP, were optimized, with RT-RPA at 42 °C using primer pair 129F/412R showing superior performance. Six crRNAs targeting the N gene were designed and tested, among which crRNA 144F produced the strongest detection signal. The CRISPR-Cas12a reaction was optimized at a crRNA concentration of 2 µM and validated for specificity against common canine viruses. Lyophilized reagent mixtures with trehalose and a simple heat-based RNA release method were also standardized to enable portable, field-ready detection. The optimized CRISPR-Cas12a–RT-RPA assay successfully detected rabies virus RNA within 50 minutes, showing high fluorescence intensity and no cross-reactivity with other canine pathogens. Validation using 60 field samples demonstrated complete concordance with PCR results, while additional positives indicated enhanced sensitivity. The lyophilized formulation maintained stability and efficiency under field conditions, confirming its suitability for on-site surveillance without requiring advanced laboratory infrastructure. The developed CRISPR-Cas12a–RT-RPA assay offers a rapid, specific, and low-resource diagnostic solution for effective rabies surveillance and control in field-level settings.

Molecular studies and characterization of proteins involved in *Orthoflavivirus* antiviral innate immune responses

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Orthoflaviviruses represent an enormous group of emerging and reemerging arboviruses such as dengue virus (DENV), West Nile virus (WNV), Spondweni virus complex consisting of Spondweni virus (SPOV) and Zika virus (ZIKV), TBEV, and Usutu virus (USUV), to name a few, that hold the immense potential to not only dominate human existence but also adversely affect agriculture and livestock, including cattle, goat, sheep, and birds globally. There are neither specific antivirals available nor effective vaccines to mitigate the burden associated with these infections. The vaccines frequently encounter drawbacks such as antibody-dependent enhancement (ADE) and cross-reactivity due to shared epitopes upon repeated infections. The intricate interactions between the host and viral proteins play a key role in pathogenesis and disease progression. The cross-talk between stress granule assembly and innate immunity has been studied in several RNA viruses. The viruses direct the host proteins, including the components of stress granule assembly, to direct their replication by subverting the innate immune response. The objective of the study was to identify the key host factors that interact with viral proteins, thereby blocking the innate immune response and disrupting the stress granule pathway. Host factor-1 was identified by multiple-sequence alignment and conserved-motif analysis. Coding sequences were cloned into bacterial expression vectors with N-terminal His-tags, expressed in *E. coli* BL21(DE3), and purified by Ni-NTA affinity chromatography followed by size-exclusion chromatography. Direct binding to viral protein was quantified by ITC (micro- to low-μM range, 25°C) to obtain Kd, ΔH, and stoichiometry. Cellular relevance to be tested in human hepatoma cells: infections/transfections, RT-qPCR for IFN-β and ISGs, immunofluorescence for G3BP1/TIA-1 stress granules, and functional perturbation via siRNA/overexpression and IFN-β luciferase reporter assays. Our study characterized Host Factor-1 (HF-1), a host protein of the stress granule pathway that acts as an interacting partner of flavivirus capsid. Sequence alignment studies have shown that key interacting residues of Host Factor-1 and orthoflavivirus capsid are conserved amongst orthoflaviruses to develop pan-orthoflavivirus therapeutics. Biophysically, ITC revealed binding affinity with a dissociation constant of 3 μM for this interaction. Molecular therapeutics targeting these host-pathogen interactions to restore the interferon response to normal are being investigated further for host-targeted antiviral-based therapy.

Gene cloning and expression of recombinant glycoprotein (G) of Nipah Virus (NiV) using prokaryotic expression system

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Nipah virus (NiV), a zoonotic pathogen belongs to the genus *Henipavirus* and family *Paramyxoviridae*, is classified as a Biosafety Level-4 pathogen due to its high pathogenicity. Epidemiological surveys have shown that NiV is distributed across Asia including India, and is transmitted by its natural reservoir, Pteropus bats. It causes severe respiratory illness and encephalitis with a high fatality rate (40–75%) in humans. Pigs are reckoned to play a vital role in disease transmission cycle. Sentinel surveillance could help to determine the prevalence and distribution of the Nipah virus in animals in potential risk areas under the One Health strategy. The glycoprotein (G) of NiV is one among the structural proteins, having a key receptor in host cell binding, and contains the conserved immunodominant epitopes, known to produce neutralizing antibodies. Production of recombinant Nipah virus protein and evaluation of its immunoreactivity for diagnostic applications. The candidate gene encoding for Glycoprotein (G) of NiV was cloned in to prokaryotic expression vector (pET28a) and efficiently expressed in Escherichia coli Rosetta (DE3)-RIPLG host strain following chemical induction. further protein was purified using Ni-NTA affinity column chromatography. The quality and quantity of purified rG of NiV was analyzed by SDS-PAGE analysis. In addition, the preliminary immunoreactivity of rG of NiV was analysed by indirect-ELISA using field pig sera samples. The recombinant NiV-G (~51 kDa) protein with an N-terminal hexa-Histidine tag was over-expressed in Prokaryotic expression system and immunoreactivity analysis by western blot showed significant result. Nipah virus Glycoprotein was expressed successfully in prokaryotic expression system and its immunoreactivity was analysed by indirect-ELISA using field pig sera samples. This protein may aid in early detection and surveillance of Nipah virus infections.

Indirect ELISA using recombinant mpl17 of *Leptospira interrogans* for the detection of anti-leptospiral antibodies

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Leptospirosis is an important zoonoses affecting animals and humans. Microscopic agglutination test (MAT) is the current gold standard assay for diagnosing leptospirosis, but its limitations call for a safer and quicker test. The objectives of this study were the expression of recombinant MPL17 protein and its evaluation in indirect ELISA for the diagnosis of leptospirosis. Expression of recombinant protein rMPL17 of *L. interrogans* was done using the pPROEx-HTa vector in BL21 *E. coli* cells. The expressed protein was purified using Ni-NTA affinity chromatography and the recombinant protein was confirmed by SDS-PAGE and western blotting techniques. Purified rMPL17 protein was used in the development of an indirect ELISA. The collected serum samples from bovine and canine were tested for anti-leptospiral antibodies using the microscopic agglutination test (MAT) and rMPL17 protein-based indirect ELISA. Subsequently, receiver-operating characteristic (ROC) curve analysis was performed to assess and compare the diagnostic performance (sensitivity, specificity, and cut-off values) of the ELISA relative to MAT. When tested with canine sera, the rMPL17 protein-based indirect ELISA detected antibodies in 8 of 100 samples. In contrast, when applied to bovine sera, it detected antibodies in 14 of 100 samples. Receiver-operating characteristic analysis showed that indirect ELISA has a sensitivity of 100% and a specificity of 85.0% in canine samples, whereas it has 91.7% sensitivity and 90.9% specificity for the bovine serum samples. Samples positive or negative in MAT were considered as true positive or negative for ROC analysis. The study demonstrated that the rMPL17 is a promising antigen for the diagnosis of leptospirosis and can complement MAT, which requires live cultures and is not available in all labs.

Comparing the infectivity and gene expression of different *Brucella* strains in the macrophage raw 264.7 cell line

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Brucellosis is a significant zoonotic disease in many developing countries, including India. The virulence factors play a crucial role in establishing and maintaining infection of *Brucella* spp. This study aimed to examine the infectivity of vaccine, reference, and field strains of *Brucella* in RAW 264.7 macrophages and to compare the expression dynamics of virulence-associated genes Omp19, Ure, VirB, and BvfA during the infection process. The RAW 264.7 cells were infected at a multiplicity of infection (MOI) of 100 with *B. abortus* strains (S19, S99, 544), *B. melitensis* strains (16M, Rev1), and a field isolate (P5) were examined at 0, 3, 6, 12, 24, 36, and 48 hours post-infection using the MZN (Modified Ziehl-Neeelsen) staining to assess bacterial internalization, followed by DNA extraction for qPCR-based detection of virulence gene expression. Microscopy confirmed intracellular localization of *Brucella* from 6 hours post-infection, with peak infection at 36 hours. *B. melitensis* 16M exhibited delayed internalization as compared to other strains. Gene expression profiling revealed that vaccine and reference strains (S19, S99, 544, Rev1) consistently upregulated all four target virulence genes, correlating with enhanced intracellular survival. Conversely, strain 16M showed downregulation of Omp19, Ure, VirB, and BvfA, suggesting the utilization of alternative virulence mechanisms. The field strain P5 exhibited a mixed expression pattern, with upregulation of Ure and BvfA but downregulation of Omp19 and VirB, indicating strain-specific adaptations. Notably, S19 displayed the highest expression of Omp19, BvfA, and Ure, whereas 16M showed the lowest levels of Omp19, VirB, and Ure. VirB expression peaked in Rev1, while BvfA expression was lowest in S99. These differences in

gene expression likely influence the infectivity and pathogenic potential across strains. Many virulence factors can mediate at each step of infection, and each strain may have a unique combination of these factors that affect the rate of bacterial pathogenesis. These findings enhance our understanding regarding the pathogenesis of *Brucella* spp. and the development of novel diagnostics and therapeutic strategies.

Post-vaccination antibody response in dogs and cats against Rabies virus: A seroprevalence study

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Rabies is a lethal, zoonotic, and neurological disease with a 100% case fatality rate, but it is vaccine preventable. Post-vaccination protective antibody titre is essential for effective disease control and surveillance. The study aims to determine the Seroprevalence of rabies virus-neutralizing antibodies in vaccinated dogs and Cats, evaluating protective antibody titres in the animals, and understanding the effect of factors such as age, sex, and their distribution within different titre ranges. A total of 533 post-vaccination serum samples, comprising 399 from Dogs and 134 from Cats, were received after vaccination for antibody titre reports from Mumbai (mostly), U.P., Goa, and Hyderabad from January 2023 to September 2025. The antibody titres were analyzed using PLATELIA™ RABIES II ASSAY Ad Veterinarian (Ref: 3550180) BIORAD ELISA kit. The ELISA test was performed as per the manufacturer's procedure. Serum titres were expressed in equivalent units per millilitre (IU/ml). Out of 533 serum samples, the overall seropositive percentage observed in the present study was 98.6%, comprising 73.7% of dogs and 23.95% of cats. Sex wise distribution revealed, male Dogs 99.2% (260/262), Bitches 97.08% (133/137), Tomcats 100% (65/65), Queens 98.5% (68/69) showed protective titre. In terms of age-wise distribution, the highest positive percentage of antibody titre was observed in the 1-month to 6-month age group (100%), and the lowest in the 6-month to 1-year age group. The highest antibody titre observed is 2.67 IU/ml. More samples are within the titre range of 0.5 IU/ml to 1 IU/ml (410/533), 7 samples showed unprotective titre <0.5 IU/ml. The study revealed a high seroprevalence of anti-rabies antibody titres in both dogs and cats, indicating adequate vaccination coverage and awareness among pet owners. Sex did not show a significant influence on the rabies antibody response. The age group between 1 month and 6 months showed the highest percentage of titre, possibly due to maternal antibody interference. Most animals under study have titres in the 0.5-1IU/ml range, which indicate the protective titre.

Seroprevalence of brucellosis in buffaloes of commercial farm of Bharatpur metropolitan city, Nepal

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Livestock farming significantly contributes to Nepal's economy, accounting for 24.01% of AGDP and 11% of GDP. However, brucellosis, a major zoonotic disease, threatens livestock productivity and public health. The study was undertaken to study the seroprevalance of brucellosis in buffaloes of commercial farm of Bharatpur Metropolitan city. Study was conducted in Bharatpur during winter season of 2024 from last week of November to Third week of December. The site is located at southwestern belt of Nepal at latitude: 27.5291° N, longitude: 84.3542°E. Cross-sectional study along with questionnaire survey using face to face interview pattern was done before taking 46 serum sample from 4 different farm. 5-7 ml blood was collected from the jugular vein, serum separated and stored at −20°C (AFU). Indirect ELISA (IDvet, France) was performed at CVL. Data was analyzed using with

Chi-square test. Out of 46 samples taken only one (2.1%) sample tested positive for *Brucella* organism by ID Screen® Brucellosis serum Indirect Multispecies kit. Since, only one sample gave positive result for Brucellosis test, no any statistical tool can be use and compare them with risk factors. Only one sample tested positive in the study area by iELISA. Problems such as repeat breeding, abortions, retention of placenta and other various signs that were addressed during the questionnaire survey by the farmers may be because of other causative agents and not due to brucellosis.

Detection, isolation and genotypic characterization of Rotavirus A from calves and poultry in Gujarat with successful adaptation in MA-104 cell line

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Rotavirus A is an important cause of neonatal diarrhoea in livestock and poultry. This study aims to detect and characterize its presence in diarrhoeic faecal samples across Gujarat. Objectives of the study was to detect, isolate, and molecularly characterize Rotavirus A from calves and poultry in Gujarat and evaluate its adaptation efficiency in MA-104 cell culture system. A total of 74 diarrhoeic faecal samples from cattle calves, buffalo calves, foals, and poultry chicks were collected from various organized and unorganized farms in Junagadh, Rajkot, Anand, and Godhra regions of Gujarat. Samples were screened using Latex Agglutination Test (LAT), followed by RT-PCR targeting the VP6 gene. Positive samples were processed for virus isolation in MA-104 cell line, and cytopathic effects were monitored. Molecular genotyping of positive isolates was performed using RT-PCR for VP7 (G typing) and VP4 (P typing) genes. Typing was done using type-specific primers. Adaptation efficiency was evaluated based on the number of passages required to observe CPE. Of the 74 samples, five (four bovines and one poultry) tested positive by LAT, RT-PCR, and cell culture, indicating a 6.76% prevalence. The highest detection was in Anand (8.82%). CPEs were observed in all LAT-positive samples, with two adapting in the first passage. G6 (50%), G10 (25%), and G8 (25%) were identified, along with P [1] and P [11] types. G6P [11] was the most common combination. The poultry isolate, although positive, remained untypeable. The study highlights the diversity of Rotavirus A strains in Gujarat and supports MA-104 as a reliable system for primary isolation, aiding in regional surveillance and vaccine planning.

RNA-PAGE analysis and molecular characterization of Rotavirus A isolates from diarrhoeic livestock and poultry in Gujarat

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Rotaviruses, Reoviridae members, cause severe diarrhoeal disease in livestock and poultry, with their segmented dsRNA genome driving genetic diversity and new strain emergence.This study utilized RNA-PAGE and molecular genotyping to detect and characterize Rotavirus A (RVA) from a total of 74 diarrhoeic faecal samples collected from cattle calves, buffalo calves, foals, and poultry chicks across four Gujarat regions in India: Junagadh, Rajkot, Anand, and Godhra. Initial screening of the diarrhoeic faecal samples using the Latex Agglutination Test (LAT) and RT-PCR identified 5 positive samples for Rotavirus A (RVA): 4 from cattle calves and 1 from poultry chicks. All samples from buffalo calves and foals were negative. RNA-PAGE analysis of these five positive isolates

revealed the characteristic 4:2:3:2 migration pattern typical of mammalian Rotavirus A. Surprisingly, the single poultry isolate also displayed this mammalian-type electropherotype, rather than the expected 5:1:3:2 avian pattern. This unexpected finding is significant, suggesting the potential occurrence of cross-species transmission or genomic reassortment events involving Rotaviruses within the studied region. Molecular characterization of the VP7 (G typing) and VP4 (P typing) genes was performed on the bovine Rotavirus A isolates. The analysis revealed G6 (50%), G10 (25%), and G8 (25%) types for the VP7 gene. For the VP4 gene, P[1] and P[11] types were identified in equal proportions (50% each). The most prevalent genotype combination observed was G6P [11], which accounted for 50% of all characterized bovine Rotavirus A isolates. These findings provide crucial data on the circulating RVA strains in the region. This study confirms the genetic diversity of Rotavirus A (RVA) strains in Gujarat, India, and validates RNA-PAGE as a valuable initial epidemiological tool alongside advanced genotyping methods.

Immunological and molecular detection of Bovine Rotavirus from diarrhoeic calves

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Rotaviruses are recognized as a primary cause of gastroenteritis in both animals and humans. Rotavirus belongs to the family Reoviridae and the genus Rotavirus. In calves, Bovine Rotavirus is a significant pathogen responsible for considerable economic losses in the dairy and meat industries. In India, the prevalence of Rotavirus-associated diarrhoea in calves under one month of age varies widely, with estimates ranging between 10% and 52%. This study was undertaken to detect Bovine Rotavirus in diarrhoeic calves using both immunological and molecular diagnostic techniques, namely the Lateral Flow Assay (LFA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR).A total of 50 faecal samples were collected from diarrhoeic calves around Junagadh region. Samples were collected based on age, sex, species, season, feeding and symptoms (Colour of diarrhoea). All samples were first screened using a commercially available LFA kit (Anigen Rapid Rota Ag Test Kit- BioNote) for the presence of rotaviral antigens. Subsequently, RNA was extracted by using commercial kit (RNA Sure® Virus Kit- Genetix) & cDNA was synthesis by using commercial kit (First Strand cDNA Synthesis Kit) and then subjected to RT-PCR targeting the VP6 & VP7 gene, which are highly conserved genes among group A Rotaviruses. The results revealed that 4 out of 50 samples (8.00%) tested positive by LFA. Similarly, RT-PCR also detected rotavirus in 4 samples (8.00%). However, due to the inherently higher sensitivity and specificity of RT-PCR, it remains the preferred method for confirmatory diagnosis. The present study confirms the presence of Bovine Rotavirus infection in diarrhoeic calves in the Junagadh region, with a detection rate of 8% by both Lateral Flow Assay (LFA) and RT-PCR. The concordance between the two diagnostic methods indicates that LFA can serve as a useful rapid screening tool in field conditions, while RT-PCR, owing to its higher sensitivity and specificity, remains essential for confirmatory diagnosis.

Indian Fruit Bats (*Pteropus medicus*) Act as Reservoir of Drug-Resistant *Klebsiella pneumoniae*: A Serious Concern to One Health

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Bats are the unique non-terrestrial wild mammals that are the primitive but most successful species to continue to live on the earth. Owing to their flying habit and exposure to different ecological niches, the bats can acquire and spread a number of zoonotic bacteria with or without antimicrobial determinants. This study aimed to investigate the role of the Indian flying fox or fruit bat (*Pteropus medius*), if any, in harbouring zoonotic pathogens relevant to human and animal health. Samples from guano/faeces and habitat (tree bark, leaves, partially eaten fruits, and soil) of bats, roosted at 'Tala Park' of North Kolkata, were collected and analyzed culturally, biochemically, and molecularly to detect the presence of *Klebsiella pneumoniae*, a major zoonotic pathogen of concern. Phenotypic and genotypic screenings of the isolates were carried out for the presence of antibiotic resistance. Analysis of 40 samples resulted in the isolation of *K. pneumoniae* (n= 30; 75%), with confirmation based on cultural characteristics, biochemical profiling, and molecular (16S rRNA PCR) methods. Double disc synergy testing revealed that all 30 isolates were phenotypically positive for extended-spectrum β-lactamases (ESBL). PCR screening demonstrated that 11 (36.7%) of 30 isolates carried blaTEM, 5 (16.7%) carried blaSHV, 1 (3.3%) possessed blaCTX-M, and 7 (23.3%) isolates possessed blaAmpC genes. Antibiotic susceptibility testing indicated that all *K. pneumoniae* isolates were resistant to Enrofloxacin and Ticarcillin/Clavulanate, while significant resistance was noted for Tetracycline (80.2%), Cefixime (78.1%), Doxycycline (50.6%), and Amoxicillin (50.2%). In contrast, remarkable sensitivity was observed against Piperacillin (86.5%), Chloramphenicol (80.2%), Gentamicin (72.6%), and Cotrimoxazole (62.5%). The findings suggest that the roosting of fruit bats in highly populated North Kolkata's 'Tala Park' area-a close human-bat interface-may facilitate the transmission of multi-drug resistant *K. pneumoniae* to park visitors and their pets, posing a critical One Health concern.

Exploring the Lytic potential and Host Range of *Salmonella Typhimurium* Phages Combating Emerging Zoonoses: One Health Perspective

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The global rise of multidrug-resistant pathogens necessitates alternatives to antibiotics, and lytic bacteriophages offer a promising, targeted approach to control *Salmonella* infections and ensure food safety. The study was done to isolate and characterize strictly lytic bacteriophages against *Salmonella typhimurium*, determine their host range, and evaluate their potential as natural biocontrol agents to combat emerging zoonotic infections. Six bacteriophages specific to *Salmonella typhimurium* were isolated from environmental and animal-derived samples using standard enrichment procedures. Lytic activity was initially screened through turbidity reduction assays and confirmed via the double agar overlay method. Phages were tested against multiple *Salmonella* serovars-including *Typhimurium, Enteritidis, Choleraesuis*, and *Tennessee*, to assess host range. Non-Salmonella bacterial strains were included to ensure specificity and prevent disruption of beneficial microbiota. Plaque morphology, propagation efficiency, and lytic characteristics were recorded. The study aimed to explore the lytic potential and host range of these phages as sustainable interventions, aligning with the One Health approach to reduce zoonotic transmission, food contamination, and antimicrobial resistance. All six

isolated phages exhibited strict lytic activity against *Salmonella Typhimurium*, with broad host-range activity against other serovars such as *Enteritidis*, *Choleraesuis*, and *Tennessee*. No activity was detected against non-Salmonella species, highlighting high specificity. Turbidity reduction assays demonstrated rapid bacterial lysis, while plaque morphology confirmed efficient propagation. Host-range profiling indicated suitability for targeted applications in both clinical and food safety settings. The phages' strict specificity ensures minimal impact on beneficial microbiota. These findings emphasize their potential as natural biocontrol agents and therapeutic candidates, offering a promising one health strategy to combat emerging zoonoses and reduce the global threat of multidrug-resistant *Salmonella.* Exploring the lytic potential and host range of these phages demonstrates their promise as targeted biocontrol agents, supporting one health strategies for food safety, zoonotic disease prevention, and antimicrobial resistance mitigation.

Climate change-driven shifts in livestock disease dynamics in antimicrobial resistance: A one health perspective

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Climate change has emerged as a critical determinant of livestock health, influencing the occurrence, distribution, and virulence of infectious diseases. Altered temperature, humidity, and rainfall patterns are reshaping vector habitats and pathogen survival, leading to the emergence of novel infections and re-emergence of endemic ones. The present study investigated the relationship between climatic variability and emerging livestock diseases, with a specific focus on altered disease transmission dynamics, antimicrobial resistance (AMR), and implications for sustainable livestock health management. Climatic and epidemiological datasets were analyzed to identify correlations between temperature, precipitation, and the prevalence of major livestock diseases across selected agro-climatic zones. Disease incidence records were obtained from national veterinary health databases, while climate parameters were sourced from meteorological agencies. Statistical modeling was employed to assess associations between climatic anomalies and disease outbreaks. The One Health framework was used to integrate environmental, microbial, and host-level determinants contributing to disease emergence and AMR patterns. The analysis revealed a significant rise in vector-borne and water-borne diseases, including bluetongue, Rift Valley fever, and helminth infections, in regions experiencing higher temperatures and variable rainfall. Seasonal shifts extended vector breeding periods and pathogen transmission windows. Extreme weather events were correlated with increased incidences of enteric and respiratory diseases. Additionally, higher ambient temperatures and altered moisture regimes promoted AMR by influencing microbial gene transfer and antibiotic usage patterns in livestock. The findings underscore that, climate change is a key driver of emerging livestock diseases and antimicrobial resistance. Strengthening adaptive livestock management, disease surveillance, and cross-sectoral interventions under the One Health framework is essential to enhance resilience, ensure food security, and mitigate zoonotic risks in a changing climate.

Session 2:

One Health Approach to Antimicrobial Resistance, Microbiome, Food Safety and Biosecurity

Predict, Prevent, Protect: Harnessing Epidemiology and Informatics for Health Security

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India's livestock sector, comprising over 536 million animals-including cattle, buffaloes, sheep, goats, and pigs, is a cornerstone of food security, rural livelihoods, and contributes approximately 5.5% to the national GDP. However, the sector remains vulnerable to both endemic and emerging diseases such as Foot and Mouth Disease (FMD), Lumpy Skin Disease (LSD), African Swine Fever (ASF), and Avian Influenza. These diseases pose persistent threats to productivity, trade, and public health. In this context, harnessing epidemiology and informatics through the framework of "Predict, Prevent, Protect" is vital to safeguard livestock health and national resilience.

India has made notable progress in this direction. Epidemiological tools and data-driven informatics platforms have enhanced the country's capacity to foresee and respond to disease threats. The NADRES v2 system, developed by ICAR-NIVEDI, delivers early warnings up to two months in advance for around 15 priority livestock diseases, covering more than 750 districts nationwide. Simultaneously, under the flagship National Animal Disease Control Programme (NADCP), reported FMD outbreaks dropped from 132 in 2019 to just 49 in 2023. These measurable outcomes underscore the value of integrating statistical modelling, weather-based forecasting, and real-time informatics to inform vaccination strategies and guide targeted disease responses.

The "Predict" pillar uses epidemiological analysis and machine-learning algorithms to identify hotspots, risk periods and likely outbreak scenarios, enabling pre-emptive action. Informatics systems such as NADRES v2 democratise access to predictive alerts, delivering thousands of SMS/email advisories to veterinarians and farmers. The "Prevent" component is realised through data-guided mass vaccination campaigns and surveillance-such as the NADCP's nationwide FMD/ Brucellosis drive. Finally, "Protect" represents the tangible impact: reduction in disease incidence, improved trade access, and strengthened One-Health linkages.

Despite these achievements, significant challenges persist. Transboundary animal diseases continue to bypass surveillance infrastructure, data systems are often fragmented, and capacity in veterinary informatics remains uneven. Moreover, the growing threat of antimicrobial resistance (AMR) risks undermining disease control gains.

Looking ahead, the roadmap calls for full national roll-out of predictive informatics, integration of artificial intelligence, GIS and genomics in livestock health monitoring, capacity-building of field veterinary personnel and embedding a formal One Health framework linking animal, human and environmental surveillance. In sum, by systematically harnessing epidemiology and informatics, India can transition from reactive to predictive disease management—thus safeguarding the health of its vast livestock populations, protecting farmers' livelihoods and reinforcing national bio-security.

A Cross-Sectoral Framework for Food Safety: The One Health Connection

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Food safety represents a central pillar of public health, economic stability, and sustainable development. Despite advancements in food control systems, global estimates from the World Health Organization (2024) indicate that nearly 600 million people suffer from foodborne illnesses annually, underscoring persistent vulnerabilities in agrifood systems. These challenges are increasingly complex due to the convergence of human, animal, plant, and environmental health determinants. Conventional, sector-specific approaches have proven insufficient to address emerging threats such as antimicrobial resistance (AMR), zoonotic pathogens, and environmental contamination. The One Health approach**,** by contrast, emphasizes systemic interdependence and collaborative governance across sectors and disciplines. This Framework proposes a cross-sectoral framework for food safety grounded in One Health principles. The framework integrates five key components—risk assessment and surveillance, governance and legislation, capacity and infrastructure, multi-stakeholder collaboration, and preventive control measures. Drawing upon global policy instruments such as the FAO–WHO–WOAH–UNEP Quadripartite One Health Joint Plan of Action (2022–2026), the World Bank One Health Operational Framework (2018)**,** and OECD's economic analysis of AMR (2023), the framework highlights pathways for harmonizing data, policies, and institutional actions across the agrifood continuum. Empirical insights are illustrated through India's and South Asia's agrifood systems**,** including the ILRI One Health Initiative in Uttar Pradesh**,** which demonstrates the benefits of integrated AMR surveillance and improved biosecurity in livestock supply chains. The paper argues that a coordinated, One Health–based approach enhances the efficiency and sustainability of food safety systems while contributing to the Sustainable Development Goals (SDGs)**.** In conclusion, operationalizing food safety through a cross-sectoral One Health framework advances both scientific understanding and policy coherence, enabling resilient agrifood systems that safeguard human health, protect ecosystems, and promote sustainable economic development.

One Health in Nepal: Status, Challenges and Opportunities

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The One Health concept recognizes the interconnectedness of human, animal, and environmental health and is increasingly adopted worldwide. In Nepal, the One Health movement has gained momentum over the past few decades as the country faces growing challenges of zoonotic diseases, antimicrobial resistance (AMR), pesticide use, food safety issues, and climate change. A review of published and grey literature was conducted to assess the situation, challenges, and opportunities of One Health in Nepal. The formal endorsement of the National One Health Strategy in 2019 provided a policy framework to advance this agenda, establishing multisectoral high-level steering and technical committees at national level. Provincial One Health committees have also been formed across all seven provinces in the country.

The One Health approach has been applied in several priority areas. The growing threat of AMR is being addressed through the Multisectoral National Action Plan on AMR. Likewise, avian influenza outbreaks—first detected in 2009—have been managed under the Bird Flu Control Regulation using a One Health framework. The approach is also applied in rabies control programs, while ten zoonotic diseases were identified as national priorities in 2021 through a One Health process. Capacity-building initiatives involving multisectoral stakeholders have expanded significantly with the support of development partners such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

Despite this progress, several challenges remain. Sectoral silos still exist, and Nepal lacks a dedicated institutional mechanism to operationalize the One Health approach. The environmental sector is yet to be fully integrated, and efforts need to extend to the local level to ensure sustainability. Nevertheless, with a formal policy in place and multiple ongoing initiatives, Nepal has a strong foundation and growing opportunities to institutionalize and expand the One Health approach nationwide.

Reinventing Food Safety: Natural Solutions for the Antimicrobial Resistance Era

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Antimicrobial resistance (AMR) is now recognized as one of the greatest threats to global public health, food security, and sustainable development. The widespread and often indiscriminate use of antibiotics in animal agriculture has been a major contributor to this crisis. For decades, antibiotics have been used not only to treat infections in livestock but also as growth promoters and preventive measures. While these practices improved productivity, they also created an environment that encouraged bacteria to evolve resistance mechanisms. Resistant strains originating from farms may reach humans through meat, dairy, and eggs, as well as through environmental routes such as water and soil contamination. Consequently, strategies to reduce antibiotic use while maintaining animal health and food safety have become a global priority. Natural antimicrobials have emerged as promising alternatives. They are derived from plants, microbes, or natural fermentation processes and act through mechanisms different from conventional antibiotics. Many of them disrupt bacterial membranes or interfere with multiple cellular targets, making resistance development less likely. Their potential to reduce microbial loads in meat, poultry, and dairy products offers a way to protect consumers and extend shelf life while lowering the reliance on antibiotics. The growing crisis of antimicrobial resistance has made it essential to reduce the reliance on conventional antibiotics in livestock production and processing. Natural antimicrobials provide a wide range of effective alternatives, from plant-based essential oils to bacteriocins, organic acids, probiotics, and bacteriophages. When integrated across the food chain using a multi-hurdle approach, these agents can significantly reduce microbial loads, enhance food safety, and contribute to global AMR mitigation strategies. Continued research, innovation in delivery technologies, and supportive regulatory policies will be crucial for unlocking the full potential of natural antimicrobials in sustainable livestock processing.

Integrons: Genetic Architects of Antimicrobial Resistance

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Integrons, specialized segments of double-stranded DNA (dsDNA) are known as microbial evolutionary engines with the capabilities to mediate the acquisition, storage, and expression of modular DNA units known as gene cassettes including encoding of antimicrobial resistance genes (ARGs). The functionality of integrons is attributed to synergistic action of site-specific recombinase gene (intI), a primary recombination site (attI), and a promoter (Pc) which catalyzes the integration and expression of novel resistance factors, facilitating the transfer of ARGs from environmental reservoirs into clinically relevant pathogenic strains. Integrons are broadly categorized based on the sequence of their intI gene and their overall location, leading to a division between environmental (Super-Integrons) and clinically relevant classes. (RI-I, II, III). They use precise, site-specific recombination mechanism mediated by the IntI enzyme for the distribution and transmission of drug resistance genes. Thus, the present chapter briefs about integrons, their types, mechanism and function to have better understanding of integron in driving antimicrobial resistance and to develop strategies to mitigate integrons mediated AMR transmission.

Genomic Insights and One-Health Approaches for Controlling Zoonotic Tuberculosis in India

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Animal tuberculosis (TB), caused by members of the *Mycobacterium tuberculosis* complex (MTBC), continues to present a significant challenge to both animal and human health. Traditionally, *M. bovis* has been regarded as the primary causative agent of bovine tuberculosis (bTB). However, advances in molecular epidemiology have revealed a far more intricate landscape, with *M. orygis*, *M. caprae* and even *M. tuberculosis* increasingly identified in livestock infections across South Asia. These emerging genomic findings are reshaping our understanding of the disease's ecology and transmission dynamics.

Once considered a rare or geographically confined MTBC lineage, *Mycobacterium orygis* has now been reported in 14 countries spanning five continents, infecting a wide array of mammalian hosts. Evidence from South Asia indicates that *M. orygis* may now rival, or even surpass, *M. bovis* in contributing to zoonotic and reverse zoonotic infections. The detection of *M. orygis* associated tuberculosis in India's wildlife populations further suggests the existence of hidden reservoirs that may facilitate interspecies transmission.

India, home to one of the world's largest cattle populations, harbours an estimated 21.8 million bTB infected cattle. This poses a serious threat to animal productivity, livelihood security and public health. Yet, the actual burden of animal tuberculosis remains grossly underestimated due to limited surveillance, diagnostic bottlenecks and insufficient genomic profiling of circulating strains. The recent identification of *M. orygis* in human extrapulmonary TB cases marks a pivotal moment, implying potential host adaptation and the emergence of new ecological niches.

Whole-genome sequencing (WGS), phylogenomic analyses, and spatial-epidemiological modeling have begun to unravel critical insights into the evolving epidemiology of animal TB in India. Genomic studies of *M. orygis* isolates from livestock have revealed distinct phylogenetic clades closely related to strains circulating in neighbouring countries such as Nepal and Bangladesh, highlighting patterns of transboundary transmission and regional adaptation. Comparative genomic clustering further suggests that dairy buffaloes and certain wildlife species may serve as maintenance reservoirs, sustaining undetected transmission cycles within mixed farming and wildlife interface ecosystems.

Achieving India's target of eliminating TB will be unattainable without addressing the animal and wildlife components of disease transmission. Bridging these critical gaps requires integrated surveillance, enhanced genomic capacity and robust cross-sectoral collaboration under the One Health framework, uniting veterinary, medical and environmental disciplines toward a shared goal of tuberculosis control.

Oral Presentations

Detection of multidrug resistance, methicillin resistance, biofilm production, and virulence determinants in mastitis-associated *Staphylococcus aureus* by phenotypic and genotypic methods

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Staphylococcus aureus, a common cause of bovine mastitis, relies on several virulence factors, with biofilm formation being a key contributor to its pathogenicity. The present study investigated occurrence of *S. aureus* in bovine mastitis with a focus to detect virulence factors and resistance status. One hundred twenty (n=120) mastitic milk samples were collected from Uttar Pradesh and West Bengal state and subjected to isolation of *S. aureus.* Isolates were confirmed by conventional biochemical tests and PCR. *S. aureus* isolates were characterized for presence of various virulence factors, antibiotic resistance pattern, biofilm formation ability and efflux pumps by phenotypic and genotypic methods. Of 120 milk samples, 36 *S. aureus* strains were confirmed (30%). Phenotypic analysis revealed hemolysin (55.55%) and coagulase production (36.11%) while presence of leukotoxin (19.44%), hemolysin (58.33%), coagulase (63.88%), and toxic shock syndrome toxin (30.55 %) gene was detected by PCR. Biofilm production was detected in 97.22% (crystal violet assay) and 86.11% (Congo red assay) of S. aureus with biofilm-associated icaA (80.55%), icaB (75%), icaC (69.44%), icaD (86.11%) and MSCRAMM genes clfA (58.33%), clfB (75%), fnbA (75%), fnbB (55.55%), bap (38.88%), bbp (83.33%), ebps (69.44%), eno (66.66%), fib (41.66%) and cna (8.33%). Antibiotic resistance was observed in 88.88% isolates, with 72.22% exhibiting MDR. Among the isolates, 83.33% were MRSA and mecA, femA, and femB were present in 76.66% of the isolates. Efflux pump genes viz. norA, norB, norC, mdeA, mepA, and sepA were also detected. 61.53% of MDR-MRSA isolates were harboring all the six efflux pump genes. The study concludes that mastitogenic S. aureus harbors various virulence, antibiotic resistance, biofilm-forming forming and efflux pump genes and poses a significant public health risk. Multidrug-resistant MRSA, carrying various virulence factors, requires critical attention to formulate strategies for its control and prevention of transfer to the human food chain.

Chemosensory proteins as mediators of insecticide binding and resistance in *Anopheles culicifacies* and *Anopheles stephensi*

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Olfaction regulates key mosquito behaviors such as host-seeking, mating, and oviposition, mediated by chemosensory proteins (CSPs) and associated receptors. Emerging evidence also suggests a non-canonical role of CSPs in insecticide resistance, which poses a significant challenge for malaria vector control. This study aims to investigate the involvement of CSPs in insecticide binding and resistance mechanisms in *Anopheles culicifacies* and *Anopheles stephensi*, two major malaria vectors in India. Seven CSPs from *An. culicifacies* and *An. Stephensi* were heterologously expressed and purified. Their binding interactions with insecticides (pyrethroids, carbamates, organophosphates) were measured by microscale thermophoresis (MST). Two conserved CSPs- Ac_CSP1 and Ac_SAP2- were further analyzed for differential expression between deltamethrin-resistant and susceptible *An. Stephensi* strains at larval and adult stages using transcript profiling and whole-mount

immunofluorescence imaging. MST revealed moderate affinities (Kd = 0.7-3.3 μM), demonstrating specific interactions between CSPs and insecticides. Ac_CSP1 and Ac_SAP2 share over 95% sequence identity with their orthologs in *An. Stephensi* which exhibits higher expression in the third instar larvae and adult females of the resistant strain compared to the susceptible one. Immunofluorescence imaging confirmed elevated protein localization in key olfactory tissues of resistant mosquitoes. CSPs contribute to insecticide resistance in *Anopheles* mosquitoes by binding insecticides in addition to their role in chemosensory function. These findings highlight CSPs as potential molecular targets for novel vector control strategies aimed at mitigating insecticide resistance and enhancing malaria control in endemic regions.

Mastitis in dairy cattle of an organized farm due to co-infection of pathogenic *Staphylococcus aureus* and *Escherichia coli* in Meghalaya

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Mastitis caused by co-infection of pathogenic *Staphylococcus aureus* and *Escherichia coli* leads to significant economic losses for dairy farmers and represents a source of threat to raw milk quality and dairy food-chain safety. In this case study, we report an outbreak of mastitis in an organized farm in Umsning, Ri-Bhoi District of Meghalaya, which involves *Staphylococcus aureus* and *Escherichia coli* possessing pathogenic genes from 8 individual milk samples of lactating cattle The milk samples were first enriched in Brain Heart Infusion broth and, after 24 hours, were grown on selective media of Baird-Parker agar for *S. aureus*; McConkey and Eosin-methylene blue (EMB) agar for *E. coli*, respectively. Pure cultures of isolated and biochemically confirmed *S. aureus* and *E. coli* were molecularly characterized by Polymerase Chain Reaction (PCR) to demonstrate the presence of selected pathogenic genes. The positive isolates having pathogenic genes were subjected to an antibiotic sensitivity test using Mueller Hinton agar. Molecular detection by PCR revealed the amplicons representing 16s rDNA (228bp), nuc gene (280bp) & eap gene (230bp) for 5 isolates of *S. aureus* and in the case of *E. coli*, eae gene (831bp) belonging to EPEC could be confirmed in 3 isolates. Antibiotic sensitivity of *S. aureus* isolates revealed high sensitivity to gentamicin and cefoxitin; and high resistance to penicillin and tetracycline; whereas the 3 EPEC isolates showed high sensitivity to azithromycin, colistin, meropenem and high resistance to ampicillin, ciprofloxacin, cefotaxine and tetracycline. Recovery of pathogenic *S. aureus* and *E. coli* is a serious issue, particularly to milk quality and a public health concern encountered in the reckless dairy food chain. Control measures, such as adopting biosecurity steps and on-farm practices, such as antibiotic treatment, will result in the recovery of the affected herd and elimination of the pathogens in the farm environment.

Artificial designing of a novel antimicrobial peptide

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Artificial short antimicrobial peptide (sAMP) has emerged as a new class of AMP to overcome the shortcomings of the natural AMPs. In this study, we have developed a short, compositionally simple antimicrobial peptide utilizing three distinct amino acids, which we characterized using various in-silico methodologies. The peptide showed promising antimicrobial efficacy against a range of bacterial pathogens, including antibiotic-resistant strain. To design and characterize a short compositionally simple antimicrobial peptide using artificial designing approach An artificial 12-mer AMP was constructed using a knowledge-driven approach, considering the critical physicochemical properties of AMPs like net positive charge, helicity, hydrophobicity, and amphipathicity. The peptide was synthesized on rink amide MBHA resin using Fmoc-chemistry and was purified via RP-HPLC.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the synthesized peptide were assessed according to CLSI guidelines. The molecular docking study was conducted utilizing the online docking server MDockPeP. The stability of the peptide was tested under different environmental conditions. The cytotoxicity of the designed AMP was measured spectrophotometrically by quantifying haemoglobin release from the red blood cells (RBCs). The designed peptide exhibits an amphipathic nature with a net charge of +7. The synthesis of the peptide was accomplished through Fmoc-chemistry via Solid Phase Peptide Synthesis (SPPS), followed by characterization using RP-HPLC and mass spectrometry. The peptide showed promising antimicrobial efficacy against a range of bacterial pathogens, including antibiotic-resistant strain. Molecular docking analyses indicated that the peptide exhibits strong interactions with aerolysin, a key virulence factor of Aeromonas spp. The antimicrobial activity of the peptide was evaluated under various environmental conditions, including elevated temperatures, the presence of serum, and physiological salts. Notably, the peptide displayed low hemolytic activity even at higher concentrations. This study presents a novel artificially designed short and compositionally simple AMP. The designed AMP demonstrated promising antimicrobial activity. The results suggest that this design strategy could be beneficial for development of new peptide-based antimicrobial agents.

Isolation, identification and antimicrobial resistance profiling of bacterial isolates from pharmaceutical wastewater and surrounding environmental samples in south India

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Antimicrobial resistance is a leading one-health issue among the community. The reasons for antibiotic resistance development are numerous, and which are essential to investigate them and to find suitable remedial or control measures to contain the AMR problems in society. The study was undertaken to isolate and characterize aerobic and anaerobic bacteria from pharmaceutical industry wastewater, surface water, and groundwater collected from areas surrounding pharmaceutical industries in South India. A total of 55 samples were collected from Chennai, Nellore, Hyderabad, Kerala, and Bangalore. Bacteria were isolated under aerobic and anaerobic conditions and identified using biochemical characterization, VITEK 2 Compact, and VITEK MS systems. Antibiotic susceptibility testing was conducted against 16 antibiotics to determine phenotypic resistance. From the 55 samples, 48 isolates representing ten bacterial species were identified: *Klebsiella pneumoniae* (19%), *Pseudomonas aeruginosa* (22%), *Enterobacter aerogenes* (12%), *Corynebacterium* sp. (2%), *Acinetobacter* sp. (5%), *Aeromonas punctata* (3%), Ralstonia picketti (2%), *Staphylococcus aureus* (28%), *Stenotrophomonas maltophilia* (3%), and *Citrobacter freundii*. Phenotypic resistance was observed to up to 14 of 16 antibiotics. The study highlights the prevalence of multidrug-resistant bacteria in water sources impacted by pharmaceutical effluents. Effective effluent treatment and antibiotic stewardship are crucial to curb the spread of antimicrobial resistance from industrial discharges.

Qualitative and quantitative phytochemical analysis of different extracts from medicinal plants of Himalayan region of Uttarakhand

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The Himalayan region of Uttarakhand is a biodiversity hotspot, rich in medicinal plants with significant therapeutic potential due to their diverse phytochemicals. These bioactive compounds, known for antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, are the basis for their use in traditional and modern medicine. This study was aimed to conduct a qualitative and quantitative phytochemical analysis of selected medicinal plants from this region. Qualitative screening identified bioactive compounds like alkaloids, flavonoids, and phenols using standard chemical tests (Mayer's, Wagner's, Lead acetate, Ferric chloride). Quantitative analysis determined the concentration of key phytochemicals, including total phenolic content (TPC) and total flavonoid content (TFC), alongside assessments of antioxidant potential via Ferric Reducing Antioxidant Power (FRAP) and Ascorbate-iron (III) catalyzed phospholipid peroxidation (AICPP) assays. The analysis of aqueous and ethanolic extracts revealed pronounced bioactivity. Qualitative tests showed highly positive alkaloids in *Cannabis sativa* (aqueous) and *Zanthoxylum armatum* (ethanolic) leaf extracts. Quantitatively, the FRAP assay indicated superior antioxidant activity in ethanolic extracts of *Pinus roxburghii* bark (434.15 mmol Fe^{2}□/g) and *Rhododendron arboreum* leaves (471.1 mmol Fe^{2}□/g). The AICPP assay showed the highest hydroxyl radical scavenging activity in *Rhododendron arboreum* leaves (78.98% inhibition) and *Berberis aristata* roots (77.19%). Furthermore, *Pinus roxburghii* bark extracts exhibited the highest TFC (71.79 mg CE/g), while its aqueous extract recorded the highest TPC (7823.4 mg GAE/g). T he results validate the ethnobotanical uses of these plants and highlight their potential as rich sources of antioxidants and bioactive compounds for pharmacological applications.The varied phytochemicals found in these plants likely explain their effectiveness in traditional medicine. This highlights the need for more research to fully understand their therapeutic benefits and develop them into modern herbal medicines.

One health microbiome surveillance of pathogenic and commensal microorganisms in *Hyalomma* ticks from Haryana state

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Ticks host a diverse microbiome comprising bacteria, fungi, viruses, and protozoa, which influence their physiology, vector competence, and the transmission risk of diseases to livestock and humans. As key vectors of livestock diseases, understanding the tick microbiome is crucial for predicting zoonotic threats and developing effective control strategies. To comprehensively characterize the bacterial and non-bacterial microbial communities of Hyalomma ticks and to identify potential pathogens and symbionts relevant to animal and public health. Different life cycle stages of Hyalomma ticks were collected from Haryana state and identified morphologically. Genomic DNA was extracted, and bacterial communities profiled by high-throughput sequencing of the 16S rRNA gene using Illumina platform. Sequence data were quality-controlled and taxonomically assigned to the species level. Microbial diversity indices were calculated, and relative abundances compared between tick species. Pathogenic taxa were identified and results statistically assessed using R. Unique and core bacterial taxa for each species were determined to elucidate potential vector-pathogen relationships. Distinct bacterial profiles were found between different stages of Hyalomma ticks. Hyalomma ticks exhibited higher microbial diversity, with both pathogenic and symbiotic bacteria detected. Notably, Burkholderia, Corynebacterium, Clostridium species, including Babesia, Rickettsia, etc, were identified. Adult ticks harbored varied taxa that were not found in partial fed and male ticks. Shared core microbiome constituents were also observed, suggesting ecological adaptation and sensitive detection of zoonotic threats. Comprehensive tick microbiome surveillance enables early detection of emerging pathogens and informs tick control strategies, improving animal and public health outcomes

Poster Presentations

Antimicrobial potential of selected essential oils and essential oils' active components against food spoilage bacteria and foodborne pathogens and anti-biofilm potential against multi-drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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The rising concern of multi-drug resistant (MDR) pathogens and unavailability of effective antibiotics has generated the need of alternative approaches for their control. Essential oils (EOs) and essential oil active components (EoCs) have been reported for their antimicrobial effect since time immemorial. In this study we examined eleven EOs and four EoCs for their antimicrobial and antibiofilm activity against multi-drug resistant Staphylococcus aureus and Pseudomonas aeruginosa. Antibacterial effect was determined by disc diffusion method while anti-biofilm activity was determined by crystal violet assay. Among all the tested EOs, oregano, cinnamon, and cassia and among EoCs, carvacrol, cinnamaldehyde and thymol were found to carry broad spectrum antibacterial activity against the Gram positive and Gram-negative pathogens. Only cinnamon and cassia EOs were found to be effective against P. aeruginosa. Anti-biofilm potential of EO and EoCs was analyzed against P. aeruginosa and S. aureus, which was found at concentration 0.031% for cinnamon EO and cinnamaldehyde and 0.015%-0.007% for carvacrol. The study highlights that EOs and EoCs can be used as alternative to antimicrobials against drug resistant bacterial pathogens.

Comprehensive analysis of the occurrence, virulence genes, and antibiotic resistance of thermophilic *Campylobacter* in Uttarakhand, India, spanning fourteen years

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Campylobacter is a common Gram-negative bacterium and a leading cause of bacterial gastroenteritis worldwide, primarily linked to consuming contaminated poultry. Infection can sometimes lead to serious complications like Guillain-Barré syndrome. The study aimed to investigate the occurrence, antimicrobial resistance, and molecular characteristics of *Campylobacter* spp. isolated from various sources, including poultry, livestock, wildlife, and humans, in northern India. The study employed a comprehensive approach, screening a total of 19,469 samples collected from diverse sources, including poultry, livestock, wildlife, and humans, in Northern India. The investigation spanned fourteen years, from 2009 to 2022. The primary methodological focus was to estimate the prevalence (proportion positive) of *Campylobacter* spp. among collected samples, characterize their AMR profiles, and perform molecular characterization. This included genetic analysis to identify the presence of specific virulence and antibiotic resistance genes, providing critical data on the public health significance of the isolated strains. Analysis of the 19,469 samples confirmed a significant presence of Campylobacter. The study successfully identified confirmed positive cases across all sampled

populations. The predominant *Campylobacter* species identified were *C. jejuni* and *C. coli*. Furthermore, the isolates exhibited high antimicrobial resistance against several key antibiotics, specifically Nalidixic acid, Erythromycin, Tetracycline, Ciprofloxacin, and Ampicillin. Crucially, the genetic analysis revealed the presence of both virulence and antibiotic resistance genes, which highlights the significant public health implications of *Campylobacter* contamination in the region. Campylobacter, predominantly *C. jejuni* and *C. coli*, poses a significant public health risk due to its high prevalence, notable resistance to key antibiotics, and the presence of virulence genes. Continuous surveillance and improved antimicrobial stewardship are urgently needed, and molecular investigations can lead to a reduction in *Campylobacter* incidence, ultimately helping in achieving the goals of one health initiative.

Isolation and characterization of lytic bacteriophages targeting MDR *Escherichia coli* associated with bovine endometritis

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The rise of multidrug-resistant *Escherichia coli*, a key cause of bovine endometritis, threatens dairy herd health and productivity, necessitating alternative therapies such as lytic bacteriophages. The study was done to isolate and characterize lytic bacteriophages from environmental sources and evaluate their efficacy against clinical MDR *E. coli* isolates from bovine uterine infections. Phages were isolated from diverse environmental sources including animal waste (n=1), TVCC effluents (n=2), city sewage (n=8), and the Yamuna river (n=1). Enrichment was performed using standard ATCC *E. coli* strains. Lytic activity was initially assessed using spot assays and confirmed with double agar overlay (DAL) assays, where clear, well-defined plaques indicated successful isolation. Phages were purified through three successive passages and stored at 4°C. Host range studies were conducted against pathogenic bacterial isolates, including *E. coli*, Salmonella Typhimurium, Proteus mirabilis, Klebsiella pneumoniae, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Efficacy was further evaluated against ten clinical *E. coli* isolates from bovine uterine infections, calculating the multiplicity of infection (MoI). A total of 12 distinct lytic phages were successfully purified from environmental sources. Host range analysis revealed strong, specific lytic activity exclusively against E. coli, with no activity against other tested pathogens, indicating narrow-spectrum specificity. MoI values for the purified phages ranged from 1×10^5-4.3×10^5, with a mean MoI of 2.6×10^5, demonstrating efficient bacterial lysis. Phages produced clear, reproducible plaques in DAL assays, confirming their bacteriolytic potential. These findings highlight the value of environmental reservoirs for novel phages and demonstrate their targeted efficacy against MDR *E. coli* isolates implicated in bovine endometritis. Lytic bacteriophages offer a promising, targeted, and sustainable alternative to antibiotics against MDR *E. coli* in bovine endometritis, supporting phage therapy as a One Health approach to antimicrobial resistance.

Isolation and characterization of lytic anti-pseudomonas phages from animal waste: For public and animal health interventions

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The rapid rise of multidrug-resistant *Pseudomonas aeruginosa* poses serious threats to human and animal health, making lytic bacteriophages a promising alternative for targeted and sustainable infection control. The present study was undertaken to isolate and characterize lytic anti-Pseudomonas phages from animal waste and evaluate their potential as eco-friendly interventions to mitigate antimicrobial resistance and safeguard public and animal health. Animal waste samples were collected and screened for the presence of anti-Pseudomonas bacteriophages using standard enrichment techniques. Two representative samples yielded distinct lytic phages, which were purified and propagated for characterization. The lytic activity of the isolated phages was initially assessed via spot assay, observing plaque formation, and further validated using the double agar overlay (DAL) method. Clear, well-defined plaques confirmed strong bacteriolytic activity. Morphological features, plaque size, and propagation efficiency were recorded. The study emphasized assessing phage specificity, stability, and consistency of lytic activity. These investigations aimed to explore animal waste as a reservoir for novel phages and to establish their potential for one health interventions in antimicrobial resistance, food safety, and biosecurity. Two distinct lytic phages were successfully isolated from animal waste and demonstrated potent activity against *Pseudomonas aeruginosa*. Spot assay and DAL method confirmed consistent plaque formation, indicating effective bacteriolytic potential. The phages displayed specificity towards *Pseudomonas aeruginosa*, with no observable activity against other bacterial species, suggesting minimal disruption to beneficial microbiota as a spectrum phage type. Clear and reproducible plaques indicated stable propagation and strong lytic efficacy. These findings advocate the value of animal waste as a reservoir of novel bacteriophages and highlight the applicability of lytic anti-Pseudomonas phages for reducing bacterial loads in clinical, veterinary, and environmental contexts. The results align with one health objectives for antimicrobial resistance mitigation and biosecurity. Lytic anti-Pseudomonas phages from animal waste offer targeted, sustainable interventions for controlling infections, supporting One Health strategies in antimicrobial resistance, food safety, microbiome management, and biosecurity.

Assessment of ESBL-producing *E. Coli* in faecal samples of cattle from western Uttar Pradesh, India

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The worldwide dissemination of extended-spectrum-lactamase (ESBL)-producing *Escherichia coli* has emerged as a serious global public health concern. These strains also act as potential reservoirs for the horizontal transfer of antimicrobial resistance determinants. ESBL-producing *E. coli* infections are associated with high mortality and mobility rate in developing countries due to less susceptibility to antibiotics. The present study aimed to elucidate the occurrence and genetic characteristics of ESBL producing *E. coli* in the cattle of Western Uttar Pradesh, India. A total of 100 faecal samples of cattle were collected from three districts viz. Meerut, Amroha, and Bijnor. ABST (antibacterial sensitivity test) was performed on 36 isolates which were previously confirmed as *E. coli* by PCR using *uidA* specific primer specific to *E. coli*. Phenotypic test was conducted by using ESBL specific antibiotics.

Further, PCR was performed to know the presence of ESBL-producing antimicrobial resistance genes *bla*TEM, *bla*SHV, *bla*CTXM-1and *bla*OXA-48. Among the isolates, 20 % isolates showed resistance to Aztreonam, 25 % to Cefoperazone, 25% to Cefotaxime, 16% to Cefixime, and 8.3 % to Meropenem. Further, phenotypic test using ESBL-specific antibiotics revealed 55.5 % isolates resistant to antibiotics. PCR analysis confirmed that 11.1% of isolates carried one or more ESBL genes. Among these, *blaTEM* was the most prevalent (88.8%), followed by *bla CTX-M-1* (44.4%), *blaSHV* (11.1%), and *blaOXA-48* (11.1%). Overall, a high occurrence of ESBL genes was found in cattle probably due to the high usage of antimicrobials. This study revealed the daunting state of occurrence of ESBL producing *E. coli* and its infection dynamics in Western Uttar Pradesh and underscores the need for prudent antimicrobial stewardship in livestock production systems in western Uttar Pradesh

Evaluation of *Pongamia pinnata* leaf extract as an antiviral candidate against Classical swine fever virus

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Antiviral therapeutics are urgently needed since antibiotics are ineffective against viruses. Plant extracts may slow viral replication, reduce disease severity and support host immune responses. The study was done to evaluate the cytotoxicity and antiviral efficacy of *Pongamia pinnata* leaf extracts prepared at different temperatures against GFP tagged classical swine fever Virus (CSFV) in PK 15 cells. Aqueous extracts of *Pongamia pinnata* leaves were prepared at 37 °C, 60 °C, and 90 °C. Cytotoxicity assays identified safe concentrations for antiviral testing. A GFP tagged CSFV stock (10^5.64 FFU/mL) was used to infect PK 15 cells. Viral spread was monitored by GFP fluorescence for nine days, followed by qPCR quantification of viral RNA and luciferase assay validation. The 90 °C extract showed the lowest cytotoxicity (<62.5 µg/mL). Treated and untreated cultures were compared for delay in cytopathic effect and viral propagation. The 90 °C extract delayed complete CSFV spread until day 9, compared to day 7 in untreated controls, indicating a two day inhibition of viral progression. Cytotoxicity profiling confirmed safety at working concentrations. qPCR did not show significant RNA reduction, but fluorescence tracking revealed clear suppression of viral spread. Luciferase assays corroborated inhibitory activity. Observing such effects is notable, as most crude plant extracts poorly penetrate cells where viral replication occurs. Aqueous *Pongamia pinnata* extract delayed CSFV replication in vitro, suggesting potential to mitigate disease severity. Purification of active compounds may enhance antiviral efficacy and translational value

Assessment of antimicrobial resistance pattern of bacterial pathogens isolated from canine urine samples

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Urinary tract infections (UTIs) are among the most common bacterial infections affecting dogs. Early diagnosis and identification of the causative agent are essential for initiating effective treatment and preventing recurrence. The present study was designed to isolate and identify bacterial pathogens from urine samples collected from dogs exhibiting clinical signs suggestive of urinary tract infections. A total of 10 urine samples were collected aseptically from dogs presented to VCC, College of Veterinary Science, and A.H., NDVSU, Jabalpur (MP). The collected samples were subjected to

standard bacteriological culture techniques using selective and differential media, followed by characterization for bacterial identification. The antibiotic susceptibility pattern of each isolate was assessed by the Kirby–Bauer disc diffusion method using a panel of commonly employed antibiotics. The bacteriological analysis revealed the presence of both Gram-negative and Gram-positive organisms. Among the isolates, *Escherichia coli* emerged as the predominant uropathogen, followed by *Staphylococcus* spp. For *Escherichia coli*, the highest resistance was observed against amikacin (100%), ampicillin (100%) and cefotaxime (100%), and *Staphylococcus* spp., isolates were showing resistance to ampicillin (100%), whereas all the isolates comparatively showed higher sensitivity against tetracycline (50%) and ciprofloxacin (50%). The study emphasizes the importance of routine bacteriological examination of urine samples in suspected canine UTI cases, as it provides crucial insights into the distribution of pathogens. Antibiotic sensitivity will be helpful for accurate treatment and reducing the development of antimicrobial resistance

Prevalence of *Pseudomonas spp*. isolated from bovine mastitis milk samples and their antibiotic resistance pattern

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Mastitis is one of the most prevalent diseases in the dairy industry, causing huge economic loss to livestock owners. There are many pathogens primarily responsible for causing bovine mastitis, but nowadays, *Pseudomonas* spp. significantly gaining importance. In the present study, the prevalence of mastitis caused by Pseudomonas spp. was studied using conventional methods, and the antibiogram profile was evaluated. A total of 1278 milk samples (492 from cows and 786 from buffaloes) were analyzed in the Department of Veterinary Microbiology, Kamdhenu University, Junagadh, Gujarat, received from different parts of the Saurashtra region, Gujarat, over a period of 6 months. In order to facilitate the growth of bacteria for investigation, the samples were cultured on Brain Heart Infusion agar and Pseudomonas agar base. The isolates were identified based on their morphological characteristics and various biochemical tests. The overall prevalence of *Pseudomonas* spp. was 5.5%. Out of 492 milk samples collected from cows, 24 (4.8%) were positive for *Pseudomonas* spp., while 47 (5.9%) of the 786 milk samples collected from buffaloes were found to be positive... The antibiogram profiles of the isolates were determined using the disc diffusion method on Mueller–Hinton agar against eight different classes of antibiotics. The results revealed a 100% multidrug-resistant (MDR) pattern among the isolates. Among the tested antibiotics, gentamicin (76.5%) and levofloxacin (87.5%) were found to be the most effective against *Pseudomonas* spp. isolated from buffaloes and cows, respectively. The least effective antibiotics were ceftizoxime and ampicillin/sulbactam (2.1% each) for buffalo isolates, and chloramphenicol and oxytetracycline (4.1% each) for cow isolates. The findings highlight the alarming prevalence of MDR *Pseudomonas* spp., posing a serious threat to therapeutic management of bovine mastitis, and underscore the urgent need for prudent antibiotic use and improved control strategies

Isolation and identification of antibiotic resistance bacteria from nasal swab of small ruminants in Gujarat, India

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This study focuses on isolating and identifying antibiotic-resistant bacteria from nasal swabs of small ruminants in Gujarat, India, to assess prevalence and potential public-health risks. A total of 50 nasal swab samples were collected aseptically from small ruminants presented to the veterinary clinical

complex (VCC), Junagadh, Gujarat. The swabs were inoculated into modified brain heart infusion (BHI) broth supplemented with penicillin, streptomycin, and fetal calf serum (FCS), followed by sub-culturing on similarly modified BHI agar plates. These supplements were intended to suppress contaminant bacteria and promote mycoplasma growth. Unexpected bacterial colony growth was noted, indicating the presence of organisms resistant to both penicillin and streptomycin. The isolates were subjected to gram staining, antibiogram profile, and biochemical tests for identification and characterization of bacteria. Gram staining of the samples revealed the presence of gram-positive bacilli, gram-negative bacilli, *Staphylococcus* spp., *Corynebacterium* spp., and *Streptococcus* spp. The ability of these bacteria to proliferate in media containing widely used antibiotics suggests the presence of strong resistance mechanisms. The study revealed a high prevalence of resistance to chlorpromazine (60%), levofloxacin (50%), penicillin (100%), and streptomycin (100%). The study revealed high prevalence of antibiotic-resistant bacteria in small ruminants' nasal swabs in Gujarat, emphasizing the need for prudent antimicrobial use and continuous resistance monitoring.

Exploring the potential of *Lawsonia inermis* green nanoparticles to mitigate quorum sensing in ESBL-producing *E.coli*

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The green synthesis of nanoparticles (NPs) using biological systems, particularly plant extracts, offers an ecofrindly and cost-effective alternative to conventional chemical and physical method. In this study, zinc oxide nanoparticles (ZnO-NPs) were synthesized using *Lawsonia inermis* (Henna) leaf extracts prepared in aqueous, methanolic, and ethanolic solvents, with zinc nitrate as the precursor and the potential of green nanoparticles to mitigate quorum sensing in MDR bacteria was investigated. The synthesized nanoparticles were characterized by Ultraviolet–Visible spectroscopy (UV–Vis), Fourier Transform Infrared Spectroscopy (FT-IR), and Transmission Electron Microscopy (TEM). A total of 42 clinical samples (pus, wound, and faeces) were collected from various clinics and hospitals of western Uttar Pradesh (U.P.) for the isolation and identification of MDR ESBL-producing *E. coli*. The antimicrobial potential of nanoparticles was evaluated by determining the minimum inhibitory concentration (MIC) against multidrug-resistant *E. coli*. Aqueous Henna nanoparticles were further tested at the same concentration across different time intervals, and the real-time expression of quorum-sensing gene luxS, which regulates cell-to-cell communication in AMR bacteria. UV-Vis spectra showed typical absorption peaks at around 330 nm, 340 nm, and 360 nm of ethanolic, methanolic, and aqueous ZnO-NPs, respectively. Chemical bond formations of zinc oxide were confirmed by FT-IR analyses, and TEM showed that the NPs synthesized were 16nm in size and spherical in shape. The aqueous ZnO-NPs exhibited the lowest MIC (62.5 µg mL^{-1}) and caused significant downregulation of the quorum-sensing gene *luxS*, which encodes AI-2 synthase responsible for intercellular communication. These results suggest that Henna-mediated ZnO-NPs, especially those derived from aqueous extracts, hold potential for mitigating quorum sensing and antimicrobial resistance in pathogenic bacteria. Further studies with a larger set of MDR isolates are warranted to validate these findings.

Prevalence and Identification of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) from Bovine Mastitis Milk Samples

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Bovine mastitis is a major infectious disease of dairy animals, causing significant economic losses in the dairy sector due to reduced milk yield and quality. Among the etiological agents, *Staphylococcus aureus* is one of the most prevalent pathogens responsible for both subclinical and clinical mastitis. In recent years, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has raised serious concern because of its zoonotic potential and limited therapeutic options. MRSA not only complicates treatment in animals but also poses a risk to public health through the food chain. The study was done to investigate the prevalence of *Staphylococcus aureus* in bovine mastitis milk samples and to identify methicillin-resistant *S. aureus* (MRSA) isolates using phenotypic and genotypic methods. A total of 50 milk samples were aseptically collected from bovines suspected of mastitis. Samples were cultured, and presumptive Staphylococcus isolates were identified based on colony morphology, Gram's staining (Gram-positive cocci in clusters), and biochemical tests (catalase positive, oxidase negative). Colonies producing golden yellow pigmentation on nutrient agar and fermenting mannitol on MSA were considered presumptive *S. aureus*. The coagulase test was further performed to confirm pathogenic *S. aureus*. DNA was extracted using the traditional heat method. Species confirmation was done by PCR targeting the 23S rRNA gene (894 bp amplicon). Methicillin resistance was determined by cefoxitin disk diffusion and PCR amplification of the *mecA* gene (162 bp fragment). From 50 milk samples, 22 isolates of *Staphylococcus* spp. were obtained. Among these, 10 isolates were identified as *S. aureus* (golden yellow colonies, mannitol fermentation). 7 isolates were coagulase positive, while 3 isolates were coagulase negative. PCR confirmed all 10 isolates as *S. aureus* by 23S rRNA gene amplification (894 bp). Cefoxitin disk diffusion detected 3 isolates as methicillin-resistant. PCR detection of the *mecA* gene revealed 5 isolates harboring methicillin resistance (162 bp amplicon). The study highlights the occurrence of *Staphylococcus aureus* in bovine mastitis milk samples, with a considerable proportion exhibiting methicillin resistance. The detection of MRSA (5 out of 10 *S. aureus* isolates) emphasizes the potential risk of antimicrobial resistance in dairy herds, which may threaten both veterinary and public health. The combined use of phenotypic and molecular methods provides a reliable approach for accurate detection of MRSA.

Assessment of Comparative Efficacy of *Convolvulus Pluricaulis* Nanoparticles and Commonly used Antibiotics Against Clinical MDR Bacterial Isolate

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The rapid emergence of antimicrobial resistance (AMR) in pathogenic bacteria poses a potential threat to global public and veterinary health, necessitating the development of novel therapeutic strategies. This study reports the eco-friendly and cost-effective green synthesis of zinc oxide (ZnO) nanoparticles utilizing the aqueous extract of *Convolvulus pluricaulis* (Shankhpushpi) as a natural reducing and stabilizing agent and explore the antibacterial potential of Shankhpushpi-mediated ZnO nanoparticles. The plant extract of Shankhpushpi, rich in phytochemicals, was used to facilitate the controlled reduction of zinc ions and to provide a protective capping layer to prevent agglomeration to obtain Zn nanoparticles. Successful synthesis was confirmed by UV–visible spectroscopy and Fourier-

transform infrared (FTIR) spectroscopy. Clinical isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from human and veterinary sources and confirmed through cultural, biochemical, and molecular methods, antibiotic susceptibility testing (Kirby–Bauer method). The plant extract facilitated the controlled reduction of zinc ions and provided a protective capping layer to prevent agglomeration. UV–visible spectroscopy showed a characteristic absorption peak at 373–386 nm, and Fourier-transform infrared (FTIR) spectroscopy indicated functional groups responsible for capping and stabilization. Clinical isolates of *E. coli* and *S. aureus* obtained from human and veterinary sources revealed that, over 70% of isolates exhibited resistance to commonly prescribed β-lactams and fluoroquinolones. The antimicrobial activity of Shankhpushpi-mediated ZnO nanoparticles demonstrated a minimum inhibitory concentration (MIC) of 62.5 µg/mL against MDR *E. coli*. These findings underscore the superior antibacterial potential of Shankhpushpi-mediated ZnO nanoparticles. Shankhpushpi-derived ZnO nanoparticles demonstrate potent efficacy against MDR bacteria and represent a promising eco-friendly alternative or adjunct to conventional antibiotics in the fight against AMR.

Synergistic potential of heavy metals (lead and chromium) on thiamethoxam induced renal cell toxicity: *In-vitro* cytotoxicity model

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Lead (Pb) and Chromium (Cr) are recognized as nephrotoxicants, disrupting kidney functions in mammals on chronic exposure, even at low to moderate levels, resulting in chronic kidney disease (CKD). Thiamethoxam (TMX), a widely used 2nd generation neonicotinoid insecticide, causes dose-dependent nephrotoxicity in mammals. The present study aimed to determine the extent of renal cytotoxicity induced by either toxicants (Pb, Cr, TMX) or in combination using the NRK-52E (Normal Rat Kidney cells) cell line as an in vitro cytotoxicity model. Kidney cell line of rat origin was procured from the National Centre for Cell Science (NCCS), Pune, Maharashtra, India. The cell line was cultured using DMEM media containing fetal bovine serum and penicillin–streptomycin solution at 37ºC with 5% CO2 in the carbon dioxide incubator. The MTT, Resazurin, Trypan blue assays, and Antioxidant biomarkers (TAS, TTH, GSH, MDA, and AOPP) were performed to assess cell viability and cytotoxicity. Individual exposure of Pb (10-160 µM), Cr (10-60µM), and TMX (0.012-0.188mM) produced a dose-dependent cytotoxicity in the NRK-52E cell line. These observations further corroborated with a dose-dependent reduction in TAS, GSH, and TTH levels and increased levels of MDA and AOPP in cell homogenate. The median inhibitory concentration (IC50) values for Pb, Cr, and TMX were 95.25µM, 3.97µM, and 0.0372µM, respectively. Co-exposure of Pb and Cr potentiated renal cell damage induced by TMX as indicated by antioxidant biomarkers and cytotoxicity assays. Observations of cytotoxicity assays and cellular antioxidant status revealed that Pb potentiated Cr induced renal cell damage. Simultaneous exposure of Pb and Cr in combination with TMX acts synergistically to damage the renal cell line, which may be due to altered cellular antioxidant status.

Clinical management of mastitis in a cow using herbal formulation as a supportive treatment

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The present era is witnessing the challenge of antimicrobial resistance (AMR) which has already started showing devastating effects. At present, various alternatives such as ayurvedic, homeopathic preparations *etc.* are being explored to combat AMR. Here, the ayurvedic treatment is often based on content and action of different herbs. It is either used directly for treatment or as supplement to standard therapy. Livestock sector is considered as one of the major backbones of rural economy. The present case study highlights a case of mastitis in a Holstein Friesian cattle brought at College of Veterinary Science & Animal Husbandry, Kamdhenu University, Rajpur (Nava), Himmatnagar which had not recovered despite multiple attempts of treatment using allopathic medicines at field level. The animal was subjected to diagnostic tests such as udder examination, california mastitis test (CMT), pH measurement, electric conductivity measures, catalase test, Bromothymol Blue (BTB), lactate dehydrogenase (LDH strip test), gram staining, cultural isolation and antibiotic sensitivity test (ABST). Considering the time spent after treatment, it was decided to combine ABST based antibiotic (*viz.,* Ceftriaxone+Tazobactam) for 03 days and oral supplementation of herbal formulation (Trieto Mustfree, Trieto Biotech, Gandhinagar) containing Aloevera, Satavari and Jivanti @ 250 ml b.i.d. for 15 days. No other supportive medicines (e.g., anti-inflammatory, intramammary antibiotics) were given. The cattle recovered after 03 days while improvement in milk quality and quantity was observed due to herbal formulation resulting in faster recovery. The present case study elaborates detailed approach.

Tackling a Global Threat: A One Health Analysis of Antimicrobial Resistance in Livestock Systems

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Antimicrobial resistance (AMR) presents a critical global health challenge, with the unregulated use of antibiotics in food-producing animals being a significant driver. This study examines the drivers of AMR in livestock systems and explores evidence-based mitigation strategies through a coordinated, multisectoral One Health lens. The study was undertaken to examine the drivers of antimicrobial resistance (AMR) in livestock systems and explore evidence-based mitigation strategies using a coordinated, multisectoral One Health approach. The research employed a comprehensive methodology involving a review of current literature, global surveillance data, and policy reports. The analysis placed particular emphasis on identifying key challenges in low- and middle-income countries (LMICs) and scrutinizing cross-sectoral gaps within the AMR surveillance and monitoring framework. The findings indicate that the use of antimicrobials in livestock-particularly for non-therapeutic purposes like growth promotion and metaphylaxis-remains high and is often poorly regulated in many regions. Surveillance systems were identified as fragmented, operating in silos that rarely integrate data from human, animal, and environmental sectors, thereby hindering coordinated response. In LMICs, these issues are exacerbated by a confluence of factors, including a scarcity of trained veterinarians, unregulated over-the-counter access to antibiotics, and limited awareness among farmers about AMR risks. Conversely, the study found that effective antimicrobial stewardship programs demonstrate significant promise. Their success is consistently linked to strong policy backing, farmer education, and robust intersectoral

collaboration between veterinary, medical, and environmental authorities. The study concludes that a unified One Health approach is not merely beneficial but essential to curb the rise of AMR. This requires integrated surveillance, stringent regulation of antibiotic use, and the promotion of sustainable alternatives such as improved biosecurity and animal husbandry practices. The implications are clear: protecting public health, ensuring animal welfare, and securing sustainable livestock production systems depend on our ability to foster synergistic action across all relevant sectors.

Reproductive Microbiome: The Next Revolution in Livestock Fertility

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The reproductive tract of livestock harbors complex microbial ecosystems in the vagina, uterus, and semen, that play a pivotal role in fertility, conception, embryo implantation and pregnancy outcomes. The present study aimed to investigate the emerging role of vaginal and uterine microbiomes in regulating fertility, implantation and pregnancy outcomes in livestock while developing and evaluating innovative biotechnological interventions to enhance reproductive efficiency. The study included the collection of vaginal and uterine discharges, DNA extraction and molecular identification, specific microbial diversity analysis, identification of probiotics, fertility trials and pregnancy diagnosis. It was observed that, dysbiosis in reproductive microbiomes is linked to endometritis, repeat breeding and reduced conception rates, while beneficial microbes enhance implantation, sperm survival and pregnancy maintenance. The reproductive microbiome represents an untapped frontier in veterinary biotechnology. Its targeted modulation through artificial microflora could revolutionize fertility management, complementing traditional, hormonal and genetic approaches.

Session 3:

Omics and Innovation in Vaccines, Diagnostics and Therapeutics

Food Systems as Drivers of Species Spillover of Animal Viruses

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With growing human population, global demand for food has been growing particularly in low and middle income countries. The rising of consumption of animal-sourced foods has driven agricultural intensification, land-use change, wildlife exploitation, and complex food systems. These changes reshape ecological interfaces among wildlife, livestock and humans, creating numerous scenarios for animal viruses to emerge and jump species barriers. This chapter describes pathways linking food and nutrition security demands and climate change to viral spillover. The recent examples of avian influenza, Nipah virus, corona virus spillover amply demonstrate the association with wet markets and wildlife trade and vector-borne pathogens influenced by livestock and agriculture expansion. We emphasize that food and nutritional security objectives must be aligned with biosafety, ecosystem management, and social protections to reduce emergent risk. Conserving critical habitats, integrating climate-sensitive surveillance, and investing in 'One Health' research is imperative. Coordinated global efforts are needed on equitable food systems that protect livelihoods while minimizing risky animal–human interfaces and prevent the next viral spillover.

A Futuristic Vision on Status of Trans Boundary Diseases of Livestock in India: Linking it with "Viksit Bharat" @ 2047

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The Government of India is working hard to control important transboundary animal diseases of livestock and poultry. At the same time efforts are also directed towards the elimination and eradication of some of most important livestock viral diseases. These include FMD of cattle and buffalo, PPR of sheep and goat, and classical swine fever of pigs. However, the disease control efforts are jeopardized due to the entry of new transboundary animal diseases of livestock in the geography, such as LSD of cattle and African Swine Fever of Pigs during recent years. The success of these efforts largely depends on how promptly we accept the presence of a disease and react to eliminate it at the source of origin. Delayed detection and acceptance of the presence of disease affects the disease control and subsequent virus elimination due to unique socio-economic and socio-cultural reasons to India. Due to socioeconomic barriers and regulations, we cannot always practice test and slaughter method/stamping out policy for disease affecting cattle and buffalo and even sheep and goat. Due to this fact, there is hardly any example of disease elimination immediately after its entry has been reported in India. Of the important livestock transboundary animal diseases like, FMD, LSD, PPR, CSF, ASF, goatpox, sheeppox & rabies, some of them are targeted for disease elimination and eradication during the coming years by the end of 2030 and beyond. These include FMD of cattle /Buffalo, sheep/goat and pigs for disease elimination and PPR of goat/sheep for disease eradication. Global geographical distribution of TADs shows that the countries which maintain freedom from important transboundary animal diseases (TADs) try to do so at continental/at least regional level and not only at national/country level. The efforts of Goverement of India are largely focused on national level, and therefore sustainability of freedom from TADs is a big question mark. For a country like India, the elimination of a disease like FMD will require a lot of coordinated efforts with neighboring countries, as these neighboring countries do not give equal emphasis to such efforts due to differences in priorities and poor socio-economic status. Further, the global distribution of FMD and LSD remains almost similar. This indicates that the presence of either of these in India will restrict the trade to FMD/LSD free countries. Therefore, a parallel effort to eliminate both these will be required along with the prevention of any of the exotic FMD serotypes to Indian Territory to boost the trade and export of livestock products. Similarly, the global distribution of PPR and goat/sheep pox is almost similar in nature and therefore export of small ruminant based products will require to tackle all these three important diseases of small ruminants together. Due to wider host range FMD is also present in South America and rabies is present in almost all the continents except Australia. However, dog-mediated rabies does not occur in major part of North America and Europe.

Elimination of several of these diseases with vaccination will be a strong indicator of Vikshit Bharat@ 2047. However, eradication from most of these from Indian Geography will be difficult preposition, as the neighboring countries like Nepal, Bangladesh, Bhutan,, Pakistan do not put equal effort to control such diseases. In order to demonstrate eradication for any of these, the Government of India needs to conduct a very strong advocacy and capacity-building program in the neighboring countries and support disease control efforts of neighboring countries with coordinated vaccination and disease prevention activities in the interest of India. All these efforts can directly be justified with the enhanced trade and export of livestock and livestock products. Detailed data will be presented during deliberations.

Unraveling Respiratory Pathogenesis in Buffalo: Cellular Perspectives for Livestock Health and Protection

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The water buffalo is the backbone of India's dairy industry and rural economy, contributing almost 50% of the nation's total milk production and supporting millions of smallholder farmers. Beyond milk, buffaloes provide draught power, meat, and manure, making them indispensable to India's livestock-based livelihoods. Despite being a hardy species, buffaloes are highly susceptible to acute respiratory inflammation caused by *Pasteurella multocida*, highlighting the need to understand their immune responses for improving herd health and productivity. Our research provided the first evidence of pulmonary intravascular macrophages (PIMs) in water buffalo. Interestingly, the CD68 antibody, which labels septal macrophages in cattle, did not react with buffalo macrophages. Instead, the MCA874G antibody identified septal macrophages, indicating distinct cellular diversity. Depletion of macrophages using gadolinium chloride (GC) significantly elevated mRNA expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-8), while GC pretreatment followed by *P. multocida* challenge reduced cytokine expression. Histological analysis revealed an abundance of monocytes/macrophages and relatively fewer neutrophils in alveolar septa during infection, suggesting a need to explore neutrophil dynamics further.

We also generated the first data on neutrophil viability and apoptosis in water buffalo. Freshly isolated neutrophils showed 98.4% purity and 98.44±0.35% viability, which declined significantly after 24–72 hours. Lipopolysaccharide (LPS) challenge decreased apoptotic neutrophils compared to controls, indicating prolonged activation. Notably, neutrophils from buffalo with mastitis or metritis exhibited delayed apoptosis, suggesting sustained inflammatory activation. We also report first data on the potential of buffalo neutrophils to form neutrophil extracellular traps (NETs) with or without co-culture with *Pasteurella multocida.* Freshly isolated buffalo neutrophils showed a significant ($p<0.05$) time dependent increase in apoptosis. There was a significant increase in apoptosis post 120 minutes incubation of neutrophils with *P.multocida* compared to the control group.

Together, these findings highlight the unique cellular landscape of the buffalo immune system and emphasize the importance of elucidating macrophage and neutrophil responses to design effective disease management strategies, thereby enhancing buffalo health, productivity, and rural livelihood sustainability.

Molecular Profile of Antimicrobial Resistance in Methicillin-resistant *Staphylococcus aureus* Isolates from Various Sources

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Staphylococcus aureus is part of the normal flora of the human body, commonly found in the nose, respiratory tract, and on the skin, and often causes infections in humans and animals. As the organism commonly develops resistance against β-lactam antibiotics by acquiring resistance genes, penicillinase-resistant penicillin (PRP), like methicillin, a semi-synthetic PRP, was used against *S. aureus* infections. However, methicillin-resistant strains have emerged quickly, and this has become a marker for evaluating multidrug resistance, known as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA also secretes various toxins encoded by multiple virulence genes. MRSA strains exhibit genotypic differences, and their characteristics differ across hosts and regions. Based on these facts, the present study focused on isolating MRSA from human and animal hosts, evaluating their antibiograms, profiling their virulence genes, identifying their resistance gene profiles, and genotyping the isolates using appropriate molecular typing methods. Samples were collected from the anterior nares and wound sites of patients admitted to a hospital ward for hospital-associated MRSA (HA-MRSA), from the anterior nares of apparently healthy individuals for community-associated MRSA (CA-MRSA), and from milk, skin infection sites, and wounds of livestock animals for livestock-associated MRSA (LA-MRSA) isolation. Molecular identification of MRSA was performed by detecting the *nuc*, *coa*, *aroA*, and *mecA* genes, and through *16s rRNA* gene sequencing. Antibiogram was evaluated using the Kirby-Bauer disc diffusion method against 28 antibiotics from 19 classes. Multiple antibiotic resistance (MAR) indexing was calculated. The Epsilometer test (E-Test) was conducted to determine the minimum inhibitory concentration (MIC) for 12 antibiotics. Virulence gene profiling was performed by polymerase chain reaction (PCR) for *tst-1*, *lukPV*, *hla*, *hlb*, *eta*, and *etb*. Resistance gene profiling was conducted by PCR for *blaZ1*, *blaZ2*, *smr*, *aacA-aphD*, *tetK*, *tetM*, *vatA*, *vatC*, *msrA*, *msrB*, *linA/linA'*, *ermA*, *ermC*, *vat*, *vatB*, *cfr*, *fexA*, *vga*, and *vgb*. Molecular typing of the MRSA isolates was achieved through SCCmec typing and MLST.

A total of 154 hospital-associated, 253 community-associated, and 204 livestock-associated samples were collected. From these, 20 (12.99%) were HA-MRSA, 32 (12.65%) were CA-MRSA, and 52 (25.49%) were LA-MRSA. HA-MRSA resistance was primarily observed against oxacillin (100%), methicillin (85%), cefoxitin (100%), fusidic acid (85%), cefaclor (70%), cefdinir (100%), cefotaxime (85%), cefepime (70%), ciprofloxacin (75%), kanamycin (55%), trimethoprim (70%), and ticarcillin+clavulanic acid (95%). CA-MRSA showed resistance mainly to oxacillin (87.5%), methicillin (50%), fusidic acid (65.6%), ciprofloxacin (93.8%), kanamycin (56.3%), erythromycin (53.1%), trimethoprim (71.9%), and ticarcillin+clavulanic acid (75%). LA-MRSA resistance was mostly observed against oxacillin (100%), methicillin (86.5%), cefoxitin (90.4%), fusidic acid (86.5%), cefdinir (69.2%), ciprofloxacin (80.3%), and ticarcillin+clavulanic acid (69.2%). A total of 20 isolates (100%) from HA-MRSA, 30 isolates (93.75%) from CA-MRSA, and 52 isolates (100%) from LA-MRSA were identified as multidrug resistant (MDR). Additionally, 4 isolates (20%) from HA-MRSA, 3 isolates (9.36%) from CA-MRSA, and 3 isolates (5.77%) from LA-MRSA were classified as extensively drug resistant (XDR).

Among 20 HA-MRSA isolates, 9 (45%) tested positive for *luk PV*. In CA-MRSA, 7 (21.88%), and in LA-MRSA, 18 (34.61%) isolates harboured the *luk PV* gene. In HA-MRSA, 5 (25%) of the 20 isolates carried the *tst-1* gene. In CA-MRSA, 2 (6.25%), and in LA-MRSA, 2 (3.84%) isolates carried it. In HA-MRSA, 9 (45%) isolates possessed the *hla* gene. In CA-MRSA and LA-MRSA, 22 (68.75%) and 26 (50%) isolates harboured the *hla* gene, respectively. In HA-MRSA, 9 (45%) isolates carried the *hlb* gene. In CA-MRSA and LA-MRSA, 21 (65.63%) and 31 (59.62%) isolates carried the *hlb* gene, respectively. One isolate from CA-MRSA (3.12%) and one from LA-MRSA (1.9%) contained the *eta* gene, while none of the isolates from HA-MRSA, CA-MRSA, or LA-MRSA carried the *etb* gene.

In HA-MRSA, a total of 4 (33.33%) isolates tested positive for the *tetM* gene, while none tested positive for the *tetK* gene. In CA-MRSA, 2 (8.69%) isolates were positive for the *tetM* gene, and 5 (21.73%) for the *tetK* gene. In LA-MRSA, 14 (41.17%) isolates harboured the *tetM* gene, and 6 (17.64%) harboured the *tetK* gene. In HA-MRSA, 10 (83.33%) isolates tested positive for *vatA*, 2 (16.66%) for *msrA*, and 2 (16.66%) for *msrB*. In CA-MRSA, 16 (69.56%) isolates were positive for *vatA*, 10 (43.47%) for *ermC*, 8 (34.78%) for *msrA*, and 8 (34.78%) for *msrB*. In LA-MRSA, 30 (88.23%) isolates tested positive for *vatA*, while 2 (5.88%) each were positive for *msrA* and *msrB*. All isolates carried the *blaZ1* and *blaZ2* genes across the hosts. None tested positive for *vatB*, *vatC*, *ermA*, *vat*, *cfr*, *fexA*, *vga*, or *vgb* genes across the hosts. Resistance gene profiling reveals that most MRSA strains resist via ribosomal modification, followed by efflux-mediated mechanisms.

A total of 20 HA-MRSA, 30 CA-MRSA, and 24 LA-MRSA isolates were subjected to SCCmec typing, while 36 selected MDR isolates underwent MLST analysis. Three different SCCmec types and only one class of S. aureus were identified in this study through three types of multiplex PCR. SCCmec type I and SCCmec type II are specific to humans, and finding them in livestock suggests zoonotic transmission of MRSA. In MLST, a total of 18 different sequence types (ST) were identified among these isolates, while no clonal complex from the database was linked to 22 isolates recovered in the present study. Among these, eight were identified as ST-672, and one as ST-88. The remaining 13 isolates had STs that are new to the MLST database, indicating genetic diversity of MRSA clones in this region.

Omics and Innovations in Viral Disease Diagnostics

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The advent of omics technologies has revolutionized viral disease diagnostics, offering unprecedented sensitivity, specificity, and systems-level insight into pathogen detection and host responses. This chapter explores the transformative role of genomics, transcriptomics, proteomics, metabolomics, and integrative multi-omics in veterinary virology, with a focus on economically and epidemiologically significant viruses such as Rotavirus, Bluetongue virus (BTV), Peste des petits ruminants virus (PPRV), Bovine coronavirus (BCoV), and Equine herpesvirus (EHV). Through detailed case studies, we illustrate how omics platforms enable comprehensive viral characterization, early detection, and surveillance, while also uncovering host biomarkers and immune signatures. The chapter highlights emerging biosensor technologies, including electrochemical and nanomaterial-based platforms, which offer rapid, field-deployable diagnostics. We further discuss integrated diagnostic workflows that combine point-of-care testing, high-throughput sequencing, and host response profiling to enhance decision-making and outbreak management. Finally, we address implementation challenges ranging from infrastructure and bioinformatics to data sharing and explore future directions such as portable sequencing, CRISPR-based assays, AI-driven analytics, and One Health harmonization. Together, these innovations position omics technologies as critical enablers of precision diagnostics and global health resilience in the face of emerging and re-emerging veterinary viral threats.

Identifying Universal Principles of Host-Pathogen Interactions: Lessons from Morbilliviruses

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Morbilliviruses are known to induce acute and profound immune-suppression in host which renders infected host more susceptible to secondary bacterial and viral infections, and are responsible for most of the virus-related morbidity and mortality. However, the mechanisms and responses that are induced upon infection that are either protective or pathological have not been elucidated till date. We investigated the role of PPRV coded structural and non-structural proteins on modulation of host proteins critical for inflammation and autophagy pathways. The cell culture model system was used to investigate PPRV coded proteins interaction with host proteins for which we used various tools to identify, validate and characterize the functions significance of these interactions. These include pull-down assays, IP and Co-IP assays, reporter assays, confocal and electron microscopy. In addition, various in-silico approaches were used for structural and functional characterization of PPRV coded membrane proteins. Our studies have indicated that PPRV non-structural proteins can directly interact with host transcription factors modulating transcription of host genes critical for disease pathogenesis. Our studies have also identified host proteins that directly interact with viral coded proteins resulting in modulation of host immune responses, and are also critical for induction of cellular autophagy induction. A thorough understanding of how viral protein hijack cellular machinery resulting in disease and death of host can help immensely in development of intervention strategies.

Assessing the Efficacy of Inactivated, Adjuvanted *Brucella suis* Vaccine in Piglets

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Brucellosis is a worldwide zoonosis caused by gram-negative bacteria of the genus *Brucella*. Many studies pointed out that brucellosis vaccines based on *B. abortus* S19 and *B. melitensis* Rev1 in swine do not provide complete protection. There is an acute need for the development of *B. suis*-specific vaccine based on the circulating biovar in a region. An attempt was made in this study to assess the efficacy of the vaccine in piglets, as a mouse model study was done earlier. The study was carried out to assess the efficacy of the inactivated, adjuvanted *Brucella suis* vaccine in piglets. *B. suis* isolate was obtained from the aborted fetal tissue samples collected from a private farm, which had an abortion problem during October 2020 in the Nagapattinam district of Tamil Nadu, which was typed as biovar 1. A simple inactivated vaccine was developed using this local isolate by incorporating a novel agent, imiquimod, to enhance cell-mediated immune response. Two groups (6 piglets per group) were made, comprising Group I–bacterin+Montanide ISA 201 VG+imiquimod and Group II - unvaccinated control, and were given the optimal dose of the respective vaccine (2.0 ml/ piglet, i/m route). After 48 h of vaccination, whole blood in EDTA was collected to assess the cell-mediated immune response, and again, 21 days post vaccination, blood for serum was collected in all the animals. A booster dose was given after the 21st day of vaccination. Blood for serum was collected 21 days after the booster dose. The serum samples were subjected to assess the serum antibody levels by using the BruAlert kit available at TRPVB, TANUVAS. The ELISA results showed that the mean antibody titre at 21 days post vaccination was 74.71±7.20, and after the booster dose, the mean antibody response was 94.25±0.85 when compared with the unvaccinated control group, where the titre was 6.94±0.75 and 7.13±1.02, respectively. Similarly, the CMI response also showed promising results as expected. The role of adjuvants and imiquimod agents in conferring required immune responses will be discussed in the presentation. The indigenous B. suis strain-based vaccine gave sufficient immunity in terms of humoral and cell-mediated immunity and a necessary challenge study needs to be done.

Oral Presentations

Development of serological assays for the diagnosis of *Mycoplasma agalactiae, Mycoplasma mycoides* subsp. *capri* and *Mycoplasma capricolum* subsp. *capricolum* among goats and sheep

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Mycoplasma infections are a major constraint to small ruminant health, with *Mycoplasma agalactiae* (Ma), *M. mycoides* subsp. *capri* (Mmc), and *M. capricolum* subsp. *capricolum* (Mcc) being the primary etiological agent of contagious agalactia, caprine pleuropneumonia, and related syndromes in goats and sheep. These pathogens cause mastitis, arthritis, keratoconjunctivitis, pneumonia, and reproductive losses, leading to significant economic impact in India. Early and reliable diagnosis is vital for surveillance and control; however, molecular tools like PCR have limited field applicability. Development and standardization of two complementary serological assays—an indirect ELISA (iELISA) using whole-cell sonicated supernatant antigens of Ma, Mmc, and Mcc, and a coloured antigen-based serum agglutination test (SAT) for Ma was carried out. Whole-cell supernatant antigens of Ma, Mmc and Mcc were used to develop indirect ELISAs and Rose Bengal colored Ma whole-cell antigen was prepared for the serum agglutination test (SAT). Standardization of iELISA was done by checkerboard titration using known positive and negative sera. Diagnostic cut-off values were determined by ROC analysis. For the SAT, formalin-inactivated Ma antigen was stained with Rose Bengal dye at optimized concentrations. The coloured antigen's reactivity and stability were evaluated using field and reference sera samples. The developed iELISAs were optimized with antigen and primary antibody concentrations of 1:8 (1.25 µg/well) and 1:800 for Ma, 1:8 (1.25 µg/well) and 1:400 for Mmc, and 1:16 (0.625 µg/well) and 1:200 for Mcc, respectively. Cut-off OD values were ≥0.24, ≥0.26, and ≥0.21, respectively, with diagnostic sensitivity ranging from 73.68–89.66% and specificity from 96.23–97.37%. No cross-reactivity was observed with PPR or Pasteurella multocida positive sera. The SAT antigen, prepared with 3% *Mycoplasma agalactiae* cells and Rose Bengal at a 1:3000 dilution, gave satisfactory reaction with known positive and negative sera as well and remained stable under refrigeration. The Rose Bengal colored Ma antigen and iELISAs developed offer valuable tools for sero-surveillance and disease control programs targeting *Mycoplasma* infections in goats and sheep in India.

Optimization of iron oxide nanoparticle conjugated peptide-based enrichment process for *Salmonella* detection

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Salmonellosis is one of the most significant zoonotic diseases, primarily caused by *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium. The study introduces a superior alternative to traditional horizontal bacterial culture for diagnosing Salmonellosis. The study was carried out to develop a simplified *Salmonella* detection system in which iron oxide nanoparticles synthesized by the co-precipitation method were used. The iron oxide nanoparticles were functionalized with *Salmonella*-specific peptide aptamers. FTIR confirmed peptide activation on the iron oxide

nanoparticle. For *Salmonella* Typhimurium enrichment studies, 100µl of peptide-activated nanoparticles were incubated with 1ml of a standardized *Salmonella* Typhimurium culture (OD 0.5 MCF) and subsequently separated magnetically. As per FSSAI standards, the detection of viable *Salmonella* from a food sample is required. To address this, we devised a simple method for *Salmonella* enrichment. Bacterial cells were incubated with *Salmonella*-specific iron oxide nanoparticles. The nanoparticle-bound bacterial cells were counted by the colony count method. Quantification of the bound bacterial cell showed excellent capture efficiency, with 9.8×10^5 CFU/ml retention from $5X10^8$ CFU/ml culture stock. The method reduces the time for confirmation of *Salmonella* to 12-16 hours in comparison to the FSSAI method, which requires 96-120 hours. The binding of iron oxide nanoparticles with the bacterial cell was specific. This method overcomes the drawbacks of the culture, time-consuming, labour-intensive method by delivering results in approximately 23.5 hours while maintaining high specificity and a low risk of false positives, positioning it as a powerful new tool for rapid pathogen detection. However, further studies are needed to understand the enrichment efficiency in various biological matrices.

Sero-diagnosis of toxoplasmosis in sheep and goats

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Toxoplasma gondii is an obligate intracellular protozoan parasite and is known to cause abortion, stillbirth, and neonatal losses in various types of livestock, particularly in sheep and goats. To diagnose toxoplasmosis in sheep and goats by using recombinant microneme protein-3 (MIC-3) and surface antigen-1 (SAG-1) proteins by ELISA The full-length coding sequence of MIC3 and SAG-1 was PCR-amplified from genomic DNA and expressed in the heterologous host Escherichia coli. The recombinant proteins from both genes were confirmed by Western blot using sera from Ni-NTA conjugate and from experimentally infected mice. The serodiagnostic capabilities of the rMIC3 protein were compared with those of the rSAG-1 using known positive and negative samples from experimentally infected mice, as well as field sera from sheep and goats in Uttarakhand, Himachal Pradesh, Jammu and Kashmir, and Punjab. The sensitivity of the ELISA tests was 86.9% for rMIC3 and 88.3% for rSAG-1, while the specificity was 90.6% for rMIC3 and 93.9% for rSAG-1. Out of 1,227 goat serum samples, 562 (45.68%) tested positive for Toxoplasma antibodies using rMIC3. Among the 571 sheep serum samples, 109 (37.71%) were also positive for *Toxoplasma* antibodies. The highest seropositivity among goats was recorded in Uttarakhand at 47.4%, while for sheep, it was in Himachal Pradesh at 43.68%, based on the rMIC3 ELISA results. Additionally, using the SAG-1 ELISA, the highest seropositivity was observed in goats in Jammu and Kashmir (48.76%) and in sheep in Punjab (38.96%). These findings indicate a high prevalence of *Toxoplasma* infection, which requires immediate attention due to its zoonotic importance.

Comparative evaluation of recombinant protein-based indirect ELISAs for sero-diagnosis of lumpy skin disease virus (LSDV)

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Lumpy skin disease virus (LSDV) causes substantial economic losses in cattle due to reduced productivity and trade restrictions, necessitating reliable serological assays for disease surveillance. To express immunogenic recombinant proteins of LSDV and comparatively evaluate their performance in indirect ELISAs for serological detection of LSDV infection. Five LSDV genes-A4L, A27L (full length), and truncated A36R, P32, and B5R-were amplified and cloned into pET-33b(+)/pET-32a(+)

vectors using Nco I and Xho I restriction sites. Recombinant proteins were expressed in E. coli BL21 Codon plus cells and confirmed by SDS-PAGE and Western blot using anti-His HRPO conjugate and anti-LSDV serum. Purified proteins were evaluated as coating antigens in indirect ELISAs standardized with LSDV-positive and negative sera. Optimal antigen concentration and serum dilution were used to compare diagnostic performance and cross-reactivity profiles. P32 and B5R proteins expressed using pET-33b(+) vector could not be purified however, these were successfully purified using pET-32a(+) vector, however, these proteins showed mild reactivity. Among the recombinant antigens expressed using pET-33b(+) vector, LSDV-A4L exhibited the highest reactivity with infected sera, followed by A27L and A36R. All proteins demonstrated minimal reactivity with vaccinated sera. The LSDV-A4L–based indirect ELISA showed the best diagnostic performance, with 93% sensitivity and 94% specificity, and no cross-reactivity with other poxviruses. The comparative evaluation identified A4L as the most suitable candidate antigen for reliable sero-diagnosis of LSDV. The LSDV-A4L–based indirect ELISA outperformed other recombinant antigen assays, showing potential as a specific and sensitive tool for LSD surveillance and epidemiological studies.

From gene to diagnostic antigen: Expression and evaluation of CDV N and H proteins

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Canine distemper is a severe and highly contagious disease of domestic dogs and wild carnivores, often associated with high mortality. It is caused by canine morbillivirus (formerly canine distemper virus, CDV), a member of the genus Morbillivirus within the family Paramyxoviridae. The disease remains endemic in many parts of the world, including India, and effective management requires sensitive and reliable diagnostic tools. Objective was to develop alternatives to whole-virus antigen use, the nucleocapsid (N) and hemagglutinin (H) genes of an Indian CDV isolate were expressed using the baculovirus expression system. The N gene (1,572 bp) encoded ~58 kDa nucleocapsid protein and the H gene (1,824 bp) encoded ~68 kDa haemagglutinin proteins were expressed using Sf9 and Tn5 insect cells. Expression was confirmed by SDS-PAGE and western blotting using anti-His and anti-CDV polyclonal sera. The expressed N-protein was also checked for its reactivity with a panel of monoclonal antibodies against CDV. Among a panel of monoclonal antibodies, only CDV-2F8 reacted with the recombinant N protein, confirming retention of immunogenic epitopes. When evaluated in an indirect ELISA, both recombinant proteins successfully differentiated a panel of 20 positive and 20 negative dog serum samples, with consistently higher OD values observed in N-protein–coated wells, indicating superior diagnostic sensitivity. These findings demonstrate that recombinant N and H proteins represent safe and effective alternatives to whole-virus antigen for serodiagnosis of CDV. By eliminating the need for live virus, recombinant protein–based ELISAs offer a scalable, cost-effective, and biosafe approach for routine diagnosis and large-scale serosurveillance in endemic regions.

Early diagnosis of canine and feline viral diseases: PCR based solution from HiGenoMB

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A variety of viruses can lead to acute and severe illnesses in dogs and cats, two of the most prominent pet animals in India and globally. Emergency and critical care veterinarians often encounter infections such as canine parvovirus (CPV), canine distemper virus (CDV), canine influenza virus (CIV) in Dogs and Feline Calicivirus (FCV), Feline Herpesvirus-1 (FHV-1), Feline Panleukopenia Virus (FPV) in Cats. Early detection of these viral infections is crucial for effective treatment and improving the chances of survival for affected animals. Therefore, probe based PCR kits were developed for sensitive and specific detection of viral pathogens infecting dogs and cats. Real-time reverse transcription polymerase chain reaction (RT-PCR) is considered the gold standard for diagnosing and monitoring viral infections due to its high specificity, sensitivity, and rapid results. The Hi-PCR® Canine Viral Pathogens Probe PCR kit (MBPCR248) and Hi-PCR® Feline Viral Pathogens Probe PCR kit (MBPCR249) are in vitro multiplex probe based PCR assays designed for the qualitative detection of CPV, CDV and CIV infection in dogs and FCV, FHV and FPV infections in cats. Target specific primers and probes were designed for each pathogen followed by optimization of primer and probe concentrations for the assay. Additionally, an exogenous internal control (IC) amplification system was incorporated to ensure efficient PCR amplification and to detect any inhibition arising from sample extraction. The MBPCR248 and MBPCR249 kits were found to be highly sensitive and specific for the viral pathogens in a two-tube assay format. Analytical sensitivity of MBPCR248 and MBPCR249 was determined to be between 1-25 copies/µL for the targeted pathogens. Additionally, no significant cross reactivity with other related organisms were observed with MBPCR248 and MBPCR249 as verified by wet lab testing and in silico analysis. The MBPCR248 and MBPCR249 offer highly sensitive and specific assays in a two-tube format, tailored to the sample types needed for accurate detection of CPV, CDV and CIV infection in dogs and FCV, FHV and FPV infections in cats.

Sequential distribution and persistence of Goatpox virus in goats: insights from experimental infection with the Indian Mukteshwar strain

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Goatpox virus (GTPV), a double-stranded DNA virus belonging to the genus Capripoxvirus of the family Poxviridae, causes severe skin lesions, fever, and significant economic losses in small ruminants across Africa, Asia, and the Middle East. Despite its endemicity in India, limited data exist on the tissue distribution and pathogenesis of indigenous GTPV strains. This study investigates the tissue tropism and temporal distribution of the virulent Mukteshwar strain of GTPV in experimentally infected goats, employing molecular and immunohistochemical approaches to elucidate the dynamics of viral replication and dissemination. Sixteen clinically healthy, GTPV-seronegative goats were intradermally inoculated with the virulent Mukteshwar strain of GTPV to mimic natural infection. At 3, 5, 8, 11, 14, 21, 28, and 35 days post-infection (dpi), two animals were humanely euthanized for comprehensive analysis. Samples of blood, secretions, urine, and multiple organs were collected to trace

infection kinetics. Quantitative PCR (qPCR) was performed for viral genome detection and quantification, while immunohistochemistry (IHC) localized viral antigens in tissues. This systematic approach enabled detailed characterization of the temporal and spatial progression of GTPV infection and its organ-specific localization pattern. GTPV infection progressed sequentially from local replication to systemic dissemination. Initial viral replication occurred at the inoculation site, predominantly in follicular and epidermal epithelial cells, followed by hematogenous and lymphatic spread. Highest antigen loads were observed in the skin, respiratory tract, and intestinal crypts from 8-14 days post infection. By 14 dpi, lesions evolved from focal epithelial immunoreactivity to extensive involvement of epidermal and glandular structures. Viral clearance began after 21 dpi, yet persistent immunoreactivity in respiratory and intestinal epithelium suggested prolonged viral shedding potential. This study provides the first comprehensive report on tissue-specific localization of an Indian Goatpox virus strain, revealing persistent viral presence in respiratory and gastrointestinal epithelia, critical for transmission and disease maintenance.

Poster Presentations

Comparative evaluation of in-house developed Taqman and SYBR green based real-time PCR diagnostic assays for the detection of bovine ephemeral fever virus.

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Bovine ephemeral fever (BEF), caused by the bovine ephemeral fever virus (BEFV), remains a significant transboundary disease of cattle and buffaloes, characterized by acute febrile illness, reduced milk yield, and substantial economic losses to the livestock industry. The virus, belonging to the genus Ephemerovirus within the family Rhabdoviridae, is primarily transmitted by arthropod vectors, with outbreaks often coinciding with the rainy season. Sensitive and rapid diagnostic tools are crucial for early detection and control of the disease. To evaluate the in-house developed TaqMan and SYBR green-based real-time PCR diagnostic assays for the detection of bovine ephemeral fever virus. The present investigation was carried out during the peak rainy months of July–August 2025 in Punjab, India, to detect BEFV from field samples using in-house-developed real-time PCR assays based on both TaqMan probe and SYBR Green chemistry. A total of 40 blood samples were collected from clinically suspected cattle exhibiting signs of BEF. Viral RNA was extracted and reverse-transcribed into cDNA, followed by amplification of the G gene (glycoprotein gene), which is highly conserved and considered a reliable target for molecular diagnostics of BEFV. Out of 40 samples tested, 18 (45%) were confirmed positive by the TaqMan assay, while 15 (37.5%) were positive by the SYBR Green assay. The slightly higher detection rate observed with the TaqMan assay highlights its greater sensitivity and specificity compared to the SYBR Green approach, although both methods proved effective in identifying BEFV. The present study demonstrates the efficiency of real-time PCR assays targeting the G gene for identification of BEFV from suspected samples. The results highlight the critical role of in-house developed TaqMan and SYBR green based real-time PCR diagnostic assays in endemic areas during the rainy season, facilitating timely disease management and reducing economic impacts.

Development of a duplex Taqman real-time PCR assay for the simultaneous detection of African Swine Fever and Classical Swine fever virus

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African Swine Fever Virus (ASFV) and Classical Swine Fever Virus (CSFV) are among the most economically important pathogens impacting swine populations globally. The similar clinical presentations of these two diseases pose a significant challenge for differential diagnosis. Single-plex PCR assays, although dependable, require significant time and resources for concurrent detection. Recent advancements in multiplex real-time PCR tests have provided an effective solution for the simultaneous identification of several pathogens in a single test. In response to this critical diagnostic challenge, we developed a TaqMan probe duplex real-time PCR assay for the simultaneous detection

of both ASFV and CSFV. To develop a duplex TaqMan real-time PCR assay for the simultaneous detection of African Swine Fever and Classical Swine Fever Virus. The primers and probes for ASFV (based on the P72 gene) and CSFV (based on 5' UTR) were designed in-house and synthesized commercially as a means for the assay development. Single-plex and duplex real-time PCR assays were developed for the detection of both viruses. Both single-plex and duplex real-time PCR assays were developed for the detection of both viruses. The analytical sensitivity of the TaqMan real-time duplex assay was comparable with the single-plex real-time PCR assays. The assessment of specificity was done with other notable swine viruses, such as Porcine Parvovirus 2, Porcine Circovirus 2, and Porcine Astrovirus, and no cross-reactivity was observed. The developed assay was compared with the standard PCR/real-time PCR assay and showed high sensitivity. The assay demonstrated 100% sensitivity and specificity when its applicability was evaluated with known positive and negative samples. The developed duplex real-time PCR assay provides a reliable, rapid, and cost-effective method for the differential diagnosis of ASFV and CSFV.

Cloning and expression of truncated glycoprotein B (gB) of Bovine alphaherpesvirus-1 (BoAHV-1) in *Escherichia coli*

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Bovine alphaherpesvirus-1 (BoAHV-1), belonging to the genus *Varicellovirus* and family *Herpesviridae*, is an economically important pathogen of cattle responsible for a wide range of clinical manifestations, including respiratory, reproductive, ocular, and systemic disorders. A characteristic feature of BoAHV-1 infection is its ability to establish latency soon after infection, with reactivation leading to viral shedding through nasal secretions and semen. This latent nature hinders effective control and makes antibody detection crucial for diagnosis and surveillance. Among the ten envelope glycoproteins encoded by BoAHV-1, glycoprotein B (gB) is highly conserved and immunodominant, conferring strong diagnostic potential and playing a key role in immunodiagnostic applications. To clone and express the truncated glycoprotein B(gB) of Bovine Alphaherpesvirus-1 (BoAHV-1) in Escherichia coli. Primers targeting the UL27 gene of Bovine alphaherpesvirus-1 (BoAHV-1) were designed based on the GenBank sequence and custom synthesized. BoAHV-1 DNA was extracted from virus cell-culture supernatant (ICAR-NIVEDI isolate) using the Qiagen DNA Isolation Kit. The gB gene fragment was amplified by conventional PCR and visualized on agarose gel. Amplified products containing restriction sites were ligated into the pET vector and transformed into *E. coli* BL21 cells. The recombinant clones were confirmed by colony PCR, restriction digestion, and sequencing. Protein expression was induced with IPTG, analyzed by SDS-PAGE, and its immunoreactivity verified by Western blot using IBR-positive bovine sera. The recombinant protein was purified using Ni-NTA affinity chromatography and stored at –80°C. The 720 bp truncated fragment of the BoAHV-1 gB (UL27) gene was successfully amplified, cloned into the pET-32b(+) vector, and transformed into *E. coli* BL21 (DE3)pLysS cells. Recombinant clones were confirmed by colony PCR, restriction digestion, and sequencing. Following IPTG induction, SDS-PAGE analysis revealed a distinct ~47 kDa protein band corresponding to the recombinant gB. Western blotting showed strong reactivity with both anti-His antibodies and IBR-positive bovine sera, confirming presence of immunoreactive domain of gB protein. The recombinant protein was purified under denaturing conditions using Ni-NTA affinity chromatography and stored at –80°C for subsequent immunodiagnostic applications. The truncated gB protein of BoAHV-1 was successfully expressed and shown to be immunoreactive, indicating strong potential as a cost-effective antigen for IBR serodiagnostic assays.

Designing chimeric Circumsporozoite Protein (CSP) construct: a promising vaccine candidate against *Plasmodium vivax* and *Plasmodium falciparum*

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Plasmodium vivax is a major contributor to global malaria cases, posing unique challenges, such as relapse due to hypnozoites and increasing drug resistance. Although artemisinin-based combination therapies (ACTs) are used to treat *P. vivax* malaria, the lack of an effective vaccine remains a significant hurdle. This study focuses on developing a chimeric Circumsporozoite Protein (CSP) construct to advance vaccine research targeting multi-Plasmodium species. The objectives of the study were to select B and T cell epitopes of the Circumsporozoite Protein of all human malaria parasites, designing and computational assessment of the chimeric construct, expression and purification of the chimeric construct and assessment of seroreactivity of the chimeric CSP construct against *Plasmodium*-infected patient sera samples. A chimeric CSP construct was engineered using immunogenic sequences to enhance antigenicity and structural stability. The designed construct consists of 324 amino acids, with a molecular weight of 34.9 kDa and a theoretical isoelectric point (pI) of 9.28. Structural modelling and refinement were performed using I-TASSER and Galaxy web server (C-score = -2.83, TM-score = 0.39±0.13, RMSD = 13.1±4.1 Å). Linear antibody epitopes were predicted using ElliPro. Immune simulations using C-ImmSim predicted strong immune responses, identifying epitopes interacting with MHC class I and II alleles. Cytokine analysis revealed IL-2 and other interleukins indicating effective immune activation. The construct was expressed in a heterologous system, and its expression was confirmed via western blotting with anti-His antibodies under pre- and post-refolding conditions. Additionally, indirect ELISA was performed using sera from individuals infected with P. falciparum (Pf) and P. vivax (Pv) to assess cross-reactivity. The CSP chimeric construct demonstrated no similarity to human proteins, mitigating potential risks of autoimmunity. Structural analyses revealed significant antigenic regions suitable for eliciting robust immune responses. C-ImmSim simulations confirmed a strong immunogenic profile, with epitopes recognized across diverse HLA alleles and cytokine production consistent with effective immune activation. Western blot analysis confirmed successful protein expression at 37°C, with stronger recognition post-refolding, indicating proper folding and structural integrity. Indirect ELISA showed that the chimeric construct elicited a response against both Pf and Pv sera, demonstrating its cross-reactive potential across *Plasmodium* species. The CSP chimeric construct showed promise as a vaccine candidate, addressing the challenges of P. vivax while exhibiting cross-reactivity with P. falciparum, enhancing its utility in regions co-endemic for both species. The absence of similarity to human proteins reduces the risk of adverse immune reactions. The successful expression, refolding, and serological reactivity highlight the construct's suitability for further immunogenicity studies. Future in vivo experiments are needed to validate its protective efficacy and potential to mitigate the burden of malaria caused by *P. vivax* and *P. falciparum.*

Comparative evaluation of subunit and heat-killed map vaccines: highlights of protection and histopathological resolution

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Paratuberculosis continues to compromise livestock productivity worldwide due to the limited efficacy of existing vaccines and diagnostic challenges. Innovative vaccine strategies are needed to enhance immune protection and tissue-level control of the pathogen.to the study was carried out to compare the immunogenicity, protective efficacy, and histopathological outcomes of an adjuvanted recombinant subunit vaccine (MAP1693, MAP2677, MAP3547, MAP4308) and a heat-killed MAP (HK-MAP) vaccine. Mice were immunized with either the subunit vaccine emulsified with mineral oil adjuvant or with the HK-MAP vaccine. Humoral and cell-mediated responses were assessed via IgG1 titers, IFN-γ and IL-17A secretion. Tissue protection was evaluated through histopathological analysis of liver, spleen, and intestinal tissues. Booster doses were administered to assess the enhancement of immunity and tissue protection. The adjuvanted subunit vaccine induced robust and sustained immune responses, including high IgG1 titers and elevated IFN-γ and IL-17A production, whereas HK-MAP generated moderate immunity with partial cytokine activation. Histopathology demonstrated near-complete suppression of granulomas in subunit-vaccinated mice, while HK-MAP immunization resulted in mild to moderate granulomatous reactions. Booster doses improved immune responses and reduced tissue lesions in all groups, particularly in the subunit vaccine cohort. Adjuvanted subunit vaccine surpassed heat-killed vaccine in immunogenicity, cytokine induction, and tissue protection, representing a promising, safe, and effective strategy for large-scale Johne's disease control.

Evaluation of Classical Swine fever (CSF) vaccine virus stability in different bait forms for development of CSF oral vaccine

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Classical swine fever (CSF) is among the highly prevalent viral diseases of pigs in India, inflicting high mortality and significant economic loss to farmers. Being viral disease, the effective control strategy is vaccination which is currently done through parenteral route. Parenteral vaccination has limitation in free-ranging swine and wild-boar population, which act as reservoir of the disease, and highlight the importance of oral-bait vaccination for effective immunization in such animals. For development of oral vaccine, the stability of vaccine virus in various oral-bait vaccine formulations at different temperatures is crucial to determine the shelf-life of the vaccine. In the present study, three different CSF oral-bait vaccine formulations viz., Cereal based bait, Plant based bait and Wax-coated plant-based bait were evaluated for stability at various temperatures (Room temperature i.e 25°C, 4°C and -20°C). The different bait forms containing the CSF vaccine virus were freeze dried and exposed to different temperatures (-20°C, 4°C, 25°C) for different time periods. Following the recovery of vaccine virus from the lyophilised baits, the titre of the vaccine virus was evaluated using FAT to assess the loss in virus titre. The plant-based and the cereal-based bait formulation were stable for 24 hours at room temperature, 15 days when stored at 4°C, and up to 45 days at −20°C, highlighting its suitability for field-deployment as well as storage at the given temperatures. In contrast to these, the wax coated

plant-based bait was found to be less stable than other formulations at different temperatures with only 6 hours at room temperature, 7 days at 4°C and 15 days at -20°C. Thus, it was concluded that plant-based and cereal-based baits offer a promising choice for formulation of CSF oral bait vaccine; so that vaccine coverage can be ensured in all pig population including backyard and wild without need for restraining.

Immunological signatures of protection induced by recombinant subunit vaccine against Paratuberculosis

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Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic enteric granulomatous infection causing significant economic losses in livestock. Vaccination strategies targeting both humoral and cellular immunity are critical to achieving effective protection. The study was done to evaluate the immunogenicity and protective efficacy of subunit (MAP1693, MAP2677, MAP3547, and MAP4308) and heat-killed MAP vaccines, with or without adjuvant/booster, including DIVA potential analysis of MAP0862 for subunit vaccine. C57BL/6 mice were divided into six experimental groups, receiving either subunit or heat-killed MAP vaccines with or without adjuvant and booster doses. Serum IgG titers were measured via ELISA at 7, 14, 21, 28, and 35 DPV. Splenocytes were analyzed at 35 DPV for T-cell subsets (naive, effector, central memory- CM, effector memory-EM, activated memory) and cytokine expression (IFN-γ, TNF-α, IL-17A, IL-2, IL-4, and IL-10) using flow cytometry and qPCR. Myeloid-derived suppressor cells (MDSC) and macrophage populations were also evaluated, with non-specific (PMA+Ionomycin) and PPD-specific stimulation. Seroconversion was observed by 21 DPV in all vaccinated groups, with Group 6 (subunit+adjuvant) showing the highest IgG titer at 35 DPV (OD□□□ = 2.45±0.12, $p<0.01$), correlating with reduced spleen MAP load (Ct 18.5±0.3). Throughout the trial, all animals remained negative for antibodies against the DIVA marker. Group 6 also displayed significant IL-17A upregulation pre- and post-booster, while Group 3 (heat-killed without adjuvant) exhibited the highest IFN-γ expression. EM and CM, CD4□ and CD8□ T cells were elevated in Groups 3, 4 (heat-killed+adjuvant), and 6. Post-booster, activated memory CD4□ T cells increased across all vaccinated groups. PPD stimulation confirmed the strongest antigen-specific IFN-γ responses in Group 6. MDSC levels increased across all vaccinated groups; macrophage populations were significantly higher in groups 3, 4, and 6. Adjuvanted subunit vaccine induced robust humoral and cellular immunity, early seroconversion, enhanced T-cell memory, cytokine production, and macrophage activation, highlighting its potential to improve protection against JD through coordinated adaptive and innate immune mechanisms and have DIVA potential.

Development and Optimization of an Indirect ELISA for The Detection of Antibodies Against Canine Parvovirus

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Canine parvovirus (CPV) causes severe gastroenteritis in dogs. Developing an indirect ELISA enables rapid, specific, and economical detection of CPV antibodies for effective disease monitoring To clone, express, and purify the recombinant VP2 protein of Canine Parvovirus and to develop and optimize an indirect ELISA for sensitive detection of CPV-specific antibodies. The VP2 gene of *Canine Parvovirus* was cloned into a prokaryotic expression vector and transformed into *E. coli* BL21 (DE3) cells for recombinant protein production. The expressed VP2 protein was purified using Ni-NTA affinity chromatography and confirmed by SDS-PAGE and Western blot analysis. The purified recombinant VP2 (rVP2) protein was used as the coating antigen to develop an indirect ELISA for detecting CPV-specific antibodies in dog serum samples. Various assay parameters, including antigen concentration, serum and conjugate dilutions, and incubation conditions, were optimized to achieve maximum sensitivity and specificity and it was compared with HI and commercially available ELISA. The developed ELISA was further validated using known positive and negative sera. The VP2 gene of Canine Parvovirus was successfully cloned, expressed in *E. coli* BL21 (DE3) cells, and purified under denaturing conditions using Ni-NTA affinity chromatography, yielding a recombinant VP2 protein of ~81 kDa. The purified rVP2 served as an antigen in the developed indirect ELISA. Optimization yielded an antigen concentration of 4500 ng/well, serum dilution of 1:200, and conjugate dilution of 1:16000. The assay showed a diagnostic sensitivity of 93.6% and specificity of 96.6% with substantial agreement ($\kappa = 0.697$) compared to the haemagglutination inhibition and commercial ELISA, confirming its reliability for CPV antibody detection. The developed indirect ELISA using recombinant VP2 protein is sensitive, specific, and cost-effective, suitable for large-scale serodiagnosis and surveillance of Canine Parvovirus infection in dogs.

Recombinant Protective Antigen Based Indirect-ELISA for Sero-Monitoring of Anthrax Specific Antibodies in Sheep

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Anthrax is an acute, bacterial (*Bacillus anthracis*) zoonotic disease that primarily affects herbivorous animals, including small ruminants, and also accidentally infects humans. Anthrax is prevalent in many geographical regions of India, and outbreaks occur during the early or post-monsoon season among various livestock. The pathogenesis is mediated by three exotoxins encoded by plasmids. Of which, Protective antigen (PA) is a key exotoxin, also known to induce protective neutralizing antibodies. Anthrax live spore vaccine is used to prevent the disease in endemic areas. Despite regular vaccinations, repeated outbreaks are being reported in various regions, and there is no assay available to monitor the antibody levels in animals. An indirect ELISA utilizing a well-defined recombinant PA antigen (rPA) was optimised. The recombinant protective antigen (~67 kDa) was produced using a

prokaryotic expression system, and the rPA-based indirect ELISA was optimized using a known positive and known negative sheep sera panel. The assay was found to possess good diagnostic sensitivity and specificity, without any cross-reactivity with commonly occurring disease-specific sera. Further, the assay was used for screening of randomly collected sheep sera samples from anthrax endemic and non-endemic areas for the detection of anthrax-specific antibodies. The study indicated the potential utility of rPA-ELISA for sero-monitoring of anthrax / PA-specific antibodies in sheep.

Development of DNA vaccine for chicken infectious anemia (CIA) and its immunogenicity studies

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Chicken infectious anemia (CIA), an emerging disease mainly of young chicken, characterized by poor weight gain, severe anemia, aplasia of the bone marrow, lymphoid atrophy, subcutaneous and muscular hemorrhages and increased mortality, has been responsible for considerable health problems and economic losses to the poultry industry. In present study DNA vaccine was developed for chicken infectious anemia (CIA) and its immunogenicity was studied. Development of DNA vaccine for Chicken infectious anemia (CIA) its immunogenicity was studied. CIAV VP1 and VP2 genes were used together as DNA vaccine. Primers were designed in a way to amplify VP1 and VP2 whole genes. Amplified and purified CIAV VP1 and VP2 cloned in pTARGET (a mammalian expression vector). These recombinant plasmid pTARGET-VP1 and pTARGET-VP2 were characterized by colony PCR, restriction endononuclease (RE) digestion. Further confirmation of genes in vector was done, employing PCR by using T7 promoter primer with reverse primer of insert and sequencing. pTARGET-VP1 and pTARGET-VP2 were extracted in bulk and there purity and concentration was checked by NANODROP. In vitro expression of pTARGET-VP1 and pTARGET-VP2 were studied in Vero cell line by RT–PCR and indirect fluorescent antibody technique (IFAT). pTARGET-VP1 and pTARGET-VP2 were used as DNA vaccine. Efficacy of developed vaccine was studied in specific pathogen free (SPF) chicks. Chicks were tested for CIAV specific antibodies before injecting vaccine. Cell mediated immune response (CMI) and humoral immune responses (HIR) were studied weekly by fluorescent activated cell sorting (FACS) and enzyme linked immunosorbent assay (ELISA), respectively. At 1 WPV, the primary DNA vaccine injection induced a lower moderate protection titre of 2724.29±271.84, which rose sharply at 2 WPV, being maximum at 3 WPV (5746.22±215.89) and both were moderately protective, thereby declining gradually with a moderate protection titre of 5273.33±457.51 maintained at 6 WPV. However, booster vaccine group showed a very sharper increase in antibody titres at 1 week post booster (WPB) (7193.58±406.78) which is supposed to be a good moderately protective titre as per IDEXX laboratories ELISA titre correlation with the virus neutralization antibody titres. Also in the booster group, antibody showed a gradual decrease in antibody titres and a good moderately protective antibody titre of 6526.17±428.81 was maintained compared with the primary vaccination group titre of 5273.33±457.51. In the present study, the cell mediated immunity was measured by CD4+ and CD8+ ratio of peripheral blood mononuclear cells on 4, 5, 6 and 7 weeks age of chicks. The result indicated that CD4+ and CD8+ ratio of groups A, B and C differed significantly from the unvaccinated control (group D) on 4, 5, 6 and 7 weeks age of chicks. Group B and group A showed significantly lower CD4+ and CD8+ ratio than group C and group D on 4, 5, 6 and 7 weeks age of chicks. In group-B, lowest CD4+ and CD8+ ratio was found on 7 weeks age of chicks (1.662±0.085). So DNA booster dose lead to more increased CD8+ T-cells. Result also indicated group C showed lower CD4+ and CD8+ ratio in comparison with group D on 4, 5, 6 and 7 weeks age of chicks. This developed DNA vaccine giving moderate antibody level protective titres can further be improved by vaccination using gene gun, help in direct transfection of dendritic cells and favouring effective antigen presentation. Also, by using cationic lipid complexes and cytokine adjuvants, the efficacy could be significantly improved.

H5N1 flu multiplex probe PCR kit for sensitive and specific detection of Avian Influenza

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Influenza is a highly contagious respiratory disease caused by influenza viruses. These viruses are a leading cause of morbidity and mortality worldwide. Influenza viruses belong to the Orthomyxoviridae family and are classified into three types, Influenza A, Influenza B and Influenza C. Influenza A virus can infect humans as well as variety of mammalian species including horses, swine and numerous avian species while Influenza B virus primarily infects humans. Both Influenza A and Influenza B viruses are responsible for annual epidemics and occasional pandemics in humans. The H5N1, a subtype of Influenza A, is a primary cause of Avian influenza, which spreads globally and poses a serious threat to wildlife, poultry and dairy. In recent years, the H5N1 virus has increasingly affected mammals, with reports of severe human infections as well. Early detection of the H5N1 viral infections is crucial for effective treatment and improving the chances of survival for affected animals. Therefore, a probe-based PCR assay was developed for sensitive and specific detection of H5N1 infection in clinical samples. Real-time reverse transcription polymerase chain reaction (RT-PCR) is considered the gold standard for diagnosing and monitoring viral infections due to its high specificity, sensitivity, and rapid results. Hemagglutinin (HA) and Neuraminidase (NA) gene-based primers and probes specific for H5N1 were designed followed by optimization of primer and probe concentrations for the assay. An exogenous internal control (IC) amplification system was incorporated to ensure efficient PCR amplification and to detect any inhibition arising from sample extraction. Additionally, the assay included detection of generic Influenza A virus gene to validate influenza A infection and the presence of any other influenza infection. The sensitivity of the assay was estimated using ATCC standards for H5N1.Analytical sensitivity of MBPCR280 was determined to be 1 copy/µL for H5N1_HA, 2 copy/µL for H5N1_NA and 1 copy /µL for generic Influenza A target. MBPCR80 assay did not show any cross reactivity with related organisms/ viruses as verified in wet lab testing and in silico analysis. The MBPCR280 offers a highly sensitive and specific one tube multiplex assay for early detection of H5N1 virus in clinical samples.

Poly:IC adjuvanted PLGA nanoparticles: A next generation vaccine strategy against Equine herpes virus 1

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Equine herpesvirus-1 (EHV-1) is a major pathogen of horses, responsible for respiratory illness, abortion, neonatal foal mortality, and equine herpesvirus myeloencephalopathy (EHM). Conventional vaccines offer partial protection and fail to induce long-lasting immunity, underscoring the urgent need for next-generation vaccine platforms. Nanotechnology-based delivery systems provide novel opportunities to enhance vaccine stability, targeted delivery, and immunogenicity.In the present study, we developed a PLGA (poly lactic-co-glycolic acid) nanoparticle-based vaccine encapsulating formalin-inactivated EHV-1 virus, co-formulated with Poly I:C, a synthetic double-stranded RNA analogue and potent Toll-like receptor-3 (TLR3) agonist. The formulation was optimized for particle size, surface charge, encapsulation efficiency, and antigen release kinetics. In vitro evaluation using equine peripheral blood mononuclear cells (PBMCs) demonstrated efficient cellular uptake of nanoparticles and a significant induction of antiviral cytokines, including IFN-α and IL-6, indicating robust activation of innate immune pathways. Furthermore, the presence of Poly I:C enhanced immunostimulatory potential, suggesting synergistic activation of both innate and adaptive responses.

This PLGA-based nano-formulation represents a promising next-generation vaccine strategy against EHV-1, offering controlled antigen release, enhanced antigen presentation, and potent adjuvant activity. By overcoming the limitations of current vaccines, such a nanoplatform has the potential to improve protective immunity in horses and reduce the economic and welfare burden of EHV-1 outbreaks. Future studies will focus on in vivo immunogenicity and protection efficacy in equine models.

Session 4:

Genomics, Disease Surveillance and Epidemiology

Policy Approaches for Bluetongue Control and Management

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Bluetongue (BT) is a vector-borne viral disease of major economic importance, affecting sheep, goats, cattle, and wild ruminants. The disease, caused by *Bluetongue virus (BTV)* and transmitted by *Culicoides* midges, leads to productivity loss, trade restrictions, and high morbidity in susceptible livestock. The disease is enzootic in India, and its outbreaks are correlated with post monsoon & winter season in Southern India and post monsoon & summer season in some parts of Northern India. Of the 29 reported serotypes of the virus, 21 have been shown to be prevalent serologically, while 12 have been successfully isolated in cell culture. Globally, bluetongue incidences have been on the rise and in the year 2024, it was ranked to be the third most reported disease. Given its complex epidemiology and transboundary potential, a comprehensive policy framework is essential for effective control. Following are some of the policy approaches needed for its control.

1. **Strengthened Surveillance and Diagnostics:** A robust surveillance system is the cornerstone of BT control. Regular sero-surveillance, virus isolation, and molecular detection (RT-PCR, sequencing) should be carried out to monitor circulating serotypes and emerging strains. Establishing regional reference laboratories equipped for BTV diagnosis and genome sequencing will facilitate early detection and molecular epidemiology studies. Data integration through a national disease information system can support rapid response and risk mapping.
2. **Vector Surveillance and Control Measures:** Systematic monitoring of *Culicoides* populations and vector ecology is vital. Policy support is needed for establishing vector surveillance units in high-risk zones. Integrated vector management (IVM) practices—including habitat management, insecticide use in animal shelters, and improved housing—should be incorporated into livestock development schemes.
3. **Vaccination Policy and Research Support:** Region-specific vaccination strategies using inactivated or multivalent vaccines should be encouraged based on circulating serotypes. Support for indigenous vaccine development and production, with emphasis on DIVA-compatible and thermostable vaccines, is crucial. Policy guidance should ensure pre-monsoon vaccination drives in endemic regions.
4. **Livestock Movement Control and Biosecurity:** Implementation of movement regulation and quarantine protocols for animals moving from one state to another is necessary. Certification systems and border check-posts should be strengthened, particularly in inter-state and cross-border trade corridors.
5. **Climate-Based Risk Mapping and Predictive Modeling:** Climate change influences vector distribution and outbreak dynamics. Integration of meteorological, ecological, and GIS-based tools can aid in mapping high-risk areas. Policies should support the use of remote sensing and predictive modeling to guide resource allocation and preparedness planning.
6. **Capacity Building and Awareness Generation:** Training programs for field veterinarians, para-vets, and farmers should focus on early disease recognition and control measures. Public awareness campaigns through state departments can enhance community participation. Institutional strengthening and human resource development in epidemiology, diagnostics, and vector biology must be prioritized.
7. **Inter-Sectoral Collaboration:** Coordination between animal health, environmental, and policy agencies will ensure integrated management. Collaboration with research institutes and international partners can enhance surveillance, data sharing, and technology transfer.

In brief, formulation of **National Bluetongue Control Plan** encompassing surveillance, vector management, vaccination policy, and capacity building, backed by dedicated funding and inter-institutional coordination is required to reduce the socio-economic impact of the disease.

Assessment of Vaccine Efficacy and Population Immunity in Goats and Sheep in Odisha: Strategy for Advancing Towards PPR Eradication in India

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The study focuses on evaluating the status of population immunity within the Peste des petits ruminants (PPR) eradication programme implemented in Odisha, India. It specifically assesses seroconversion/vaccine efficacy and population immunity following annual vaccination. The study aligns with the World Organisation for Animal Health (WOAH) and the Food and Agriculture Organization (FAO) guidelines for post-vaccination Evaluation (PVE) under the Global Control and Eradication Strategy (GCES), with the ultimate goal of global PPR eradication.

The present cross-sectional study was conducted during 2023 and 2025, in the pre-vaccination phase in 2023, 3466 random serum samples were collected from goats and sheep across 120 epidemiological units (epi-units) spanning 82 taluks in 28 districts. The PPR seropositivity rates in the were: 6–12 months: 60%; 1–2 years: 62% and 2 years: 66% with overall, average seroprevalence was 61.1%, suggesting a considerable level of prior vaccination, with 43% of the epi-units demonstrating antibody prevalence rates of ≥70%. Following the first round of vaccination in 2023, 1125 serum samples were collected from goats and goats in the 6–12-month age group within 90 days post-vaccination across 119 epi-units, covering 64 taluks in 23 districts. The observed seroconversion in this age group was 76.9%, confirming the efficacy of the vaccine. Additionally, over 68% of the epi-units achieved a response of ≥70% seroprevalence, marking a notable improvement from 43% during the pre-vaccination phase. Further PVE conducted after the second-round mass annual vaccination in 2024 showed continued progress in herd immunity, with vaccine effectiveness reaching 81.85%. A total of 2650 serum samples were collected across all three age groups, covering 90 epi-units in 77 taluks in 28 districts, revealing that over 80% of the epi-units achieved an immunity response of ≥70%. Post third round of annual mass-vaccination in 2025 showed expected outcome so far with vaccine efficacy of ~75% with vaccine effectiveness of 74% and population immunity of 78% (testing ongoing). A total of 1748 serum samples were collected from all three age-groups, covering 49 epiunits so far from 44 taluks in 20 districts, revealing >74% of the epiunits achieved immunity response of ≥70%. The findings emphasize the importance of continuing three successive mass vaccination campaigns to achieve herd immunity. All four plans implemented in the State of Odisha have effectively progressed towards eradication of PPR by achieving a vaccination coverage rate of over 95% targeting small ruminants aged >3-4 months, to achieve a herd immunity threshold of ~80%. This study provides key insights into post-vaccination population immunity and PPR vaccine efficacy, guiding national strategies for a PPR-free India and supporting global eradication efforts. The continued implementation of the control and eradication strategies, along with intensive surveillance and sentinel surveillance in cattle, will provide the required momentum to achieve PPR-FREE status as per schedule, enhancing small ruminant health and productivity, and benefiting India's agricultural economy and affected regions.

Evolutionary Dynamics of Equine Influenza H3N8 outbreaks in India

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Influenza A viruses are segmented, negative-sense RNA viruses that circulate in many hosts, evolve rapidly through error-prone replication and reassortment, and diversify into multiple hemagglutinin (HA)/neuraminidase (NA) subtype combinations. Among these, equids have historically been affected by two subtypes: H7N7, which has not been detected for decades and is presumed extinct in horses, and H3N8, which persists globally. It is H3N8 that underpins equine influenza (EI) today. This subtype adapts steadily via antigenic drift in haemagglutinin and then exploits horse-movement networks-breeding farms, racecourses, fairs, and pilgrimage routes-to ignite fresh waves of disease. This paper brings the evolution into sharp focus and grounds it in three Indian milestones: the first confirmed nationwide epizootic in 1987, the Florida clade 2 (FC2)-driven resurgence across multiple states in 2008–09, and the 2025 outbreak in the North Indian Himalayan State. To delineate H3N8 evolution and understand India's 1987, 2008–09, and 2025 EI outbreaks, by reconstructing lineages, quantifying antigenic change, and mapping space–time transmission, and to translate findings into actionable guidance for sentinel surveillance, vaccine updates, movement controls, and policy implementation. Integrated analysis combining genomics, antigenic phenotyping, and field epidemiology across India's 1987, 2008–09, and 2025 equine influenza events was carried out, and findings were benchmarked against the global H3N8 landscape. Infection in diseased animals was confirmed by qRT-PCR targeting the influenza virus A and M gene, and a rise in antibody titres in the paired serum samples. Viruses were isolated in SPF embryonated eggs and HA (± whole-genome) were sequenced. Lineage dynamics were inferred using maximum-likelihood phylogenies and time-scaled. HA1 substitutions at antigenic and receptor-binding sites were mapped, and selection pressures were evaluated. Antigenic phenotypes were measured by haemagglutination inhibition assay, with antigenic cartography estimating distances to vaccine/reference strains. India's equine influenza record shows three clear turning points, and phylogenetic analysis links them into one evolutionary arc. In 1987, a nationwide epizootic swept North India, and two recovered H3N8 isolates represented distinct genetic backgrounds, indicating co-circulation of divergent lineages during the same wave. After a comparatively quiet period, equine influenza re-emerged in mid-2008 at Katra (Jammu & Kashmir) and spread across about fourteen states through 2009; viruses from this episode clustered within the Florida clade 2 lineage, mirroring contemporaneous Asian circulation. In March 2025, the virus was detected near the pilgrimage site in North Indian Himalayan state - Uttarakhand, which has international borders, prompting an immediate standstill and rapid deployment of diagnostic facilities in nearby areas, enabling large-scale screening and village-level quarantine with phased reopening. Approximately 25,000 samples were tested overall, yielding about 1,030 HAI-positive and 155 qRT-PCR-positive results, with case involvement estimated at 4,500–5,000 animals; Uttarakhand accounted for most positives, and a secondary cluster in Bijnor, Uttar Pradesh, was linked to return routes. Maximum-likelihood and time-scaled phylogenies placed all 2025 egg-grown isolates firmly within Florida clade 2, with strong branch support and short internal branches consistent with recent expansion. HA1 analysis showed substitutions at key receptor-binding/antigenic positions, including a distinctive Gly→Cys change near 227/228, consistent with ongoing antigenic drift shaping transmission along high-traffic routes. Taken together, the 1987, 2008–09, and 2025 Indian episodes show that equine influenza's impact is the product of two inseparable forces: gradual antigenic change in H3N8 and the structure of horse mobility. Phylogenetic reconstructions link these events into one trajectory, with the 2025 Uttarakhand–Uttar Pradesh cluster sitting squarely within FC2 and displaying HA1 changes at receptor-binding/antigenic sites, consistent with ongoing drift in the Florida Clade 2 viruses, which are restricted currently to Asia mostly. Operationally, the 2025 response demonstrates that diagnostics placed near the front line, rapid standstills, village-level quarantine, and phased reopening can bend the epidemic curve, helping in the control of such highly contagious infections.

Prevalence of Zoonotic and Transboundary Animal Diseases in West Bengal, India: A One Health Initiative

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West Bengal is located at the eastern part of India sharing international border with Bangladesh. The state is also thickly populated in terms of human and animals. Exchange of zoonotic pathogens between the animals and human and transmission of major transboundary diseases through domestic and wild animals is reported previously. The present study was undertaken to estimate the prevalence of selected important zoonotic and transboundary diseases of domestic livestock in West Bengal. Total four zoonotic bacterial (brucellosis, tuberculosis, salmonellosis, listeriosis), one rickettsial (Q-Fever), two parasitic (cryptosporidiosis, cysticercosis) and five transboundary viral pathogens (African swine fever, porcine reproductive and respiratory syndrome, Japanese encephalitis, lumpy skin disease and swine influenza) were studied using serological, microbiological and molecular techniques. Sample size was calculated based on the population of animals and the existing prevalence data to generate a statistically valid data. Total 8.18% (14/171) cattle were found to be reactant to tuberculosis by single intradermal test. Similarly, 1.87% (7/374) bovines were found to be positive for brucellosis by blocking ELISA. Through bacteriological technique and specific PCR assay, 9.12% (73/800) samples (rectal swab, cloacal swab and raw eggs) was found to be positive for salmonellae and 6.74% (44/652) of fresh meat and meat products were recorded as positive for *Listeria* spp. *Cryptosporidium* antigen was detected (4.79%, 9/188)) in the feces of young calf (up to 12 months) by sandwich ELISA. Q-fever was detected in 0.32% (2/624) of cattle and buffalo by indirect ELISA. Total 0.24% (1/416) pigs of West Bengal were positive for cysticercosis. ASF and PRRS could not be detected by ELISA and PCR in West Bengal. Seroprevalence of LSD, JE and swine influenza was 20.35% (70/344), 41.1% (37/90) and 33.54% (54/161), respectively.

Antimicrobial-Resistant, Virulent and Biofilm-Forming *Salmonella*: Molecular and Phenotypic Evidence from Duck at the Human-Animal-Environment Interface

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Salmonella, one of the prominent Gram-negative bacteria, stands as a paramount concern in the realm of public health due to its profound pathogenic potential. The present study aimed for the comprehensive molecular and phenotypic characterization of antimicrobial-resistant, biofilm-forming, and virulent Salmonella spp. Isolated from apparently healthy ducks and their environments in West Bengal, India. A total of 462 samples from Indigenous, Khaki Campbell, and Pekin ducks and their environment were used to isolate *Salmonella* spp., and their ESBL, virulence, and biofilm-forming genes were determined. Phylogenetic analysis of ESBL gene sequences was carried out. Resistance/susceptibility of the isolated strains to different antibiotics was determined by an antibiotic sensitivity test (AST). Outer membrane proteins of selected isolated strains having antibiotic resistance, virulence, and biofilm-forming ability were separated, and immunogenic polypeptides were assessed by SDS-PAGE and subsequently by Western blot. The collected samples yielded 436 isolates, of which 42.2% were ESBL producers carrying *bla*TEM (36.5%), *bla*CTX-M (20.6%), *bla*SHV (17.7%), and *bla*AmpC (32.6%). Sequence analysis revealed multiple clinically relevant alleles, including *bla*TEM-164, *bla*CTX-M-15, and *bla*SHV-45, underscoring their potential public health significance. The isolates were also screened for biofilm genes (*csgA*, *sdiA*, *rpoS*, *rcsA*) and the virulence gene *invA*. Biofilm-associated genes were widely distributed (*csgA:* 54.59%, *sdiA*: 52.52%, *rpoS*: 80.28%, *rcsA*: 63.76%), while 141 (32.34%) of isolates possessed the *invA* virulence marker. Of 26 selected strains, high multidrug resistance was detected, mainly against tetracycline and cefixime. Phylogenetic analysis of ESBL gene sequences showed clustering across avian, animal, and clinical (human) Salmonella isolates, indicating potential interspecies transmission and evolutionary divergence. Notably, strong positive correlations were observed among biofilm formation, multidrug resistance, and virulence (τ = 0.656, ρ = 0.765, $p<0.001$). Western blotting further identified two unique polypeptide markers (69 and 35 kDa) with diagnostic potential for detecting resistant, virulent, and biofilm-forming *Salmonella*. In conclusion, these findings highlight, for the first time, ducks as silent reservoirs of high-risk Salmonella strains, posing a menace to the human-animal-environment interface.

Initiatives and Strategies for the Eradication Plan for Peste des Petits Ruminants (PPR-EP) in India by 2030: A Comprehensive Approach to Combatting Small Ruminants Plague

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Peste des Petits Ruminants (PPR), is caused by a morbilivirus closely related to rinderpest virus and affects goats, sheep and some wild relatives of domesticated small ruminants as well as camels and known as 'Small Ruminants Plague,'. This disease, caused by the PPR virus, presents symptoms such as fever, eye and nasal discharges, ulcers, gastroenteritis, and bronchopneumonia. It has significant economic implications, due to severe morbidity and mortality rates especially in areas where it is endemic. To address this, global initiatives like the PPR Global Control and Eradication Strategy (GCES) and the PPR Global Eradication Programme (PPR-GEP) have been introduced with the goal of eradicating PPR by 2030. In India, where PPR remains prevalent among sheep and goats, efforts to combat the disease began even before the global PPR-GEP was established in 2017. This program emphasizes rapid diagnosis, surveillance, monitoring, and vaccination. India's vaccination efforts commenced in 2002, with nationwide campaigns launching in 2011. In 2022, the Department of Animal Husbandry and Dairying (DAHD) of the Government of India introduced the PPR Eradication Programme (PPR-EP) under the Livestock Health and Disease Control (LHDC) for PPR Eradication. The PPR-EP main features include comprehensive vaccination of sheep and goats until 2025-26, achieving herd immunity and halting the spread of the virus by 2027-28, and declaring freedom from PPRV infection by 2029. The strategic plan also involves mass vaccination targeting specific populations, such as those above 4 months of age, to achieve an immunity level of 80-90%. Additionally, there will be sero-monitoring, outbreak investigations, and surveillance in various hosts, including wildlife. Importantly, due to the threat of the disease spreading from bordering states, strategic vaccination efforts will be targeted at migratory populations and border regions to ensure comprehensive coverage and containment. The program will also provide training for veterinarians, vaccinators, and support staff, and conduct awareness programs for different stakeholders. Real-time monitoring of vaccination data, vaccine logistics, and cold chain maintenance will be emphasized. The eradication of PPR will have a positive impact on low to medium-income farmers who rely on sheep and goat farming, leading to increased income and food security. The consistent supply of products from these animals will also benefit consumers.

Oral Presentations

Understanding the genetic and antigenic variations in Foot-and-mouth disease virus during its persistence in bovine under natural conditions

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The naïve population is at risk due to the FMD virus (FMDV) persistence in bovine, which also affects control programs and international trade. The present investigation aimed to understand the genetic and antigenic variations of FMDV during its persistence in bovines under natural conditions to provide the basis for effective control strategies. A longitudinal study of FMDV persistence in cattle and buffalo was carried out after the diagnosis of FMD for a period of 24 months. Regularly vaccinated, clinically affected, or asymptomatic cattle and buffalo were sampled during and after the outbreak in the study site. Oesophageal-pharyngeal fluid (OPF) and serum were regularly collected at 2-month intervals. Antibody to nonstructural protein (NSP-Ab) was tested by 3AB3 indirect ELISA, while nucleic acid detection was carried out by real-time RT-PCR (target 3D gene) and multiplex RT-PCR (target VP1 gene). Virus isolation (VI) from the clinical samples was carried out in LFBKαvβ6 cells, and antigenic variation was carried out by two-dimensional microneutralization test. (2D-MNT). The important findings of this study are that (i) in cattle, the NSP reactor varied from 68.75% to 100% for asymptomatic and 83.33% to 100% in clinically affected animals. In Buffalo, in both categories, the proportion of NSP-antibody-positive animals was less (varying from 0 to 37.5%) than cattle despite having a higher proportion of genome-positive OPF samples. (ii) FMDV persists in cattle and buffalo up to 7 months post infection (mpi) and 13 mpi, respectively, at their sites of persistence. (iii) The mean nucleotide substitution was estimated to be 1.816×10^{-2} substitution/site/year (s/s/y) with a 95% credibility interval of $1.362\text{-}2.31 \times 10^{-2}$ s/s/y. This clearly indicates higher selection pressure on the virus genome during persistent infection, which may result in the generation of genetic and antigenic variants, and (iv) antigenic and genetic variants are generated during the persistent phase of the disease, and during a natural outbreak, the generation of antigenic variants may be more frequent in carrier buffalo than in cattle. This study helps in the understanding of the evolution of FMDV in a host and at a herd during the persistence of FMDV in bovine.

Identification of recombinant Porcine Astrovirus strain from Haryana provide insights into viral evolution

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Recombination in RNA viruses is a driving force in evolution and emergence of novel viruses that could lead to changes in virulence of the virus, higher genetic diversity and the likelihood of adaptation to new hosts. Porcine Astrovirus (PAstV) is a non-enveloped spherical virus having positive-sense, single-stranded RNA genome of about 6.4-7.3 kb in length. The viral genome comprises three open reading frames (ORFs), namely ORF1a, ORF1b, and ORF2. In India, however, genetic

characterization of PAstV remains limited, primarily based on partial sequences of the ORF1b and ORF2 regions. The present study was undertaken to characterize the complete genome of PAstV in pigs from Haryana, India. In this investigation, 70 fecal samples were screened for the presence of PAstV using RT-PCR, of which 22 (31.42%) samples tested positive for PAstV. Three PAstV-positive samples were selected, and the complete genome was amplified by RT-PCR in two overlapping segments. Whole genome sequencing was conducted using the Illumina NovaSeq6000 platform, and recombination analysis was performed utilizing RDP v4.101 and SimPlot v3.5.1 software. The analysis identified two recombination events in the ORF1a region of PV247173 (LUVAS/2023/PAstV_20), with PV247174 (LUVAS/2023/PAstV_21) as the major parent and OL689632 (PAstV1/Ah-1/China/2022) as the minor parent. The recombination observed in PAstV strains from geographically distant locations suggests that pig trade and fecal–oral transmission have likely facilitated the exchange of viral genetic material. This study reports the first whole-genome molecular characterization and identification of recombinant PAstV from Northern India, contributing to our current understanding of PAstV epidemiology, diversity, and evolution.

Molecular characterization of emerging Ungulate tetraparvovirus 3 in swine herd of Punjab, India

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The swine industry is an important livestock sector across the world, often affected with various pathogens including certain emerging viral infectious agents. Porcine parvoviruses (PPVs), members of the Parvoviridae family belongs to different genera. Apart from PPV1 (Ungulate protoparvovirus 1), other novel PPVs are gradually emerging in many parts of the world. Porcine parvovirus 2 (PPV2) i.e., Ungulate Tetraparvovirus 3 is among the novel and emerging porcine parvoviruses in many parts of the world including India, which is often associated with respiratory and reproductive affections. The tetraparvoviruses are among the emerging viral pathogens in the Indian subcontinent. There are sparse/no systematic reports on characterization of these viruses from the country. Similarly, lack of molecular epidemiological datasets pertaining to these viruses in swine herds from Northern part of the country including Punjab, India necessitates molecular characterization of these emerging pathogens. The strategy focused on the amplification and molecular characterization of ORF2 gene through PCRs using in house designed primers targeting six overlapping regions of the genome. The purified PCR products were sequenced by Sanger's dideoxy sequencing technology in both forward and reverse directions to ensure bidirectional coverage and accuracy of the obtained nucleotide sequences. The overlapping sequences were aligned and analyzed with the reference sequence data. Further, pairwise distance was calculated as well as the phylogenetic tree were constructed using the maximum likelihood method with 1000 bootstrap replicates, and the best evolutionary model to determine the ancestral linkages. Present study reported molecular characterization of nearly complete capsid protein encoding gene (~3060 base pair) of the virus. There were 5 missense mutations observed including three transitions at the positions 770th, 3049th and 3050th as well as two transversions at the positions 640th and 743rd. A total of 6 amino acid substitutions were observed at the position 209th, 214th, 248th, 257th, 714th and 1017th. Pairwise distance analysis revealed high sequence similarity to Chinese, Brazilian and Hungarian sequences. Phylogenetic analysis based on ORF2 gene sequences on the basis of Maximum Likelihood and Bayesian Inference methods also revealed clustering of the local PPV2 sequence with the Chinese, Brazilian and Hungarian strains. The

present study reported the characterization of PPV2 from India based on the nearly complete capsid protein encoding gene sequence. This will ensure strengthening the viral gene pool datasets from the country. Phylogenetic clustering indicated a potential evolutionary link between geographically distinct PPV2 isolates, suggesting possible shared viral ancestry. Such findings are suggestive of possible global circulation patterns and underline the importance of international genomic comparisons in understanding PPV2 epidemiology.

Studies on Porcine teschovirus (PTV) infection in piglets of Madhya Pradesh, India

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Pigs are important farm animals and are potentially profitable for farmers than other livestock species owing to high feed conversion efficiency, high fecundity, early maturity, and short generation interval. The occurrence of diseases can significantly reduce the profitability of the pig farming due to high piglet mortality and reduced reproductive performance. One such infection is the porcine teschovirus (PTV) group under porcine enteric picornaviruses, which can cause neurological problems and diarrhoea leading to significant losses for the farmers. Keeping the above facts into consideration the study was planned to find out the prevalence and pathology of porcine teschovirus (PTV) in organized and un-organized pig farms of Madhya Pradesh. Porcine teschovirus (PTV) group under porcine enteric picornaviruses, can cause neurological problems and diarrhoea leading to significant losses for the farmers. Keeping the above facts into consideration the study was planned to find out the prevalence and pathology of porcine teschovirus (PTV) in organized and un-organized pig farms of Madhya Pradesh. A total of 150 fecal samples/rectal swabs were collected from pig farms of Vindhya and Jabalpur regions of Madhya Pradesh. In addition, tissue samples from lungs, tonsils, brain, intestine and lymph nodes were collected form naturally fallen piglets presented for necropsy at department of Veterinary Pathology, College of Veterinary Science and A.H., Rewa, Madhya Pradesh. All the samples were subjected to RT-PCR and histopathology. A total of 17 samples out of 150 faecal samples and rectal swabs were found positive for PTV using RT-PCR, yielding an 11.3% PTV positivity rate. A total of two Indian isolates from the current study were matched with the reference PTV sequences and blast analysis was performed for phylogenetic analysis. Histopathology revealed interstitial pneumonia, haemorrhagic enteritis, sloughing of villi, meningeal congestion, swelling of endothelial cells, perivascular cuffing and neuronophagia. The current study revealed the prevalence and pathology of highly neglected PTV in piglets of Madhya Pradesh. The study underlined the significance of constant surveillance and monitoring of porcine teschoviruses to contain this economically significant class of porcine pathogen.

Molecular detection and phylogenetic analysis of *Dichelobacter nodosus*, *Fusobacterium necrophorum* and *Treponema* spp. associated with footrot disease in sheep and goats in Himachal Pradesh

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Footrot disease, prevalent worldwide in sheep and goats, is a contagious bacterial disease caused by Gram-negative, anaerobic, rod-shaped *Dichelobacter nodosus* and *Fusobacterium necrophorum*. It causes pain, lameness, and impaired ability to graze, reproduce, and contribute to reduced wool and meat production leading to significant financial losses. This study was conducted to detect *Dichelobacter nodosus*, *Fusobacterium necrophorum*, and *Treponema* spp. in Footrot-suspected sheep and goats in Himachal Pradesh and to study their phylogenetic relationship.For this, a total of 45 investigations (34 migratory Gaddi sheep & goat flocks and 11 slaughter point visits) were conducted during a period of one year (October 2024 to September 2025). Foot swabs were collected from 153 animals, followed by the extraction of bacterial DNA. PCR based on primers targeting the species-specific 16S rRNA (783 bp amplicon), lktA gene (405 bp), and 16S-tRNAII region of ISR2 (335 bp) was performed for the molecular identification of *D. nodosus*, *F. necrophorum* and *Treponema* spp., respectively. PCR amplicons of D. nodosus (6 no.), F. necrophorum (4 no.), and Treponema spp. (3 no.) were commercially sequenced and obtained NCBI accession numbers. Nucleotide BLAST analysis, multiple sequence alignment using MegAlign Pro, DNA-STAR software, and phylogenetic analysis by the Neighbor-Joining method using MEGA4 software were carried out.Out of the total 45 investigations, 36 flocks'/slaughter point animals (80%) showed characteristic signs of Footrot, with 25 flocks'/slaughter point animals (55.55%) found positive based on PCR detection. The infection patterns were as follows - Solitary infections: *D. nodosus* (4), *F. necrophorum* (24), and *Treponema* spp. (9). Coinfections: *D. nodosus*+*F. necrophorum* (22), *D. nodosus*+*Treponema* spp. (3), *F. necrophorum* and *Treponema* spp. (6) and all 3 organisms (17). The sequence alignment and phylogenetic tree analysis of *D. nodosus* isolates revealed genetic similarity to each other, followed by isolates from France and Sweden. *F. necrophorum* isolates were found to be similar to each other, followed by isolates from New Zealand, India (J&K), Pakistan, China, and Iraq. *Treponema* spp. isolates were also found to be similar to each other, followed by isolates from J&K in India, Denmark, and Norway.In conclusion, *F. necrophorum* alone was responsible for the highest number of Footrot cases, followed by co-infection with *D. nodosus* and *F. necrophorum* and co-infection with *D. nodosus*, *F. necrophorum*, and *Treponema* spp. The above findings point towards the presence of multiple bacterial etiologies contributing to the higher incidence and severity of Footrot disease. The phylogenetic analysis showed their genetic similarity to isolates from J&K in India and other parts of the world. Therefore, continued surveillance is required to know the genetic similarity between isolates, which would play a role in the development of an effective vaccine against Footrot disease.

Decoding the molecular landscape of Canine adenovirus type 1: Evidence of strain diversification in Indian dog population

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Canine Adenovirus Type 1 (CAV-1) is the etiological agent of infectious canine hepatitis (ICH), a highly contagious disease affecting domestic and wild canids. Belonging to the genus Mastadenovirus within the family Adenoviridae, CAV-1 is a non-enveloped, double-stranded DNA virus that primarily targets hepatocytes and endothelial cells, leading to hepatic necrosis, vascular injury, and disseminated intravascular coagulation. Infected dogs typically exhibit fever, abdominal pain, corneal opacity ("blue eye"), and jaundice, with severe cases resulting in acute hepatic failure. The virus is transmitted via urine and other secretions and can persist in the environment for extended periods, complicating control measures. Molecular characterization of the CAV-1 isolates of dogs in India. Molecular characterization of the partial E3 gene regions of Indian CAV-1 isolates was undertaken using PCR and sequencing approaches. Phylogenetic analysis revealed distinct clustering of Indian CAV-1 sequences, forming a separate lineage from established global strains. Unique genetic insertions identified in the E3 region may influence immune modulation and host adaptation mechanisms of the virus.

Establishment of experimental animal models to investigate the pathogenesis of Canine adenoviruses

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Canine adenoviruses (CAVs) are significant viral pathogens responsible for fatal diseases in dogs. Two distinct types of CAVs have been identified: CAV type 1 (CAV-1), the causative agent of infectious canine hepatitis, and CAV type 2 (CAV-2), associated with infectious tracheobronchitis. These are highly contagious viruses and transmitted through contact with the saliva, urine, or feces of infected animals. CAVs infect a broad range of hosts, including canines, bears, seals, raccoons, ferrets, and walruses. However, suitable laboratory animal models for studying CAV pathogenesis are still limited. The present study aimed to identify an appropriate laboratory animal model for CAV research by evaluating pathological, virological, and immunological responses following infection. Dunkin-Hartley guinea pigs, Syrian golden hamsters, BALB/c mice, and Wistar rats (2–3 weeks old) were inoculated with CAV-1 via intravenous and intraperitoneal routes. Animals were euthanized at sequential intervals between 1 and 21 days post-infection for pathological assessment. Guinea pigs

infected intravenously exhibited severe neurological signs, high mortality, and marked gross and histopathological lesions, including prominent intranuclear inclusion bodies, findings more severe than those observed via the intraperitoneal route. Syrian hamsters infected intraperitoneally displayed clinical signs, mortality, and pathological changes, although intranuclear inclusion bodies were absent in both routes. BALB/c mice showed no mortality, mild clinical signs, and limited pathological alterations, with enlarged lymph nodes but without intranuclear inclusion bodies. Wistar rats exhibited neither clinical signs nor mortality, and only mild pathological changes were observed in both infection routes. The susceptibility ranking to CAV-1 infection was: Dunkin-Hartley guinea pigs>Syrian hamsters>BALB/c mice>Wistar rats. In conclusion, guinea pigs appear to be the most suitable laboratory animal model for studying the pathogenesis of CAVs, particularly during the acute phase of infection. They also provide a valuable system for investigating endotheliotropism, neuropathology, and vaccine-induced immune responses against CAVs.

One Health approach to investigate a Japanese Encephalitis Outbreak in Jalpaiguri, West Bengal

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Japanese encephalitis (JE) remains a major public health concern in India, with periodic outbreaks reported from endemic states such as West Bengal. The present investigation aimed to elucidate the source and circulation of JE virus (JEV) during a human outbreak in Jalpaiguri, West Bengal, in July 2025. To assess the potential role of pigs as amplifying hosts, concurrent surveillance was conducted in nearby villages and adjoining district, covering all seven blocks of Jalpaiguri and four blocks of Cooch Behar district. A total of 630 pig serum samples were tested for anti-JEV IgM and IgG antibodies using rNS1-based indirect ELISA(s). Molecular detection of JEV RNA from serum samples was performed using real-time RT-PCR, followed by nested RT-PCR targeting the C/prM gene for sequencing and phylogenetic analysis. The sero-positivity rates for IgM and IgG antibodies were 71.43% and 58.25%, respectively, with an overall seroprevalence of 75%, indicating both recent and past exposure to JEV among the pig population. The phylogenetic analysis revealed circulating strain as genotype III of JEV. These findings confirm active viral transmission in the affected area, with pigs serving as sentinel indicators of JEV circulation. The results, along with recommended control measures, were communicated to the State Animal Husbandry and Health Departments, aiding in containment of the human outbreak. The study underscores the importance of integrated One Health surveillance involving humans and animals for the early detection and control of JE outbreaks in endemic regions.

Cytokines expression Analysis in Lumpy Skin Disease Affected Cattle

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Lumpy Skin Disease (LSD) is a vector-mediated disease that affects cattle and buffaloes, including some wild ruminants, caused by Lumpy Skin Disease Virus (LSDV), which belongs to the genus *Capripoxvirus*. The present study was conducted to understand the clinical presentation and to study the expression of pro-inflammatory and anti-inflammatory cytokine genes in LSD affected cattle. The clinical signs were recorded during the field investigation, along with the collection of samples such as blood, serum, nasal swabs, and skin tissues. Haematology, histopathology, and serum samples were tested for LSDV antibodies using an indirect ELISA developed at ICAR-NIVEDI, Bengaluru. The LSDV was confirmed in the skin tissues by immunohistochemistry and PCR. The peripheral blood mononuclear cells were isolated from whole blood and used for gene expression analysis. Varied clinical

signs were observed such as fever, nasal and lachrymal discharge, brisket and limb edema, decreased milk production, skin nodules at different stages of disease presentation, including generalized farm in late stages. The total leukocyte count and absolute lymphocyte count were significantly higher in the late stages of LSDV-affected cases compared to controls. The microscopic changes were associated with vasculitis and myositis in the superficial and deep dermis regions in affected skin. The virus was predominantly found in epithelial cells of the basal layer of the epidermis, hair follicles, and the epithelial cells of sweat and sebaceous glands. The gene expression revealed reduced levels of cytokines (IL-2, IL-4, IL-10, IFN-gamma, and GM-CSF) in PBMCs and skin tissues from LSDV-affected cattle. These systemic cytokine reductions reflect LSDV strategies to evade host immunity, facilitating its persistence and enhancing disease severity. In conclusion, the LSD was presented in different forms in the field conditions, and cytokines play a major role in viral pathogenesis, which needs further detailed studies in controlled experimental conditions for designing therapeutics and antiviral targets in the future.

Poster Presentations

Effusive feline infectious peritonitis in India: Prevalence and diagnostic recommendations

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Feline Infectious Peritonitis (FIP) is a severe, immune-mediated condition in cats caused by a mutated form of Feline Coronavirus (FCoV). While FCoV typically causes mild gastrointestinal symptoms, around 12% of cases progress to fatal FIP, which presents in either wet (effusive) or dry (non-effusive) forms. Diagnosing FIP remains challenging due to nonspecific clinical signs and the high cost of molecular diagnostics. This study aimed to determine the prevalence of effusive FIP in India and assess the diagnostic utility of the cost-effective Rivalta test in clinical settings. The study was conducted on 159 effusion fluid samples from cats suspected of having FIP. Data on age and gender was recorded. Rivalta test was conducted on all samples. A sensitive in-house SYBR-based real-Time RT-PCR served as the gold standard test to confirm FIP. Our findings showed that the overall prevalence of FIP among the suspected effusion fluid samples (n= 159) was 42.8%. FIP was more prevalent among younger cats (under 3 years old) at 53.1%, compared to older cats (over 3 years old) at 16.7%. Male cats were more susceptible (49.5%) to FIP effusions than females (32.7%).

The Rivalta test, a simple and affordable diagnostic tool, had a high sensitivity of 97%, meaning it was very effective at identifying cats with the disease. However, its specificity was low at 49.5%, indicating it frequently returned positive results for other conditions causing effusions, such as septic peritonitis/pleuritis or lymphoma. The positive predictive value (PPV) was also low at 58.9%, suggesting that a positive Rivalta test alone is not a strong indicator of FIP. On the other hand, the Rivalta test had a high negative predictive value (NPV) of 95.7%, making it highly reliable for ruling out the disease. In conclusion, while the Rivalta test cannot definitively confirm FIP due to its low specificity, its high NPV make it a valuable tool to rule out FIP. Its affordability, speed and ease of use support broader adoption in clinical settings. When combined with cytology, Total Protein levels, and the Albumin: Globulin ratio, the Rivalta test can guide decisions on whether confirmatory RT-PCR testing is warranted.

Pathological and molecular characterization of porcine circovirus associated diseases in pigs

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Porcine circoviruses (PCVs) are significant viral pathogens that pose a major challenge to swine production, leading to substantial economic losses. The present study was undertaken to investigate the occurrence and pathology of PCV associated diseases (PCVAD) and to perform molecular characterization of circulating virus. A total of 219 clinical and necropsy samples collected from pigs in the Bareilly district were screened for Porcine Circovirus 2 (PCV2) using gene-specific PCR primers. Hematobiochemical parameters and pro-inflammatory cytokine profiles were analyzed to assess the systemic impact of infection. Immunohistochemical examination of PCR-positive tissues was carried out to localize viral antigens within affected organs. Additionally, molecular characterization of the ORF2 gene from two representative isolates was performed to study genetic variation and phylogenetic relationships. PCV2 was confirmed in 59 samples (26.94%), of which 27 were mono-infections and 32

were co-infections with porcine parvovirus (PPV1), *Streptococcus suis*, and *Haemophilus parasuis*. Age wise analysis showed highest prevalence in grower pigs (33.92%), followed by suckling piglets (25%), weaners (24.35%), and adults (23.07%). Lungs, lymph nodes, spleen, tonsils, intestines, kidneys, and liver were major organs positive for PCV2. Clinically, infected pigs displayed progressive wasting, poor body condition, respiratory distress, anorexia, and sudden death in some cases. Hematobiochemical analyses revealed anemia, neutrophilia, lymphopenia, and elevated liver enzymes (ALT, AST), indicating systemic immunosuppression and organ dysfunction, respectively. Cytokine profiling demonstrated stage-dependent immune responses: early infection marked by strong IFN-γ activity, while late stages showed elevated IL-10, suggesting immunoregulation favoring viral persistence. Gross lesions included emaciation, hydrothorax, hydropericardium, pulmonary edema, hemorrhage, and enteritis. Microscopically, lymphoid depletion, bronchointerstitial pneumonia, interstitial pneumonia, myocarditis, hepatic necrosis, nephritis, and enteritis with lymphoid depletion in GALT were observed. Immunohistochemistry confirmed broad tissue distribution of PCV2 antigen in lymphoid cells, epithelial cells of multiple organs, and mononuclear cells, corroborating systemic viral dissemination. Molecular characterization of the ORF2 gene from two isolates showed high genetic similarity (97.8–99.0%) with Asian PCV2 strains, clustering within the PCV2d genotype, consistent with current global epidemiological trends.

It is inferred from the study that porcine circovirus associated disease is significantly prevalent in the region, often with co-infection by PPV1 and the circulating porcine circovirus belongs to PCV2d genotype causing diverse pathological lesions in pigs.

Characterization of Lumpy Skin Disease (LSD) virus from an outbreak in cattle in Meghalaya, India

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Lumpy Skin Disease (LSD) virus is a rapidly emerging pathogen causing significant economic impact on the livestock farmers. The disease it caused in cattle affects production due to high morbidity rate and low mortality. Here, we presented cases of two outbreaks of LSD in non-vaccinated herd from two districts in Meghalaya where the scabs and sera samples were received for diagnosis in the laboratory. The molecular detection of LSD virus specific gene by PCR method and correlating with the clinical signs and lesion to confirm the disease. The representative scab and serum samples were processed for isolation of virus in MDBK cells for its characterization. The molecular detection of LSD virus specific p32 envelope protein gene by PCR method and correlating with the clinical signs and lesion confirms the disease. Serial 5 blind passages were done for the first isolate LSDV-NS/B-16/05/25 till the apparent cytopathic effect (CPE) appears while the second isolate LSDV-SK-16/07/25 gives CPE after 3 blind passages. The CPE in the form of irregular shapes of cell with thin long projections, shrinking and aggregation of cells, degeneration and detachment of monolayer were observed. Intermittent plaques developed in subsequent serial passages by 10 passages for first isolate and 6 passages for second isolate. However, the plaques were not consistent in plaque assay for titration of virus. Therefore, virus titration was done by TCID50 in 96 well cell culture plate and calculated by Reed and Munch method. Characterization of virus is important to understand the possible emergence of mutated strains of virus where the new variants may become more virulent or may not be effectively controlled by existing vaccines, leading to more outbreaks and potential economic losses for livestock production.

Understanding of foot-and-mouth disease virus ecology at wildlife-livestock interface

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Foot and Mouth Disease (FMD) is a highly contagious transboundary viral disease of cloven-hoofed animals (livestock and wildlife). The disease is caused by FMD virus (FMDV). In India, there was a direct loss of USD 3159 million (INR 221,110 million) due to FMD. The Government of India has been implementing an intensive FMD control program (FMDCP), aiming to control and eliminate the disease by 2030. But due to broad host range, multiple transmission factors, and the virus persistence FMD control program complicated. Wildlife-livestock interfaces (WLI) are areas where the ranges and resource use of wild and livestock species overlap and this may increase in their interactions and may lead to the emergence of FMDV or any other infectious disease. The role of wild ungulates and livestock in the spread and maintenance of FMDV has not been studied in India, although disease has been reported in captive elephant, deer, gaur etc. 1428 serum samples were collected randomly from livestock (cattle and buffaloes) at WLI and tested for presence of NSP antibody against FMD. It was found that 19.09 % and 2.27 % of cattle and buffalo, respectively were found positive of 3AB3 anti-NSP antibody against FMDV. Subsequently, from NSP reactors oropharyngeal fluid (OPF) were collected from 159 animals and tested for FMD viral genome by mRT-PCR and real time RT-qPCR. Out of 159 OPF, 12 and 47 samples found positive of FMD viral genome by mRT-PCR and real time RT-qPCR, respectively. Out 47 FMD positive samples, 3 FMD virus serotype A was isolated. Detection of anti-NSP antibodies, viral genome, and isolation of FMD virus form domestic animals indicate circulation of FMD virus at WLI and it has a real threat for wildlife and it may complicate the FMD control program. Therefore, there is a need of FMD control strategies implementation at WLI like regular biannual vaccination and systematic FMDV surveillance.

Serological Responses against Crucial *Plasmodium vivax* Antigens from Geographically Diverse Malaria Endemic Regions of India

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Malaria remains a significant public health concern in India, with Plasmodium vivax contributing substantially to the overall malaria burden. Despite declining transmission rates, P. vivax continues to cause recurrent infections due to its ability to form hypnozoites and relapse. Understanding naturally acquired immunity against P. vivax antigens in endemic populations is critical for identifying immune correlates of protection and prioritizing vaccine targets. Serological surveillance provides a valuable tool for assessing exposure patterns and immune responses within populations from diverse transmission settings. The objectives of this study was to identify P. vivax antigenic regions of full-length proteins reported in previous studies, recombinant expression & purification of selected P. vivax antigens in heterologous expression system and validation of functional immune response against the selected antigens.

1. Identification of P. vivax Antigenic Regions- Previously reported P. vivax proteins were reviewed to identify immunogenic regions based on published serological data and predicted B-cell epitopes. Antigens representing distinct parasite life stages—PvCSP, PvRAMA, and Pvs48/45—were selected for further experimental evaluation.

2. Recombinant Expression and Purification- Selected antigenic regions were cloned into suitable expression vectors and expressed recombinantly in *E. coli*. Purified proteins were obtained using affinity chromatography, and their integrity confirmed by SDS-PAGE and Western blot analysis with anti-His antibodies.
3. Validation of Functional Immune Response- Purified recombinant antigens were evaluated by ELISA using sera collected from individuals in malaria-endemic regions of India. IgG antibody responses were measured to determine seropositivity, antigen immunogenicity, and naturally acquired immune recognition patterns across study populations

   A significant prevalence of IgG antibodies was observed against all three P. vivax antigens, indicating sustained exposure to malaria parasites in these populations. Among the tested antigens, PvCSP elicited the highest seropositivity rate and antibody titers, suggesting frequent exposure to sporozoite stages. PvRAMA and Pvs48/45 showed moderate responses, reflecting immune recognition of blood-stage and transmission-stage antigens, respectively. The observed variations in antibody levels across regions likely correspond to differences in local transmission intensity and exposure history. The study demonstrated significant naturally acquired immune responses against PvCSP, PvRAMA, and Pvs48/45, indicating ongoing P. vivax exposure in endemic regions. These findings highlight their potential as serological biomarkers for surveillance and as promising candidates for inclusion in multi-stage malaria vaccine development and elimination strategies

Molecular Characterization of *Peste Des Petits Ruminants Virus* circulating in Uttarakhand

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Goats are an essential part of India's livestock sector due to rapid reproduction, high fertility and easy marketability. Among infectious diseases of small ruminants, Peste des petits ruminants (PPR) is most devastating, leading to nearly 100% morbidity and up to 90% mortality. The aim of this study was to determine the prevalence of PPR in Uttarakhand and molecular characterization of *Peste des petits ruminants virus* circulating in Uttarakhand. In this study, 105 suspected animals were sampled from eight sites in six districts of Uttarakhand. Virus was isolated in Vero cells, showing cytopathic effects. Swab samples were confirmed for PPRV through a 350 bp N gene fragment. Selected positive samples were subjected to full-length N (1578 bp) and M (1008 bp) genes amplification followed by Sanger sequencing. Bioinformatics analysis was performed. In this study, 105 suspected animals were sampled from eight sites in six districts of Uttarakhand. Of these, 39 were tested positive by partial N gene RT-PCR (37.14% prevalence). Virus was isolated in Vero cells, showing cytopathic effects starting with cell rounding at 24 hpi, cell fusion and synctium formation at 48 hpi, and cell death by 72 hpi, confirmed by RT-PCR targeting partial N gene (350 bp amplicon). Swab samples were confirmed for PPRV through a 350 bp N gene fragment. Selected positive samples were subjected to full-length N (1578 bp) and M (1008 bp) genes amplification followed by Sanger sequencing. Bioinformatics analysis included mutation profiling, pairwise distance, percent identity, selection pressure and phylogenetics. Molecular characterization revealed that Uttarakhand isolates belong to Lineage IV, clustering with contemporary Indian sequences. Molecular characterization revealed that Uttarakhand isolates belong to Lineage IV, clustering with contemporary Indian sequences. Several synonymous and non-synonymous substitutions were identified as temporal and geographical markers, while selection pressure analysis indicated most codons of N and M genes are under purifying selection.

Emergence of Lumpy skin disease virus clade 1.2b in India: Changing disease dynamics vis-a-vis molecular characterization

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Lumpy skin disease (LSD) is a transboundary viral disease causing substantial economic losses. Effective diagnosis, surveillance, and control requires detailed molecular characterization of LSDV strains currently circulating in India. Such genetic insights are essential to understand virus evolution, trace transboundary incursions, and design effective diagnostics and vaccines for emerging LSDV variants. To perform comprehensive molecular characterization of recent Indian LSDV isolates to elucidate their genetic diversity, identify lineage-specific mutations, and differentiate emerging field strains for effective surveillance and control. For molecular characterization, seven virulence-associated genes-GPCR, RPO30, A36R (ORF126), C7L (ORF067), B22R (ORF134), ORF019, and ORF144, were amplified, cloned into pGEMT-Easy vector, sequenced, and edited. The obtained sequences were aligned with representative global LSDV strains to identify nucleotide polymorphisms, insertions, and deletions, and to determine their phylogenetic clustering patterns. Comparative sequence analysis of RPO30, C7L, B22R, ORF019 genes enabled clear differentiation between the classical Kenyan-type (1.2a) and the newly emerging wild-type (1.2b) LSDV strains circulating in India. Whereas, GPCR and A36R genes can be targeted for PCR-based differentiation of 1.1 (vaccine)/1.2a (Kenyan)/1.2b (wild) LSDV strains. Recent LSDV-RJ/22, LSDV-Mukt/23, LSDV-Ntl/UK/22 samples clustered with 1.2b wild type LSDV strains, whereas older LSDV strains of 2019-2021 clustered with 1.2a Kenyan type LSDV strains with most of the genes. These consistent genetic variations act as molecular markers for rapid differentiation of emerging field strains. The findings strongly suggest a recent transboundary incursion of LSDV 1.2b into India, likely through the western border regions, highlighting the need for continuous genomic surveillance and strengthened biosecurity measures to monitor and mitigate future outbreaks. As there is new strain circulating in India, cross-protection of current vaccines against the new strains needs to be determined.

Molecular Detection of Tick-borne Hemoparasites in Dogs: A Quantitative PCR Approach

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Vector-borne hemoparasites such as *Babesia gibsoni*, *Babesia vogeli*, and *Ehrlichia canis* pose significant health threats to dogs, causing a wide spectrum of clinical manifestations that may progress to severe, life-threatening illness. These pathogens are primarily transmitted by ticks, making their detection critical for effective clinical management and disease control. In the present study, clinical samples from dogs were screened using a real-time PCR assay to determine the occurrence of these infections. The results revealed frequent detection of *B. vogeli* and *E. canis*, with an occurrence of 30.76% (8/26) and 26.92% (7/26), respectively. *B. gibsoni* was detected at a lower rate [3.84% (1/26)]. Notably, co-infections were identified in 11.53% (3/26) of cases, highlighting the complexity of canine vector-borne diseases. The study underscores the importance of routine molecular surveillance and the adoption of sensitive diagnostic tools for accurate detection of hemoparasite infections in endemic areas, along with the implementation of effective tick-control strategies.

Genetic characterization and cross-neutralization study of bluetongue virus serotype 2 isolates from India

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Bluetongue (BT) is an infectious, non-contagious, and economically important viral disease of domestic and wild ruminants, most commonly in sheep, transmitted by Culicoides midges. In the present study, BTV-2 isolates from India were characterized to study the phenotypic antigenic and phylogenetic relationship. The neutralization behavior of BTV-2 isolates (BTV-2/CHN/IND, BTV-2/HYD/IND, BTV-2/IND/92, BTV-2/IND/93) was studied using the beta method of Serum Neutralization test with its Hyper Immune Serum (HIS) raised against these isolates in rabbits. The neutralization titer was calculated using the Reed and Muench method, and mean anti-log values of titers were used for calculation of antigenic relationship (r) Full genome sequence was carried out for BTV-2 isolates (n=4) by Next Generation Sequencing (NGS) method. The phylogenetic relationship and nucleotide identity of all four BTV-2 isolates were compared with previously reported Indian and global BTV-2 isolates. Nucleotide sequence data were aligned using the CLUSTAL W algorithm. Phylogenetic analyses were carried out using MEGA software with the best nucleotide substitution model. BTV-2/HYD/IND contributed the lower range of 'r' mean value (0.1 to 0.42) which showed higher magnitude of neutralization resistance with all the isolate specific heterologous HIS. BTV-2/CHN/IND, BTV-2/IND/92, BTV-2/IND/93 isolates showed wide antigenic relationship where r mean ranges from 0.40 to 0.96 was observed against the isolate-specific heterologous HIS. Phylogenetic analysis based on the VP2 and VP5 encoding genes segregates BTV-2/CHN/IND, BTV-2 HYD/IND, BTV-2/IND/92, and BTV-2/IND/93 in a distinct cluster with Indian BTV-2 with proximity to the Eastern topotype. The neutralization behaviors of BTV-2 isolate-specific HIS suggest probable minor subtype antigenic variation. Full-genome sequencing and phylogenetic analysis indicated that BTV-2 isolates might have evolved from a common Eastern topotype ancestors.

Prevalence and sero-surviellance study of Bluetongue disease with special reference to Northern hilly states of India

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Bluetongue virus (BTV), a vector-borne pathogen affecting domestic and wild ruminants that poses a significant threat to livestock health and productivity in India. This study was conducted to evaluate the seroprevalence and active circulation of BTV among sheep, and goat populations in Northern hilly states of India during the year 2024–2025. A total of 1045 serum samples and 112 whole blood samples were collected from sheep and goats of Himachal Pradesh, Uttrakhand and Jammu and Kashmir. The serum samples were analyzed using in house developed competitive ELISA to detect BTV-specific antibodies, while the blood samples were tested using an antigen capture ELISA to identify active infections. Results revealed that 41 serum samples (3.92%) were positive for BTV antibodies, indicating prior exposure, and 5 blood samples (4.46%) tested positive for BTV antigen, suggesting ongoing viral circulation. These findings confirm the presence of BTV in Northern hilly region of India where earlier vector population was unnoticed. In the climate change scenario, the seroprevalence and antigenic prevalence observed in the present study caution us about the future chances of the clinical disease in hilly region of the country. Furthermore, isolation of virus, serotyping, molecular and phylogenetic characterization is required to obtain the epidemiology of BTV and to implement proper control and preventive measures.

Mapping of protective passive immunity in calves of dams vaccinated with combined vaccine

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Foot and mouth disease (FMD) is a highly contagious viral disease with well-established devastating socio-economic and environmental impacts in the affected regions. For the prevention and control of FMD, multiple vaccinations are performed along with vaccinations against other diseases. These multiple vaccinations impose an extra financial burden on animal owners and cause stress in animals, including pain, discomfort, or sickness. These factors discourage animal owners from vaccination and also induce stress on vaccinators. To avoid this, a combined vaccine may be a good alternative. With such vaccines, the stress of vaccination on animals, the cost of vaccines for animal owners, and stress on vaccinators can be minimized with a suitable vaccination strategy. However, there is limited information regarding the calfhood vaccination with the use of a combined vaccine. The study was designed to estimate the level, duration of transmission, and protective titer of maternal antibodies in newborn cattle calves of dams regularly vaccinated with a combined vaccine of Foot and Mouth Disease (FMD), Hemorrhagic septicemia (HS), and Black quarter (BQ). Twenty (20) newborn calves were included in the assessment of vaccination status by Solid-phase competitive ELISA and virus neutralization test (VNT) during initial 0-180 days. The health status of all the calves were measured by routine biochemical tests and calves maintained healthy parameters throughout the study period. The SPCE revealed protective titre in all the calves at 0 days (immediately after birth). The majority of samples continued to exhibit a protective status up to day 120 (n=18), followed by 135 days (n=9). However, from day 135 onwards, a sharp decline was observed, and by 180 days, only one calf showed a protective titre, suggesting waning immunity over time. Estimation of protective antibodies by VNT demonstrated high titres (>2.6 log□□ SN50%) between 15-45 days of age and maintained a protective threshold up to 120 days. Beyond 120 days, antibody titres progressively dropped, with levels at 135 days remaining protective for serotypes A and Asia-1 only. Thus, it can be concluded that use of combined vaccine may provide passive immunity up to 120 days. However, passive immunity is maintained up to 150 days that can neutralize the active immunization.

Molecular confirmation of canine parvovirus in the suspected clinical cases of canines

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Canine parvovirus is a DNA virus that comes in two strains: CPV-1 and CPV-2. The CPV-2 virus is a small, round, non-enveloped virus with a single standard negative-sense RNA. According to the taxonomic categorization, CPV-2 belongs to the genus *protoparvovirus* and subfamily *Parvovirinae* of the family *Parvoviridae*. It causes haemorrhagic enteritis, one of the most significant and infectious disease that affect dogs which is characterized by anorexia, vomiting, depression, lethargy, foul-smelling diarrhoea, dehydration, and fever. CPV-2 causes severe gastroenteritis, as well as morbidity and mortality rates in domestic dogs and other carnivore domestic or wild animals. This deadly and highly contagious disease is brought on by CPV and is typified by myocarditis and consequent heart failure in puppies younger than three months old. One of the main reasons of CPV2 infection in dogs is vaccination failure. Dogs can contract the disease from one another by coming into direct or indirect

contact with infected faeces. Because of its high sensitivity and specificity, PCR is used for early and confirmatory laboratory diagnosis of the disease. Thirty (n=30) samples of CPV-2 suspected dogs were collected from the university clinic, and then the samples were stored at -20°C. For DNA isolation, PCI (Phenol, chloroform, isoamyl alcohol) method was employed and then, PCR using CPV-2 primers (pCPV-2) was used for confirmatory diagnosis. Out of 30 faecal samples examined, five (n=5) samples showed the presence of CPV-2 with an amplicon of 680bp size. The low confirmation rate suggests that all symptom-based diagnosis needs confirmatory diagnosis for treatment.

Molecular Detection and Characterization of Bacterial Pathogens Causing Urinary Tract Infections in Dogs

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Urinary tract infections (UTIs) are common in dogs, causing discomfort and renal complications, highlighting the need for accurate detection and characterization of causative bacterial pathogens. The bacterial pathogens causing canine UTIs were investigated using molecular and conventional techniques for detection, pathogen identification, and to guide timely therapeutic interventions. A total of 76 urine samples were aseptically collected from dogs via catheterization and cystocentesis. Urinalysis assessed physical, biochemical, and microscopic parameters, including color, pH, specific gravity, glucose, protein, leukocytes, erythrocytes, and crystals. For bacterial isolation, samples were inoculated in nutrient broth and cultured on selective and differential media, including EMB agar for *Escherichia coli* and Mannitol Salt Agar for *Staphylococcus* spp. Microbiological identification involved Gram staining and biochemical tests such as IMViC, catalase, oxidase, coagulase, and hemolysis. Molecular confirmation was performed using species-specific PCR, targeting virulence and pathogen-identification genes (e.g., uidA for E. coli, nuc for *S. aureus*). PCR-based molecular detection confirmed the presence of *E. coli* and *S. aureus*, validating and complementing traditional culture and biochemical findings. Molecular assays demonstrated higher sensitivity, particularly in samples with mixed infections or low bacterial loads. Urinalysis parameters correlated with bacterial presence, aiding in initial diagnosis. The combined application of urinalysis, bacterial culture, and molecular techniques improved diagnostic accuracy and provided detailed characterization of the pathogens associated with canine UTIs. These results emphasize the importance of integrating molecular tools in routine veterinary practice for precise detection and targeted treatment strategies.

Microbial analysis of feces in dogs with hemorrhagic gastroenteritis

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Dogs are considered 'man's best friend' and are the most preferred animal as pets or in-house companions. It is also perceived that our sensitive and animal-loving citizens often come in direct or indirect contact with secretions and excretions of stray or pet dogs, which leaves a possibility of zoonotic disease transmission. Among all the diseases, dogs tend to suffer more from digestive and dermatological disorders. Digestive disorders often exhibit vomiting or diarrhea as conspicuous clinical signs. Hemorrhagic gastroenteritis is a digestive disorder that can be caused by a wide range of etiological factors. The blood-mixed feces/diarrhea from dogs often come in contact with owners, rescuers, caretakers, veterinarians, and hospital staff. Hence, the present study was conducted on 138 cases of hemorrhagic gastroenteritis of varying origins (e.g., bloody feces caused by foreign body ingestion, ancylostomiasis, canine parvoviral infection, food origin, iatrogenic, crushing injuries, and

mixed infections) to check the presence of bacterial pathogens. Swab samples were collected by inserting the sterile swab directly into the rectum, taking all aseptic precautions. Bacteria were isolated using general and specific media for identification. Overall, seven different types of bacterial isolates could be recovered in faecal samples of dogs with hemorrhagic gastroenteritis. Among 138 cases, the overall presence of *Escherichia coli* was highest (95.65%), followed by *Staphylococcus* spp. (81.16%), *Streptococcus* spp. (55.80%), *Salmonella* spp. (33.33%), *Clostridium* spp. (28.26%), *Klebsiella* spp. (21.74%) and *Enterobacter* spp. (00.72%). This data indicates that multiple organisms can be found in dogs with hemorrhagic gastroenteritis. Further studies can be carried out on the molecular detection of pathogenic and zoonotic bacteria as part of the 'One Health Initiative'.

Evaluation of FMDV antibodies using solid-phase competitive ELISA (SPCE) in western Uttar Pradesh, India

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FMD is still an important risk to the global livestock sector due to its rapid spread and major economic effects. In India, all three FMDV serotypes: O, A, and Asia 1 are endemic and pose a great threat to the existing population of bovines. Considering its socio-economic impact, the Government of India is running a biannual vaccination programme across the country. In spite of continuous vaccination, reports of FMD outbreaks are common. One of the reasons may be failure of vaccination. So, pre- and post-vaccination seromonitoring is conducted to establish the status of vaccination. This study presents the seromonitoring data of pre- and post-vaccination of 25 districts of western Uttar Pradesh for the year 2024-2025. The level of antibodies was determined by using a Solid-Phase Competitive Enzyme-Linked Immunosorbent Assay (SPCE-ELISA). A total of 4619 sera samples collected from apparently healthy vaccinated cattle and buffaloes were used to determine antibodies against FMD. Serotyping of sera revealed the presence of protective circulating FMD antibodies against serotypes O, A, and Asia-1 in 31.64%, 26.86%, and 29.10% animals, pre-vaccination, and 67.29%, 54.75%, and 56.69% animals, post-vaccination. The overall protection level due to the vaccination program was 62.1% and 60.9% in cattle and buffaloes, respectively. The present study confirmed the presence of circulating antibodies in the vaccinated animals. However, the percentage of animals carrying protective antibodies titre is a serious concern and needs urgent action for the success of the ongoing vaccination programme.

Prevalence and Antifungal Susceptibility Profile of *Candida* spp. Isolated from Bovine Mastitis

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The increasing prevalence of fungal pathogens in bovine mastitis cases presents a significant challenge to dairy production worldwide. *Candida* spp. has emerged as important etiological agents, particularly in mastitis cases that are unresponsive to antibacterial treatments. In view of the increasing relevance of fungal mastitis in dairy production, the present study was undertaken to investigate the prevalence and antifungal susceptibility of *Candida* spp. isolated from bovine mastitis cases in Junagadh, Gujarat, India. A total of 608 mastitic milk samples were collected from the Veterinary Clinical Complex (VCC), Junagadh, and screened for fungal pathogens. Identification of *Candida* spp. was presumptively based on colony morphology, Gram staining, and germ tube test, and

further confirmed by PCR using genus-specific primers. Antifungal susceptibility testing was performed using the standardized disc diffusion method. Fungal pathogens were detected in 10.5% (64/608) of the samples, with *Candida* species specifically accounting for 1.64% (10/608) of bovine mastitis cases. None of the isolates produced germ tubes. Molecular characterization supported the phenotypic findings, with all presumptive *Candida* isolates yielding amplicons in the expected range of 250–350 bp. Antifungal susceptibility testing revealed complete sensitivity to fluconazole (100%), followed by miconazole (80%), clotrimazole and itraconazole (50% each), and the least sensitivity was found against ketoconazole (40%).These findings highlight the emerging role of *Candida* spp. in bovine mastitis and emphasize the need for routine mycological testing to improve treatment outcomes and reduce economic losses in dairy production.

Session 5:

Future Poultry in Viksit Bharat @2047 and Industry-Academia Interface

Envisioning Poultry Production and Protection under the One Health Landscape in India

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The Indian poultry sector has emerged as a key driver of food and nutritional security, rural livelihood generation, and agricultural growth. However, its rapid expansion and intensification have also amplified One Health challenges involving the interconnected domains of animal, human, and environmental health. Diseases such as avian influenza, salmonellosis, and Newcastle disease, along with the growing threat of antimicrobial resistance (AMR), highlight the urgent need for an integrated approach to poultry production and protection in India.

The One Health framework offers a comprehensive solution by promoting collaboration among veterinary, medical, and environmental sectors. Strengthening disease surveillance, laboratory diagnostics, and real-time data sharing between human and animal health systems can facilitate early detection and containment of zoonotic diseases. Improved farm-level biosecurity, vaccination, hygienic slaughter practices, and regulated live-bird markets are critical to reducing disease transmission and ensuring food safety. Moreover, responsible antimicrobial use and adoption of stewardship programs are essential to combat AMR and preserve the efficacy of existing treatments.

Policy coherence and institutional coordination are central to operationalizing One Health in poultry production. Establishing national and state-level One Health coordination mechanisms, harmonizing legislation, and investing in research and innovation-particularly in genomics, eco-epidemiology, and sustainable waste management-can enhance preparedness and resilience. Equitable inclusion of smallholder and backyard poultry farmers through awareness, training, and compensation mechanisms will ensure that disease prevention efforts are both effective and socially just.

Envisioning poultry production and protection under the One Health landscape in India thus means creating a biosecure, sustainable, and inclusive poultry ecosystem that balances productivity with public health and environmental safety-contributing to national goals of food security, One Health resilience, and the vision of "Viksit Bharat 2047."

Studies on characterization of fowl pox virus (FPV) from outbreaks in backyard poultry clusters of West Bengal

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Fowl pox is frequently reported in India, particularly in backyard and free-range poultry systems where biosecurity measures are limited. A study in Tirupur, Tamil Nadu, recorded a prevalence of 7.66% in native desi chickens. Outbreaks have also been documented in several states, including West Bengal, where persistent infections occur in both vaccinated and unvaccinated flocks. According to a study, circulating FPV strains may possess the ability to evade vaccine-induced immunity. The causative agent, *Fowlpox virus* (FPV), belonging to the *Avipoxvirus* genus, is associated with reduced egg production, poor growth performance, impaired feed conversion, immunosuppression, and, in severe cases, blindness and mortality. The emergence of new FPV variants has heightened concern for disease control, especially as limited genomic data constrain understanding of *Avipoxvirus* evolution, virulence, and host adaptation. The present study aimed to characterize and establish the phylogenetic profile of FPV isolates from field outbreaks in backyard poultry clusters of West Bengal, and to investigate the presence of Reticuloendotheliosis virus (REV) provirus integrated within FPV genomes. A total of 25 suspected FPV samples were collected from affected birds and analyzed by PCR targeting the 4b core protein gene, followed by sequencing. Screening for REV genome integration was performed using FPV–REV junction-specific PCR, REV–LTR PCR, and REV–ENV gene PCR, while attempts were also made to isolate and propagate FPV in embryonated chicken eggs (ECE) and cell culture. All field isolates tested positive for the FPV 4b gene (578 bp), confirming FPV infection. Evidence of REV integration, including amplification of the env gene (807 bp), 5–3′ repeat junctions (291 bp), and 5′LTR regions (370 bp), confirmed the presence of full-length integrated REV provirus in the FPV genomes (FPV/1/2025, FPV/2/2025, FPV/3/2025). No active or free REV virions were detected. Phylogenetic analysis of the 4b gene revealed that Indian isolates clustered into a distinct sub-clade under purifying selection, indicating localized circulation, with two major clusters identified—Sonarpur/Canning and Dighari. The 4b gene remained highly conserved, validating its reliability as a molecular marker for diagnosis and phylogenetic analysis. Integration of REV within FPV was associated with immunosuppression and increased disease severity, while FPV transmission occurred not only via aerosols but also through fomites and arthropod vectors. Overall, the study confirmed the presence of REV-integrated FPV strains in backyard poultry of West Bengal, underscoring the need for continuous molecular surveillance and genomic monitoring to mitigate the emergence and spread of recombinant FPV variants in low-biosecurity poultry systems.

Isolation and Molecular Characterization of Fowl Adenovirus Serotype 11 Associated with Inclusion Body Hepatisis from India

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Fowl adenovirus (Fadv) infections are prevalent in poultry industry especially in broilers. Fowl adenovirus serotype 11 is a common causative agent of Inclusion body hepatitis (IBH) in broilers. The study was designed with a objective of isolation, identifcation and molecular characterization of Fowl adenovirus-11 from India. Virus isolation was attempted from all the suspected cases in chicken embryo liver cells made from 14 days old SPF embryos. Viral DNA was extracted from liver tissues of IBH suspected birds using Qiagen DNA mini kit. The partial hexon gene amplified with hexon A and hexon B primers from all the five samples were gel purified using gel extraction kit (Qiagen) and sequenced. Restriction digestions of amplicons were done with BsiW1, Sty 1, Mlu 1, Asp 1, Sca 1, Bgl 1. Sequence was analyzed using Bioinformatics tools to construct phylogenetic relationship. All the isolated strains showed characteristic cytopathic changes such as rounding of cells, refractivity and detachment of cells from the first passage onwards. Sequence analysis confirmed all the strains as Fadv-11. The virus was characterized using hexon gene loop-1 region. The isolated strains share nucleotide identity of 94 % to 100 % with other Fadv-11 Indian isolates. The hexon Loop-1 demonstrated 100 % identity between the strains. Phylogenetic analysis of the nucleotide sequence showed isolated strains grouped under Fadv-D into a different group along with other Indian isolates. The secondary structure prediction of hexon Loop-1 region compared to the prototype sequence revealed lack of helix in the examined region. The Loop-1 consensus derived from five isolates demonstrated 19 nucleotide substitutions of which 8 are non-synonymous. The predicted protein binding sites demonstrated 18 regions in the strains whereas prototype sequence exhibited 13 regions. Except two mutations, N to V at 478,479 and K to N at 744 other mutations exhibited functional conservation. The two substitutions in hyper variable regions 1 and 3 has altered the isoelectric point, in comparison to the reference sequence. The hexon protein of adenovirus carrying antigenic determinants being a larger protein has prominent role to play in the host immune response. This study revealed variations in hexon loop-1 region of Fadv-11 that may have implications in the development of vaccine strain selection and design of vectors to study the host pathogen interactions.

Oral Presentations

Exploring trained immunity in augmentation of vaccine-mediated immune response in chicken

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Trained immunity refers to the epigenetic and metabolic reprogramming of innate immune cells, resulting in an enhanced and more rapid response upon subsequent exposures to unrelated stimuli. This emerging concept offers a novel approach to boosting nonspecific host defense mechanisms, potentially improving resistance to a broad range of pathogens. The present study was aimed to evaluate trained immunity in chickens, with a specific focus on assessing the immunomodulatory potential of β-glucans derived from *Euglena gracilis* (EBG). Day-old SPF chicks (n = 60) were randomly allocated into two groups: (1) Control (PBS) and (2) Treatment group (EBG @ 5 mg/kg) on day 0 and day 3 through the i/m route. To evaluate the induction of a trained immunity phenotype, PBMCs were isolated from EBG-primed birds on day 7 post-priming. Cells were *ex vivo* stimulated with either the LaSota strain of Newcastle disease virus (NDV) vaccine or lipopolysaccharide (LPS), and the expression of relevant trained immunity markers was assessed. In a parallel in vivo experiment, EBG-primed birds were vaccinated with the LaSota strain of NDV on day 7 post-priming. Afterward, the expression of immune response genes was examined, and serum antibody titers were assessed to determine the impact of EBG priming on vaccine-induced immune responses. Chickens primed with EBG displayed enhanced cytokine expression in monocytes upon ex vivo re-stimulation with NDV vaccine or LPS. Notably, NDV stimulation of monocytes collected from EBG-primed birds significantly upregulated TNF-α and HIF1-α expression, suggesting the engagement of HIF1-α in the glycolysis pathway. Immunoprofiling of EBG-primed, NDV-vaccinated chickens revealed systemic upregulation of innate (e.g., TNF-α, IL-6, iNOS) and adaptive (e.g., IFN-γ, IL-4, MHC-II, CD40, CD80) immune markers, indicating induction of trained immunity involving both Th1 and Th2 polarization. Further, these molecular changes translated into enhanced humoral responses, with significantly higher hemagglutination inhibition (HI) titers in the EBG primed group vaccinated with NDV vaccine ($p < 0.01$). Collectively, the findings of the present study provide the first comprehensive understanding of trained immunity in the augmentation of vaccine-mediated immune response and highlight the potential of β-glucans as an effective vaccine adjuvant.

Generation of chicken scFv library against Newcastle disease virus genotype XIII

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Newcastle disease (ND) is an acute, highly contagious viral disease of poultry, which can cause up to 100% morbidity and mortality in unvaccinated birds, depending on the virulence of the strain. Although vaccines are available, due to genotype diversity, severe outbreaks are routinely observed. The major concern for the disease is the lack of diagnostic and therapeutic agents. Antibody phage display (APD) technology has revolutionized the field of immunovirology with its application in viral disease diagnostics and antiviral therapy. In the present study, we have developed a chicken scFv phage display library from NDV genotype XIII immunized animals. A total of four specific pathogen-free

(SPF) birds were immunized with inactivated NDV XIII virus mixed with Freund's Complete Adjuvant and subsequently 3 booster doses with Freund's Incomplete Adjuvant. Blood serum was regularly monitored for antibody production using a commercially available kit. After immunization, spleen and bone marrow samples were collected and processed for RNA isolation and cDNA synthesis. The chicken variable heavy chain (~450bp) and light chain (~350bp) immunoglobulin genes were amplified and joined together by a short (G4S)3 flexible linker. The VH- (G4S)3-VL amplicon was cloned into the pADL-22c phagemid vector using *Bgl* I and *Sfi* I restriction enzymes and transformed into bacterial cells. The colonies of transformants were harvested and stored at -80℃. The quality of the library has been assessed by colony PCR and sequencing. The constructed library will serve as a resource material for NDV disease diagnostics and therapeutics.

Molecular detection and genetic characterization of Newcastle disease virus sub-genotype VII.2 and co-infecting low pathogenic Avian influenza (H9N2) in poultry flocks of Gujarat, India

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Newcastle disease (ND) and low pathogenic avian influenza (LPAI) remain major threats to poultry health and production worldwide. Avian Influenza Virus (AIV) and Newcastle disease virus (NDV) are the major respiratory pathogens, causing high mortality in chickens Avian Influenza virus (AIV) and Newcastle disease virus (NDV) are the major respiratory pathogens, causing high mortality in chickens Currently, the fifth NDV panzootic, caused by sub-genotype VII.2, has begun and is affecting many countries in Asia and Africa. Genotypes II, IV, VI, VII, XIII, and XVIII are circulating in India and sub-genotypes XIIIc and XIIIb are in Gujarat. The present study was formulated to screen NDV associated with disease outbreaks in vaccinated chickens through different diagnostic tests and their pathotyping and genotyping, with a possibility to obtain sub-genotype VII.2 of NDV. The objectives of the study were Molecular detection of Newcastle disease virus and LPAI from clinically suspected chicken, Isolation, identification of Newcastle disease virus and LPAI and Molecular characterization of Newcastle disease virus using F gene sequencing to ascertain the genotype. Samples were collected from total 37 flocks, comprised of 12 layer and 25 broiler farms located at different geographical areas of Gujarat. Isolation of NDV and LPAI in embryonated chicken eggs by inoculation in 9 days old SPF embryonated eggs through allantoic route. Identify by HA HI test and Molecular Detection of NDV by RT-PCR using NDV partial and full F gene amplification where as LPAI detection by H9N2 specific primer pair. Molecular characterization of NDV, Out of 24 samples, only 12 samples were found positive by this RT-PCR 1661. Among RT-PCR 1661 positive field isolates, ten (7 broiler and 3 layer isolates) representative isolates were selected based on various criteria such as mortality, location, and flock type for sequencing for sequencing.

The obtained sequences were edited and aligned using different bioinformatic software and submitted to NCBI. The present research records a massive outbreak of Newcastle Disease in four districts of Gujarat state, India, despite following regular vaccination. In Gujarat, genotype VII.2 of NDV was found responsible for these outbreaks. Such a wider geographical spread and quantum of mortality indicate that India is now in the grip of the fifth NDV panzootic due to genotype VII.2. Higher prevalence of ND occurred during the transition from winter to summer and hot weather conditions and 3rd to 4th week age of broiler birds. The passage of the suspected NDV sample in eggs increases the RT-PCR positivity. Therefore, a minimum of two passages in ECE were is required for an accurate RT-PCR-based diagnosis. The MDT calculation, primer combination PCR and amino acid sequence of the partial 'F' gene showed motif 112R/K-R-Q-K-R*F117 at FPCS, confirming the velogenic type of NDV in the present study. The study identified that out of 24 RT-PCR positive samples, only 12 showed complete F gene coverage, so the additional primer pairs may be required to amplify the other non-amplified F gene sequences of NDV isolates. The present study sequences of genotype VII.2 showed many amino acid changes compared to widely used NDV vaccines, indicating that vaccines should be

prepared considering these marked changes. These nucleotide and consequent amino acid substitutions compared to the nearest phylogenic isolates of VII.2 sequences, especially the additional six and four amino acid substitutions in two isolates, strongly indicate that genotype VII.2 is in a rapid and continuous evolution process. These findings underscore the constant monitoring and genetic analysis for effective disease management and control. The study identified a significantly higher prevalence of the H9N2 virus in 67.56% of the tested samples, with a notable rate of co-infection with the Newcastle disease virus in 51.35% of cases, indicating widespread viral infections and co-infections among poultry flocks. The study confirms co-circulation and co-infection of NDV VII.2 and LPAIV H9N2 in vaccinated poultry, emphasizing genetic variation, diagnostic challenges, and need for ongoing molecular surveillance.

Molecular detection, antimicrobial resistant gene profiling and multi-locus sequence typing of non-typhoidal *Salmonella* serovars (enteritidis and typhimurium) from broiler chickens at meat outlets in southern India

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Antimicrobial resistance (AMR) in *Salmonella enterica* non-typhoidal serovars (NTS), particularly Enteritidis and Typhimurium, is a growing public health concern, especially in poultry, where antibiotics are commonly used for growth promotion and disease prevention. This study aimed to isolate and characterize S. Enteritidis and *S. Typhimurium* from broiler meat swabs and faecal samples collected across six geographical locations in southern India and to assess their antimicrobial resistance profiles using both phenotypic and molecular approaches. A total of 288 samples (24 meat and 24 faecal swabs per location) were collected from Tirupati, Hyderabad, Chennai, Thrissur, Mysore, and Puducherry. Samples were pre-enriched in Buffered Peptone Water (BPW) and selectively enriched in Rappaport Vassiliadis Soya Broth (RVS). Species identification was performed by Real-Time PCR targeting the invA gene, while fliC and safA primers were used for *S. Typhimurium* and *S. Enteritidis* detection, respectively. AMR gene screening was conducted using SYBR Green-based PCR. Representative isolates were subjected to antibiotic susceptibility testing (ABST), minimum inhibitory concentration (MIC) determination, and Multi-Locus Sequence Typing (MLST) according to CLSI guidelines. Among 288 samples, *Salmonella* spp. was detected in 82 (56.9%) meat and 24 (16.7%) faecal samples. *S. Typhimurium* was identified in 74 (51.4%) meat and 10 (6.9%) faecal samples, while *S. Enteritidis* was found in 9 (6.3%) meat and 7 (4.9%) faecal samples. Other serovars accounted for 9 (6.3%) isolates in both meat and faecal samples. Multiple AMR genes and phenotypic multidrug resistance were observed, and MLST indicated genetic variations and similarities among representative isolates. The study emphasizes the need for continued AMR monitoring and responsible antibiotic use in poultry production.

BHKin1™ and CELLin1™: Redefining Serum-Free Platforms for Modern biomanufacturing

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The reliance on fetal bovine serum (FBS) in cell culture presents persistent challenges in biomanu facturing-including cost volatility, contamination risks, and ethical concerns. In response, HiMedia has developed BHKin1™ and CELLin1™, two animal component-free, chemically defined media specifically optimized for BHK-21 and Vero cell platforms, respectively. BHKin1™ supports both

adherent and suspension cultures of BHK-21 cells, enabling highdensity growth with superior viability and recombinant protein expression. In comparative studies, BHKin1™ demonstrated up to 191% higher cell proliferation and sustained >95% viability relative to commercial alternatives.

CELLin1™, tailored for WHO-approved Vero substrates, ensures consistent growth kinetics and enhanced viral yields across vaccine applications. It also supports scalable performance in related mammalian lines such as MDCK and MDBK, reinforcing its versatility for viral vector and biologics production.

Both media are supported by full traceability documentation and are produced under GMP-compliant conditions, aligning with global regulatory expectations. Collectively, BHKin1™ and CELLin1™ empower the transition toward serum-free, ethical, and reproducible biomanufacturing, marking a critical advancement for the vaccine and therapeutic protein industries.

Poster Presentations

Standardization of Infectious bursal disease virus-anti-DEC205 receptor antibody conjugation for targeted antigen delivery in dendritic cells

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Infectious bursal disease virus (IBDV) is a major immunosuppressive pathogen in poultry, responsible for significant economic losses and reported outbreaks even in vaccinated flocks, indicating the need for improved vaccination strategies. Dendritic cell (DC) targeting has been proposed as a means to enhance antigen uptake and presentation, thereby improving immune response and protection. One such approach is to target antigen delivery into dendritic cells through the major endocytic receptor, DEC 205. In this study, we aimed to develop a stable conjugate of a cell culture–adapted IBDV vaccine strain with anti-chicken DEC205 antibody, so that the conjugate can effectively bind with the DC receptor and aid in effective antigen uptake through receptor-mediated endocytosis. The vaccine virus, adapted in chicken embryo fibroblast (CEF) cells, was propagated, purified by sucrose density gradient ultracentrifugation, and chemically modified by thiolation with Traut's reagent. Cross-linking with Sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) facilitated conjugation of antigen with the DEC205 antibody. Different ratios of virus and antiDEC 205 antibody were used for this conjugation (1:1, 2:1, and 1:2). The conjugates were characterized by SDS-PAGE and Western blotting, confirming successful antigen–antibody linkage. Among different antigen-to-antibody ratios tested, the 1:2 ratio yielded the highest conjugation efficiency, as evidenced by distinct molecular weight shifts and strong immunoreactive bands specific to IBD antigen. These findings demonstrate the feasibility of generating IBDV–DEC205 antibody conjugates for DC targeting, and the standardized protocol provides a foundation for exploring further advances in immunology for improved vaccine delivery and subsequently potentiating vaccine efficacy.

Evaluation of *Salmonella typhimurium* outer membrane vesicles (OMVS) as vaccine candidate against salmonellosis in broiler poultry

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Poultry is one of the fastest-growing essential sectors, meeting nutrition security and the Indian poultry industry ranks second and fifth in global egg and meat production, respectively. However, poultry is highly susceptible to non-typhoidal *Salmonella* infections, especially caused by serovar Typhimurium also accounts for the highest zoonotic transmissions and food risks. Vaccination approaches applicable for broilers are limited, as many live attenuated vaccines are not suitable for young chicks, and killed vaccines are limited to parenteral administration. Therefore, there is an urgent requirement for safe and effective vaccines that can be delivered at an early age through the oral route. The purpose of the current study was to develop and assess oral vaccines based on outer membrane vesicles (OMVs) and outer membrane proteins (OMPs) of *S. typhimurium*. OMVs and OMPs were isolated, while OMPs were conjugated to chitosan nanoparticles to improve stability and delivery. Both

vaccine candidates were evaluated via biochemical assays, electrophoresis, surface charge (zeta potential), and electron microscopy. One-day-old broiler chicks received a prime dose orally at 3 days old and a booster at 3 weeks. Immune responses were measured via indirect ELISA (with OMP as antigen) for humoral immunity and lymphocyte proliferation assays for cell-mediated immunity. Both OMV and OMP-CHNP vaccines generated good immune responses; however, the OMV vaccine performed better overall, likely due to its multicomponent structure and inherent adjuvant effect. These results suggest that OMV-based oral vaccines may be a viable option in the establishment of early protection of broilers against *S. typhimurium*, giving benefit to poultry health, food safety, and reducing antimicrobial resistance.

Development and Characterization of Recombinant Fowlpox Virus gene construct containing gB gene of infectious laryngotracheitis virus

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A safe and effective indigenous infectious laryngotracheitis (ILT) virus (ILTV) vaccine is urgently needed to meet the demands of poultry farmers for ILTV control. Therefore, a study was planned to initiate the development of a recombinant fowlpox vector-based ILT vaccine. Study aimed to develop a recombinant gene construct for the generation of a fowlpox virus (FPV)-vectored vaccine expressing the gB protein of Infectious Laryngotracheitis Virus (ILTV). A synthetic gene was designed with multiple restriction sites and three distinct fowlpox virus promoters. The recombinant construct was generated through step-by-step cloning of the amplified ILTV gB gene and selection marker genes (GFP and GPT), flanked by the amplified left and right homologous arms (LHA and RHA) of the FPV gene, into the cloning vector. The fowlpox vaccine virus was adapted to CEF primary cells to facilitate homologous recombination between the recombinant construct and the FPV genome. The gB gene (2670 bp), GPT (460 bp), and GFP (720 bp) were cloned into this synthetic cassette at appropriate sites. Cloning was confirmed by colony PCR and restriction enzyme digestion. Separately, the LHA (1324 bp) and RHA (1384 bp) of the FPV gene were amplified from the fowlpox virus genome and cloned into the pMD20 vector. An empty transfer vector was constructed by ligating LHA and RHA fragments. The final recombinant transfer plasmid was assembled by inserting the gB-GPT-GFP cassette into the LHA-RHA backbone. Successful cloning was confirmed through PCR, yielding expected band sizes of 1384 bp (RHA), 2760 bp (gB), and 460 bp (GPT). Additionally, restriction digestion released the gB insert (2882 bp with promoter) and selection marker cassette (1500 bp), confirming the construct. The fowlpox vaccine virus was successfully adapted to chicken embryo fibroblast (CEF) cells for homologus recombination with recombinant gene construct. This study establishes a foundation for rescuing recombinant fowlpox virus and contributes towards the development of a safer and more effective ILTV vaccine in the country

Study on the prevalence of *Mycoplasma gallisepticum* in chronic respiratory disease (CRD) suspected birds

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Mycoplasma gallisepticum (MG), the most economically important avian *Mycoplasma* and the causative agent of CRD in poultry, remains largely understudied in terms of surveillance and prevalence. This study was carried out to determine the incidence of CRD in broiler chickens from two representative districts of Uttar Pradesh and Maharashtra. A study was carried out from January to September 2025 to determine the incidence of CRD in broiler chickens from two representative districts, Bareilly (Uttar Pradesh) and Pune (Maharashtra). A total of 119 birds (51 from Bareilly and 68 from Pune) exhibiting respiratory symptoms were examined for CRD. Among these, ante-mortem samples (tracheal, nasal, and ocular swabs) were collected from 39 chickens, while post-mortem samples (lung, trachea, and air sac tissues) were obtained from 90 birds. All samples were processed for DNA extraction using the Qiagen MiniSpin Kit and screened by polymerase chain reaction (PCR) employing *Mycoplasma gallisepticum*-specific primers recommended by the World Organisation for Animal Health (WOAH). A higher positivity rate was recorded in Bareilly district (15/51; 29.4%) compared to Pune district (12/68; 17.6%). The findings indicate a moderate prevalence of CRD among the broiler birds, suggesting an active circulation of the pathogen within the population. This highlights the need for comprehensive epidemiological surveillance for effective control and prevention.

Assessment of Aluminium Hydroxide Adjuvanted Inactivated Trivalent Vaccine Candidate against Avian Colibacillosis

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APEC causes severe poultry losses; rising antimicrobial resistance necessitates effective vaccines. This study evaluates trivalent inactivated vaccines using different adjuvants. Preparation and Standardization of inactivated adjuvanted trivalent vaccine (O78, O1, and O2) and evaluation of the immunogenicity of inactivated adjuvanted trivalent vaccine. Formalin-inactivated *E. coli* antigens of serogroups O1, O2, and O78 were formulated into trivalent vaccines using Aluminium hydroxide or Montanide ISA adjuvants (30:70 ratio). Twenty-three-week-old laying hens were divided into three groups—Aluminium hydroxide, Montanide, and control—and vaccinated subcutaneously with two doses, two weeks apart. IgY antibodies were extracted from egg yolks using PEG precipitation. SDS-PAGE and Western blot analysis confirmed the integrity and specificity of the antibody. Humoral responses were quantified by indirect ELISA using APEC-specific antigens, and antibody titres were measured up to 42 days post-vaccination. Vaccination was well-tolerated without adverse effects. Western blot confirmed intact (~180 kDa) antigen-specific IgY antibodies in vaccinated groups. Both formulations induced strong humoral responses compared to the control. Montanide-adjuvanted vaccine showed slightly higher ELISA titers than Aluminium hydroxide adjuvanted vaccine across all APEC serogroups (O1, O2, O78). Control birds showed minimal antibody response up to 42 days. Indirect ELISA of egg yolks revealed significant anti-APEC IgY levels, confirming successful maternal antibody transfer. These findings highlight the immunogenicity and safety of both adjuvanted trivalent formulations in laying hens. Formalin-inactivated trivalent APEC vaccines induced strong humoral and maternal responses, with Montanide showing superior immunogenicity over Aluminium hydroxide.

Isolation, antimicrobial susceptibility, and histopathological assessment of *Salmonella* spp. from poultry meat sold in local shops of Uttarakhand, India

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Salmonella spp. are major foodborne pathogens globally, with poultry meat serving as an important source of infection for humans, posing serious public health challenges in India. This study aimed to determine the occurrence of *Salmonella* in poultry meat from Uttarakhand retail markets, their antimicrobial resistance patterns, and histopathological changes in experimentally infected tissues. in from A total of 20 poultry meat samples were randomly collected from retail shops in Haldwani, Kiccha, and Pantnagar regions of Uttarakhand. Samples were processed using standard bacteriological isolation and biochemical identification techniques for *Salmonella*. Confirmed isolates were subjected to antimicrobial susceptibility testing against commonly used antibiotics using the disc diffusion method. Histopathological examination was performed on experimentally infected tissues to assess organ-specific lesions. Liver, spleen, and intestinal tissues were fixed, sectioned, and stained to study microscopic alterations associated with *Salmonella* infection. Observed lesions included hepatic congestion, hepatocellular necrosis, Kupffer cell hyperplasia, and lymphoid depletion in the spleen. Data were analyzed to evaluate the prevalence, resistance profiles, and pathogenic potential of isolated strains. *Salmonella* was successfully isolated from poultry meat samples collected in the study areas. Antimicrobial susceptibility testing revealed a high prevalence of resistance against commonly used antibiotics, highlighting the threat of multidrug-resistant strains. Histopathological analysis showed significant pathological changes in vital organs, including hepatic congestion, necrosis of hepatocytes, Kupffer cell proliferation, and lymphoid depletion with necrosis in the spleen. These findings confirm both the circulation of resistant Salmonella in retail poultry meat and their ability to cause severe tissue damage, reflecting their pathogenic potential. The study highlights the urgent need for routine Salmonella surveillance, strict antimicrobial stewardship, and hygienic poultry handling to mitigate public health risks in Uttarakhand.

Assessment of antibacterial property of bacteriophage against bacterial pathogens associated with chicken meat

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Bacteriophage has been proposed for the variety of uses in market, including bio preservation, bio control, and pathogen detection and as an alternative of antibiotics in animal health. The potential applications of phages are widespread throughout the entire food production process, aim to enhance food quality, prevent foodborne illnesses and improve production efficiency. Present study was planned to investigate the antibacterial efficacy of a bacteriophage against pathogens, specifically *S. aureus* and *E. coli* associated with chicken meat samples. Bacteriophages were isolated from sewage samples by DAL Methods. Initially the samples were. processed for microbial detection to confirm the presence of bacteria (mainly *S. aureus* and *E. coli*). These isolates were subjected for antimicrobial resistance pattern and phage sensitivity assay for assessment of biocontrol activity. Bacteriophages were isolated from sewage samples. Good quality bacteriophage was selected to assess their antibacterial effect bacterial pathogens on chicken meat. Initially the samples were processed for microbial detection to confirm the presence of bacteria (mainly *S. aureus* and *E. coli*). The phage cocktail treatment effectively eliminated E. coli from all meat samples but not showing any effect on *S. aureus*. This study highlights the use of a bacteriophage cocktail against bacterial pathogens to control the pathogens present on food product. Bacteriophage can be effective alternative of antimicrobials used in food industry.

Mitigation of *Salmonella Gallinarum* infection by *Alstonia scholaris* in broiler chickens

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Fowl typhoid, caused by *Salmonella enterica* serovar Gallinarum (*Salmonella Gallinarum*), causes significant morbidity and mortality in broilers. Since, multi drug resistance has been reported against *S. Gallinarum,* the present investigation was conducted with the objectives to evaluate the potential of *Alstonia scholaris* extract in modulating hemato-biochemical, oxidative and pathomorphological alterations induced by experimental *S. Gallinarum* infection in broiler chickens. Culture of *S. Gallinarum* was procured from NCVTC, ICAR-National Research Centre on Equines, Hisar (Haryana). A total of 120 day old broiler chicks (DOC) were divided in total four groups. Group I served as a control, group II was kept as infected control. At two weeks of age, group III and IV birds were provided *A. scholaris* @ 200 mg/L in drinking water for two weeks. At three weeks of age, groups II and IV were infected with 2 x 10^9 CFU intraperitonially. Sequential sacrifice of birds from each group was carried out at 3, 6,9, 12 and 15 days post infection. Additionally, birds were observed for any clinical abnormality throughout the experimental period and spontaneous mortality was also recorded. The mortality observed in group II (infected control) was 43.33%; however, only 20% mortality was seen in birds in group IV (extract supplemented group). *S. Gallinarum* induced significant alterations in haematological, biochemical and oxidative stress parameters which were alleviated by extract administration. The pathological lesions recorded in group II included hepatomegaly, bronze discoloration of liver, splenomegaly, multifocal necrosis in liver and spleen, whitish nodules on heart, myocardial necrosis and heterophilic infiltration. *A. scholaris* significantly ameliorated severity of these lesions. Moreover, a significant decrease in bacterial counts in liver, spleen and heart blood was observed in group IV as compared to group II. Overall, *A. scholaris* significantly ameliorated *S. Gallinarum* infection.

Effect of supplementation of oligosaccharides based prebiotics on haemato-biochemical and carcass parameters in kuroiler chicken

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The study explored the potential of oligosaccharide-based prebiotics as alternatives to antibiotic growth promoters in poultry, focusing on their effects on haemato-biochemical and carcass parameters of Kuroiler chickens. A total of 160 day-old Kuroiler chicks were randomly assigned into four groups (n=40; four replicates of 10 each). The groups included: control (CON, no additives), Chlorotetracycline Hydrochloride 15% (CTC), Fructo-oligosaccharides (FOS), and Mannan-oligosaccharides (MOS). Experimental diets were provided as per planned feeding schedules. Blood samples were collected to estimate total protein, creatinine, cholesterol, glucose, blood urea nitrogen (BUN), albumin, globulin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). At slaughter, carcass traits were recorded, including dressing yield, eviscerated yield, ready-to-cook yield, giblet yield, cut-up parts, and feather with skin loss. Data were statistically analyzed to assess treatment effects. Most blood parameters remained unaffected by dietary treatments, except cholesterol, which differed significantly ($P<0.001$). Cholesterol levels were highest in CTC and lowest in the control, while FOS and MOS showed intermediate values. Dressing yield improved significantly in prebiotic-

supplemented groups, with MOS recording the highest (72.98%), followed by FOS (70.39%), CTC (69.84%), and CON (67.95%). Gizzard yield was highest in FOS, giblet yield was lowest in CTC and highest in CON, while feather with skin loss was lowest in MOS, indicating enhanced carcass quality. Oligosaccharide-based prebiotics, particularly MOS, enhanced carcass yield and reduced slaughter losses, while FOS supported gizzard development, offering safe and effective alternatives to antibiotic growth promoters in poultry.

Successful management of Avian influenza in Gorakhpur zoological park through biosecurity measures

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Avian influenza (AI) mostly regarded as one of the most feared diseases of birds. During May 2025 Shaheed Ashfaq Ullah Khan Zoological Park, housing 400 wild animals of more than 53 species, became epicenter of AI as eight animals (big cats and birds) along with few feral crows were tested positive for H5N1. Incidentally, only three animals succumbed before being diagnosed due to acuteness of the disease. However, immediate additional biosecurity measures implementations helped in successful control of the disease. Key biosecurity measures included (1) Immediate closing of zoo for visitors. (2.a) Weekly sanitization of the whole zoo premises including animal keeper gallery, animal enclosures and kraals with (i) triple salt solution containing potassium mono persulfate, potassium hydrogen sulfate and sodium chloride, (ii) glutaraldehyde, dodecyl dimethyl ammonium chloride and ethylene dioxy dimethanol solution (2.b) The iron grills, walls and floors of the animal night/feeding houses were heat treated by blow torching methods weekly. (2.c) Visitor's area/service roads were sanitized using sodium hypochlorite. (2.d) Aviary was sanitized using didecyldimethyl ammonium chloride, additionally same solution was given in drinking water of birds also. (3) All construction activities in the zoo were paused. (4) Movement of all zoo animal handlers from one enclosure to other were restricted. (5) All zoo staff were screened twice for AI. (6) Almost all zoo carnivores and representative bird species swabs were sent to referral labs at the interval of fifteen days for examination as per protocol. (7) Disposable personal protective equipment such as long aprons, gloves, head covers and shoe covers were used (8) Meat for the carnivores was sourced from the poultry free area. (9) Zoo animals were given immuno-boosters, Vitamin C, B-complex, A, D, E regularly. (10) Whole zoo campus was thoroughly screened twice daily for presence of any dead or diseased free ranging birds especially crows. All the measures resulted in successful management of AI inside the zoo premises.

Session 6:

Genome Editing and Data-Driven Smart Livestock Farming and Production

Microbiome and resistome profiling in bovine subclinical mastitis and harnessing probiotics as antibiotic alternative for sustainable mastitis management: A one health approach

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Subclinical mastitis (SCM) significantly reduces dairy productivity and accelerates antimicrobial resistance (AMR) due to excessive antibiotic use. This study employed a One Health framework, integrating metagenomic profiling and functional validation of native probiotics, to advance sustainable mastitis management and mitigate AMR. Whole metagenome shotgun sequencing of 30 milk samples from healthy (HM), SCM, and antibiotic-treated (ANT) crossbred cows revealed distinct microbial dynamics. HM samples exhibited significantly higher microbial diversity ($p<0.05$). SCM samples were dominated by opportunistic pathogens like *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus uberis*, while ANT samples showed enrichment of *Pseudomonas aeruginosa*, *P. putida* and *Raoultella ornithinolytica*, indicating antibiotic-driven selection. Conversely, HM samples harbored beneficial taxa such as *P. lundensis*, *Actinoalloteichus* spp., *Acinetobacter* spp., *Staphylococcus epidermidis* and *Lactococcus lactis*. Resistome profiling demonstrated significant enrichment of antibiotic resistance genes (ARGs) conferring resistance to macrolides, aminoglycosides, and multidrug efflux pumps in ANT samples ($p<0.01$). Importantly, ARGs were also detected in HM samples, highlighting potential hidden AMR reservoirs with zoonotic implications. To explore safe and effective alternatives, three probiotic strains namely *Pediococcus pentosaceus* (MBBL4, MBBL6) and *Enterococcus faecium* (MBBL3)—were isolated from healthy milk and subjected to genomic characterization. Genome analyses confirmed their metabolic versatility, stress tolerance, and revealed multiple bacteriocin clusters (bovicin, penocin A, enterolysin A) with antimicrobial potential. Safety profiling verified the absence of major virulence genes and broad antibiotic susceptibility. *In-vitro* assays confirmed their inhibitory activity against *S. aureus, E. coli*, and *K. pneumoniae*. Further in-silico analyses predicted robust bacteriocin–pathogen interactions, notably targeting transcriptional regulatory proteins, and *in-vivo* models corroborated these pathogen-suppressive effects. These findings underscore mastitis- and antibiotic-driven microbiome dysbiosis and associated ARG enrichment. The study highlights indigenous probiotics as promising, sustainable alternatives for improved mastitis management, emphasizing the need for precision diagnostics and prudent antimicrobial use within a One Health context.

Genomic Insight of Mastitis causing ESBL (Extended Spectrum Beta Lactamase) Producing *E coli.*

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The composition of microbiomes in bovine mastitis vary according to different forms of mastitis (clinical, subclinical and recurrent). This diversity comprises both contagious udder pathogens including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Mycoplasma* spp., *Escherichia coli*, *Klebsiella* spp. etc. *E. coli* is an environmental pathogen that commonly associated with mastitis, but most strains are harmless bacteria found in the gut. Pathogenic strains can spread through contaminated food or water, contact with infected human and animals. Recent studies suggest that mastitic milk contain a significant amount of antibiotic-resistant *E. coli*, specifically extended-spectrum beta-lactamase-producing *E. coli*. The resistomes (the total collection of antibiotic resistance genes) act as a potential key factor in disease complication, recurrence, complete damage to the udder and transmission of the pathogen to the human food chain via milk and milk product processing loop holes. The genomic diversity of the indicator Escherichia coli in a bovine mastitis is huge. We relate it dynamic genetic makeup and related to the isolates' antibiotic resistance and possible transmission to human. *E. coli* isolates from mastitis have a surprisingly large pan-genome, which harbors virulence genes and genes correlating with resistance. We analysed more than 700 E coli (mastitis isolates) based on phenotypic PCR data for better understanding the pathogen and subsequently to mitigate them. The antimicrobial susceptibility testing of these *E. coli* isolates showed 100% of isolates resistant to oxacillin, along with resistant to erythromycin, and ciprofloxacin. The isolates were susceptible to chloramphenicol (100%), gentamycin (~90.00%), and ciprofloxacin (~30.00%). The presence of ESBL genes such as blaSHV, blaCTX-M-1, blaTEM, blaOXA-1, and AmpC CMY-2, was observed in ~70.00% of E. coli isolates. A total of ~20% to 40% of E. coli isolates were found as multi-drug resistant (MDR) (resistance to ≥3 class of drugs). Among three Pathogenicity Islands (HPI, SerU, and pks island), only High Pathogenicity Island (HPI) was detected in ~30% of isolates. *In silico* studies on global carbapenem and cephaloporin-resistant E. coli genomic sequences poses a severe global public health risk. Three major ST types were found to be dominant: ST131, ST10 and ST410. Among the most dominant lineages, ST131 was almost exclusively associated with phylogroup B2 and represented the major human-associated clone worldwide, with particularly high prevalence in high-income countries such as the USA, UK and France. B2/ST131 expanded rapidly after 2008, peaking around 2020. Temporal of *E. coli* resistance genes revealed a sharp rise in extended-spectrum β-lactamases (ESBLs) and carbapenemases over the past two decades. The blaCTX-M-15 emerged as the dominant ESBL globally. We also proposing a point of care diagnostics for a rapid, sensitive and precise method to identify microbial DNA coming from veterinary clinical samples, such as milk of cows affected with mastitis. An amplification-free visual assay for rapid and sensitive detection of specific DNA from *E. coli*, particularly uidA gene encoding for beta-glucuronidase, isolated in milk of cows affected with mastitis. This test can be performed in 1–1.5 h after template DNA preparation and can be visualized with naked eyes. Regarding mitigation, a work was directed towards isolating bacteriophages from sewage against multidrug-resistant mastitis causing ESBL *E. coli* bacteria.

Author's Responsibility in Scientific Communication

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Scientific communication - data, results, and interpretations - is the product of research work conducted by scientists. The author wants to communicate the valid results of analysis of data to relevant audiences. Publish-or-perish climate also pressurize the scientists to publish articles timely. Besides scientific writing is the basic currency must for academic development of a scientist.

In the changing scenario of online availability of articles (pre-print as well as printed) with no time-lag in printing and availability to readers have brought global visibility as well transparency to scientific publications. Under such penetrating visibility and scrutiny of readers and peers the authors are to be more careful about— plagiarism, authorship (inclusion of author, deletion of names, ghost author, gifting authorship), slicing of articles, conflict of interest and acknowledgement.

Artificial intelligence (AI) is bringing different type of challenges in science communication domain. AI is here to stay and authors, reviewers, editors and publishers are going to use it making scientific communication management more complicated.

The journals, conferences and symposiums have to develop AI Policy, which is able to develop organically vis-à-vis the fast developing AI uses. Policy must have— (i) framework defining do's and don'ts in AI use in scientific communication, and (ii) when AI use is to be disclosed.

Authors, reviewers, editors, and publishers have to declare the extent of AI-use and the App used. They have to declare that AI help was taken only for grammatic corrections and not for creating the content.

In case of conferences, the organizers should have —clear guidelines for authors on extent of AI use, disclosure of AI tool; and they should complete AI checks before accepting articles for conference.

The authors, editors and reviewers should be aware that the Big Brother that helped you in generating the content will co-ordinate with the authorities to detect it. So, work hard and be an honest citizen of the scientific world.

Addressing Anti Microbial Resistance (AMR) using Indigenous Means

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Antibiotics remain a cornerstone of modern medicine; however, their effectiveness is increasingly undermined by the rise of antimicrobial resistance (AMR) in both hospital (nosocomial) and community settings. Among drug-resistant pathogens, the most critical threat comes from the *ESKAPEE* group—an acronym representing *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species, and *Escherichia coli*. These organisms are recognized by the World Health Organization as Priority I and II pathogens due to their high to critical levels of drug resistance.The unchecked misuse and overuse of antimicrobial agents in humans, animals, and agriculture have accelerated the emergence and spread of resistant strains.

Understanding AMR

AMR refers to a reduction in a microorganism's susceptibility to an antimicrobial agent, clinically observable as an increased minimum inhibitory concentration (MIC) or complete insensitivity to treatment. This reduced susceptibility or resistance can arise naturally over time through genetic or phenotypic adaptations. Genetic resistance develops when bacterial populations survive and proliferate even at antibiotic concentrations several times higher than the MIC. Phenotypic resistance, in contrast, occurs without genetic alteration. It represents a reversible state within certain bacterial cells that transiently display reduced sensitivity. Phenotypic AMR can range from partial tolerance—where higher antibiotic doses may still be effective—to total resistance, resulting in treatment failure. Although the evolution of AMR is inevitable, inappropriate prescribing practices and suboptimal dosing significantly accelerate the process. Resistance is no longer confined to older, widely used antibiotic classes; even the newest and most potent agents, such as carbapenems, are increasingly compromised.

Molecular Mechanisms of Resistance

Mechanistically, resistance arises through spontaneous mutations or horizontal gene transfer from resistant bacteria. These genetic changes enable bacteria to

- Produce enzymes that inactivate antibiotics,
- Modify antibiotic targets,
- Alter membrane permeability through porin loss,
- Overexpress efflux pumps to expel drugs, or
- Protect themselves via biofilm formation.

Additionally, various xenobiotics can influence AMR development by disrupting membrane integrity, DNA/RNA synthesis, intermediary metabolism, cytoplasmic stability, or quorum sensing (cell-to-cell communication).

From a microbiological perspective, AMR arises both through bacterial evolutionary mechanisms and human-driven selection pressures. Bacteria use *quorum sensing* (QS) to coordinate group behavior: when population density reaches a threshold, QS can trigger expression of genes for biofilm formation, virulence factors, efflux pumps, or antibiotic-degrading enzymes, all of which enhance survival under antibiotic stress.These resistance traits were already developed through genetic mutation and natural selection long before the use of antibiotics. Studies of ancient DNA—from permafrost and other pristine environments—have uncovered resistance genes for β-lactams, glycopeptides, tetracyclines, and more, confirming that AMR predates clinical antibiotic use. These ancient resistance mechanisms have diversified over billions of years, giving bacteria many molecular tools to evade antibiotics. When intensive use of antibiotics started for medicine, agriculture, etc.,strong selection pressures were created. This, combined with interactions among environmental bacteria, animal microbiomes, and human pathogens, has facilitated the transfer of ancient resistance genes into disease-causing bacteria, undermining our ability to treat infections.

Natural Antimicrobial Defences

"Every action provokes a reaction — it's a fundamental law of nature." Nature itself is a rich source of antimicrobial agents—think penicillin, streptomycin, and lysozyme—originally evolved for microbial self-defence. These molecules enable small organisms to compete and help larger ones resist infections. In the gut, commensal microbes can perform both protective and regulatory roles.Scavengers present a remarkable case: despite consuming bacteria-laden carcasses, they remain largely disease-free. Animals like vultures, hagfish, and blowflies eat decaying matter teeming with pathogens, yet avoid illness. This resilience is enabled by multiple defences.

Natural species specific physiological defences are powerful. Wolves, for example, keep food in their stomachs twice as long as humans do, allowing stronger acid exposure to kill bacteria before they reach the intestines. In insects such as dermestid beetles, a chitin-lined gut adds a physical antibacterial barrier.

Immune surveillance also plays a key role. Scavengers' immune systems are fine-tuned for bacterial detection—more precise than in many non-scavengers. Moreover, scavengers produce new chemical defenses: blowflies synthesize antimicrobial peptides, lipids, and proteins to protect their outer surfaces and circulatory systems.

Microbial allies assist internally. In vultures and sexton beetles, resident microbes secrete bacteriocins and cyclic lipopeptides that help guard the host's gut. While transient microbes from food (bacteria, fungi, viruses) may colonize temporarily, the host-microbiome ecosystem adapts rapidly.

In the gut microbiome, diet and antibiotic exposure are crucial modifiers. Dietary imbalance can lead to dysbiosis—that is, a disruption of the normal microbial community—creating environments that favor resistance gene expression. Interestingly, shifts in gut microbiota—such as the rise of *Bilophila wadsworthia* on animal-rich diets link diet, bile acid metabolism, and risks of inflammatory bowel disease. Together, these insights underscore how natural antimicrobial strategies are multi-layered, dynamic, and closely tied to ecology, diet, and coevolution.Meanwhile, antibiotics exert strong selection pressure, killing susceptible bacteria and allowing resistant ones to proliferate.

Together, the interaction among quorum sensing, biofilm formation, diet□induced dysbiosis, and antibiotic use creates a feedback loop promoting resistant strains. Understanding these molecular and ecological processes highlights targets—for instance, QS inhibitors, probiotics to restore microbiome balance, or interventions that reduce biofilm establishment—that may help curb the rise of difficult□ to-treat bacterial infections. Altered diet and antibiotic use can both significantly impact the development of AMR in gut microflora. The interplay between indigenous resources, antibiotics, and gut microflora underscores the importance of maintaining anevolutionary balance and judicious usage of antibiotics to manage and mitigate the risks associated with AMR.

Overcoming Insecticide Resistance; A Critical Step Towards Eradication of Vector- Borne Diseases

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Chemical insecticides like Indoor Residual Spraying and Long-Lasting Insecticide-Treated Nets remain key tools for malaria control, but widespread resistance in Anopheles mosquitoes threatens the long-term success of these interventions. Resistance to current insecticides has increased the risk of vector-borne diseases such as malaria, dengue, and chikungunya. This study aims to identify and functionally characterize a novel protein involved in insecticide resistance in *Anopheles stephensi* to uncover new potential targets for vector control. Field-caught and laboratory-reared *Anopheles stephensi* mosquitoes resistant to deltamethrin and malathion were subjected to differential proteomic analysis to identify proteins associated with insecticide resistance. Among the identified candidates, a unique esterase-like protein, named CSDIR (Cub and Sushi Domain containing Insecticide Resistance protein), was selected for detailed characterization. The CSDIR gene fragment (amino acids 913–1190) was cloned, expressed recombinantly, and purified to assess its biochemical properties. Enzymatic assays were performed to evaluate esterase-like activity and insecticide-binding affinity. Localization studies determined the tissue-specific distribution of CSDIR protein in mosquitoes. Functional validation was conducted through RNA interference (RNAi)-mediated knockdown of the CSDIR gene, followed by insecticide exposure bioassays to assess changes in mortality and enzymatic activity compared to control groups. Statistical analysis was performed to determine the significance of observed differences. The recombinant CSDIR913–1190 protein exhibited strong esterase-like activity and high binding affinity toward deltamethrin and malathion. Immuno-localization revealed predominant expression in female ovaries and salivary glands. RNAi-mediated silencing of the CSDIR gene resulted in over 60% mortality after one hour of insecticide exposure compared to controls. Knockdown mosquitoes also showed significantly reduced esterase activity against α- and β-naphthyl acetate substrates, confirming CSDIR's functional role in metabolic resistance.

CSDIR is a novel esterase-like protein contributing to insecticide resistance in *Anopheles stephensi* and represents a promising molecular target for next-generation mosquito control interventions. CSDIR inhibitors may be complemented with current insecticides to overcome the resistance problem.

Oral Presentations

Isolation and genomic characterization of *Bacillus anthracis* from clinical anthrax in animals

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Anthrax is a well-recognized zoonotic disease of herbivorous animals caused by *Bacillus anthracis*, a capsulated, non-motile, aerobic, Gram-positive, spore-forming bacterium. The present study focused on the isolation and genomic characterization of two Indian virulent *B. anthracis* strains recovered from anthrax cases in cattle and sheep. The two isolates were obtained in pure culture, and their identity was confirmed through culture characteristics, Gram staining, phage typing, and molecular assays. Comparative genomic analysis was performed with available *B. anthracis* strains of animal origin. Microscopically, Gram-positive rod-shaped bacilli were observed, typically arranged in long chains with squared ends. Both strains tested positive for the McFadyean reaction and were susceptible to gamma phage. PCR amplification yielded specific amplicons of 596 bp (pag), 846 bp (cap), and 185 bp (rpoB). Antibiotic susceptibility testing of both strains identified resistance to penicillin. The draft genomes were predicted to have sizes of approximately 5.4 Mb and 5.5 Mb with GC contents of 34.92% and 35%, respectively. Multi-locus sequence typing (MLST) assigned the strains to *B. cereus* sequence type ST1, *B. anthracis* cgMLST ST284, and *B. anthracis* plasmid ST12 based on the typing scheme. A total of 5,217 orthologous clusters between the strains and the Ames ancestor strain were identified. Canonical SNP (canSNP) analysis classified both strains as A.Br.003 (A.Br.Aust94 sub-lineage). The ANI phylogenetic tree clustered all 57 isolates into three groups. In conclusion, the study emphasises the circulation of diverse *B. anthracis* sub-lineages in livestock.

Whole genome sequencing analysis of carbapenem resistant *E. coli* isolated from bovine mastitis milk sample

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Carbapenem-resistant *Escherichia coli* (CREC) poses a growing threat to public health, particularly when emerging from animal reservoirs such as dairy cattle. This study aimed to characterize CREC isolates recovered from bovine mastitis cases in Gujarat, India, using a combination of phenotypic antibiotic susceptibility testing and whole-genome sequencing (WGS). Out of 130 confirmed *E. coli* isolates from 790 mastitic milk samples, 33 (25.38%) were resistant to imipenem. Of these, nine exhibited multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-drug-resistant (PDR) phenotypes. WGS was performed on four representative isolates (SKN144, SKN685, SKN687, SKN926), revealing genome sizes ranging from 4.7 to 5.4 Mb and GC content between 50.4% and 50.8%. Annotation identified numerous resistance determinants, including carbapenemase genes (*blaNDM, blaOXA-48, blaTEM, blaCMY, blaCTX-M*), aminoglycoside-modifying enzymes (APH,

AAC), macrolide resistance genes (mphA, ermB), and multiple efflux pump systems (AcrAB-TolC, EmrAB, MdtEF-TolC). Functional genes associated with replication, repair, stress response, and mobile genetic elements (integrases, transposases, CRISPR-Cas) were also detected, indicating high genomic adaptability. Phenotypic testing revealed alarming resistance to key antimicrobials, including ampicillin (56.15%), amikacin (55.38%), ceftazidime (53.08%), and colistin (79.23%, including intermediate strains). Subsystem analysis highlighted metabolic versatility, defence mechanisms, and virulence-associated pathways. Phylogenetic analysis indicated that all isolates clustered within the same clade, suggesting possible clonal dissemination within the bovine population. The presence of CRISPR-Cas elements, integrases, and transposases suggests ongoing horizontal gene transfer and genome plasticity. These findings underscore the alarming prevalence of CREC in dairy environments and the urgent need for enhanced AMR surveillance, prudent antibiotic stewardship, and implementation of a One Health approach to prevent zoonotic transmission. This study contributes valuable genomic insights into livestock-associated CREC and highlights their close genomic parallels with high-risk human clinical clones.

Comparative genomics of Indian *Clostridium chauvoei* strain 23CCJK: insights into species lineages and virulence factors

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Clostridium chauvoei, a spore-forming bacterium, is responsible for fatal illness in cattle called black quarter (BQ) with significant economic impact in India. Despite its importance, genomic data on Indian strains remain scarce. This study aimed to sequence and characterize a *C. chauvoei* strain from an Indian outbreak and place it in a global phylogenetic context. A *Clostridium chauvoei* isolate was recovered from a black quarter outbreak in cattle in Leh, Ladakh (Oct–Nov 2023). Tissue samples were cultured anaerobically, and species confirmation was done via PCR. Genomic DNA was extracted and sequenced using Illumina NovaSeq 6000. Quality control, assembly, and annotation were performed using fastp, Unicycler, and Prokka, respectively. Comparative genome analysis of 84 global strains involved pangenome profiling (Panaroo, PanGP), virulence gene screening (BLAST, MEGA11), and CRISPR/prophage detection (CRISPRDetect, PHASTEST). SNP-based phylogeny was constructed using vSNP3 and RAxML. Core genome MLST was performed using chewBBACA and GrapeTree. Functional annotation of SNP-rich genes was done using egg NOG-mapper. The genome of *Clostridium chauvoei* strain 23CCJK (2.7 Mb, 2,557 coding genes) revealed key virulence factors including cctA, nanA, hyaluronidase, and collagenase. Pangenome analysis of 85 global strains identified 2,357 core and 381 accessory genes, indicating a closed genome. CRISPR profiles showed conserved patterns among Indian strains. Phylogenetic analysis grouped strains into three lineages, with Indian isolates clustering in L3. Novel hypermutated genes, such as those encoding discoidin domain proteins, ABC transporters, and histidine kinases, were identified, suggesting roles in metabolism, virulence, and host adaptation. These findings provide insights into *C. chauvoei* evolution and pathogenicity. This study reveals a conserved *C. chauvoei* genome with hypermutated genes, offering insights into virulence, lineage, and adaptation, and advancing genomic understanding of Indian *C. chauvoei* strains.

Non-cytopathogenic CSFV engineered as a viral vector for recombinant protein expression with optimized post-translational modifications

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Recombinant protein production in mammalian cells is efficient but costly. Viral vectors offer scalable alternatives; Classical Swine Fever Virus (CSFV) provides unique advantages due to its non-cytopathogenic phenotype and cytoplasmic replication. The study was conducted to develop and validate a CSFV based expression system as proof of concept for heterologous gene expression, and to assess its potential for safe, cost-effective recombinant protein production, a non-cytopathogenic CSFV vaccine strain backbone was engineered using an in-house customized plasmid assembled from synthetic and reported sequences. A dual reporter cassette (GFP and Renilla luciferase, ~1.8 kb) was inserted with a modified CMV promoter, kanamycin resistance marker, and 2A self cleavable peptide to ensure polycistronic expression. The construct was validated by PCR, restriction digestion, and sequencing. Functional rescue was performed in PK 15 cells, and viral progeny were monitored up to the seventh passage. Reporter expression was confirmed by fluorescence microscopy, immunofluorescence assay using CSFV specific antibodies, and RT-PCR amplification of luciferase transcripts, ensuring both genetic stability and phenotypic integrity of the recombinant virus. The recombinant CSFV successfully rescued in PK 15 cells and maintained stable replication through seven passages. Both GFP and luciferase were robustly expressed, confirmed by microscopy, immunofluorescence, and RT-PCR. Importantly, the engineered virus retained its non cytopathogenic phenotype, demonstrating that insertion of heterologous genes did not compromise viral stability. These findings establish the feasibility of CSFV genome manipulation and validate its use as a safe viral vector. The system supports authentic eukaryotic post translational modifications, including glycosylation, phosphorylation, acetylation, ubiquitination, and disulfide bond formation, ensuring functional optimization of recombinant proteins. This study establishes, for the first time, a functional non-cytopathogenic CSFV-based vector supporting stable dual reporter expression, validating its feasibility as a safe platform for recombinant protein production and future therapeutic biologic.

Role of circRNA–miRNA–mRNA crosstalk in regulating molecular events of early pregnancy in buffaloes

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Early embryonic losses in buffaloes are notably higher than in cattle, particularly during periods of long daylight hours, posing a major challenge for reproductive efficiency. A deeper understanding of the molecular events underlying early pregnancy is essential for deciphering the complex regulatory processes that influence embryo survival and implantation. Investigating circRNAs and their interactions with mRNAs and miRNAs provides valuable insights into post-transcriptional regulatory mechanisms and may help develop strategies to enhance conception and early embryonic success in

buffaloes. The present study aimed to elucidate the regulatory roles of circRNAs and their interactions with mRNAs and miRNAs during early pregnancy in buffaloes. Cotyledonary tissue was collected from early- and mid-stage pregnant buffaloes. Sequencing was performed using ribo-depleted RNA libraries on the NovaSeq 6000 platform. The raw reads were filtered, and circRNA analysis was conducted using seekCRIT, a STAR aligner-based pipeline. mRNA data from previous studies and miRNA data from published sources were selected for further analysis. Correlations between mRNAs and circRNAs were calculated in R. miRNA target identification was performed using miRANDA and miRWalk. Further analysis focused on identifying interacting RNAs with high positive correlation sharing the same miRNA. The ceRNA network was visualized in Cytoscape, integrated with ClueGO, and a protein–protein interaction (PPI) network was developed from the mRNAs included in the study. The integrated network was assessed using network analysis, cluster identification, and hub gene detection. Functional enrichment was performed using g:Profiler, DAVID, and STRING. A total of 35,260 known and 506 novel circRNAs were identified. Comparative analysis revealed 175 circRNAs with significant differential expression (log2 fold change ≥ 2, $p<0.05$). Functional enrichment analysis indicated that the enriched terms involve cell proliferation, differentiation, implantation, immune modulation, and focal adhesion. Cluster analysis identified nine distinct clusters. Notably, the clusters contained genes involved in the JAK/STAT pathway, COL5A3 and COL11A1 (extracellular matrix remodeling), and RNASEL3 (interferon signaling and apoptosis pathways). These findings provide novel insights into circRNA-mediated post-transcriptional regulation during early pregnancy in buffaloes and establish a foundation for future functional validation and strategies to enhance conception and implantation success.

Whole genome sequencing (WGS) analysis of multidrug-resistant (MDR) *Enterococcus* species isolated from milk

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Enterococcus species, particularly *E. faecalis* and *E. faecium*, are emerging as major public health concerns due to their ability to acquire and disseminate antimicrobial resistance (AMR). Milk and dairy products serve as potential reservoirs for these multidrug-resistant (MDR) pathogens, posing risks to both food safety and human health. This study aimed to evaluate the antimicrobial resistance (AMR) patterns and genomic characteristics of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from milk and dairy products, including buttermilk, curd, ice cream, and sweets, in the Anand region of Gujarat, India. According to CLSI standards, milk & milk products were collected and processed for bacterial isolation and for subsequent antibiotic susceptibility testing. The samples were inoculated in Brain Heart Infusion (BHI) broth for enrichment and incubated at 37 °C for 18-24 h. After that, the broth cultures were streaked onto BHI agar, Mannitol Salt Agar (MSA), and MacConkey Agar (MLA) to observe differential growth patterns. Following 48 h of incubation, colonies were subjected to Gram staining and observed under the microscope. Similarly appearing isolates were first identified by biochemical tests (Catalase, Coagulase, Oxidase, D-mannitol fermentation) and were then further confirmed by using MALDI-TOF mass spectrometry. A total of 205 *Enterococcus* isolates were obtained from milk and dairy products, with the highest contamination levels in buttermilk and curd. Isolates were identified using biochemical tests and MALDI-TOF mass spectrometry. The findings revealed significant multidrug resistance (MDR), particularly among E. faecium and E. faecalis, with over 95% resistance to key antibiotics, including linezolid, ciprofloxacin, cefpodoxime, and carbapenems. Many strains were classified as MDR, XDR, and PDR. This study pointed out the alarming prevalence of multidrug-resistant *Enterococcus* spp. in milk and dairy products from Anand, Gujarat. The detection of MDR, XDR, and PDR strains, along with the co-occurrence of multiple resistance determinants and efflux pump mechanisms, really signals the very serious risks for AMR transmission through the food chain.

Whole genome analysis of Lumpy Skin Disease virus isolated from natural outbreaks in Assam, India

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Lumpy skin disease virus (LSDv) is a highly infectious DNA arbovirus that causes a morbid disease in cattle populations worldwide, resulting in a devastating loss of milk production. The objective of the study was to analyze the complete genome of the circulating LSDv strain from Assam and compare it with global and reported Indian isolates to identify the genetic variations and possible origins of introduction. This study is a part of a greater study aimed at isolating and molecular characterization of LSDv from natural outbreaks in Assam. A total of 52 samples were collected from five different locations in Assam, where outbreaks had been reported. 39 samples were reported positive based on successful thermal amplification (PCR) of the partial P32 gene. A representative isolate was selected for whole-genome sequencing after successful amplification of ORF012 and ORF036 from the virus propagated in BHK-21 and MDBK cells. The sequenced raw data were processed to obtain high-quality clean reads using Trimmomatic v0.38 to remove adapter sequences, ambiguous reads, and low-quality sequences, and were compared with reference genomes (Accession nos. NC_003027 and OP297402). After alignment, the total mapping percentages were 0.83% and 0.95%, with genome coverages of 99.94% and 99.95% respectively, with the total SNPs being 143 and 147 for the reference genomes NC_003027 and OP297402, respectively. The highest number of SNPs was observed in the LSDV094 gene. When compared to the NC_003027 and OP297402 genomes, six SNPs were identified for each, respectively. Subsequently, a total of 26 and 30 insertions and deletions, and 16 and 18 intergenic insertion-deletions were observed for the sample genome when compared with NC_003027 and OP297402, respectively. Genetic divergence in our LSDV strain, marked by SNPs and indels, affects host range and immune evasion. This underscores the need for broader sequencing to refine genomic epidemiology.

Poster Presentations

Harmony in the heat: unraveling the symphony of heat shock protein 70 in Indian malarial vector- a molecular ballet shaping mosquito development and influencing *Plasmodium t*ransmission

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Heat Shock Proteins (HSPs) are critical molecular chaperones, categorized into various classes based on their molecular mass, which assist in protein folding and support organisms in adapting to extreme environmental conditions. Despite their importance, the role of HSPs in the stress response of mosquitoes remains insufficiently understood. This study investigates Heat Shock Protein 70 (HSP-70) in two primary Indian malaria vectors, *Anopheles culicifacies* and *Anopheles stephensi*, aiming to elucidate its role in stress response mechanisms and its potential application in vector control. The objectives of the study were to identify and characterize HSP70 in *Anopheles* species, role of HSP70 in mosquito development. role of anopheles HSP70 in plasmodium development inside midgut The investigation involved a comprehensive bioinformatics analysis of HSP-70 in *An. culicifacies* and *An. stephensi* using tools such as BLASTP, tBLASTn, and Pfam for sequence analysis, and phylogenetic and syntenic analyses to explore evolutionary relationships. Experimental validation included qPCR to examine HSP70 expression across developmental stages and under various stress conditions, alongside recombinant protein expression and characterization. Functional studies involved RNAi-mediated silencing of HSP70-1 in *An. culicifacies* larvae, coupled with selective inhibition of HSP70 to evaluate effects on developmental cycle of mosquito and Plasmodium development inside mosquito. Seven distinct subcellular localizations of HSP70 isoforms were identified. Notably, HSP70-1 exhibited increased expression at the L3 larval stage, underscoring its role in larval development. Silencing HSP70-1 led to significant upregulation of other HSP70 isoforms indicating compensation of stress. In *P. berghei*-infected mosquitoes, HSP70 inhibition markedly impaired oocyst development, suggesting its critical role in parasite survival within the mosquito host. This study provides novel insights into the function of HSP70 in malaria vectors, particularly how its inhibition impacts mosquito development and malaria parasite progression. The findings suggest that targeting HSP70 could serve as a potential vector control strategy. By examining the interactions between HSP70 and malaria parasites, this research identifies exploitable vulnerabilities that may aid in reducing malaria transmission.

Micro-RNAs profiling after Foot and Mouth Disease (FMD) vaccination in cattle

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Foot and mouth disease (FMD) remains a major threat to the sustainability of livestock production systems. Despite extensive vaccination efforts, frequent outbreaks have been reported in India over the past decade, impacting its 573.6 million livestock population. Achieving effective disease control and eradication necessitates a deeper understanding of the molecular mechanisms regulating host immunity, particularly the post-transcriptional gene regulation mediated by microRNAs (miRNAs).

Given their immunological relevance and potential as biomarkers for vaccine efficacy and disease progression, this study aimed to identify, characterize, and validate miRNAs expressed in peripheral blood mononuclear cells (PBMCs) of cattle following FMD vaccination. Seronegativity before vaccination and seroconversion post-vaccination were confirmed using the Virus Neutralization Test (VNT). High-throughput miRNA sequencing (miRNA-seq) identified 301 known miRNAs at 0 days post-vaccination (dpv) and 315 at 7 dpv, with 37 and 17 miRNAs uniquely expressed at 0 dpv and 7 dpv, respectively. A total of 26 miRNAs were found to be differentially expressed, including 14 upregulated and 12 downregulated miRNAs. Based on log□ fold change, two upregulated (bta-miR-421 and bta-miR-885) and two downregulated (bta-miR-148a and bta-miR-155) miRNAs were selected for downstream analyses. Target gene prediction revealed 207 and 219 potential targets for the upregulated and downregulated miRNAs, respectively. Functional annotation and enrichment analysis indicated significant involvement of these targets in pathways related to actin cytoskeleton regulation and cancer signaling. Experimental validation using stem-loop qRT-PCR confirmed the sequencing results for the selected downregulated miRNAs. Furthermore, qPCR validation of target genes (MAPK3, MARK2, MMD, and LBR) demonstrated concordant expression patterns for MARK2 (target of bta-miR-155) and MAPK3 (target of bta-miR-421) with their respective miRNAs. Upon validation in an expanded sample set, bta-miR-148a and bta-miR-155 emerged as promising biomarkers for distinguishing infected from vaccinated animals (DIVA) and hold potential for application in breeding programs aimed at developing disease-resistant livestock.

Whole genomic sequencing of archival GTPV Mukteswar strain (1946) reveals genetic diversity and phylogenetic relationships of Goatpox viruses

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Goatpox is an OIE-notifiable and economically important viral disease of goats, characterized by pyrexia, skin nodules, and internal lesions. Despite vaccination efforts, limited molecular characterization of GTPV strains exists in India. Considering the endemic nature of the disease, comprehensive genomic studies are essential to understand its genetic diversity and evolution. This study aimed to characterize the complete genome of the archival GTPV Mukteswar virulent strain (1946) using next-generation sequencing to understand its genetic composition and evolutionary significance. The genomic DNA was extracted from infected skin scab tissues and was sequenced using Illumina HiSeq technology. Reads were quality-filtered, assembled, and mapped to reference genome. Gene prediction and annotation were performed to identify open reading frames (ORFs) and structural variations. Comparative genomic and phylogenetic analyses were conducted with available GTPV, SPPV, and LSDV sequences to identify similarities, unique genes, and evolutionary relationships. The complete genome of the GTPV Mukteswar strain was approximately 150 kbp in length and encoded 150 full-length ORFs. Eight ORFs (002/155, 004/153, 009, 013, 026, 132) were fragmented in comparison with LSDV. In contrast to SPPV and other GTPV strains, ORF136 remained intact. Phylogenetic analysis revealed the existence of three distinct sub-lineages among GTPV strains, with the Mukteswar strain clustering closely with isolates from India, China, and Vietnam, indicating regional genomic conservation. These findings enhance understanding of the genetic diversity and evolutionary relationships among capripoxviruses. In the future, this study will support comprehensive genomic surveillance of GTPV strains circulating in India and serve as a reference for one of the earliest (historical) GTPV isolates. This information will be valuable for designing effective diagnostic tools, understanding epidemiological patterns, and guiding control strategies to manage goatpox in endemic regions.

Multi-omics integration of sperm interactomes to elucidate sex-specific molecular regulation in livestock reproduction

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The multi-omics approach is a cutting-edge technique in biomarker discovery. The role of the Multi-Omics approach in drug discovery and cancer studies by using an integrated analysis of mRNA, lncRNA, miRNA, and proteomics has led to the identification of specific molecules in terms of disease. In the case of reproductive biology and biotechnology, Multi-Omics studies revealed a prominent information helped in deciphering infertility and helped in embryo production success. The main objective of the study was to develop a network analysis between X and Y chromosome-bearing spermatozoa of cattle by integrating miRNA, mRNA, lncRNA, and proteomics data, for identifying sex-specific pathways and biomarkers for a better reproductive success in cattle production. Data related to X and Y bearing spermatozoa of cattle mRNA, miRNA from NCBI; Proteomics LC-MS/MS data (prteomeXchange) retrieved and analyzed to identify the DEGs, Differential miRNA, DEPs, and DElncRNA done by using command line by using miRDeep2, salmon, bowtie2, cuffcompare, and finally the DESeq2 RStudio package for DE analysis. Further integration was done to find out the co-differential expression patterns of all data types, gene target prediction, and functional enrichment analysis by using RStudio packages like dply, BiomaRt, limma, and GenomicRanges. In the end, a comprehensive ceRNA Network Construction was done to know the effects on each other's molecules in terms of fertilization and embryo development. In differential analysis, a total of 81 genes were significantly down and 16 genes were significantly up in mRNA; in lncRNA analysis, 31 were significantly downregulated and 18 were significantly upregulated; in miRNA,13 were significantly down and 8 were significantly up. By using TargetScan prediction, we found out the miRNA targets, and further integration for co-expression analysis showed major reproductive pathways regulation in terms of motility and fertilization. The ceRNA network analysis revealed the major interactions, and enrichment analysis helped to find sex-specific reproductive molecular Interactomes in cattle, both in X and Y chromosome-bearing spermatozoa. In conclusion, Interactome and interwork analysis by using a Bioinformatics approach helped in finding major sex-specific regulatory mechanisms in either X/Y spermatozoa of cattle.

Homology arm length and donor size dictate HDR mediated site specific CRISPR/Cas9 knock in efficiency in Vero cells

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CRISPR/Cas9 mediated HDR enables precise gene integration, but knock in efficiency is strongly influenced by donor design. We investigated homology arm length and donor size effects in Vero cells. The objective of the study was to define an optimal homology arm window and assess donor size impact on HDR mediated knock in efficiency at the thymidine kinase locus in Vero cells. A TK targeting sgRNA and Cas9 were co transfected with linear dsDNA donors carrying a ~2 kb GFP cassette flanked

by symmetric HAs of 600, 800, 1000, 1500, or 2000 bp. BrdU selection enriched TK loss populations. Integration was validated by locus specific PCR, dual junction sequencing, and GFP fluorescence. HDR efficiency was quantified as GFP positive knock in clones relative to total transfected cells. Off target risk was assessed at predicted sites by PCR. GFP expression was monitored across passages to distinguish transient from integrated signals, with stable integration evident after extended culture. Knock in efficiency increased with HA length up to 1000 bp, achieving ~37%. Shorter arms (600 bp) yielded <8%, while extension beyond 1000 bp (1500–2000 bp) did not further enhance HDR. Larger donor constructs reduced transfection efficiency, lowering edited fractions, but longer HAs consistently favored HDR over NHEJ, improving integration precision. BrdU selection enriched TK loss populations, and stable integration was confirmed by dual junction PCR and sequencing. GFP signals stabilized after serial passages, consistent with integration rather than episomal persistence. Off target PCR screening revealed no disruptive edits at predicted sites. An optimal HA window of 800–1000 bp enables efficient HDR knock in of ~2 kb donors in Vero cells, providing actionable design parameters for vaccine and functional genomics platforms.

Genetic Parameters, Gains, and Trends of First-Parity Production and Reproductive Traits in Nili-Ravi Buffaloes

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The Nili-Ravi buffalo is among the world's top dairy breeds. Analysis of first-parity records enables early identification and selection of superior animals, reducing the time needed for genetic evaluation. While buffaloes typically reach peak performance from the third parity, traits with high heritability allow effective early selection. Traits with low heritability are more influenced by management but can be improved via correlations with highly heritable traits. Genetic trends indicate long-term progress, and trait correlations guide selection to achieve favorable responses while minimizing undesired correlated effects. To estimate genetic parameters, genetic and phenotypic trends, genetic gains, and correlations for production and reproduction traits in first parity animals. Data on production and reproduction traits were obtained from herd history sheets and historical records spanning 1980 to 2023, comprising performance data of 811 females and 158 bulls. The effects of non-genetic factors were evaluated using the Least Squares Method in SPSS 26.0, considering Birth Season Group (BSG), Birth Year Group (BYG), Parity, Calving Season Group (CSG), Calving Year Group (CYG), and Age at First Calving Group (AFG) as fixed factors. The significant factors were subsequently incorporated into the mixed model for estimating genetic parameters using the Restricted Maximum Likelihood (REML) approach in WOMBAT. An animal model was employed for the estimation of genetic parameters for the selected first-parity traits. Heritability estimates were moderate for most traits: FPY (0.318±0.073), F305LY (0.302±0.014), FTLY (0.236±0.012), FLL (0.204±0.066), FGP (0.172±0.075), AFC (0.310±0.076), FSP (0.105±0.059), FDP (0.096±0.059), and FICP (0.108±0.060). The phenotypic trends showed annual improvements of 0.126 kg for FPY, 30.08 kg for F305LY, and 13.05 kg for FTLY, whereas FLL decreased by –1.58 days/year, AFC by –8.48 days/year, FSP by –3.15 days/year, FDP by –3.45 days/year, and FICP by –3.33 days/year. Genetic trends were positive but low for FPY (0.0086 kg/year), F305LY (1.00 kg/year), FTLY (0.14 kg/year), and FLL (0.14 days/year), while AFC (–0.06 days/year) and FDP (–0.51 days/year) exhibited a slight decline. Environmental trends followed a similar direction to the phenotypic trends, indicating steady genetic progress supported by improved management practices. Phenotypic correlations were strong and positive among F305LY–FLL, F305LY–FPY, FDP–FICP, FDP–FSP, FLL–FICP, and FLL–FSP,

suggesting concurrent improvement potential. Genetic correlations between F305LY and FLL, FPY, FSP, and FICP were also strong and positive, indicating shared genetic control over these traits. Conversely, FGP showed negative genetic correlations with F305LY (–0.47±0.23), FICP (–0.78±0.35), FLL (–0.60±0.29), and FSP (–0.80±0.34), implying that deviations in gestation length adversely influence production and fertility traits. Production traits (FPY, F305LY, FTLY, FLL) showed high heritability, allowing early selection and stable genetic gains, though genetic trends were slightly positive with low R^2 due to small herd size. Reproductive traits had low to very low heritability, with flat to slightly positive or negative genetic trends, heavily influenced by environmental factors. The FSP, FDP, and FICP phenotypic and environmental trends are in the desired and required negative direction. High-heritability production traits may benefit from intensified selection, including advanced reproductive techniques like IVF-OPU, to accelerate genetic improvement.

Prevalence and molecular characterization of methicillin -resistant *Staphylococcus* species from skin infections of bovines and canines

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Antibiotic misuse has contributed to the emergence of methicillin-resistant *Staphylococcus* spp. (MRS). These pathogens, isolated from animal skin infections, pose veterinary and zoonotic risks due to their strong antimicrobial resistance. The study aimed to evaluate the prevalence, phenotypic resistance, and molecular characterization of methicillin-resistant *Staphylococcus* spp. from bovine and canine skin infections, highlighting their zoonotic importance and alternative resistance mechanisms. A total of 57 skin infection samples, including 24 bovine and 33 canine specimens, were processed using standard microbiological protocols. Initial screening involved culture on Brain Heart Infusion (BHI) agar, Gram staining, biochemical identification, and tube coagulase testing. Phenotypic resistance was determined using cefoxitin and oxacillin susceptibility tests. Molecular confirmation was performed through polymerase chain reaction (PCR) using genus-specific *mecA* genes. Isolates were further classified into *Staphylococcus aureus*, *Staphylococcus pseudointermedius*, and coagulase-negative staphylococci (CoNS) based on coagulase activity and molecular assays, enabling accurate prevalence estimation and antibiotic resistance profiling. Out of 57 samples, *Staphylococcus* spp. were detected in 11 (45.8%) bovine and 20 (60.6%) canine cases. Among *Staphylococcus* Spp Coagulase activity was present in 10 canine (50%) and 3 bovine (12.5%) isolates. PCR confirmed 10 bovine and 18 canine isolates as *Staphylococci*. *Staphylococcus aureus* occurred in 33.3% of bovine and 22.2% of canine coagulase-positive strains, while *Staphylococcus pseudointermedius* predominated in canines (61.1%). CoNS represented a significant proportion. Methicillin resistance was observed phenotypically, with 42.85% cefoxitin and 89.28% oxacillin resistance. The *mecA* gene was detected in 12.5% bovine and 21.2% canine isolates, indicating potential alternative resistance mechanisms beyond *mecA*. CoNS and coagulase-positive Staphylococci significantly contribute to bovine and canine skin infections. High phenotypic methicillin resistance and variable *mecA* presence highlight emerging resistance mechanisms needing advanced molecular investigation.

Phytochemical characterization and cytotoxicity analysis of *Tinospora Cordifolia* aqueous extract: Exploring its potential as a natural therapeutic agent

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Tinospora cordifolia (Guduchi) is a large, smooth, perennial, deciduous climbing shrub with a weak, fleshy stem that is widely distributed throughout India. This plant has been extensively used in traditional Ayurvedic and folk medicine due to its diverse therapeutic properties. In the present study, the significance of *T. cordifolia* was investigated by evaluating its aqueous stem extract. The aqueous extract of *T. cordifolia* was prepared and subjected to qualitative phytochemical screening to detect bioactive compounds with potential antiviral and immunomodulatory effects. The extract was further characterized using various physicochemical and analytical techniques, including UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy, to identify functional groups and molecular structures. Additionally, the cytotoxicity of the extract was assessed through percent hemolysis assays to ensure its safety. Cytotoxicity was assessed via hemolysis assays to determine its safety profile. Results indicated that the aqueous extract was non-cytotoxic to red blood cells within the tested range. These findings suggest that, aqueous extract of *T. cordifolia* is safe for mammalian cells, supporting its potential development as a natural antiviral and immunomodulatory therapeutic agent.

Comparative assessment of Cre recombinase mediated cassette exchange and CRISPR/Cas9 directed homology repair for site specific gene knockin

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Precise genome editing underpins modern biotechnology. This study compared CRISPR/Cas9 mediated HDR and Cre recombinase mediated cassette exchange (RMCE) for targeted knock in using an engineered Vero cell model. The aim of the study was to evaluate efficiency, specificity, and applicability of CRISPR/Cas9 HDR versus Cre RMCE in a customized Vero clone, highlighting their relative strengths and limitations for site specific genome engineering. Experiments were conducted in an in house engineered Vero clone (C308), which expresses GFP, is BrdU resistant due to TK inactivation, and carries unique loxA/loxB sites flanking the GFP cassette integrated at the TK locus. For CRISPR/Cas9, a sgRNA targeting the TK region was co-transfected with a donor plasmid carrying GFP flanked by homologous arms. For Cre-RMCE, a lox flanked donor cassette and transient Cre recombinase expression mediated cassette exchange at the integrated lox sites. Both systems were optimized for transfection and evaluated under identical conditions. Knock in events were confirmed by PCR, sequencing, and fluorescence microscopy, with Renilla luciferase normalization. CRISPR/Cas9 achieved reproducible knock in efficiencies of 9–10%, while Cre RMCE yielded ~8–9% but only in lox engineered C308 cells. CRISPR/Cas9 showed slightly reduced viability (~85%) compared to Cre RMCE (~90%) but offered broader applicability without prior genome modification. Off target insertions occurred in ~2–3% of CRISPR clones, whereas Cre RMCE showed none, confirming its high specificity. GFP expression was stronger in CRISPR-edited clones, suggesting favorable genomic positioning. Both systems demonstrated reliable performance, with CRISPR offering flexibility and Cre RMCE providing precision in pre agged lines. CRISPR/Cas9 enables versatile, locus independent knock-in with modest off target risk, while Cre RMCE ensures high specificity in engineered lines. Hybrid strategies may combine CRISPR's flexibility with Cre's precision.

Comparative genomic analysis of resistance and virulence determinants of milk derived extensively drug resistant (XDR) pathogens of *Escherichia coli* and *Staphylococus ureilyticus*

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Dairy products, particularly raw milk and fermented beverages, serve as potential reservoirs for antimicrobial-resistant bacteria that can enter the human food chain. *Escherichia coli* and *Staphylococcus* spp. are common milk-borne pathogens with the ability to acquire multidrug resistance and virulence traits. Whole genome sequencing (WGS) provides a powerful tool for identifying resistance and virulence determinants at the molecular level. In this context, comparative genomic analysis of extensively drug-resistant (XDR) isolates is critical for understanding the spread of resistance and formulating effective control measures. This study was undertaken to isolate, identify, and perform comparative genomic analysis of extensively drug-resistant (XDR) *Escherichia coli* SKN 649 and *Staphylococcus ureilyticus* SKN 217 derived from raw milk and buttermilk samples, with emphasis on their antimicrobial resistance profiles and genetic determinants. 100 raw milk and buttermilk samples were collected from cattle farms in Anand, Gujarat, India (January 2022 - December 2023). Initial isolation was performed on selective agar media, followed by species-level confirmation using MALDI-TOF mass spectrometry. Antibiotic sensitivity was tested against multiple drug classes using standard CLSI protocols. XDR isolates (*E. coli* SKN 649 and *S. ureilyticus* SKN 217) were sequenced, and genomic analysis was performed to identify antimicrobial resistance (AMR) genes and mechanisms. *E. coli* SKN 649 showed resistance to penicillin G, oxacillin, erythromycin, amikacin, and ampicillin, while remaining sensitive to tetracycline and chloramphenicol. *S. ureilyticus* SKN 217 exhibited high resistance to penicillin, cefpodoxime, and cefoxitin; 90% of isolates were resistant to two or more drugs, and 75% were non-susceptible to linezolid. The study demonstrates the presence of extensively drug-resistant *E. coli* and *S. ureilyticus* in raw milk and fermented dairy beverages from Anand, Gujarat. Comparative genomic analysis revealed multiple resistance determinants associated with efflux pumps, target modification, and drug inactivation. These findings highlight the genetic adaptability of dairy-derived pathogens in acquiring AMR traits and emphasize the urgent need for genomic surveillance, prudent antibiotic use, and improved biosecurity measures in dairy farming to mitigate AMR risks and safeguard public health.

Aeromonas hydrophila inoculated Indian major carp, rohu (*Labeo rohita* ham.), shows dynamic changes in immune-effector activities similar to *in vitro* generated T-cell lines

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The Indian major carp, rohu (*Labeo rohita* Ham.), one of the popular species in the Indian subcontinent, is highly susceptible to motile *Aeromonas* infections. Progress in the development of prophylactic tools to control aeromoniasis in the farming systems requires a thorough knowledge of basic immune functions in rohu during *A. hydrophila* infections, which are lacking now. In this study, rohu, sensitized with live *Aeromonas hydrophila*, was used to evaluate *in vitro* immunoreactivity against *Aeromonas hydrophila* antigens. Fish were maintained in the laboratory in 4 groups, viz., two control groups, C1 (n= 10) and C2 (n= 10), and two test (treated) groups, T1 (n= 10) and T2 (n= 10). Test fishes (T1 and T2) were inoculated (100µl) intra-peritoneally (I/P) with live *Aeromonas hydrophila* @ 2 ×

10^7 cfu/ml for sensitization. At 7th, 15th, and 21st day post inoculation (DPI), fish (n=2) from each tank were sampled, heart blood was collected for serum separation to detect anti-aeromonas antibodies by enzyme-linked immunosorbent assay (ELISA) and subsequently by Western blot analysis. Further, mononuclear cells from spleen were isolated at 7th, 15th, and 21st DPI and plated in 96-well plate @ 2×10^5 cells per well. The cells were subjected to nitrocellulose membrane (NCM)-blotted polypeptides of *Aeromonas hydrophila* somatic antigens, along with crude antigen and positive control (conA). Subsequently, the in vitro proliferative and cytotoxic ability of leucocytes was determined. Two antigen-specific T-cell lines were generated from *A. hydrophila* sensitized two rohu fishes, one each of the T1 and T2 groups, randomly, on the 7th day post-sensitization. On the 14th day of T-cell line generation, lymphoproliferation assay and cytotoxicity assay were performed to characterize the antigen-specific T-cell lines. Dynamic changes in seroreactivity were observed in sensitized fish, with the highest seroreactivity at 15DPI as assessed by ELISA. Three polypeptides having molecular weights of 57, 67, and 92 kDa were identified in a Western blot when the pooled serum of sensitized rohu of 15DPI was used. The stimulation indices (S.I.) of leucocytes stimulated by crude somatic antigens were observed to be much higher in the sensitized fish than in the control at all DPI. In vitro stimulation of leucocytes was observed to peak at 15 DPI. Further, S.I. of NCM-blotted polypeptides was found to be much higher in the molecular weight range of 90-95 kDa at 15 DPI. The in vitro percent cytotoxicity of sensitized leucocytes was observed to be 65.8% at 7 DPI, 38.1% at 15 DPI, and 13.21% at 21 DPI. Antigen-specific in vitro-generated T-cell lines also showed high lymphoproliferation and cytotoxicity on the 14th day of development. In conclusion, as the characteristics of in vitro-generated T-cell lines were observed to be similar to spleen leucocytes collected from sensitized rohu, antigen-specific T-cell lines might be considered as a model for studying immunology of fish (rohu), corroborating the in vivo situation.

Healing assessment of canine surgical wound treated with allogeneic stromal vascular fraction and xanthosine: A preliminary result

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This study evaluated the efficacy of allogeneic canine stromal vascular fraction (SVF) and xanthosine (Xs), administered individually and in combination, for improving surgical wound healing in dogs, compared with standard post-operative treatment. Client-owned dogs undergoing surgery were treated immediately after suturing with subcutaneous injections of SVF, Xs, SVF+Xs, or standard wound care as controls. Wound healing was assessed through photographic monitoring, evaluation of wound redness, subjective hair regrowth scoring, and measurement of serum levels of vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) via ELISA on Days 0, 7, and 14. Hair regrowth scores were significantly higher in the SVF-treated group (Day 7: 1.16±0.16; Day 14: 1.6±0.16) compared to control (0.6±0.09; $p<0.05$). Wound redness decreased markedly in the Xs group (Day 14: 0.3±0.16) versus control (1±0.2; $p<0.05$). VEGF levels were elevated with Xs (231.2±31.75 ng/mL; $p<0.001$), SVF (147.7±1.31; $p<0.001$), and the combined Xs+SVF therapy (99.91±0.42; $p<0.05$), correlating with improved vascularization. TGF-β concentrations increased at Day 14 with Xs (19.94±2.23 pg/mL; $p<0.001$) and SVF (15.2±0.02; $p<0.01$), supporting their role in collagen synthesis and wound remodeling. The combination therapy demonstrated attenuated TGF-β elevation compared to individual treatments. Both SVF and xanthosine significantly enhanced wound healing parameters in canine surgical cases, confirming their potential as regenerative therapies. These findings highlight promising avenues for integrating SVF and nucleoside-based therapy into veterinary wound management protocols.

Pipeline-dependent variation in microbial diversity estimation: Metagenomic analysis of bovine uterine discharge

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Metritis is associated with disturbance in uterine environment leading to anestrous, irregular estrous, no conception, early embryonic death and many more reproductive disorders. These can only be addressed through restoration of uterine environment. To understand the uterine environment understanding of uterine microbiota is essential. The present study tried to understand uterine microbiota of healthy and metritic uterus of sahiwal cows. The study, characterized and compared the uterine microbial communities of healthy and metritic Sahiwal dairy cows using two distinct bioinformatics pipelines, DADA2 (in R) and MG-RAST, to assess microbial dysbiosis and evaluate analytical consistency. Uterine flush samples (n=8 healthy; n=8 metritic) were subjected to 16S rRNA gene sequencing, targeting the V4 region (Illumina HiSeq 2000) for DADA2 analysis and the V3–V4 regions for MG-RAST analysis. Total DNA was extracted and processed to infer Amplicon Sequence Variants (ASVs) using DADA2, while MG-RAST provided taxonomic classification and α-diversity metrics (Chao1, Richness, Shannon, Simpson). Both platforms revealed clear microbial distinctions between health states. DADA2 analysis indicated that, the healthy uterine microbiota was dominated by archaeal taxa, primarily Methanobacteriota and Halobacteriota, with Methanocorpusculum and Methanobrevibacter as major genera, reflecting a stable anaerobic ecosystem. In contrast, metritic samples showed decreased richness but higher evenness, characterized by a shift toward pathogenic bacterial phyla such as Bacillota, Pseudomonadota, and Actinomycetota. Pathogenic genera including Klebsiella, Escherichia-Shigella, Streptococcus, and Clostridium were markedly enriched, indicative of microbial dysbiosis. MG-RAST corroborated these findings but reported higher bacterial diversity in metritic samples (α-diversity 42.08 vs 3.08 in healthy), with additional detection of Fusobacteriaceae and Mycobacteriaceae families potentially linked to uterine pathology. Comparatively, DADA2 provided finer ASV resolution and quantitative precision through its error correction capabilities, whereas MG-RAST offered broader community profiling and functional annotation. The concordance in key pathogenic trends across both platforms strengthens confidence in cross-validated metagenomic approaches. Overall, metritis in Sahiwal cows was associated with the disruption of a stable archaeal-dominant uterine niche and the proliferation of opportunistic bacterial pathogens. The combined use of DADA2 and MG-RAST enhances accuracy in microbiome interpretation and underscores the diagnostic potential of multi-platform validation in reproductive health.

Session 7:

Frontiers in Translational Biotechnology

The Indian One Health Compass: Navigating Zoonoses, AMR and Smart Livestock Production for a Secure Future

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The 'One Health' paradigm is no longer a conceptual ideal but a strategic imperative, profoundly recognized by the Government of India through its pioneering initiatives. This keynote address will chart a visionary course for leveraging these national programmes to build a resilient and productive livestock sector. India's efforts within the global landscape positions India as a leader and provides a guiding metaphor, highlighting the integration of animal, human, and environmental health as the cornerstone of national security and economic prosperity.

The twin challenges of Emerging Zoonoses and Antimicrobial Resistance (AMR) are essentially the two sides of the same coin. The national missions, such as the National One Health Mission and National Mission on Biodiversity, are creating a cohesive framework for climate change and disaster risk reduction, coordinated disease surveillance, rapid diagnostics, and containment. The critical role of advanced genomics and molecular epidemiology in predicting spillover events and tracing pathogen evolution shifts the stance from reactive to a proactive one. The immense opportunities presented by innovation in livestock production itself pivot the integration of 'Omics' technologies, genomics, and data-driven smart farming is revolutionizing animal health, productivity, and welfare. The convergence of genome editing for disease resistance and the application of AI for precision livestock farming will be a transformative force. This not only enhances biosecurity and food safety but also directly contributes to climate-smart agriculture and farmer livelihoods, aligning with national goals for sustainable development.

Towards a visionary path emphasizing a forward-looking agenda, collaboration, leadership and, strengthening the translational pipeline-from groundbreaking biotechnology research in labs to deployable solutions in the field–are the need of the hour. By fostering deeper collaboration between veterinarians, medical professionals, environmental scientists, and data experts as well as the industry partners, the "One Health Compass" can be calibrated to navigate the complexities of the 21st century. India, with its integrated programmes and scientific prowess, is uniquely positioned to lead this charge, ensuring a healthy future for its people, its animals, and its environment.

In silico mRNA Vaccine Candidate for Avian Influenza Virus H5N1: Comparative Evaluation of Stalk and Head Domains of Neuraminidase N1

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Influenza A Virus (IAV), in family *Orthomyxoviridae*, originate almost in wild aquatic birds of different types, and hence also named as Avian Influenza Virus (AIV). The AIVs are highly zoonotic, transmit from birds to human, and to other terrestrials. The virus has two glycoproteins, HA and NA, hemagglutinin (H) and neuraminidase (N), respectively. Both HA and NA are antigenically diverse, HA>NA, and require Sialic Acids (SAs) residue as substrate. Both H and N are expressed on the viral envelope, and virus strains are named as per antigenic types of H and N. Of late the H5N1 AIV that caused highly zoonotic Bird Flu pandemic in 1995 and epidemic in India in 2006, has now spread to cows in certain parts of the Globe since 2024. Both HA and NA elicit protective antibody response in infected/ vaccinated ones. We have targeted NA as it is less divergent than HA. The present study compares the in-silico antigenicity and immunogenicity of two overlapping regions in the Neuraminidase (NA) N1 (H5N1), consensus of both bovine and avian N1(H5) sequences, viz., amino acid residue stretches 41–351 (AA311) and 115–469 [end] (AA355) to develop a novel mRNA vaccine candidate for Cattle/ Bovine. The cytoplasmic tail (CT) and trans membrane (TM) domains of NA were truncated. Amino acid residues 1-6 comprise cytoplasmic tail (CT), 7 -29 comprise transmembrane domain (TMD), 30-75 comprise Stalk domain (SD) and 76- 469 comprise Head domain (HD) of the NA. Both polypeptides (AA311 and 355) had good pTM values, 0.85 and 0.97 respectively, in AlphaFold analysis. Both are also predicted good antigenic by Vaxijen and AntigenPro. Aliphatic index of AA311 was higher (64.57) compared to AA355 (58.45), and instability index of 38.45 and 40.21, respectively. In RNAfold analysis, bovine mRNA construct with AA311 (mRNA1324) had MFE (minimum free energy) of 620kcal/mol, and that of mRNA1456 with AA355 was 725kcal/mol. Variation in MFE is related to the size of the mRNA construct.

AA355 is devoid of SD, and lacks first 74 residues from N terminus of HD. AA311 encompasses partial SD (short of first 10 residues) and 276 residues from the N terminus of HD. Both the truncated N1, AA311 and AA355 were subjected to CTL (cytotoxic T Lymphocytes/MHC I) and HTL (helper T lymphocytes/ MHC II) epitope prediction for 8 MHC I/ BoLA alleles (BoLA HD6, D18.4, T2a, T2b, T2c, T5, JSP.1 and AW10) and 5 MHCII/BoLA-DRB3 (BoLA-DRB3*1101, 1201, 14011, 1501 and 0101) alleles, using NetMHCpan 4.1 and Net MHC II pan 2.1 servers, respectively. For HTL, strong binding epitopes and for CTL both weak and strong binding epitopes were selected for analysis. The AA311 segment has 11 CTL (MHC I/Tc) and 6 HTL (MHC II/ Th) antigenic epitopes, whereas, the AA355 has 15 CTL and 3 HTL antigenic epitopes, respectively for the given BoLA alleles. AA311 MHC II epitopes (06) covered 5 alleles (BoLA-DRB3*1101, 1201, 14011, 1501 and 0101), compared to one allele (BoLa-DRB3*1101) by 03 epitopes of AA 355. Liner B cell epitopes were predicted using ABCPred server. The AA311 has 6, and AA355 has 14 B-cell epitopes. Altogether, AA 311 has less number (23) of epitopes than AA355 (32), and 44 residues shorter in length. Though both polypeptides were also found very good in Immune simulation (Tc, Th and B cell responses), both CMI and humoral, the polypeptide AA 311 was predicated to be much more stable in mammalian cells (30h), yeast (>20h) and *E. coli* (>10h), compared to AA 355 (1.1h, 3min and 2 min, respectively).

The AA311 polypeptide is composed of partial SD and partial HD regions, having four Arginine (R) residues at the positions 118, 152, 225 and 293 that are supposed to interact with carboxylate of the SAs, and other important residues of Asp^{151} and Glu^{277}. These residues made the inner shell. The SAs interaction site of NA is highly conserved, both spatially and in sequence, making it an ideal target for drug/ vaccine development. In addition, AA 311 has the framework residues of Glu^{119}, Arg^{156}, Trp^{179}, Ser^{180}, Asp^{199}, Ile^{223}, Glu^{228}, Glu^{278} and Asn^{295}, around the SAs interaction site, that do not directly interact with the SAs. The present in-silico analysis results favour AA311 as a suitable antigen target for design of N1- mRNA vaccine for NA- based immune protection against AIV H5N1.

Enhancing Large Viral DNA Genome Editing using Stable Cell Line Co-expressing CRISPR/Cas9 and gRNAs

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The CRISPR/Cas9 system has revolutionized genome editing through its efficiency and simplicity of design and implementation. CRISPR/Cas9 is a two-piece system, comprising the Cas9 nuclease and a guide RNA (gRNA), which form a ribonucleoprotein (RNP) complex. The gRNA guides the complex to a specific site in the genome where the Cas9 nuclease cleaves the DNA, resulting in a double-strand break. The introduction of the double-strand break is a critical step in any genome editing experiment and the success of such an experiment hinges on being able to maximize the cleavage efficiency of the CRISPR-Cas9 complex. One of the keys to maximizing the cleavage efficiency is to achieve high-efficiency delivery of the CRISPR-Cas9 complex into the cells.

By expressing the Cas9 nuclease and suitable guide RNAs (gRNAs) in mammalian cells, a double-strand break can be introduced at a locus of interest. The cell then has multiple options for repairing that break. If a suitable template is provided, the cell can use homology-directed repair to integrate a novel transgene at the targeted site. Alternately, the cell can repair the lesion via nonhomologous end joining (NHEJ), an error-prone process that commonly results in an insertion or deletion (indel) mutation at the double-stranded DNA break (DSB) location. In this way, CRISPR can be used to introduce stable, non-revertible alterations to genes.

Recent studies have shown that CRISPR/Cas9 can increase the efficiency of mutagenesis of large viral DNA genomes to generate knockout/in. Here, a CRISPR/Cas9 and gRNA-expressing MDBK cell line enables the isolation of recombinants in herpesvirus genome. Homologous recombination (HR) is a rare event, and it occurs primarily during the S and G2 phases in mitotic cells. During S and G2 phases, DSB occurs and DNA cleavage can be repaired by HDR or NHEJ. However, NHEJ is faster and a more favourable repair pathway than HDR. DSBs can reduce parental and undesired non-insertion introduced virus growth, which is critical to isolate desired recombinant virus progeny, because parental virus levels are often several orders of magnitude higher than recombinant virus in viral progeny. This high background of parental virus hampers isolated pure recombinant virus growth, which requires several cycles of plaque isolation. Although NHEJ is known to be an error-prone pathway, the actual rate of mutation frequency is not high, and most repairs by NHEJ are error free. Therefore, efficient gRNAs can continuously induce DSB during the several cycles of NHEJ-mediated repair, which can significantly reduce parental or undesired mutant virus growth. Furthermore, by blocking the NHEJ pathway by inhibitors, knock out, or knock down of proteins in NHEJ pathway can potentially improve the efficiency of CRISPR/Cas9-mediated mutagenesis of large herpes viral genome.

Designing Multi-epitope Vaccines against Emerging and Re-emerging Human Viruses

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In recent decades, the global rise in emerging and re-emerging human viruses has posed significant public health challenges, often with limited therapeutic or preventive options. These pathogens cause a wide range of human diseases, including respiratory infections (e.g., SARS-CoV-2, influenza). An advancement of vaccine development is the identification of antigenic epitopes, specific molecular structures on viral pathogens that trigger immune recognition and response, essential for conferring protective immunity. Multi-epitope vaccines (MEV) represent an innovative strategy, designed to incorporate multiple immunogenic epitopes to activate both cellular (T-cell-mediated) and humoral (antibody-mediated) immune responses. By minimizing antigenic load, these vaccines enhance safety and efficacy through rational design. MEVs offer a promising solution by focusing immune responses on critical viral pathogen components, reducing the risk of adverse effects associated with whole-pathogen vaccines. These vaccines are developed using in-silico modelling to predict epitope efficacy, combined with biotechnological synthesis for precise production. Recently, our research team has utilized computational approaches to design a MEV candidate targeting SARS-CoV-2. We employed immune-informatics to design a MEV targeting SARS-CoV-2 VOCs. MHC-I ($CD8^+$), MHC-II ($CD4^+$), and CTL epitopes were predicted from spike, membrane, and envelope proteins, with conservation assessed across VOCs. The MEV combined conserved spike-derived CTL and $CD4^+$ epitopes, peptide linkers, and an adjuvant. Molecular docking showed strong binding to immune receptors, with stable complexes confirmed by molecular dynamics and normal mode analysis. Immune simulations suggest robust innate and adaptive responses. This MEV candidate offers potential for broad protection against VOCs and emerging strains. Apart from SARS-CoV-2, we have designed a multi-epitope-based vaccine candidate for herpes simplex virus-1. This strategy not only addresses current pandemics but also holds promise for future viral disease challenges, offering a scalable, safe, and effective platform for vaccine development.

Regional Disease Management: Challenges and Prospects of Immuno-molecular Interventions

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The profound impact of regional diseases on public health, socio-economic well-being, and the healthcare system underscores their significant importance in India. These diseases are often linked to specific geographical regions, climate conditions, and socioeconomic factors. It's essential to note that the prevalence and distribution of the regional diseases may change over time due to various factors, including public health efforts, climate change, and socioeconomic conditions. Some of the regional diseases in India are Kala-azar (Visceral Leishmaniasis), prevalent in parts of Bihar and eastern Uttar Pradesh. Lymphatic Filariasis (Elephantiasis) is widespread in many parts of India, primarily in states like Uttar Pradesh, Bihar, Andhra Pradesh, and Kerala. The animal diseases mainly included in India are dog heart worm infection, caused by *Dirofillaria immitis* recorded in specific areas (mainly North Eastern region of the country). Here we will discuss the challenges and the role of immune-molecular interventions for the sustainable management of Goat Warble Fly Infestation, a regional disease affecting goats in UT of Jammu and Kashmir. Goat warble fly infestation is a myiasis caused by larvae of fly, *Przhevalskiana silenus*. This disease is characterized by presence of subcutaneous warbles (swellings) on the dorsal and lumbar region of domestic and wild ruminants. It causes heavy economic losses in terms of decreased meat and milk production. Its presence also necessitates carcass trimming and downgrading with depreciation of hides. The obligatory life cycle of the disease for about 9-10 months delays its diagnosis and so treatment by filed vets. Our researcher team took the challenge and based on recombinant antigen hypodermin C (HYc) developed an indirect-ELISA kit for field surveillance of the disease. It can be summarized that the major challenge faced by the regional diseases are paucity of base line data, negligence by policy makers and so by funding agencies. The strategic planning by regional researchers to overcome these obstacles for diagnosis and management of these diseases is the need of the hour. The immuno-molecular tools like- ELISAs, PCRs, gene editing etc. are the arsenals for diagnosis and exploring vaccine candidates for the regional diseases. Sustainable management of the regional diseases is a challenge for the field professional, policy makers and also the researchers working in different laboratories of the area but needs coordinated efforts for improved livestock productivity.

Development of multi-epitope DIVA vaccine constructs against brucellosis: An immuno-informatics and recombinant approach

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Brucellosis poses a major zoonotic threat affecting livestock and humans. Existing live vaccines hinder serodiagnosis and raise safety issues; thus, a DIVA-compatible multi-epitope subunit vaccine offers a safer, effective alternative. The study was done to design a safe, DIVA-compatible multi-epitope subunit vaccine against *Brucella* using immunoinformatics, recombinant expression, and antigenic evaluation for improved protection and diagnostic differentiation in One Health control. Immunodominant outer membrane proteins (OMPs); OMP10, OMP19, OMP22, OMP25, OMP28, and OMP31b were selected through comparative genomics and antigenicity profiling. B- and T-cell epitopes were predicted using immunoinformatics tools including Phobius, Protean, and Swiss-Model to identify antigenic domains. Two multi-epitope fusion constructs were designed with flexible G/S linkers and cloned into pET-28a(+) vectors. Recombinant fusion proteins and the DIVA marker protein BtpB were expressed in *E. coli* BL21(DE3) and analyzed using SDS-PAGE and dot-blot immunoassays. The expressed recombinant fusion proteins (~35 kDa) exhibited high and antigenicity, with yields of 0.55–0.57 mg/mL. Structural predictions confirmed stable β-sheet-rich conformations favorable for immune recognition. Dot-blot assays demonstrated strong immunoreactivity of the recombinant proteins, confirming successful expression and antigenic potential suitable for further immunogenicity evaluation. Computational epitope mapping and recombinant expression enabled development of safe, DIVA-compatible multi-epitope Brucella vaccine. Inclusion of BtpB allows diagnostic differentiation, supporting further in vivo evaluation of immunogenicity and protective efficacy.

Oral Presentations

Development of perosamine synthetase (per) gene–based recombinase polymerase amplification-SYBR green I assay for rapid detection of *Brucella* spp. from milk samples

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Brucellosis is one of the most prevalent zoonotic infections, causing significant reproductive and economic losses in livestock and posing public health concerns due to its transmission to humans through contaminated animal products. Rapid and accurate diagnosis of *Brucella* infection is crucial for its effective control and eradication. Conventional diagnostic methods, such as culture and serology, are time-consuming and require biosafety level-3 facilities. Although PCR and real-time PCR offer improved sensitivity and specificity, they are limited by dependence on thermal cyclers and technical expertise. Recombinase Polymerase Amplification (RPA), an isothermal nucleic acid amplification technique, provides a rapid, sensitive, and equipment-free alternative suitable for field conditions. This study focuses on the development and validation of an RPA–SYBR Green I–based assay targeting the perosamine synthetase (per) gene, which is conserved across Brucella species, for rapid detection directly from milk samples. The study was carried out to design and optimize RPA primers targeting the conserved per gene of *Brucella* spp; develop a SYBR Green I–I-based visual detection platform for rapid diagnosis; evaluate the analytical sensitivity and specificity of the developed RPAS assay and assess the diagnostic performance of RPAS in artificially spiked and field milk samples and compare it with conventional and real-time PCR. Genomic DNA from standard *Brucella* reference strains and field isolates was extracted using a commercial kit. The per gene–specific primers were designed and optimized following the TwistDx assay development guidelines. RPA reactions were carried out at 37 °C for 30 minutes, and the end-point detection was achieved through SYBR Green I dye, allowing visual interpretation under blue LED illumination. Analytical sensitivity was determined using 10-fold serial dilutions of *B. abortus* DNA and compared with PCR and real-time PCR assays. Specificity was evaluated using DNA from *Brucella* and non-*Brucella* bacterial species. Artificially spiked samples (milk, blood, semen, and fetal tissues) were tested to assess diagnostic applicability. Cohen's kappa (κ) statistics were used to determine agreement among assays. The RPAS assay successfully amplified the per gene within 30 minutes at 37 °C without requiring a thermocycler. It detected as low as 38.3 fg of B. abortus DNA (≈21 gene copies), exhibiting a 1000-fold higher sensitivity than conventional PCR and comparable performance to real-time PCR. The assay demonstrated complete specificity, with no amplification in non-Brucella species. Among 81 artificially spiked samples, RPAS detected 71 positives, while PCR and real-time PCR identified 60 and 70 positives, respectively. Agreement analysis showed good concordance with PCR (κ = 0.734) and strong agreement with real-time PCR (κ = 0.828). The visual fluorescence readout using SYBR Green I provided a clear distinction between positive and negative samples. The developed per–gene–based RPAS assay offers a rapid, sensitive, and specific molecular diagnostic platform for Brucella detection from milk and other biological samples. Its isothermal nature, minimal equipment requirement, and visual detection capability make it ideal for field-level or point-of-care brucellosis surveillance. This assay holds promise for integration into national control programs for early and reliable disease monitoring in both veterinary and public health sectors.

Exploring the egg-yolk derived IgY biomolecules for developing diagnostic assay against *Brucella* organism

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Immunoglobulin Y (IgY) biomolecules offer exciting possibilities in livestock health and disease diagnosis, as it has several advantages over traditional mammalian antibodies in terms of their production and uses in diagnostics and therapeutics. Here, we present the research work on the production of egg yolk-derived IgY and developing ELISA-based diagnostics against the *Brucella* organism, which is an economically important pathogen for livestock with zoonotic implications in humans. The production of IgY antibodies in egg yolks was done by hyperimmunization of poultry birds against heat-inactivated *Brucella* organisms (*Brucella abortus* - BAA 465 and *Brucella melitensis* - BAA 466). IgY extracted from the vaccinated egg was characterized using 10% SDS-PAGE separation, visualization, protein concentration (mg/ml) and purity (260/280 ratio) estimation.IgY production kinetics has been determined, and it was found that day 10 post 2nd dose immunization yielded the highest concentration. Specificity of IgY against *B. abortus* and *B. melitensis* was demonstrated with the Ag-Ab complex growth inhibition method. Up to 100 colony-forming units/ml of *B. abortus* and *B. melitensis* have shown complete inhibition of growth with 100 µg of anti-brucella IgY protein in blood agar incubated at 370 °C for 48 hrs. The isolated IgY protein has been used in developing an ELISA-based assay for the detection of *Brucella* antigen and antibody. Developing an affordable diagnostic kit for screening of brucellosis in livestock is much desirable and required for surveillance and serological testing, and more so needed with the ongoing National Brucellosis Eradication Programme to eliminate brucellosis in animals.

PNA based visual biosensor for rapid pathogen detection

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Rapid and accurate detection of pathogens is critical for effective disease management. In recent years, biosensor technologies have emerged as powerful alternatives for rapid diagnosis. This study focuses on the development of a visual biosensor utilizing peptide nucleic acid (PNA) probe for the specific identification of *Saprolegnia parasitica*, an important oomycete pathogen of fish. The PNA probe was designed specifically to target the internal transcribed spacer region of S. parasitica. The number and types of nucleobases as well as melting temperature were taken into considerations while designing the PNA probe. PNA tool of PNA Bio was used to verify the properties of the designed probe. The designed PNA probe was synthesized by SPPS using Fmoc chemistry and purified by RP-HPLC. The purified PNA was vacuum dried and stored in a low temperature freezer. In the visual assay, gold nanoparticles (AuNPs) were synthesized through the treatment of hydrogen tetrachloroaurate with trisodium citrate in boiling water, following a standard protocol. The protocol for the visual detection and identification of *S. parasitica* genomic DNA was optimized by making slight modifications to the existing methodology. One of the important characteristics of PNA is its ability to aggregate gold nanoparticles, resulting in a colour change from red to purple/blue. These properties make PNAs especially advantageous for the development of visual biosensor. The developed PNA based visual assay enables to detect S. parasitica and differentiate from other *Saprolegnia* species simply by observing the change in colour by naked eye. The assay could detect up to 10 ng of genomic DNA visually in a 20 µl reaction volume. The assay takes less than 20 min for identification of *S. parasitica*. The assay was validated using genomic DNA from other field isolates of *Saprolegnia*. A novel peptide nucleic acid probe was designed for the identification of the *S. parasitica* species. The probe exhibited significant specificity towards *S. parasitica*, making it suitable for the development of sequencing-free molecular approaches for species identification.

Development of a monoclonal antibody detection-based sandwich ELISA for FMD virus serotype differentiation

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Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. Rapid identification of the causative serotype is essential for effective control and vaccination strategies. The sandwich ELISA using polyclonal hyperimmune sera against FMD virus (FMDV) serves as a confirmatory diagnostic test for serotype determination. However, antigenic cross-reactivity among the serotypes is a limitation. The study was done to enhance the analytical specificity of the FMDV sandwich ELISA by using serotype-specific monoclonal antibodies (mAbs). mAbs developed against Indian vaccine strains of FMDV serotypes O, A, and Asia1 were screened and characterized for their serotype specificity. Three mAbs (O-3B12G4, A-2C4G11, and Asia1-18H12D12 and serotype-independent mAb 1G10F6) were chosen as detection reagents in a sandwich ELISA. The performance of the mAb-based assay was compared to the conventional polyclonal antibody-based ELISA using homologous vaccine strains. The mAb-based sandwich ELISA showed enhanced analytical specificity in differentiating homologous FMDV serotypes O, A, and Asia 1 vaccine viruses, as shown by the reduced cross-reactivity between serotypes. Further, the lower limit of detection in mab ELISA was 4.4, 5.2, and 6.57 log10TCID50/mL for O, A, and Asia1, respectively, compared to 5.3, 5.8, and 6.57 for the polyclonal detection-based sandwich ELISA, indicating its high analytical sensitivity as well. Incorporating monoclonal antibodies in sandwich ELISA can significantly enhance the analytical specificity and reliability of FMDV serotype identification. This improved diagnostic assay can serve as a robust confirmatory test for differentiating Indian vaccine strains of FMDV. The validation of the test for the detection and differentiation of outbreak viruses needs to be carried out for acceptance as a diagnostic assay for epidemiological surveillance and vaccine matching.

Diversity of heme-binding proteins in *Haemonchus contortus*: cellular locations, metabolic involvement, and immunological potential

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Haemonchus contortus, a blood-feeding gastrointestinal parasite causing severe economic losses in small ruminant husbandry, is increasingly resistant to anthelmintics and lacks effective vaccines, underscoring the need for novel therapeutic and prophylactic interventions. The parasite relies on the heme acquisition, transport, utilization, and detoxification pathway for survival, as it cannot synthesize heme *de novo*, making this pathway a promising target for prophylactic and therapeutic strategies. This study aimed to identify and characterize the diversity of heme-binding proteins (Hc-HBPs) in *H. contortus*, which mediate heme acquisition, transport, utilization, and detoxification. We employed a hyphenated method of hemin-agarose affinity chromatography and peptide mass fingerprinting (HAC-PMF) -based proteomic analysis to purify and identify heme-binding proteins from soluble and membrane fractions of H. contortus. The identified Hc-HPBs were subjected to gene ontology (GO) analysis to determine their molecular functions, involvement in biological processes, and distribution in cellular components. Literature mining and sequence homology studies were employed to further investigate their involvement in the parasite's metabolism, biochemical pathways, and cellular structures. Western blotting, using sera from experimentally infected sheep, was performed to assess

the immunogenicity of the purified Hc-HBP fractions. Computational prediction of heme binding motifs was conducted to examine potential regulatory roles. Around 554 putative Hc-HBPs were identified by HAC-PMF, constituting about 2.5% of the parasite's proteome. Gene ontology (GO) analyses indicated diverse cellular locations and functional roles of the Hc-HBPs across core biological processes, including catalysis, cellular transport, signalling, metabolism of carbohydrates, amino acids, lipids, and nucleotides, as well as stress responses. It also predicted extensive functional interconnections among the Hc-HBPs. Sequence homology and literature mining-based analysis revealed an association of multiple Hc-HBPs to essential metabolic pathways such as glycolysis, gluconeogenesis, the TCA cycle, protein folding, translation, and proteasomal degradation, suggesting heme's involvement in regulating major metabolic pathways of the parasite and highlighting key roles of Hc-HBPs in the parasite's biochemistry, adaptation, and survival. Sequence homology and literature mining also indicated that only a small fraction of the Hc-HBPs might be heme-responsive and heme-regulated. Immunogenicity studies indicated that some Hc-HBPs could elicit host immune response, underscoring their prophylactic potential. Computational prediction found one or more solvent-accessible canonical heme-binding motifs (HBMs) in over 96% of putative Hc-HBPs, with the majority featuring motifs based on histidine (H), tyrosine (Y), or cysteine (C) residues, indicating reversible heme coordination suitable for the mediation of transport, buffering, and detoxification of heme. The finding of cysteine-proline (CP) motifs on only a small fraction of Hc-HBPs indicated that only a few of them might be heme-responsive/regulated proteins, reinforcing the findings of the literature mining and sequence homology. The identification and characterization of Hc-HBPs revealed critical insights into heme metabolism in *H. contortus*, opening new avenues for vaccine and therapeutic development.

Poster Presentations

Development of RT-RPA-CRISPR/Cas12a based field deployable diagnostic assay for Peste des petits ruminants virus

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Peste des petits ruminants (PPR), a highly contagious viral disease of small ruminants, causes significant economic losses. Although laboratory based diagnostic tests are available, however field deployable tests are required for rapid and reliable diagnosis. In present study, a rapid, sensitive and field deployable RT-RPA-CRISPR/Cas 12a assay for PPR virus (PPRV) detection was developed aiming for improved surveillance, early diagnosis and outbreak control. The conserved regions within the N-gene in all four lineages (I-IV) were selected for designing guide RNAs using CHOPCHOP software. The most efficient RPA primer set among the designed primers was used for isothermal amplification, as verified by agarose gel electrophoresis and CRISPR/Cas12a-based detection Reaction optimization of RPA-CRISPR/Cas12a was done for isothermal amplification followed by fluorescence detection under the blue light transilluminator. Incorporation of a reverse transcription step allowed direct detection of PPRV RNA through RT-RPA/CRISPR/Cas12a system. Heat treatment was optimized to replace conventional RNA extraction to make this assay more field deployable. Analytical sensitivity and specificity of the test was analysed. The one-tube assay format integrated all separate steps leading to successful virus detection without use of any advanced instrumentation. The RPA-CRISPR/Cas12 assay could detect as low as 10 plasmid copies per reaction, demonstrating sensitivity comparable to qRT-PCR. Analytical specificity testing confirmed exclusive detection of PPRV without cross-reactivity to related viruses such as BTV, SPPV, GTPV, ORF, NDV and CDV. The RT-RPA-CRISPR/Cas12a demonstrated sensitivity down to femtogram/µL of total RNA. Heat treatment at 95°C for 5 minutes effectively replaced conventional RNA extraction protocol making it user friendly in limited resource settings. The one-tube assay format lead to detection within 40 minutes without use of any advanced instrumentation. Screening 25 field samples demonstrated its complete concordance with diagnostic RT-PCR results. This assay is a rapid and sensitive PPRV diagnostic platform for resource-limited settings. It may facilitate timely outbreak containment supporting India's commitment to the FAO-WOAH global roadmap for PPR eradication.

An innovative automated magnetic bead-based method for extraction of nucleic acid from a range of veterinary samples

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To achieve and improve animal health, production, and food security diagnosis of animal diseases, their detection, prevention and treatment play a vital role. To understand this said vital role, we need to utilize biological processes for animal health solutions, such as genetic engineering, cloning, and the development of new vaccines and diagnostics. The study was undertaken to achieve high quality yield and purity of sequencing grade Nucleic Acid using HiMedia's Automated Magnetic Nucleic Acid Extractor, from range of veterinary samples. and to develop a kit reducing wastage of reagents and consumables by using single cartridge for single sample extraction within a few minutes. Following reagents and kits were used: HiMedia's MB583PC16200 HiPurA® Pre- filled Clinical Multi-purpose

Nucleic Acid Purification Kit, MB554MPF16200 HiPurA® MultiSample Pre-filled plates for DNA Extraction, MB504PC16 HiPurA® Pre- filled Cartridges for Blood DNA Extraction, along with InstaNX®Mag16plus automated extractor have been studied for extraction of Nucleic Acid from veterinary samples. PCR was performed using LA1060 -Wee32®. Thermal Cycler & Hi-PCR® MBPCR095-18S rRNA Semi Q PCR Kit. Nucleic acid was extracted and quantified using different animal tissues such as lungs, brain, ear, tongue, skin and subjected to 18S rRNA PCR. The data was found to be better than that of the competitor kit used (QIAamp DNA Mini Kit). Also, nucleic acid obtained was of high yield and purity. The study shows that, InstaNX®Mag16plus automated platform can efficiently process a wide range of veterinary samples with a high-quality PCR amplifiable Nucleic Acid. The use of single pre-filled cartridge prevents wastage of resources. The extracted Nucleic Acid can further be used for screening purposes in downstream applications such as sequencing.

Development of indigenous quantitative real-time PCR (qPCR) assay for detection of enteric viruses of livestock in Punjab, India

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The livestock industry is a vital contributor to India's economy, accounting for about 25.6% of the national gross domestic product (GDP). Over the past three decades, this sector has grown at a rate of approximately 7% annually; however, its progress has been hindered by various viral and bacterial infections. Among these, viral gastroenteritis is a major concern, particularly in neonatal and young animals. The present study aimed to identify the viral agents responsible for gastroenteritis and to develop sensitive molecular diagnostic tools for their detection. Fecal samples were collected from pigs (n = 50) and cattle (n = 50) from various regions of Punjab, encompassing healthy and diarrheic animals from both organized and unorganized farms, across different age groups and seasons. Reverse transcriptase PCR (RT-PCR) was initially used to detect Porcine Kobuvirus (PKV), Porcine Astrovirus (PAstV), and Bovine Rotavirus (BoRV) using specific primers, resulting in 11 PAstV-positive, 24 PKV-positive, and 1 BoRV-positive samples. Subsequently, a SYBR Green-based quantitative real-time PCR (qPCR) assay was developed using in-house synthesized primers targeting specific genomic regions of the respective viruses. The qPCR assays were optimized at an annealing temperature of 55°C and exhibited high sensitivity, specificity, and strong linearity, with no cross-reactivity. Field screening showed detection rates of 15 for PAstV (30%), 26 for PKV (52%), and 4 for RV (8%), which were significantly higher than those obtained by conventional PCR. The developed qPCR assays provide a rapid, reliable, and cost-effective approach for accurate detection and differentiation of these viruses, enhancing diagnostic efficiency and supporting effective disease surveillance and management in livestock populations. This study highlights the potential of advanced nucleic acid-based diagnostics in controlling viral gastroenteritis and improving the overall health and productivity of farm animals.

Designing single domain antibodies against Orthoflavivirues

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Orthoflavivirus includes several viruses that cause significant diseases not only in humans but also in livestock and agricultural animals, representing a persistent and expanding global health threat to humans and animals, with limited therapeutic options available. The development of effective

countermeasures is often complicated by antibody-dependent enhancement (ADE), necessitating novel therapeutic strategies that can neutralize multiple serotypes and related viruses. The aim of this study was to develop a novel single-domain antibody (sdAb) targeting a conserved flavivirus E-protein epitope, validated through in silico modeling, ITC-based biophysical assays, and cell-based antiviral studies demonstrating strong neutralization potential and broad-spectrum therapeutic candidate against orthoflaviviruses. By employing a CDR-grafting strategy, we engineered an sdAb with a de novo-designed backbone specifically targeting a highly conserved epitope within the envelope (E) protein of orthoflaviviruses. The engineered sdAb and the corresponding E-protein domain were successfully expressed and purified, enabling detailed affinity studies. Binding interactions were validated in vitro using isothermal titration calorimetry (ITC), which demonstrated a strong and specific affinity of the sdAb for the recombinant E protein. Ongoing studies are further evaluating the neutralization potential of this anti-orthoflavi sdAb against multiple orthoflaviviruses in vitro. In silico interaction studies with the flavivirus envelope (E) protein revealed a highly conserved epitope that could serve as a potential therapeutic target. To experimentally validate this computational prediction, the engineered single-domain antibody (sdAb) and recombinant E protein were expressed and purified for affinity analysis. In vitro binding studies were performed using isothermal titration calorimetry (ITC), which confirmed the interaction and demonstrated a significant binding affinity of the sdAb toward the recombinant envelope protein of flavivirus. These findings underscore the therapeutic potential of the engineered nanobody as a broad-spectrum antiviral platform, providing a rationally designed therapeutic to counter flavivirus pathogenesis with high specificity and efficacy.

Evaluation of vincristine cytotoxicity in canine mammary tumors: Insights from receptor profiling and 3D tumor spheroid modeling

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Canine mammary tumors (CMTs) are among the most common neoplasms in female dogs, with over half showing malignant and aggressive behavior. Vincristine is widely used in veterinary oncology, yet its cytotoxic potential in relation to hormone receptor and HER2 status remains poorly understood. This study evaluated vincristine responsiveness in triple-positive (ER□/PR□/HER2□) and triple-negative (ER□/PR□/HER2□) primary cultured CMT cells grown under two-dimensional (2D) monolayer and three-dimensional (3D) spheroid conditions, the latter better simulating the tumor microenvironment. The objective was to determine whether receptor status influences vincristine cytotoxicity and whether 3D culture alters drug response compared with conventional 2D assays. ER/PR/HER2 receptor status was determined by real-time PCR. Primary cultures were derived from five CMTs and one normal mammary gland tissue. Cells were exposed to vincristine (0–100 µM) for 48 h, and cytotoxicity was assessed by MTT assay. Tumor spheroids (400–500 µm) were generated on agarose-coated plates and monitored for size and morphology up to 72 hr after treatment. In 2D cultures, vincristine induced a dose-dependent increase in tumor cell mortality, with near-complete cytotoxicity at 100 µM in sensitive lines (CMT2, CMT3), while normal mammary epithelial cells (N1) remained significantly less affected ($p<0.001$). Cytotoxicity was independent of ER/PR/HER2 status, although inter-line variability was observed, with CMT5 showing partial resistance. In 3D spheroids, vincristine caused progressive reductions in spheroid size and integrity, with structural collapse at 100 µM. Compared with 2D models, lower doses were less effective in spheroids, indicating greater drug tolerance in the 3D system. Vincristine demonstrates strong, dose-dependent cytotoxicity against CMT cells while sparing normal mammary cells. 3D spheroids serve as a stringent platform for evaluating drug efficacy and tumor heterogeneity. Maximum use and effectiveness of 3D model can minimize the use of laboratory animals.

Session 8

ISVIB Award Session

Viral capsid (cap) protein-based indirect enzyme-linked immunosorbent assay for detection of antibodies against Porcine circovirus type 2 (PCV2)

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Porcine circovirus type 2 (PCV2), a member of the *Circoviridae* family is a major threat to global swine health. It causes post weaning multisystemic wasting syndrome (PMWS) and other porcine circovirus-associated diseases (PCVAD). The capsid (Cap) protein, encoded by ORF2, is the only structural protein of PCV2 and the primary target of protective immunity, making it highly suitable for diagnostic applications. The objective of the study was to develop and evaluate capsid protein-based indirect ELISA for detection of PCV2 infection in pigs. In this study, the immunogenic region of the ORF2 gene (1034–1735 nt) was codon-optimized, synthesized, and expressed in Escherichia coli BL21 (DE3) using the pET-45b(+) vector. Following IPTG induction, ~35 kDa recombinant Cap protein (rCap) was obtained in inclusion bodies, purified, analysed by SDS-PAGE, and confirmed by western blotting. Hyperimmune sera generated in mice showed strong reactivity against rCap in both western and dot blot analyses. The rCap protein further served as the coating antigen for an indirect ELISA, which was successfully applied to test field sera, confirming its diagnostic robustness for routine detection of PCV2 infection. The assay demonstrated 100% diagnostic sensitivity and specificity in concordance with the commercial ELISA kit, with reproducibility and repeatability (CV<10%) across independent assays. Collectively, these findings establish rCap as a reliable diagnostic antigen and validate its large-scale production for developing sensitive, specific, and consistent serodiagnostic tools for PCV2.

Comparative studies on antimicrobial resistance in *Escherichia coli* from healthy and diseased poultry birds

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Antimicrobial resistance is a recognized global issue, and the uncontrolled use of antibiotics is considered one of the main causes. The present study was conducted to isolate and identify *Escherichia coli* from healthy and diseased poultry birds, to compare their antimicrobial resistance pattern and the resistance genes. For this purpose, a total of 120 samples (faecal and tissues) were collected from healthy (50) and diseased (70) birds from five farms in and around Ludhiana, Punjab. Each sample was processed following standard bacteriological methods, MALDI TOF, and amplification of genus-specific E. coli 23S rRNA. The prevalence of *E. coli* was 40 (80%) in healthy birds and 46 (65.71%) in diseased birds. Isolated *E. coli* were tested against 20 antibiotics to study their resistance and sensitivity patterns. All (100%) the *E. coli* isolates were resistant to fusidic acid, methicillin, and penicillin in healthy as well as diseased birds. Highest sensitivity was observed towards ceftriaxone (98%, 95%), ampicillin/sulbactam 62.5%, 75.08%), and amoxicillin (60%, 73.91%) in healthy and diseased birds. Further, all the isolates were tested to detect the presence of 10 antibiotic

resistance genes: *bla* $_{DHA-1}$, *bla*$_{MOX}$-M, *bla*$_{TEM}$, *bla*$_{SHV}$, *cmlA*, *sul1*, *aadA*, *tetA, tetB*, and *dhfrV* by PCR. Out of the total 86 E. coli isolates, 79 (91.86%) were positive for *aadA*, 72 (83.72%) for *tetA*, 66 (76.74%) for *cmlA*, 57 (66.27%) for *bla*TEM, 13 (15.11%) for dhfrV, 5 (5.81%) for tetB, and 3 (3.4%) for *sul1*. None of the isolates were positive for *bla* $_{DHA}$-1, *bla* $_{MOX-M,}$ and bla$_{SHV}$. Statistically, no significant difference was observed between healthy and diseased birds regarding antibiotic resistance pattern as well as antibiotic resistance genes. Therefore, judicious use of antimicrobial agents in poultry farms needs to be adopted to prevent further emergence of antibiotic-resistant bacteria, especially in healthy birds, as well as to prevent the failure of disease treatment due to antibiotic resistance.

Comparative *In-Vitro* Transcriptomic Profiling of Indigenous and Exotic Canine Breeds: A Systems Biology Approach

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Introduction: The domestication of dogs represents a key evolutionary process shaped by artificial selection, resulting in diverse canine breeds adapted to distinct regional environments. Indigenous breeds, such as the Gaddi dog from northern India, have evolved unique morphological and immunological traits suited to local conditions but remain largely unexplored at the molecular level. Despite their ecological and cultural importance, many indigenous breeds are underrepresented in genetic databases such as the National Bureau of Animal Genetic Resources (NBAGR).

Methods: This study investigates the transcriptomic and miRNA expression profiles of the indigenous Gaddi dog in comparison with the exotic Labrador Retriever, focusing on their immune responses to Toll-like receptor (TLR) ligand stimulation. mRNA and miRNA sequencing were performed on Illumina NovaSeq 6000 (150 bp), comparing untreated controls with Poly I:C, LPS, and CpG ODN-treated groups in both breeds. Differentially expressed genes (DEGs) (fold change>3 or<–3, $p<0.05$) were identified, followed by functional enrichment analysis to determine breed-specific pathway activation.

Results: Labrador Retrievers exhibited a higher number of DEGs across all treatment groups, with enrichment in Th1, Th2, Th17 differentiation and T-cell receptor signaling pathways. Conversely, Gaddi dogs showed significant enrichment in Wnt signaling, T-cell activation, and immune regulation pathways. miRNA-Seq analysis revealed distinct expression patterns, with Labradors showing upregulation of miR-204, miR-206, miR-106a, miR-132, miR-335, and miR-676, associated with inflammation, autophagy, and immune regulation, while Gaddi dogs expressed miR-551 and miR-1249, linked to tumor suppression and inflammation control.

Discussion: These findings demonstrate breed-specific immune transcriptomic landscapes and highlight the distinct immunological architecture of indigenous dogs. The absence of shared TLR-responsive genes underscores their evolutionary divergence. This study emphasizes the need to preserve indigenous canine genetic resources and supports leveraging systems-level transcriptomic approaches to uncover mechanisms of disease resistance and adaptive immunity.

Keywords: Gaddi dog, Labrador Retriever, Transcriptomics, miRNAome, Immune response, TLR ligands

Towards self-reliance: An indigenous Vero cell–based live attenuated vaccine candidate against Canine distemper virus

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Canine distemper virus (CDV) remains a major threat to domestic and wild carnivores in India. Vaccine failures with conventional strains highlight the need for an indigenous vaccine. To develop an indigenous Vero cell-based live attenuated vaccine candidate against the canine distemper virus (CDV) using a recent Indian field isolate A recent Indian field isolate, CDV(Dog)/Bly/Ind/2018 (India-1/Asia-5 lineage), obtained from a naturally infected dog, was characterized both at virological and molecular levels. The virus was serially passaged in Vero cells to achieve attenuation. The attenuated strain was evaluated in dogs under controlled conditions to assess safety, immunogenicity, and long-term immunity. Vaccinated and control dogs were monitored for pyrexia, clinical signs, and viral shedding. Antibody responses were measured using serological assays. Thermostability studies were conducted using various stabilizer combinations, including LAH, sucrose, and trehalose, along with diluents such as 0.85% NaCl, to identify the most stable formulation for long-term storage. The vaccine candidate demonstrated complete attenuation, with no pyrexia, virulence, or viral shedding observed in vaccinated dogs. Immunized animals showed strong protective antibody responses without adverse effects. Among the tested stabilizers, the combination of LAH, sucrose, and trehalose provided maximum thermostability, while 0.85% NaCl served as an effective diluent. Serological evaluation confirmed sustained antibody titres, indicating durable immunity lasting up to 1.5 years post-vaccination (tested so far). These findings confirm the vaccine's safety, immunogenicity, and potential suitability for use under varied field conditions. An indigenous live-attenuated CDV vaccine developed from a local strain showed excellent safety, stability, and long-lasting immunity, advancing India's self-reliance in veterinary vaccine production under the "Make in India" initiative.

Differentiating real time PCR, cloning and expression of immunogenic fusion protein of Lumpy skin disease virus

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Lumpy Skin Disease (LSD) is a vector-borne viral infection caused by the Lumpy Skin Disease Virus (LSDV), primarily affecting cattle. This disease leads to significant economic losses in the livestock industry, manifesting through reduced productivity, increased veterinary expenses and trade restrictions. The present study focuses on the detection of LSDV using conventional PCR followed by detection and differentiation of LSDV from related Capripox viruses using real time PCR. Additionally, the study focuses on cloning and expression of immunogenic fusion protein of LSDV.A total of 26 LSDV tissue samples were collected from cattle in Madhya Pradesh, India, during 2022. These samples were initially screened using conventional PCR targeting the p32 and fusion genes of LSDV. Subsequently, real-time PCR assays were employed for the detection and differentiation of LSDV from other Capripox viruses, using universal Capripox primers targeting RPO30 gene and species-specific probes for LSDV and SPPV/GTPV providing a more accurate and efficient diagnostic approach. A positive tissue sample from an LSDV-infected animal was used for cloning and expression of the recombinant fusion (A27L) protein. To clone, express and purify recombinant fusion protein; genomic DNA was extracted and a 447 bp fragment of the A27L gene was amplified by PCR, then cloned into the pJET1.2/blunt vector and transformed into E. coli DH5α. After colony PCR screening, the recombinant plasmid

(pJET1.2/blunt-A27L) was further digested with BamHI and Sal I restriction enzymes and the insert was ligated into the pQE30 expression vector, which was transformed into E. coli M15 cells. Colonies were screened for the recombinant plasmid, and expression was induced with IPTG. SDS-PAGE analysis of the induced cultures showed an approximately 17.7 kDa protein. The optimal time for harvesting the expressed protein was 12 hours post-induction. The protein was purified under denaturing conditions using nickel chelating affinity chromatography, yielding a purified 17.7 kDa recombinant protein. The diagnostic applicability of expressed protein was tested by DOT- ELISA using LSDV positive field serum sample. Molecular analysis revealed that 69.23% (18/26) of the tissue samples tested positive for the p32 gene. Among these 18 positive samples, 14 were also positive for the fusion gene. The cycle threshold (Ct) values for the eleven LSDV-positive samples ranged from 13.905 to 20.538, with the GTPV-positive sample having a Ct value of 14.362. Furthermore, a single-reaction real-time PCR assay was developed using the same universal Capripox primers combined with species-specific probes for LSDV and SPPV/GTPV. This assay successfully differentiated LSDV from GTPV, with the SPPV/GTPV probe amplifying GTPV at a Ct value of 18.096, while the LSDV probe amplified LSDV with a Ct value of 11.531. The successful cloning, expression and purification of the immunogenic fusion protein of LSDV demonstrated positive reactivity with field serum samples during initial screening by DOT-ELISA. The Real-time PCR assay effectively differentiated LSDV from GTPV, confirming its specificity with no cross-reactivity between the probes. This ensures accurate detection and differentiation of the viruses, making it a reliable tool for diagnostic applications and surveillance of Capripox virus infections. The expressed recombinant fusion protein serves as a potential candidate representing a significant advancement in the development of diagnostic tools using this recombinant fusion protein.

Building a surveillance model for detecting zoonotic spillover in increased animal-human interaction settings using a one health approach

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The ongoing multi-centric study titled *"Building a Surveillance Model for Detecting Zoonotic Spillover in Increased Animal-Human Interaction Settings Using a One Health Approach"* aims to develop a real-time surveillance framework for early detection of zoonotic diseases among slaughterhouse workers in India. The study implemented across 10 institutions and 16 slaughterhouses in Punjab, Assam, and Telangana, integrates human, animal, and environmental health surveillance through a One Health approach. Symptomatic individuals meeting syndromic case definitions for acute febrile, respiratory, diarrheal, or dermatological illnesses are enrolled following informed consent. Baseline assessments have been completed for 399 workers (Assam: 103; Punjab: 62; Telangana: 234). Weekly sample collection and testing from human, animal, and environmental sources are being conducted for priority zoonotic pathogens. Negative samples (5%) and all positive, triangulated samples are further subjected to next-generation sequencing for detailed genomic analysis. Between October 2024 and August 2025, a total of 197 human, 1605 animal, and 264 environmental samples were tested. Predominant pathogens detected include *Leptospira*, *Brucella*, *Toxoplasma gondii*, and *Coxiella burnetii* in humans; *T. gondii*, *C. burnetii*, and *Brucella* in animals; and *E. coli* in environmental samples. An automated data portal has been developed to generate real- time alerts through data triangulation for early spillover detection. Two potential spillover events *Brucella* in Assam and *T. gondii* in Telangana were identified during the study period, highlighting the operational potential of the model for proactive zoonotic surveillance in high-risk occupational settings.

Rapid detection of *Staphylococcus pseudintermedius* and *Staphylococcus coagulans* by combining fast VPCR with nucleic acid lateral flow immunoassay (NALFIA)

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In dogs, pyoderma is primarily caused by coagulase-positive *Staphylococcus pseudintermedius* (SP), *S. coagulans* (SC) and *S. aureus* (SA). Standard culture and biochemical methods for identifying these organisms are laborious, and potential misidentification is a possibility. Molecular methods, like PCR, are a viable alternative for early and accurate diagnosis of these organisms. The study was carried out to detect *Staphylococcus pseudintermedius* and *Staphylococcus coagulans* by combining fast VPCR with nucleic acid lateral flow assay. In this study, 83 swab samples were collected from pyoderma cases of dogs and compared the sensitivity and specificity of the fast VPCR in conventional agarose gel electrophoresis with Nucleic Acid Lateral Flow Immunoassay (NALFIA) in detecting SP and SC. Bacterial DNA was extracted by a simple and quick NaOH lysis method. Primers targeting the rodA gene of SC and the spsK gene of SP were used in PCR, and labelled primers of the same genes were used in NALFIA. The limit of detection of SP and SC in VPCR with gel electrophoresis was 10^2 CFU/ml and 10^4 CFU/ml for NALFIA. Though VPCR with gel electrophoresis was found to be more sensitive than NALFIA, it required specialized equipment like agarose gel electrophoretic apparatus with power pack and gel documentation system and carcinogenic ethidium bromide dye for detection of the bands in agarose gel. The VPCR, combined with NALFIA, offers sensitive and reliable detection of these bacteria in 45 minutes, even to the naked eye. The simplicity, portability, and cost-effectiveness of the NALFIA make them particularly suitable for resource-limited settings and point-of-care testing.

Immunogenicity-Based Potency Testing of IBD Immune Complex Vaccines in Chicken Immune Cells for Animal-Free Testing

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Vaccination plays a pivotal role in controlling infectious diseases in animals, with significant implications for public health and food security. However, conventional in vivo vaccine potency testing methods pose ethical, scalability, and species relevance challenges. In this context, immune cell-based in vitro assays offer a promising animal-free alternative, particularly for immune complex (Icx) vaccines, which can't be tested based on cytopathic effect in cells and rely on antigen-antibody interactions to boost immunogenicity. The present study was undertaken to develop and validate an *in vitro* immunogenicity-based potency assay for commercial IBD immune complex vaccines using primary chicken bone marrow-derived dendritic cells (chBM-DCs) and bursal B cells, correlating *in vitro* immune markers with known *in vivo* protection rates. Three commercial IBD immune complex vaccines with different protection levels (100%, 85%, and 55%) were selected for the study and evaluated for different immunogenicity parameters in vitro using primary cultures of:

- Chicken bone marrow-derived dendritic cells (chBM-DCs), to assess innate immune activation and antigen presentation
- Bursal B cells, as target cells for IBD vaccine, virus uptake, and virus-induced apoptosis

After successfully establishing the primary culture of both cells, 3 vaccines under study were inoculated in two doses (1x dose and 10x dose) and the following parameters of immunogenicity were evaluated after 48 hrs of inoculation:

In chBM-DCs: mRNA expression of different immune genes (CCR6, CCR7, iNOS, CD80, CD86, CD40, MHCII), Immunofluorescence of MHCII expression by phycoerythrin labelled anti-chicken MHCII Ab at 24h post stimulation, and nitric oxide production in culture.

In Bursal B cells: IBD virus uptake by immunofluorescence, apoptosis in cells by Caspase3 expression, and TUNEL assay (based on flow cytometry).

All results were statistically analysed, and parameters with promising results were used for establishing a correlation with 80% protection, which is the minimum standard for vaccine potency as per IP, 2022, so as to derive standards for in vitro potency testing.

1. **Evaluation of chBM-DCs as a potency test model:**
   - Among costimulatory molecules, CD80 expression at 48h post-inoculation showed a strong correlation with protection level; a relative mRNA expression value of 31.3 matched the 80% protection.
   - MHCII showed increased mRNA and protein levels across all Icx vaccines (vs. live), though not directly correlated with protection rates. Immunofluorescence was in accordance with the mRNA expression at 24h.
   - Nitric oxide (NO) production, a downstream product of iNOS activation, correlated with vaccine potency; 55.4 µM NO aligned with the 80% protection threshold.

2. **Bursal B Cell Studies:**
   - Cultures supported IBDV replication from both live and immune complex vaccines.
   - Immunofluorescence analysis revealed a dose-dependent reduction in viral signal correlating with protection levels.
   - This method shows promise for early-stage screening of vaccine viral uptake and potency.
   - Elevated caspase-3 expression and apoptosis through TUNEL assay could be observed in Immunocomplex vaccines, which are at par with that induced by IBD live vaccine, indicating that both the vaccines induce a similar response in host cells.
   - This culture revealed a suitable in vitro model for studies of host-pathogen interactions of IBD-immunocomplex vaccines

The study successfully demonstrates the feasibility of immune cell-based in vitro assays-particularly using dendritic cells and B cells from chickens for evaluating the potency and immunogenicity of IBD immune complex vaccines. These assays offer a scientifically valid, scalable, and ethically preferable alternative to traditional animal-based potency testing. Integration of such methods can enhance regulatory compliance, reduce animal use, and drive innovation in veterinary vaccine quality control.

Novel PPRV vaccine vector platform for next-generation multiplex animal vaccines (GADVASU Woman Scientist Award)

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Animal vaccine researchers worldwide have identified numerous promising candidate antigens for various pathogens. However, only a small fraction successfully translates into sub-unit vaccines, primarily because these vaccines elicit a short-lived humoral response, often requiring multiple boosters and lacking full protection. In contrast, delivering the same antigens through vaccine vector platforms can mimic natural infections, eliciting both cell-mediated and humoral responses for long-lasting immunity. This advancement could significantly impact vaccine research by providing a user-friendly method to translate candidate antigens into prophylactic vaccines. Current vector platforms predominantly rely on human viruses, which are often unsuitable for animal vaccine development, raising regulatory concerns in the one-health context. To address this, we have developed an exclusive vaccine vector platform based on the Indian strain (Sungri/96) of peste-des-petits-ruminants virus

(PPRV). This platform is designed for next-generation live attenuated multiplex vaccines targeting viral, bacterial, and parasitic diseases in livestock. PPRV, as an RNA virus, replicates in the cytoplasm, eliminating the risk of DNA integration into the host. Its remarkable genetic stability and ability to accommodate large gene inserts make it an ideal backbone for vaccine development. Using this platform, multiplex vaccines can be generated through straightforward insertion of candidate antigen genes and rescuing recombinant PPR viruses in cell culture. As the backbone originated from an attenuated vaccine, the rescued viruses can be scaled up and tested directly in animals. Sungri/96 has demonstrated proven scalability and the technology has already been acquired by several industries. Similar to the parent, Sungri/96 vectored live combination vaccines are expected to perform as adjuvant free formulations, leading to significant cost savings on adjuvants typically required for subunit/inactivated preparations. Further this internationally recognized vaccine virus has been tested extensively in Indian field conditions for several decades and is currently being employed in the national PPR control programme. Thus, the next-generation multiplex vaccines developed using this novel vaccine platform are well-positioned to obtain regulatory clearance, promising a safer, more effective, and economical approach to animal disease control.

Development of recombinant epsilon antigen-based Indirect-ELISA for detection of enterotoxaemia specific antibodies in small ruminants

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Enterotoxaemia (ET) is a severe, highly fatal, and economically significant disease of sheep and goats predominantly caused by toxinotype D/B strains of *Clostridium perfringens*. Epsilon toxin (Etx), a key exotoxin secreted by *Clostridium perfringens,* leads to 'sudden death' of affected small ruminants, and is known to induce protective antibodies. Traditionally, chemically inactivated native epsilon toxin has been used in vaccine formulations and diagnostics. The production of native epsilon toxin/toxoid has been a laborious process and needs an alternative method of production for its utility in routine assays for sero-monitoring of immune status in susceptible animals. Production of a non-toxic quadruple point mutant epsilon (rEtx) fusion recombinant protein (~36 kDa), characterization, and development of novel rEtx protein-based indirect-ELISA to detect epsilon-specific antibodies in sheep and goat sera samples. A non-toxic quadruple point mutant epsilon (rEtx) fusion recombinant protein (~36 kDa) was produced in both soluble and denatured forms using a prokaryotic expression system, and its subsequent functional characterization was performed using both *in vivo* and *in vitro* assays. Further, rEtx was utilized in the standardization of the diagnostic assay (indirect-ELISA) and evaluation of its immunogenicity. The assay reproduced significant analytical and diagnostic characteristics, and this optimized assay was employed in the sero-monitoring of ET-specific antibodies in the endemic states/areas. Furthermore, the immunogenicity and efficacy of recombinant quadruple point mutant epsilon antigen were also evaluated in mice, rabbits, and guinea pig models. Overall, the study indicated the potential utility of recombinant Etx mutant antigen in the subunit vaccine formulation and also in the immuno-diagnostics for the detection of enterotoxaemia (ET) specific antibodies in sheep and goats.

Development of IVRI-M recombinant PPR marker vaccine

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Peste-des-petits-ruminants (PPR) is a deadly viral disease affecting sheep and goats, causing global economic losses of up to $2.1 billion/year. Following Rinderpest eradication, PPR is the second animal viral disease targeted for global eradication by 2030. Current PPR vaccines, while effective, generate an immune response that resembles active infection, complicating disease-freedom verification in vaccinated countries. Conversely, maintaining disease-free status without vaccination threats re-emergence until global eradication is achieved. Hence, it is imperative that the vaccine used must differentiate infected animals from vaccinated (DIVA) to claim global freedom from PPR. The IVRI-M Recombinant PPR Marker Vaccine, developed by the ICAR-Indian Veterinary Research Institute, addresses this challenge. This vaccine uses a recombinant strain, PPRV/Sungri/Marker, created through advanced reverse genetics techniques by eliminating specific epitopes from the indigenous PPRV/Sungri/96 virus. With innovative epitope-based differentiation assays, this vaccine enables the Differentiation of Infected from Vaccinated Animals (DIVA). Our marker vaccine retains the same production scalability and all the safety and efficacy profiles of the parent vaccine, such as safety in pregnancy, 100% protection, and long-term immunogenicity of more than 3 years with single dose. In addition, the vaccine also confers the much-needed serological DIVA through innovative epitope-based differentiation assays. With the availability of the marker vaccine and companion DIVA assays developed by us, absence of PPR virus circulation within a border can still be proved to international agencies without the need to stop vaccination. As this technology permits tracking of PPR infection, even in vaccinated areas, it can be used in both endemic and PPR-free countries including cross-borders, to help neutralise the threat of re-emergence. Thus, it paves a safer and faster path towards global PPR eradication.

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Cow Milk	87.4
Chicken	82.0
Buffalo Milk	81.5
Fish	76.0
Mutton	74.0
Soyabeans	72.8
Rice (Polished)	64.0
Wheat (Whole)	64.0
Corn	60.0
Beans, dry	58.0

SUNDAY HO YA MONDAY, ROZ KHAO ANDE.

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Sample to Insight

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Our Mission: Promotion of sustainable and equitable agriculture and rural prosperity through effective credit support, related services, institution development and other innovative initiatives.

- **Research and Development on matter of importance pertaining to agriculture, agricultural operations and rural development including the provision of training and research facilities.**
- **Consultancy services related to Agriculture & Rural Development through subsidiary (NABCONS).**

Our Functions:

- Provide Credit/Refinance for production credit and investment credit to eligible banks and financing institutions.
- Development functions undertaken through Farm Sector Promotion Fund (FSPF), Financial Inclusion Fund (FIF), Watershed Development Fund (WDF), Tribal Development Fund (TDF) Rural Infrastructure Development Fund,(RIDF) etc.
- Supervisory functions in respect of Cooperative Banks and Regional Rural Banks.

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- With TANNUVAS Technology BT VAC-G (Bluetongue Vaccine with 5 Strains)
- Manufacturer of wide range of Formulation, Nutraceuticals, Companion Animal Health products

VISION

To be the world's preferred provider of cutting-edge veterinary vaccines, medicines and ascend to the zenith of leadership in veterinary health.

MISSION

To offer products of international standards with foresight of our responsibility towards protecting the precious bond between people and animals.

CORPORATE OFFICE: 6-2-1012, TGV Mansion, 5th Floor, Khairatabad, Hyderabad - 500 004
Telangana, India T: +91 40 6666 7464 / 6677 2726 **info@bbpl.co.in** | **www.brilliantbiopharma.com**

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Animal Component:
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Superior Growth & Productivity:
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Consistency & Scalability:
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Website
HiMedia Laboratories™
www.himedialabs.com

Brooke Hospital for Animals (India)

Animal health & welfare and community development organization

Our Vision

Our vision is of a world in which working horses, donkeys and mules are free from suffering and have a life worth living.

Our Mission

Our mission is to achieve immediate and lasting positive change in the lives of working horses, donkeys and mules and the communities that depend on them.

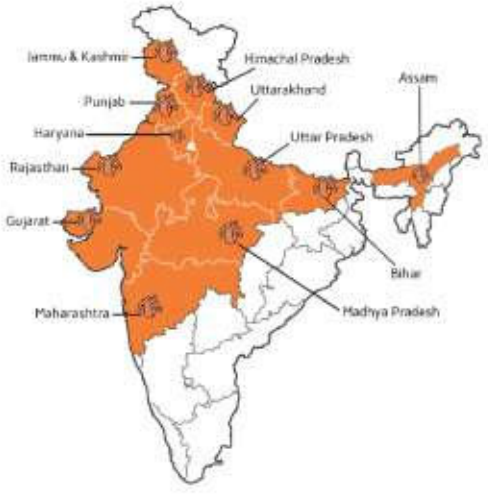
Our Theory of Change

Our Areas of Intervention

- Animal Health & Welfare
- Community Development
- Economic Resilience
- Women Empowerment
- Advocacy

Our Reach

- State covered so for - 12
- Equine Welfare Projects - 33
- Total staff strength - 292
- Equines Reached - 179,639



- Equine Welfare Groups (SHGs) - 2370
- Benefiting Equine owning communities - 316,511
- Knowledge partners- 38 (ICAR & Veterinary Institutes/universities, CSOs)
- Local Service Provider, Community Resource Person & Community Women Intern - 1412

FIND MORE ABOUT US AT:

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Brooke Hospital for Animals (India)
H.O.: Joy Tower (4th Floor), C-20/1-A/1, Sector 62, Noida - 201301 (Uttar Pradesh)
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Profile INFAH 2025

INFAH: Championing Animal Health, Empowering India

Established in 2011, the Indian Federation of Animal Health Companies (INFAH) is a dynamic coalition of over 50 leading companies dedicated to advancing animal healthcare in India. Our members are pioneers in animal healthcare & pharmaceuticals, nutritional supplements & feed additives, biologicals, diagnostics, and Ayurveda, collectively shaping the future of the industry.

Our Mission:
To foster a thriving and sustainable animal health ecosystem in India. We strive to:

- ÷ Contribute towards the development of regulatory framework for the new and existing animal health products.
- ÷ Contribute towards drafting National Health Policy of India with all stakeholders.
- ÷ Formulate a fair marketing environment through ethical business practices.
- ÷ Partnering with Global Animal Health Trade Bodies for establishing identity of Indian Animal Health Industry.
- ÷ Create a database on various segments of Animal Health Industry for business decisions.

We work together & create impact by:
Advancing Science and Innovation: Collaborating with research institutions to develop cutting-edge solutions.
Promoting Responsible Use of Antimicrobials: Actively participating in AMR initiatives to safeguard public health.
Empowering Farmers: Helping farmers increase productivity and income through healthier animals.
Ensuring Food Security: Contributing to a reliable protein supply for a growing population.

Join Us in Building a Healthier India:
By working together, we can create a brighter future for animals, farmers, and the nation.
Healthier Animals, Healthier India

Indian Federation of Animal Health Companies
Affiliated to HealthforAnimals (HFA)
311, Mastermind IV, Royal Palm, I. T. Park, Aarey Colony, Goregaon (East), Mumbai – 400065, Maharashtra (India)
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