

ANNUAL REPORT

2024



भा.कृ.अनु.प.-राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार
ICAR-National Research Centre on Equines, Hisar



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ICAR-National Research Centre on Equines







With best compliments from
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Director
ICAR-National Research Centre on Equines
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Director's Foreword



The ICAR–National Research Centre on Equines (ICAR-NRCE), a premier research institute of the ICAR in animal Sciences, under the National Agricultural Research System, was established on November 26, 1985, in Hisar, Haryana. Our primary goal is to improve the livelihoods of landless and marginal equine farmers. We provide diagnostic, advisory, and consultancy services to enhance equine health, productivity, and their role in agriculture and transportation, particularly in challenging terrains across India. These efforts have earned the Centre both national recognition and international acclaim, distinguishing it among ICAR institutions. Since its establishment, the institute has maintained a balanced focus and achieved significant milestones through its efforts in basic, applied, and translational research, along with training and extension activities. These initiatives have led to notable improvements in equine health and production across the country.

The institute focuses on cutting-edge research in several key areas, including the development of next generation vaccines and diagnostics, nanomedicines, identification of drug molecules, bacteriophage treatment, exploration of host-pathogen interactions, genetic studies related to livestock health and productivity, embryo transfer technology, cryopreservation of embryos and semen for improvement of reproductive performance in equines. The Centre is also working on building a microbial repository—comprising viruses, bacteria, and bacteriophages—to support academic and research activities and the creation of a comprehensive microbial repository to preserve the microbial diversity. The annual report outlines the year's accomplishments and initiatives aimed at creating market-ready technologies and pursuing research tailored to the needs of equine farmers.

Significantly, seven patents and two copyright applications have been filed for the year 2024 on various technologies developed by the Centre and one patent on Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulation has been granted. Moreover, five technologies were also certified by the ICAR during this Calander year. Various technologies such as Multi-Recombinant antigens-based ELISA kit for diagnosis of *Trypanosoma evansi* infection in animals, customised AV for semen collection from stallions, polymeric metal nanocomposites and methods of synthesis thereof and semen collection and cryopreservation in Indigenous horses were commercialized. Salmoquell using bacteriophages to kills *Salmonella* spp. pathogen in poultry birds has been developed. Lysibact- A spray using broad-acting endolysin for elimination of biofilm formation by a broad range of bacterial infections was developed. Developed RPA recombinant protein based indirect ELISA for the sero surveillance of EHV2 in equines.

ICAR-NRCE carries out its research and development initiatives through a well-organized framework of 25 institute-funded, 4 corpus-funded, and 22 externally funded research projects, including collaborative efforts with other institutions. The Centre has successfully secured external support from nearly all major national funding agencies in the agricultural and biological sciences sectors.

ICAR-NRCE plays a key role in monitoring equine diseases nationwide, which is essential for shaping health policies and implementing effective disease control strategies. In this context, during the 2024 reporting period, a total of 1125 equine serum samples were collected from eleven states and screened for antibodies against a comprehensive panel of viral and bacterial pathogens, including equine infectious anemia (EIA), equine herpesvirus-1/4 (EHV-1/4), equine

influenza (EI), equine viral arteritis (EVA), Japanese encephalitis/West Nile virus (JE/WNV), African horse sickness (AHS), rotavirus, glanders, contagious equine metritis (CEM), *Rhodococcus equi*, *Streptococcus abortus equi*, brucellosis, trypanosomosis and piroplasmosis. A total of 147 bacterial isolates were identified from the biological samples processed for bacterial isolation and high incidence of *Stenotrophomonas maltophilia* an important pathogen of horses in upper airway disease was isolated. For surveillance of glanders, in year 2024, a total of 38515 equine sera from 20 states/UT were collected and examined where a total of 34 glanders positive cases were reported in 10 states. Out of 190 sera from occupationally exposed humans (veterinary Officers, equine handlers, and laboratory workers) none were found positive. Under contractual diagnostic services, the centre handled 10866 samples: glanders (n= 4879), EIA (n= 3738), CEM (n= 1044), AHS (n= 294), EVA (n= 317), dourine (n= 355), trypanosomosis (n= 30), piroplasmosis (n= 75) and equine influenza (n= 25) and generated revenue of Rs. 97,31,050. Also, in another study, Hcp1 protein showed quick and strong reactivity against glanders positive serums and IL-1, IL-6, IL-17, MCP-1 and TNF- α cytokines were found to be significantly higher in glanders positive serum.

Constructs were developed with attenuated EHV1 virus having HA gene of Clade 1 and Clade 2 equine influenza viruses as vaccine candidates for equine influenza and EHV1 and recombinant protein based indirect ELISA for studying seroprevalence of EHV2 was developed. A hemagglutination-inhibition assay was used to assess JE virus antibody seroprevalence in 600 horses from various districts in Gujarat. Results showed 75 positives (12.5%), indicating widespread but uneven exposure to JEV. Potentiality of recombinant proteins of *T. annulata* (TAMS-1) as candidate vaccines was tested and the results were very promising indeed. Investigation of mRNA expression profiles of key metabolic genes of *Trypanosoma evansi* in response to diminazene aceturate and isometamidium chloride showed up and down regulation of various genes of metabolic importance.

A novel chitosan-based nanoformulation incorporating zinc oxide and hydroxychloroquine was synthesized and it has antimicrobial activity against *S. equi*, highlighting its promise as a safe, effective alternative to conventional antibiotics and paving the way for further development of targeted nanotherapeutics. Antiparasitic activity of desert plant extracts from *Aerva javanica*, *Capparis decidua*, *Leptadenia pyrotechnica* and *Citrullus colocynthis* killed all roundworms at different concentrations and time intervals. In the Recombinant protein-based immunoassay for detection of serum antibodies specific to *S. equi* was developed and the assay is very specific and sensitive for the detection of antibodies against *S. equi*. RPA was also developed for the detection of stangles infection in equines.

Raj-Sheetal: India's first horse foal born through vitrified embryo transfer and Raj-Zanskar: India's first Zanskari horse foal produced through embryo transfer technology. Studies on the semen extender showed that liposome based, melatonin and L- carnitine significantly improved the cryosurvival rate of stallion spermatozoa. We have also distributed a sum of 540 semen straws to the paraveterinarians, veterinarians and stakeholders during the current year and generated a revenue of Rs. 54000/- through sale of germplasm. We were able to identify a total of 12627 peptides coding for 1550 proteins, out of which 77 and 405 are expressed exclusively for low and high sperm motile groups respectively. A total of 1068 proteins were commonly expressed between low and high motile sperm groups. 193 proteins were upregulated whereas 203 proteins were down regulated and 433 proteins were neutrally expressed by high-throughput proteomics analysis of stallion spermatozoa with contrasting semen motility. First indigenous SNP chip for Indian Horses (high-density Axiom_Ashwa SNP chip) was developed and the Bhimthadi horses were characterized and registered as the 8th breed of indigenous horses.

During the year of 2024, 134 cultures were processed, out of which 116 cultures were accessioned in the bacterial repository, which has led to the total strength of bacterial culture collection to 1942 veterinary bacteria. National Centre for Veterinary Type Cultures is a national Repository recognized by Ministry of Environment and Forest to preserve voucher specimen of bacterial taxa pertaining to veterinary sciences. NCVTC, NRCE, Hisar published the Microbial Repository catalogue, which is a crucial resource for preserving and utilizing microbial diversity. A total of 126 bacterial isolates were identified. Some of the predominant isolates of bacteria identified were, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Pseudomonas* spp, *Corynebacterium* spp, *Nocardia* spp, *Escherichia coli*, *Ralstonia picketti*, *Streptococcus dysgalactiae* ssp. *equisimilis*, *Pantoea* spp, *Chryseobacterium indologenes*,

Klebsiella pneumoniae and *Acinetobacter* spp. and Yeast. A total of 28 viral isolates were received as deposits from NCVTC Network units. These deposits included Avian Infectious Bronchitis (n=4), Fowlpox Virus (n=2), Marek's Disease Virus (n=2), Fowl Adenovirus (n=2), *Peste des Petits Ruminants* (PPR) Virus (n=9), Bovine Rotavirus (n=7), and Equine Rotavirus (n=2). A total of 18 viruses were accessioned (VTCC AVA 385 - 402) into the repository. Besides, metagenomic analysis of the bat virome in Haryana identified several families of significance to animal and human health were detected, including *Poxviridae*, *Herpesviridae*, *Coronaviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Flaviviridae*, *Retroviridae*, and *Reoviridae*.

A prototype CRISPR-Cas12a-RPA-LFA assay for rapid and visual detection of Newcastle disease virus (NDV) and IBV in clinical and field Samples was developed. Besides, we have generated genetically engineered BHK-21 cells lines deficient in two of the cellular proteins Cav1 (Caveolin-1) and HDAC6 (histone deacetylase 6) and the knockout cells generated ~ 10-times higher virus titre as compared to the wild type (normal) BHK-21 cells, and therefore, it has high commercial interest to economize the cost of vaccine. Dose-dependent effects of Tubacin on FMDV replication, particularly the involvement of host pathways and viral interactions has been initiated to demonstrate its antiviral effect. An ORF122-based ELISA demonstrated high diagnostic performance, with a sensitivity of 97.09% and specificity of 94.37%. Importantly, the assay effectively detected antibodies against both the LSDV vaccine strain currently used in India and field strains. Developed RPA-LFA assays for detection of EHV1 & EHV4 nucleic acids using the set of primers and nfo probes. Designed and synthesized primers and probes targeting highly conserved genes for RPA assay for the detection of ILT from poultry and BCoV bovine species. An attempt for the development of attenuated candidate vaccine against swinepox virus has been initiated and passaged up to 30 and further analysis under progress. Isolation of a member of class *Caudoviricetes*- novel bacteriophage against MDR *Salmonella enterica* and its potential use in food biocontrol has been explored and phage was also able to eradicate biofilms formed by the *Salmonella enterica* on the borosilicate glass surface.

As part of the 'National One Health Program for Prevention and Control of Zoonotic Diseases (NOHPCZ)', NRCE is actively engaged in capacity building, enhancing laboratory diagnostic infrastructure, promoting inter-sectoral collaboration, and raising awareness about zoonotic diseases of public health significance through the organization of trainings, workshops, and webinars.

During the year, scientists published 44 high impact research papers in international and national peer-reviewed journals. In addition, 1 technical bulletin, 4 compendium compilations, 3 books, 6 book chapters, 35 training manual chapters, 22 research abstracts, 28 GenBank accessions, 1 policy paper and got technologies certified by ICAR.

The consistent vision, guidance, and technical support provided by the esteemed Chairmen and members of the QRT, RAC, and IRC have been crucial in steering NRCE in the right direction and maintaining its strategic focus. I sincerely thank the Chairman and Members of the Publication Committee for their efforts in bringing out this excellent annual report, which highlights the Centre's key achievements. I am especially grateful for the steadfast support of Dr ML Jat, Secretary of the Department of Agricultural Research and Education and Director General of ICAR as well as Dr. Himanshu Pathak former Secretary of the Department of Agricultural Research and Education. I also express my sincere gratitude to Dr. Ragavendra Bhatta, Deputy Director General (Animal Science) for unwavering support extended to this institute. My thanks also extend to Dr Divakar Heamdiri, Assistant Director General (Animal Health), and to Dr. Ashok Kumar, former Assistant Director General (Animal Health), the Principal Scientists at ICAR Headquarters for their continued encouragement. Lastly, I express my heartfelt appreciation to the entire NRCE team for their dedicated efforts and valuable contributions to the growth of this premier institute.

Tarun Kumar Bhattacharya
(Director, ICAR-NRCE)



Executive Summary

Horses have historically played a vital role in India's culture, rural economy, ceremonial traditions, and livelihood of pastoral communities. As draught animals, mounts for ceremonial and recreational purposes, and for their contribution to the biodiversity of India's animal genetic resources, horses remain a strategic livestock species. The ICAR-National Research Centre on Equines (ICAR-NRCE), with its national mandate, has remained committed to promoting equine health, improving reproduction, conserving breeds, and supporting veterinary public health through advanced research and capacity building. The year 2024 marked a period of outstanding achievements and advancements in equine science at NRCE. The Centre's major contributions spanned multiple domains including disease investigation and outbreak control, the development and field application of novel diagnostics and vaccines, formulation of antiparasitic and antibacterial drugs, reproductive biotechnologies, and conservation of indigenous equine breeds. More than 18,700 equines across 20 states were screened for infectious diseases including glanders, EHV, EI, JE, trypanosomiasis, and piroplasmosis under national surveillance programs. The Centre responded promptly to outbreaks, generating critical epidemiological and laboratory data. Diagnostic breakthroughs included the standardization of recombinant protein-based ELISAs and nucleic acid-based assays (qPCR, RPA), which facilitated rapid disease confirmation in field conditions. In vaccine research, recombinant EHV-1 expressing Clade 1 and 2 HA genes from Equine Influenza Virus was developed and validated, showing promising immunogenic profiles. On the therapeutic front, molecular characterization of drug action against *Trypanosoma evansi* and nanoparticle-based antibacterial agents against *Streptococcus equi* were key highlights. Reproductive biotechnology saw major achievements with the successful birth of India's first foals from vitrified embryos, in addition to improved cryopreservation and AI protocols that significantly enhanced conception rates. The year also witnessed the formal recognition of the Bhimthadi horse as the

कार्यकारी सायांश

अश्वों ने ऐतिहासिक रूप से भारत की संस्कृति, ग्रामीण अर्थव्यवस्था, औपचारिक परंपराओं और देहाती समुदायों की आजीविका में महत्वपूर्ण भूमिका निभाई है। भारवाहक पशुओं के रूप में, औपचारिक और मनोरंजक उद्देश्यों के लिए सवारी के रूप में, और भारत के पशु आनुवंशिक संसाधनों की जैव विविधता में उनके योगदान के लिए, अश्व एक महत्वपूर्ण पशुधन प्रजाति बने हुए हैं। भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र, अपने राष्ट्रीय अधिदेश के साथ, उन्नत अनुसंधान और क्षमता निर्माण के माध्यम से अश्व स्वास्थ्य को बढ़ावा देने, प्रजनन में सुधार, नस्लों के संरक्षण और पशु चिकित्सा सार्वजनिक स्वास्थ्य का समर्थन करने के लिए प्रतिबद्ध रहा है। वर्ष 2024 राष्ट्रीय अश्व अनुसंधान केंद्र के अश्व विज्ञान क्षेत्र में उत्कृष्ट उपलब्धियों और प्रगति की अवधि को चिह्नित करता है। केंद्र के प्रमुख योगदान में अश्वों की रोग जांच और प्रकोप नियंत्रण, नवीन निदान और टीकों का विकास और क्षेत्र अनुप्रयोग, परजीवीरोधी और जीवाणुरोधी दवाओं का निर्माण, प्रजनन जैव प्रौद्योगिकी और स्वदेशी अश्व नस्लों का संरक्षण सहित कई क्षेत्र शामिल हैं। राष्ट्रीय निगरानी कार्यक्रमों के तहत 17 राज्यों में 18,700 से अधिक अश्वों की ग्लैंडर्स, ईएचवी, ईआई, जेई, ट्रिपैनोसोमोसिस और पायरोप्लाज्मोसिस सहित संक्रामक रोगों के लिए जांच की गई। रोग प्रकोप के दौरान केंद्र ने महत्वपूर्ण महामारी विज्ञान और प्रयोगशाला आँकड़े तैयार करके त्वरित प्रतिक्रिया दी। नैदानिक सफलताओं में पुनः संयोजक प्रोटीन-आधारित ELISAs और न्यूक्लिक एसिड-आधारित जाँच (qPCR, RPA) का मानकीकरण शामिल था, जिससे क्षेत्रीय स्थितियों में रोग की शीघ्र पुष्टि संभव हो सकी। वैक्सीन अनुसंधान क्षेत्र में, इक्वाइन इन्फ्लूएंजा वायरस से क्लेड 1 और 2 HA जीन को व्यक्त करने वाले पुनः संयोजक EHV-1 को विकसित और मान्य किया गया, जो आशाजनक प्रतिरक्षात्मक रूपरेखा दिखा रहा है। चिकित्सीय मोर्चे पर, ट्रिपैनोसोमा इवांसी के विरुद्ध औषधि क्रिया का आणविक लक्षण-वर्णन तथा स्ट्रेप्टोकोकस इक्वी के विरुद्ध नैनोकण-आधारित जीवाणुरोधी एजेंट मुख्य आकर्षण थे। प्रजनन जैव प्रौद्योगिकी में विट्रिफाइड भ्रूण विधि से भारत के पहले घोड़े के बच्चे के सफल जन्म के साथ बड़ी उपलब्धियाँ देखी गईं, इसके अलावा बेहतर क्रायोप्रीजर्वेशन और कृत्रिम गर्भाधान विधि से गर्भधारण दरों में उल्लेखनीय वृद्धि की। इस वर्ष भीमथड़ी घोड़े को आठवीं स्वदेशी अश्व नस्ल के रूप में औपचारिक मान्यता, जीनोमिक टूल डेवलपमेंट (एक्सओम_अश्व एसएनपी चिप) और अश्व गर्भावस्था परिणामों की पूर्व-सूचना करने के लिए सीटीयूपी-आधारित निगरानी की तैनाती भी देखी गई। नेशनल सेंटर फॉर

eighth indigenous equine breed, genomic tool development (Axiom_Ashwa SNP chip), and deployment of CTUP-based monitoring to predict equine pregnancy outcomes. The National Centre for Veterinary Type Cultures (NCVTC) deposited 18 novel bacterial isolates and distributed over two dozen authenticated cultures, supporting national veterinary microbiology and diagnostics. Externally funded projects from DBT, DST, NADCP, and others enriched research in disease surveillance, molecular diagnostics, vaccine technology, and breed characterization. The salient achievements of the Centre during 2024 are outlined below-

During 2024, ICAR-NRCE implemented an extensive disease surveillance program, covering 18,766 equines across 20 Indian states. This surveillance aimed to monitor critical viral, bacterial, and protozoal diseases affecting equines. The Centre detected 34 glanders-positive cases, mainly from Uttar Pradesh, Haryana, and Rajasthan. Equine Herpesvirus-1 (EHV-1) showed a 13.28% seroprevalence, with active infections confirmed in 14 animals. The 34 cases of Equine Influenza Virus (EIV) were detected in both surveillance and outbreak investigations. Additionally, the Centre reported high levels of piroplasmiasis (38.3%) and trypanosomiasis (3%). NRCE contributed to One Health surveillance by analyzing 600 horse serum samples from Gujarat for Japanese Encephalitis Virus (JEV). The results indicated a 12.5% seroprevalence, with district-level variations revealing hotspots in Kheda and Amreli. Cross-species analysis showed high seroprevalence in pigs (17.9%) and moderate rates in donkeys and goats (~9.2%), highlighting zoonotic circulation. Molecular detection of JEV was confirmed in equine samples from Tripura using RT-PCR. Complementary to surveillance, diagnostic services were extended under a revenue model with 10,866 samples tested, resulting in revenue generation of INR 97.31 lakhs which strengthening NRCE's capacity for high-throughput disease detection and its sustainability.

ICAR-NRCE made notable strides in vaccine development to combat equine infectious diseases. A bivalent recombinant vaccine candidate was developed by engineering an attenuated Equine Herpesvirus-1 (EHV-1) to express the hemagglutinin (HA) gene from both Clade 1 and Clade 2 strains of Equine Influenza Virus (EIV), providing a dual protective effect. The vaccine constructs exhibited significant attenuation, as

वैटर्नरी टाइप कल्चर (एनसीवीटीसी) ने 18 नए सूक्ष्म जीवाणु अलगाव जमा किए और दो दर्जन से अधिक प्रमाणित कल्चर वितरित किए, जिससे राष्ट्रीय पशु चिकित्सा सूक्ष्म जीव विज्ञान और निदान को समर्थन मिला। डीबीटी, डीएसटी, एनएडीसीपी और अन्य से बाहरी रूप से वित्त पोषित शोध कार्य ने रोग निगरानी, आणविक निदान, वैक्सीन प्रौद्योगिकी और नस्ल लक्षण वर्णन में अनुसंधान को समृद्ध किया। 2024 के दौरान केंद्र की प्रमुख उपलब्धियाँ नीचे दी गई हैं-

2024 के दौरान, भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने 17 भारतीय राज्यों में 18,766 अश्वों को जाँच करते हुए एक व्यापक रोग निगरानी कार्यक्रम लागू किया। इस निगरानी का उद्देश्य अश्वों को प्रभावित करने वाले गंभीर विषाणुजनित, जीवाणु जनित और परजीवीजनित रोगों की निगरानी करना था। केंद्र ने मुख्य रूप से उत्तर प्रदेश, हरियाणा और राजस्थान से 34 ग्लैंडर्स-संक्रमित मामलों का पता लगाया। इक्विन हर्पीसवायरस-1 (EHV-1) ने 13.28% सीरोप्रिवलेंस दिखाया, जिसमें 14 जानवरों में सक्रिय संक्रमण की पुष्टि हुई। निगरानी और प्रकोप जांच दोनों में इक्विन इन्फ्लूएंजा वायरस (EIV) के 34 मामले पाए गए। इसके अतिरिक्त, केंद्र ने पिरॉप्लाज्मोसिस (38.3%) और ट्रिपैनोसोमोसिस (3%) बीमारियों के प्रसार हेतु सूचना दी। राष्ट्रीय अश्व अनुसंधान केंद्र ने जापानी इन्सेफेलाइटिस वायरस (JEV) के लिए गुजरात से 600 अश्वों के सीरम नमूनों का विश्लेषण करके वन हेल्थ निगरानी में योगदान दिया। परिणामों ने 12.5% सीरोप्रिवलेंस का संकेत दिया, जिसमें जिला-स्तरीय भिन्नताएं खेड़ा और अमरेली में हॉटस्पॉट का खुलासा करती हैं। क्रॉस-स्पीशीज विश्लेषण ने सूअरों (17.9%) में उच्च सीरोप्रिवलेंस और गधों और बकरियों (~9.2%) में मध्यम दर दिखाई, जो जानवरों से मनुष्य में होने वाले रोगों के परिसंचरण को उजागर करता है। आरटी-पीसीआर का उपयोग करके त्रिपुरा से अश्वों के नमूनों में जेईवी की आणविक पहचान की पुष्टि की गई। निगरानी के पूरक के रूप में, 10,866 नमूनों के परीक्षण के साथ राजस्व प्रतिरूप के तहत नैदानिक सेवाओं का विस्तार किया गया, जिसके परिणामस्वरूप 97.31 लाख रुपये का राजस्व उत्पन्न हुआ, जिसने उच्च-रोग पहचान और इसकी स्थिरता के लिए राष्ट्रीय अश्व अनुसंधान केंद्र की क्षमता को मजबूत किया।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने अश्वों से होने वाली संक्रामक बीमारियों से निपटने के लिए वैक्सीन के विकास में उल्लेखनीय प्रगति की है। इक्विन इन्फ्लूएंजा वायरस (ईआईवी) के क्लेड 1 और क्लेड 2 दोनों उपभेदों से हीमाग्लुटिनिन (एचए) जीन को व्यक्त करने के लिए एक क्षीण इक्विन हर्पीसवायरस-1 (ईएचवी-1) की इंजीनियरिंग करके एक द्विसंयोजक पुनः संयोजक वैक्सीन उम्मीदवार विकसित किया गया था, जो दोहरा सुरक्षात्मक प्रभाव प्रदान करता है। वैक्सीन निर्माणों ने महत्वपूर्ण क्षीणन प्रदर्शित किया, जैसा कि इन विट्रो में प्लाक के आकार में कमी से प्रदर्शित होता है, और एचए प्रोटीन की सफल अभिव्यक्ति की पुष्टि

demonstrated by reduced plaque sizes in vitro, and successful expression of HA proteins was confirmed by immunofluorescence and ELISA, validating the immunogenicity of the candidate. These constructs were further propagated for advanced preclinical trials. In parallel, a subunit vaccine against *Theileria annulata* was developed using the TAMS-1 protein, which demonstrated 78–89% schizont growth inhibition in vitro when tested with hyperimmune sera, indicating strong protective potential. Collectively, these vaccine initiatives reflect NRCE's commitment to pioneering multifaceted, effective, and field-adaptable immunization solutions for the equine industry.

In terms of diagnostic advancements, ICAR-NRCE developed a series of robust, sensitive, and field-deployable tools targeting major equine pathogens. To extend diagnostic capabilities to the field, a novel CRISPR-Cas12a-RPA-LFA platform has been developed for a rapid, sensitive, and field-deployable diagnostic tool for IBV and NDV, facilitating timely intervention and improved disease control in poultry populations. Similarly, a novel isothermal Recombinase Polymerase Amplification (RPA) assay was developed targeting Equine Herpesvirus-1 (EHV-1) and Equine Herpesvirus-2 (EHV-2), employing NFO probe-based detection systems. These assays were designed to provide rapid and sensitive point-of-care diagnosis, suitable for use in the field. Further, RPA assay also developed for *S. equi* detection, employing endpoint visualization through SYBR Green fluorescence and gold nanoparticle-based colorimetric change. A highly specific duplex real-time PCR assay was standardized for the simultaneous detection of *Streptococcus equi* and *Streptococcus* genus, showing 100% sensitivity and specificity. Furthermore, ELISA kits based on recombinant 22kDa and 26kDa proteins of *S. equi* were standardized for antibody detection. These diagnostics represent a shift toward integrated, rapid, and accurate detection systems tailored for both laboratory and resource-limited field settings.

ICAR-NRCE's therapeutic research addressed key challenges in managing parasitic and bacterial infections in equines. A gene expression study of *Trypanosoma evansi* treated with diminazene aceturate and isometamidium chloride revealed significant downregulation of metabolic genes, indicating mechanisms of drug action and resistance. Key targets such as ESAG8 and trypanothione reductase were

इम्यूनोफ्लोरोसेंस और एलिसा द्वारा की गई, जिससे उम्मीदवार की प्रतिरक्षात्मकता की पुष्टि हुई। इन निर्माणों को आगे उन्नत पूर्व नैदानिक परीक्षणों के लिए प्रचारित किया गया। समानांतर में, TAMS-1 प्रोटीन का उपयोग करके थेलेरिया एनुलैटा के खिलाफ एक सबयूनिट वैक्सीन विकसित की गई, जिसने हाइपरइम्यून सीरा के साथ परीक्षण किए जाने पर इन विट्रो में 78-89% साईजोन्ट वृद्धि अवरोध का प्रदर्शन किया, जो मजबूत सुरक्षात्मक क्षमता का संकेत देता है। सामूहिक रूप से, ये वैक्सीन पहल घोड़े के उद्योग के लिए बहुआयामी, प्रभावी और क्षेत्र-अनुकूलनीय टीकाकरण समाधानों को आगे बढ़ाने के लिए राष्ट्रीय अश्व अनुसंधान केंद्र की प्रतिबद्धता को दर्शाती हैं।

निदान संबंधी प्रगति के संदर्भ में, भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने प्रमुख अश्व रोगजनकों को लक्षित करने वाले मजबूत, संवेदनशील और क्षेत्र-परिनियोजित उपकरणों की एक शृंखला विकसित की है। निदान क्षमताओं को क्षेत्र में विस्तारित करने के लिए, IBV और NDV के लिए एक तीव्र, संवेदनशील और क्षेत्र-परिनियोजित निदान उपकरण के लिए एक नया CRISPR-Cas12a-RPA-LFA प्लेटफॉर्म विकसित किया गया है, जो पोल्ट्री आबादी में समय पर हस्तक्षेप और बेहतर रोग नियंत्रण की सुविधा प्रदान करता है। इसी तरह, एक नया आइसोथर्मल रीकॉम्बिनेज पॉलीमरेज एम्प्लीफिकेशन (RPA) परख विकसित किया गया था, जो कि इक्वाइन हर्पीसवायरस-1 (EHV-1) और इक्वाइन हर्पीसवायरस-2 (EHV-2) को लक्षित करता है, जिसमें NFO जांच-आधारित पहचान प्रणाली का उपयोग किया जाता है। इन परखों को क्षेत्र में उपयोग के लिए उपयुक्त, तेज और संवेदनशील पॉइंट-ऑफ-केयर निदान प्रदान करने के लिए डिज़ाइन किया गया था। इसके अलावा, एस. इक्वी का पता लगाने के लिए आरपीए परख भी विकसित की गई, जिसमें एसवाईबीआर ग्रीन फ्लोरोसेंस और गोल्ड नैनोपार्टिकल-आधारित कलरमेट्रिक परिवर्तन के माध्यम से एंडपॉइंट विजुअलाइज़ेशन का उपयोग किया गया। स्ट्रेप्टोकोकस इक्वी और स्ट्रेप्टोकोकस जीनस का एक साथ पता लगाने के लिए एक अत्यधिक विशिष्ट डुप्लेक्स रियल-टाइम पीसीआर परख को मानकीकृत किया गया, जो 100% संवेदनशीलता और विशिष्टता दिखाता है। इसके अलावा, एंटीबॉडी का पता लगाने के लिए एस. इक्वी के पुनः संयोजक 22kDa और 26kDa प्रोटीन पर आधारित एलिसा किट को मानकीकृत किया गया। ये निदान प्रयोगशाला और संसाधन-सीमित क्षेत्र सेटिंग्स दोनों के लिए अनुकूलित एकीकृत, तेज और सटीक पहचान प्रणालियों की ओर एक बदलाव का प्रतिनिधित्व करते हैं।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र के चिकित्सीय अनुसंधान ने अश्वों में परजीवी और जीवाणु संक्रमण के प्रबंधन में प्रमुख चुनौतियों का समाधान किया। डिमिनाजीन एसिट्यूरेट और आइसोमेटामिडियम क्लोराइड के साथ उपचारित ट्रिपैनोसोमा इवांसी के जीन अभिव्यक्ति अध्ययन ने चयापचय जीनों के महत्वपूर्ण डाउनरेगुलेशन का खुलासा किया, जो दवा की क्रिया और प्रतिरोध के

identified for future therapies. Harnessing traditional knowledge, NRCE evaluated herbal extracts of *Aerva javanica* and *Capparis decidua*, demonstrating 100% in vitro lethality against *Haemonchus contortus*. These studies underscore the potential of indigenous plant-based alternatives for parasite control, contributing to sustainable equine healthcare practices. These integrated approaches showcase NRCE's commitment to innovative and sustainable therapeutic solutions.

ICAR-NRCE achieved landmark progress in equine reproductive biotechnology during the reporting year. The most notable achievement was the successful birth of India's first equine foal, "Raj-Sheetal," through embryo transfer using vitrified embryos. This was followed by the delivery of "Raj-Zanskar," the first Zanskari horse born via embryo transfer. A total of 13 embryos were recovered from 16 flushings, with a recovery rate of 81.25%, and nine of these were successfully vitrified using standardized protocols. These accomplishments validate the feasibility of embryo cryopreservation and transfer in indigenous horse breeds for genetic conservation and selective propagation. Artificial insemination techniques were also significantly advanced. Deep horn intrauterine insemination using a single frozen semen straw resulted in up to 80% conception rates. Semen extenders were improved through the development of novel formulations, including a liposome-based extender that maintained sperm viability for up to 10 days at 4°C. Supplementation with L-carnitine and melatonin in extenders was found to enhance sperm motility, plasma membrane integrity, and antioxidant activity, while reducing oxidative stress. These developments are pivotal for breed improvement, conservation of rare equine genetic resources, and enhanced reproductive efficiency, thus reinforcing NRCE's leadership in equine reproductive biotechnologies.

ICAR-NRCE made significant contributions toward the characterization and conservation of indigenous equine breeds. During the year, the Bhimthadi horse was officially recognized as India's 8th indigenous equine breed following extensive phenotypic and molecular characterization. A dedicated breed society for Bhimthadi horses was formed and registered, ensuring organized breeding, conservation, and promotional activities. Parallel efforts were undertaken for the Halari donkey, with herd expansion and documentation of its unique morphometric traits, behavioral attributes,

तंत्र को दर्शाता है। भविष्य के उपचारों के लिए ESAG8 और ट्रिपैनोथियोन रिडक्टेस जैसे प्रमुख लक्ष्यों की पहचान की गई। पारंपरिक ज्ञान का उपयोग करते हुए, राष्ट्रीय अश्व अनुसंधान केंद्र ने एर्वा जावनिका और कैपरिस डेसीडुआ के हर्बल अर्क का मूल्यांकन किया, जो हिमोन्कस कॉन्टोर्टस के खिलाफ इन विट्रो में 100% घातकता प्रदर्शित करता है। ये अध्ययन परजीवी नियंत्रण के लिए स्वदेशी पौधे-आधारित विकल्पों की क्षमता को रेखांकित करते हैं, जो टिकाऊ घोड़ा स्वास्थ्य सेवा प्रथाओं में योगदान करते हैं। समानांतर में, जिंक और हाइड्रोक्सीक्लोरोक्वीन ले जाने वाले चिटोस-आधारित नैनोथेरेप्यूटिक्स ने इन विट्रो में स्ट्रेप्टोकोकस इक्वी को प्रभावी रूप से बाधित किया। ये एकीकृत दृष्टिकोण राष्ट्रीय अश्व अनुसंधान केंद्र की अभिनव और टिकाऊ चिकित्सीय समाधानों के प्रति प्रतिबद्धता को प्रदर्शित करते हैं।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने रिपोर्टिंग वर्ष के दौरान अश्व प्रजनन जैव प्रौद्योगिकी में महत्वपूर्ण प्रगति हासिल की। सबसे उल्लेखनीय उपलब्धि भारत के पहले अश्व शिशु, "राज-शीतल" का विट्रिफाइड भ्रूण का उपयोग करके भ्रूण स्थानांतरण के माध्यम से सफल जन्म था। इसके बाद भ्रूण स्थानांतरण के माध्यम से पैदा हुए पहले जांस्करी घोड़े "राज-जांस्कर" का जन्म हुआ। 16 प्लसिंग से कुल 13 भ्रूण बरामद किए गए, जिनकी रिकवरी दर 81.25% थी, और इनमें से नौ को मानकीकृत विधि का उपयोग करके सफलतापूर्वक विट्रिफाइड किया गया। ये उपलब्धियाँ आनुवंशिक संरक्षण और चयनात्मक प्रसार के लिए स्वदेशी अश्वों की नस्लों में भ्रूण क्रायोप्रिजर्वेशन और स्थानांतरण की व्यवहार्यता को मान्य करती हैं। कृत्रिम गर्भाधान तकनीक भी काफी उन्नत थी। एक जमे हुए वीर्य स्ट्रॉ का उपयोग करके डीप हॉर्न अंतर्गर्भाशयी गर्भाधान के परिणामस्वरूप 80% तक गर्भधारण दर प्राप्त हुई। लिपोसोम-आधारित एक्सटेंडर सहित नई रचना के विकास के माध्यम से वीर्य विस्तारकों में सुधार किया गया, जो 4 डिग्री सेल्सियस पर 10 दिनों तक शुक्राणु व्यवहार्यता बनाए रखता है। एक्सटेंडर में एल-कार्निटाइन और मेलाटोनिन के साथ पूरकता से शुक्राणु गतिशीलता, प्लाज्मा झिल्ली अखंडता और एंटीऑक्सीडेंट गतिविधि में वृद्धि हुई, जबकि ऑक्सीडेटिव तनाव कम हो गया। ये विकास नस्ल सुधार, दुर्लभ अश्व आनुवंशिक संसाधनों के संरक्षण और बढ़ी हुई प्रजनन क्षमता के लिए महत्वपूर्ण हैं, इस प्रकार अश्व प्रजनन जैव प्रौद्योगिकी में राष्ट्रीय अश्व अनुसंधान केंद्र के नेतृत्व को मजबूत करते हैं।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने देशी अश्वों की नस्लों के लक्षण-निर्धारण और संरक्षण की दिशा में महत्वपूर्ण योगदान दिया है। वर्ष के दौरान, भीमथड़ी घोड़े को व्यापक लक्षण प्ररूपी और आणविक लक्षण-निर्धारण के बाद आधिकारिक तौर पर भारत की 8^{वीं} देशी घोड़ा नस्ल के रूप में मान्यता दी गई। भीमथड़ी अश्वों के लिए एक समर्पित नस्ल समाज का गठन और पंजीकरण किया गया, जिससे संगठित प्रजनन, संरक्षण और प्रचार गतिविधियों को सुनिश्चित किया जा सके। हलारी गधे के संरक्षण के लिए समानांतर प्रयास किए गए, जिसमें झुंड का विस्तार और कठोर

and utility under harsh climatic conditions. Ethnoveterinary practices related to Halari donkey management were also recorded and submitted to NITI Aayog as part of indigenous knowledge documentation. Cryopreservation efforts continued robustly, with 5831 semen doses stored and 540 distributed to stakeholders. These scientific and field-level interventions enhance breed sustainability, promote genetic biodiversity, and align with national priorities in livestock conservation.

The Centre further initiated advanced genomic studies using the Axiom_Ashwa SNP chip to identify genetic markers of diversity and purity among Indian horse breeds. Whole genome sequencing of Marwari horses was initiated to identify ancestry-informative markers (AIMs), aiding breed traceability and purity assessment.

ICAR-NRCE emphasized fertility monitoring as a critical aspect of equine reproductive management. A comprehensive study on uterine health in mares revealed the efficacy of low-volume lavage (LVL) as the most reliable diagnostic method for detecting subclinical endometritis. Compared to uterine swabs and cytobrush techniques, LVL provided superior quality samples for cytology and microbiological evaluation, enabling precise detection of endometrial inflammation and microbial colonization. As part of comprehensive fertility assessment, NRCE also implemented the use of Combined Thickness of Uterus and Placenta (CTUP) as a key ultrasonographic parameter for evaluating fetal well-being in pregnant mares. CTUP values were monitored throughout gestation in different indigenous breeds including Marwari, Manipuri, and Zanskari horses. These measurements provided vital baseline data and reference ranges for assessing placental health and detecting placentitis.

Therapeutic management of endometritis was further enhanced through the use of Platelet-Rich Plasma (PRP) therapy. Mares treated with intrauterine PRP showed a conception rate of 91.6%, outperforming those managed with conventional antibiotic therapy. This regenerative approach not only improved uterine receptivity but also reduced the recurrence of infection, indicating a promising alternative to traditional methods. In addition to disease detection and therapy, the Centre conducted detailed studies on reproductive hormones and ultrasonographic parameters to monitor ovulation, estrous cycles, and conception status. The development of protocols for monitoring corpus luteum

जलवायु परिस्थितियों में इसके अद्वितीय रूपात्मक लक्षणों, व्यवहार संबंधी विशेषताओं और उपयोगिता का दस्तावेजीकरण किया गया। हलारी गधे के प्रबंधन से प्राकृतिक संबंधित पशु चिकित्सा पद्धतियों को भी दर्ज किया गया और स्वदेशी ज्ञान दस्तावेजीकरण के हिस्से के रूप में नीति आयोग को प्रस्तुत किया गया। भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र में अश्वों के वीर्य का हिमीकरण विधि त्रितरा से जारी है, जिसके अंतर्गत 5831 वीर्य खुराक संग्रहीत की गईं और 540 हितधारकों को वितरित की गईं। ये वैज्ञानिक और क्षेत्र-स्तरीय हस्तक्षेप नस्ल स्थिरता को बढ़ाते हैं, आनुवंशिक जैव विविधता को बढ़ावा देते हैं और पशुधन संरक्षण में राष्ट्रीय प्राथमिकताओं के साथ सरेखित होते हैं।

केंद्र ने भारतीय अश्वों की नस्लों में विविधता और शुद्धता के लिए आनुवंशिक मार्करों की पहचान करने के लिए एक्सओम_अश्व एसएनपी चिप का उपयोग करके उन्नत जीनोमिक अध्ययन शुरू किया। जिसके अंतर्गत मारवाड़ी अश्वों की संपूर्ण जीनोम अनुक्रमण की शुरुआत की गई ताकि वंशावली-सूचनात्मक मार्करों (एआईएम) की पहचान की जा सके, जिससे नस्ल का पता लगाने और शुद्धता का आकलन करने में मदद मिली।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने प्रजनन निगरानी को अश्व प्रजनन प्रबंधन के एक महत्वपूर्ण पहलू के रूप में महत्व दिया। घोड़ियों में गर्भाशय के स्वास्थ्य पर एक व्यापक अध्ययन ने सबक्लिनिकल एंडोमेट्राइटिस का पता लगाने के लिए सबसे विश्वसनीय नैदानिक विधि के रूप में कम मात्रा में लैवेज (LVL) की प्रभावकारिता का खुलासा किया। गर्भाशय के स्वाब और साइटोब्रश तकनीकों की तुलना में, LVL ने कोशिका विज्ञान और माइक्रोबायोलॉजिकल मूल्यांकन के लिए बेहतर गुणवत्ता वाले नमूने प्रदान किए, जिससे एंडोमेट्रियल सूजन और माइक्रोबियल उपनिवेशन का सटीक पता लगाना संभव हो गया। व्यापक प्रजनन मूल्यांकन के हिस्से के रूप में, राष्ट्रीय अश्व अनुसंधान केंद्र ने गर्भवती घोड़ियों में भ्रूण की भलाई के मूल्यांकन के लिए एक प्रमुख अल्ट्रासोनोग्राफिक पैरामीटर के रूप में गर्भाशय और प्लेसेंटा की संयुक्त मोटाई (CTUP) के उपयोग को भी लागू किया। मारवाड़ी, मणिपुरी और ज़ांस्करी अश्वों सहित विभिन्न देशी नस्लों में गर्भावस्था के दौरान CTUP मानों की निगरानी की गई। इन मापों ने प्लेसेंटल स्वास्थ्य का आकलन करने और प्लेसेंटाइटिस का पता लगाने के लिए महत्वपूर्ण आधारभूत डेटा और संदर्भ सीमाएँ प्रदान कीं।

प्लेटलेट-रिच प्लाज्मा (पीआरपी) थेरेपी के उपयोग के माध्यम से एंडोमेट्राइटिस के चिकित्सीय प्रबंधन को और बेहतर बनाया गया। अंतर्गर्भाशयी पीआरपी के साथ इलाज की गई घोड़ियों ने 91.6% की गर्भधारण दर दिखाई, जो पारंपरिक एंटीबायोटिक थेरेपी से प्रबंधित की गई घोड़ियों से बेहतर थी। इस पुनर्योजी दृष्टिकोण ने न केवल गर्भाशय की ग्रहणशीलता में सुधार किया, बल्कि संक्रमण की पुनरावृत्ति को भी कम किया, जो पारंपरिक तरीकों के लिए एक आशाजनक विकल्प का संकेत देता है। रोग का पता लगाने और

function and early pregnancy diagnosis has contributed to improved breeding management, particularly in elite and endangered equine breeds.

ICAR-NRCE made significant progress in converting laboratory research into practical technologies for equine breeding and health management. The Centre developed and transferred a suite of nine novel technologies, including diagnostic assays, recombinant ELISA kits, semen cryopreservation protocols, and herbal therapeutic supplements. Custom-designed artificial vaginas (AVs) for Marwari, Zanskari, and Manipuri horses improved semen collection efficiency. A liposome-based semen extender, now field-validated, enhanced sperm viability for up to 10 days under cold storage, supporting remote artificial insemination programs. The Centre also transferred its single-straw deep horn insemination protocol to private farms with 80% conception success. NRCE strengthened outreach through demonstrations of semen handling technologies at Bengaluru, Udaipur, and Khempur, and entered MoUs with private partners for technology adoption. The commercial sale of cryopreserved semen further supported genetic improvement efforts. These innovations exemplify NRCE's commitment to field-ready solutions and industry collaboration.

The National Centre for Veterinary Type Cultures (NCVTC), housed at ICAR-NRCE, continues to be a vital national repository dedicated to the preservation, authentication, and distribution of veterinary microbial resources. The Centre maintains a diverse and expanding collection of over 5400 well-characterized microbial strains, encompassing bacteria, viruses, and bacteriophages and recombinant clones relevant to animal health and veterinary research. During the year 2024, a total number of 116 bacterial strain has been accessioned including 18 novel bacterial isolates to its repository, bringing the total number of bacterial entries to 1942. These isolates include key equine pathogens such as *Streptococcus equi* subsp. *equi*, *Streptococcus equi* subsp. *zoepidemicus*, *Pasteurella multocida*, *Corynebacterium pseudotuberculosis*, *Rhodococcus equi*, and *Escherichia coli*, among others. The strains were isolated from clinical specimens collected during disease outbreak investigations and diagnostic case referrals. In addition to bacterial strains, the NCVTC currently preserves 415 viral isolates, including Avian infectious bronchitis virus PPRV, BRV, ERV etc. These viral stocks are used to support vaccine development,

उपचार के अलावा, केंद्र ने ओव्यूलेशन, एस्ट्रस चक्र और गर्भाधान की स्थिति की निगरानी के लिए प्रजनन हार्मोन और अल्ट्रासोनोग्राफिक मापदंडों पर विस्तृत अध्ययन किया। कॉर्पस ल्यूटियम फंक्शन और प्रारंभिक गर्भावस्था निदान की निगरानी के लिए प्रोटोकॉल के विकास ने विशेष रूप से कुलीन और लुप्तप्राय अश्वों की नस्लों में बेहतर प्रजनन प्रबंधन में योगदान दिया है।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने प्रयोगशाला अनुसंधान को अश्व प्रजनन और स्वास्थ्य प्रबंधन के लिए व्यावहारिक प्रौद्योगिकियों में परिवर्तित करने में महत्वपूर्ण प्रगति की है। केंद्र ने नौ नवीन प्रौद्योगिकियों का एक समूह विकसित और हस्तांतरित किया, जिसमें नैदानिक परीक्षण, पुनः संयोजक एलिसा किट, वीर्य क्रायोप्रीजर्वेशन प्रोटोकॉल और हर्बल चिकित्सीय पूरक शामिल हैं। मारवाड़ी, जांस्करी और मणिपुरी अश्वों के लिए कस्टम-डिजाइन किए गए कृत्रिम योनि (एवी) ने वीर्य संग्रह दक्षता में सुधार किया। लिपोसोम-आधारित वीर्य विस्तारक, जिसे अब क्षेत्र-मान्यता प्राप्त है, ने कोल्ड स्टोरेज के तहत 10 दिनों तक शुक्राणु व्यवहार्यता को बढ़ाया, जिससे दूरस्थ कृत्रिम गर्भाधान कार्यक्रमों को समर्थन मिला। केंद्र ने 80% गर्भाधान सफलता के साथ अपने एकल-स्ट्रॉ डीप हॉर्न गर्भाधान प्रोटोकॉल को निजी खेतों में भी स्थानांतरित किया। चिटोसन, जिंक और हाइड्रोक्सीक्लोरोक्वीन के एक नैनो-आधारित रोगाणुरोधी सूत्रीकरण ने स्ट्रेप्टोकोकस इक्वी के प्रभावी अवरोध का प्रदर्शन किया और इसे व्यावसायिकरण के लिए तैयार किया जा रहा है। राष्ट्रीय अश्व अनुसंधान केंद्र ने बेंगलुरु, उदयपुर और खेमपुर में वीर्य प्रबंधन तकनीकों के प्रदर्शन के माध्यम से आउटरीच को मजबूत किया और तकनीक अपनाने के लिए निजी भागीदारों के साथ समझौता ज्ञापन पर हस्ताक्षर किए। क्रायोप्रीजर्व्ड वीर्य की व्यावसायिक बिक्री ने आनुवंशिक सुधार प्रयासों को और अधिक समर्थन दिया। ये नवाचार राष्ट्रीय अश्व अनुसंधान केंद्र की फील्ड-रेडी समाधानों और उद्योग सहयोग के प्रति प्रतिबद्धता को दर्शाते हैं।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र में स्थित राष्ट्रीय पशु चिकित्सा प्रकार संवर्धन केंद्र (एनसीवीटीसी) पशु चिकित्सा सूक्ष्मजीव संसाधनों के संरक्षण, प्रमाणीकरण और वितरण के लिए समर्पित एक महत्वपूर्ण राष्ट्रीय भंडार बना हुआ है। केंद्र में 5400 से अधिक अच्छी तरह से चिह्नित सूक्ष्मजीव उपभेदों का एक विविध और विस्तारित संग्रह है, जिसमें पशु स्वास्थ्य और पशु चिकित्सा अनुसंधान के लिए प्रासंगिक बैक्टीरिया, वायरस और बैक्टीरियोफेज और पुनः संयोजक क्लोन शामिल हैं। वर्ष 2024 के दौरान, इसके भंडार में 18 नए बैक्टीरिया आइसोलेट्स सहित कुल 116 बैक्टीरिया स्ट्रेन को शामिल किया गया है, जिससे बैक्टीरिया की कुल संख्या 1942 हो गई है। इन आइसोलेट्स में प्रमुख घोड़ा रोगजनक जैसे स्ट्रेप्टोकोकस इक्वी उपप्रजाति इक्वी, स्ट्रेप्टोकोकस इक्वी उपप्रजाति जूएपिडेमिकस, पाश्चरेला मल्टोसिडा, कोरिनेबैक्टीरियम स्यूडोट्यूबरकुलोसिस रोग प्रकोप जांच और नैदानिक मामले रेफरल के दौरान एकत्र किए गए नैदानिक नमूनों से उपभेदों को अलग किया गया था। जीवाणु उपभेदों के अलावा, एनसीवीटीसी वर्तमान में 415

molecular epidemiology, and sero-diagnostic assay validation.

Furthermore, the Centre has expanded its collection of bacteriophages, now comprising 401 phage isolates with specificity to *Salmonella enterica*, *E. coli*, and *P. multocida*. Intensive characterization of these phages has been undertaken to assess host range, lytic potential, genetic diversity, and safety for therapeutic use. Whole-genome sequencing and electron microscopy of selected phages confirmed their virulent nature and absence of undesirable genes such as toxins or lysogenic elements. In vitro efficacy testing revealed strong bactericidal activity against multidrug-resistant isolates. A cocktail of phages targeting *Klebsiella pneumoniae* was formulated and showed reduction in bacterial load in infected cultures within 4 hours, suggesting promising application in phage therapy in poultry. Preliminary trials for their incorporation into topical formulations and biofilm disruption assays were also initiated.

In alignment with national efforts on antimicrobial resistance (AMR), NCVTC characterized more than 60 isolates for their antibiotic susceptibility profiles and deposited metadata to the ICAR-AMR surveillance platform. Whole-genome sequencing and plasmid analysis were initiated for selected multidrug-resistant strains.

NCVTC has started working on key livestock and poultry viruses Bovine Coronavirus (BCoV), Bovine Torovirus (BToV), Rotavirus and including Infectious Laryngotracheitis Virus (ILT). ILTV was isolated from poultry respiratory outbreaks and confirmed through PCR targeting glycoprotein genes, followed by embryonated egg propagation and sequencing, which revealed strain similarities with global vaccine and field isolates. In cattle, detected BCoV and BToV from diarrhoeic feces using RT-PCR and sequenced structural genes enabling strain-level differentiation and epidemiological insights. Concurrently, bovine rotavirus-positive samples were genotyped via VP6 and VP7-based RT-PCR assays, identifying circulating G10 and G6 strains. These studies provided essential genomic data and diagnostic tools for understanding the burden, diversity of viral pathogens, reinforcing NRCE's contribution to broader animal health research.

To enhance user experience, NCVTC launched a beta version of its digital catalogue, which includes

वायरल आइसोलेट्स को संरक्षित करता है। इन वायरल स्टॉक का उपयोग वैक्सिन विकास, आणविक महामारी विज्ञान और सीरो-डायग्नोस्टिक परख सत्यापन का समर्थन करने के लिए किया जाता है।

इसके अलावा, केंद्र ने बैक्टीरियोफेज के अपने संग्रह का विस्तार किया है, जिसमें अब स्ट्रेप्टोकोकस इक्वी, ई. कोली और पी. मल्टोसिडा की विशिष्टता वाले फेज आइसोलेट्स शामिल हैं। संक्रमण या परजीवीकरण की क्षमता, जीवाणु मारने की क्षमता, आनुवंशिक विविधता और चिकित्सीय उपयोग के लिए सुरक्षा का आकलन करने के लिए इन फेजों का गहन लक्षण वर्णन किया गया है। चयनित फेजों की संपूर्ण जीनोम अनुक्रमण और इलेक्ट्रॉन माइक्रोस्कोपी ने उनकी विषैली प्रकृति और विषाक्त पदार्थों या लाइसोजेनिक तत्वों जैसे अवांछनीय जीनों की अनुपस्थिति की पुष्टि की। इन विट्रो प्रभावकारिता परीक्षण में बहुऔषधि प्रतिरोधी आइसोलेट्स के खिलाफ मजबूत जीवाणुनाशक गतिविधि का पता चला। एस. इक्वी को लक्षित करने वाले फेजों का एक कॉकटेल तैयार किया गया और 4 घंटे के भीतर संक्रमित संस्कृतियों में बैक्टीरिया के भार में कमी दिखाई गई, जो पोल्ट्री में फेज थेरेपी में आशाजनक अनुप्रयोग का सुझाव देता है। सामयिक योगों और बायोफिल्म विघटन परख में उनके समावेश के लिए प्रारंभिक परीक्षण भी शुरू किए गए थे।

रोगानुरोधी प्रतिरोध (एएमआर) पर राष्ट्रीय प्रयासों के अनुरूप एनसीवीटीसी ने 60 से अधिक आइसोलेट्स को उनके एंटीबायोटिक संवेदनशीलता प्रोफाइल के लिए चिन्हित किया और आईसीएआर-एएमआर निगरानी मंच पर मेटाडेटा जमा किया। चयनित बहुऔषधि प्रतिरोधी उपभेदों के लिए संपूर्ण जीनोम अनुक्रमण और प्लास्मिड विश्लेषण शुरू किया गया।

एनसीवीटीसी ने प्रमुख पशुधन और पोल्ट्री वायरस बोवाइन कोरोनावायरस (बीसीओवी), बोवाइन टोरोवायरस (बीटीओवी), रोटावायरस और संक्रामक लैरिंजोत्राकेइटिस वायरस (आईएलटीवी) पर काम करना शुरू कर दिया है। आईएलटीवी को पोल्ट्री श्वसन प्रकोपों से अलग किया गया और ग्लाइकोप्रोटीन जीन को लक्षित करने वाले पीसीआर के माध्यम से पुष्टि की, इसके बाद अंडे के भ्रूण में प्रचारित और अनुक्रमण किया गया, जिससे वैश्विक वैक्सिन और फील्ड आइसोलेट्स के साथ स्ट्रेन समानताएं सामने आईं। मवेशियों में, आरटी-पीसीआर का उपयोग करके दस्त के मल से बीसीओवी और बीटीओवी का पता लगाया गया और स्ट्रेन-स्तर भेदभाव और महामारी विज्ञान संबंधी अंतर्दृष्टि को सक्षम करने वाले संरचनात्मक जीन को अनुक्रमित किया गया। इसके साथ-साथ ही, गोजातीय रोटावायरस-पॉंजिटिव नमूनों को वीपी6 और वीपी7-आधारित आरटी-पीसीआर परख के माध्यम से जीनोटाइप किया गया, जिससे परिसंचारी जी10 और जी6 स्ट्रेन की पहचान हुई। इन अध्ययनों से वायरल रोगजनकों की विविधता को समझने के लिए आवश्यक जीनोमिक डेटा और नैदानिक उपकरण प्रदान किए गए,

searchable strain data, antibiograms, and usage guidelines. Quality management systems were strengthened with the implementation of internal certification protocols for distributed strains, ensuring biosafety and quality assurance.

NCVTC supplied 26 authenticated microbial cultures to veterinary colleges, research organizations, and public laboratories for applications in diagnostic standardization, antimicrobial resistance studies, pathogenesis research, and vaccine seed preparation. The Centre also contributed key isolates to externally funded research programs and collaborative studies. The Centre also provided microbial support to 12 NRCE-led projects focusing on recombinant antigen development, ELISA standardization, and challenge model establishment. By integrating core biobanking functions with frontline veterinary research, NCVTC continues to serve as a national resource for advancing microbiological diagnostics, therapeutics, and translational innovations in veterinary science.

ICAR-NRCE carries out its research and development initiatives through a well-organized framework of 25 institute-funded, 4 corpus-funded, and 22 externally funded research projects, including collaborative efforts with other institutions. The Centre has successfully secured external support from nearly all major national funding agencies in the agricultural and biological sciences sectors.

ICAR-NRCE continued its impactful scientific engagement through a robust portfolio of academic and technological outputs. During the reporting year, the Centre published a total of 44 research articles in peer-reviewed journals. These encompassed key research areas including diagnostics, therapeutics, vaccine development, reproductive technologies, and epidemiology. Additionally, the Centre submitted 28 GenBank accessions representing novel gene sequences from viruses, bacteria, and protozoan pathogens isolated during surveillance and molecular characterization efforts. Additionally, 3 books, 6 book chapters, 35 chapters in training manuals, 1 technical bulletin, and 1 policy briefs were published, aiding in knowledge dissemination to field veterinarians, scientists, and policymakers. NRCE developed and documented 12 technologies, of which 6 were commercialized and transferred to users through licensing and MoUs. In the intellectual property domain, one patent has been granted and 7 patent applications

जिससे व्यापक पशु स्वास्थ्य अनुसंधान में राष्ट्रीय अश्व अनुसंधान केंद्र के योगदान को बल मिला।

एनसीवीटीसी ने उपयोगकर्ता अनुभव को बेहतर बनाने के लिए अपने डिजिटल सूचीपत्र का बीटा संस्करण जारी किया, जिसमें खोज योग्य स्ट्रेन आंकड़े, एंटीबायोग्राम और उपयोग दिशानिर्देश शामिल हैं। वितरित स्ट्रेन के लिए आंतरिक प्रमाणन प्रोटोकॉल के कार्यान्वयन के साथ गुणवत्ता प्रबंधन प्रणालियों को मजबूत किया गया, जिससे जैव सुरक्षा और गुणवत्ता आश्वासन सुनिश्चित हुआ।

एनसीवीटीसी ने नैदानिक मानकीकरण, रोगाणुरोधी प्रतिरोध अध्ययन, रोगजनन अनुसंधान और वैक्सीन विशिष्ट तैयारी में अनुप्रयोगों के लिए पशु चिकित्सा महाविद्यालयों, अनुसंधान संगठनों और सार्वजनिक प्रयोगशालाओं को 26 प्रमाणित सूक्ष्मजीव संवर्धन प्रदान किए। केंद्र ने बाहरी रूप से वित्त पोषित अनुसंधान कार्यक्रमों और सहयोगी अध्ययनों में प्रमुख आइसोलेट्स का भी योगदान दिया। केंद्र ने पुनः संयोजक प्रतिजन विकास, एलिसा मानकीकरण और चुनौती मॉडल स्थापना पर ध्यान केंद्रित करने वाली 12 राष्ट्रीय अश्व अनुसंधान केंद्र-नेतृत्व वाली परियोजनाओं को सूक्ष्मजीव सहायता भी प्रदान की। मुख्य जैव बैंकिंग कार्यों को अग्रणी पशु चिकित्सा अनुसंधान के साथ एकीकृत करके, एनसीवीटीसी पशु चिकित्सा विज्ञान में सूक्ष्मजीवविज्ञानी निदान, चिकित्सा विज्ञान और अनुवाद संबंधी नवाचारों को आगे बढ़ाने के लिए एक राष्ट्रीय संसाधन के रूप में काम करना जारी रखता है।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र में 25 संस्थान-वित्तपोषित, 4 कॉर्पस-वित्तपोषित और 22 बाह्य वित्तपोषित अनुसंधान परियोजनाएं चल रही हैं जिसमें अन्य संस्थानों के साथ सहयोगात्मक प्रयास भी शामिल हैं। केंद्र ने कृषि और जैविक विज्ञान क्षेत्रों में लगभग सभी प्रमुख राष्ट्रीय वित्त पोषण एजेंसियों से सफलतापूर्वक बाहरी समर्थन प्राप्त किया है।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने अकादमिक और तकनीकी उत्पादन के एक मजबूत विवरण के माध्यम से अपने प्रभावशाली वैज्ञानिक जुड़ाव को जारी रखा। रिपोर्टिंग वर्ष के दौरान, केंद्र ने सहकर्मी-समीक्षित पत्रिकाओं में कुल 44 शोध लेख प्रकाशित किए। इनमें निदान, चिकित्सा विज्ञान, टीका विकास, प्रजनन तकनीक और महामारी विज्ञान सहित प्रमुख शोध क्षेत्र शामिल थे। इसके अतिरिक्त, केंद्र ने निगरानी और आणविक लक्षण वर्णन प्रयासों के दौरान अलग किए गए वायरस, बैक्टीरिया और प्रोटोजोआ रोगजनकों से नए जीन अनुक्रमों का प्रतिनिधित्व करने वाले 28 जीनबैंक अभिगम प्रस्तुत किए। इसके अतिरिक्त, 3 पुस्तकें, 6 पुस्तक अध्याय, प्रशिक्षण मैनुअल में 8 अध्याय, 1 तकनीकी बुलेटिन और 1 नीति संक्षिप्त प्रकाशित किए गए, जिससे क्षेत्र के पशु चिकित्सकों, वैज्ञानिकों और नीति निर्माताओं को ज्ञान प्रसार में सहायता मिली। राष्ट्रीय अश्व अनुसंधान केंद्र ने 12 तकनीकों का विकास और दस्तावेजीकरण किया, जिनमें से 6 का व्यावसायीकरण किया गया और लाइसेंसिंग और समझौता ज्ञापन के माध्यम से

were filed for novel diagnostic tools and biological products.

ICAR-NRCE organized several institutional activities to promote wellness, integrity, and public engagement. The International Day of Yoga (21st June 2024) was celebrated with active participation in guided sessions of yoga postures and breathing techniques. Vigilance Awareness Week (30th October – 5th November 2024) was observed on the theme “Say no to corruption; commit to the Nation,” including pledge-taking, awareness talks, and exhibitions. The Centre's Foundation Day (26th November, 2024) featured expert lectures and interactions with retired scientists and stakeholders, highlighting its historical and scientific achievements. Additionally, events such as Swachhata Pakhwada (16th–31st December 2024), Hindi Pakhwada (14th–28th September 2024), Constitution Day (26th November 2024), tree plantation drives, and gender sensitization workshops were conducted, reflecting NRCE's commitment to institutional values and inclusive development.

The Centre has demonstrated significant financial sustainability by generating INR 2.32 crore in revenue. This included income from diagnostic services (INR 97.3 lakh), commercialization of diagnostic kits and semen extenders, stakeholder training programs, collaborative research projects, and licensing agreements. This revenue not only supports ongoing research and infrastructure but also reinforces the Centre's role as a self-reliant and innovation-driven institution.

In 2024, ICAR-NRCE has continued to lead the way in advancing equine science through multidisciplinary research, innovation, and stakeholder engagement. Its integrated approach—spanning disease diagnostics, molecular biology, reproductive technologies, conservation genomics, and traditional veterinary practices—has significantly contributed to improving equine health and productivity in India. The achievements of the NCVTC, combined with robust surveillance, vaccine development, and technology transfer initiatives, have enhanced the Centre's role as a national and international leader in equine research and development.

उपयोगकर्ताओं को हस्तांतरित किया गया। बौद्धिक संपदा कार्यक्षेत्र में, एक पेटेंट प्रदान किया गया है और नए नैदानिक उपकरणों और जैविक उत्पादों के लिए 7 पेटेंट आवेदन दायर किए गए हैं।

भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने स्वास्थ्य, अखंडता और सार्वजनिक सहभागिता को बढ़ावा देने के लिए कई संस्थागत गतिविधियों का आयोजन किया। अंतर्राष्ट्रीय योग दिवस (21 जून 2024) को योग आसन और श्वास तकनीक के निर्देशित सत्रों में सक्रिय भागीदारी के साथ मनाया गया। सतर्कता जागरूकता सप्ताह (30 अक्टूबर - 5 नवंबर 2024) “भ्रष्टाचार को न कहें; राष्ट्र के प्रति प्रतिबद्ध हों” विषय पर मनाया गया, जिसमें प्रतिज्ञा लेना, जागरूकता वार्ता और प्रदर्शनियाँ शामिल थीं। केंद्र के स्थापना दिवस (26 नवंबर, 2024) पर विशेषज्ञ व्याख्यान और सेवानिवृत्त वैज्ञानिकों और हितधारकों के साथ बातचीत हुई, जिसमें इसकी ऐतिहासिक और वैज्ञानिक उपलब्धियों पर प्रकाश डाला गया। इसके अतिरिक्त, स्वच्छता पखवाड़ा (16-31 दिसंबर 2024), हिंदी पखवाड़ा (14-28 सितंबर 2024), संविधान दिवस (26 नवंबर 2024), वृक्षारोपण अभियान और लिंग संवेदीकरण कार्यशालाएँ जैसे कार्यक्रम आयोजित किए गए, जो संस्थागत मूल्यों और समावेशी विकास के प्रति राष्ट्रीय अश्व अनुसंधान केंद्र की प्रतिबद्धता को दर्शाते हैं।

केंद्र ने 2.32 करोड़ रुपये का राजस्व अर्जित करके महत्वपूर्ण वित्तीय स्थिरता का प्रदर्शन किया है। इसमें डायग्नोस्टिक सेवाओं (97.3 लाख रुपये), डायग्नोस्टिक किट और सीमेन एक्सटेंडर के व्यावसायीकरण, हितधारक प्रशिक्षण कार्यक्रम, सहयोगी अनुसंधान परियोजनाओं और लाइसेंसिंग समझौतों से आय शामिल है। यह राजस्व न केवल चल रहे अनुसंधान और बुनियादी ढांचे का समर्थन करता है बल्कि एक आत्मनिर्भर और नवाचार-संचालित संस्थान के रूप में केंद्र की भूमिका को भी मजबूत करता है।

2024 में, भा. कृ. अनु.परि.- राष्ट्रीय अश्व अनुसंधान केंद्र ने बहु-विषयक अनुसंधान, नवाचार और हितधारक जुड़ाव के माध्यम से अश्व विज्ञान को आगे बढ़ाने में अग्रणी भूमिका निभाना जारी रखा है। इसके एकीकृत दृष्टिकोण - रोग निदान, आणविक जीव विज्ञान, प्रजनन तकनीक, संरक्षण जीनोमिक्स और पारंपरिक पशु चिकित्सा पद्धतियों को शामिल करते हुए - ने भारत में अश्व स्वास्थ्य और उत्पादकता को बेहतर बनाने में महत्वपूर्ण योगदान दिया है। NCVTC की उपलब्धियों ने मजबूत निगरानी, वैक्सीन विकास और प्रौद्योगिकी हस्तांतरण पहलों के साथ मिलकर अश्व अनुसंधान और विकास में एक राष्ट्रीय और अंतर्राष्ट्रीय नेता के रूप में केंद्र की भूमिका को बढ़ाया है।



Introduction

Horses have held a unique and enduring significance across civilizations, symbolizing grace, power and companionship throughout history. Since their domestication over 5,000 years ago, horses have profoundly influenced human societies through their roles in agriculture, warfare, transport, and culture. They have not only contributed to economic development but have also become powerful cultural icons, revered for their strength and nobility. Historically, horses were indispensable—serving as the primary means for plowing fields, transporting goods, and carrying soldiers into battle before the advent of mechanization. In many parts of the world, they continue to serve vital functions such as herding livestock and navigating terrains inaccessible to vehicles. In modern contexts, horses maintain their importance through equestrian sports like racing, dressage, show jumping, and polo, which highlight their athleticism and deepen the human-animal bond through discipline, trust, and communication. Culturally, horses are associated with prestige and honor. Their role in ceremonies, rituals, and traditional festivals reflects their symbolic stature in many societies. Beyond these practical and cultural contributions, horses offer proven therapeutic benefits. Equine-assisted therapies are increasingly used to support individuals with physical, emotional, and social challenges—helping reduce stress, build confidence, and enhance well-being. In the Indian subcontinent, horses continue to be cherished for their historical, cultural, and economic value. Recognizing their importance, the Indian Council of Agricultural Research (ICAR) established the National Research Centre on Equines (ICAR-NRCE) at Hisar, Haryana, in 1985. This premier institute serves as the nation's hub for equine research, equipped with state-of-the-art laboratories specializing in equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry, and biotechnology.

Supporting these research divisions are centralized facilities including animal and agricultural farms, an experimental animal facility, a microbial containment laboratory (BSL-3), AKMU cell, ATIC, a specialized library, and the Info-Equine Museum. Complementing the main campus is the Equine Production Campus at Bikaner, Rajasthan, dedicated to scientific breeding and management practices for elite indigenous equine breeds. Further strengthening veterinary microbiological research, the National Centre for Veterinary Type Cultures (NCVTC) was established in 2005 at the ICAR-NRCE Hisar campus. This national repository is tasked with the collection, preservation, and distribution of microbes of veterinary importance isolated from animal hosts. Presently, NCVTC functions through a coordinated network of 15 units across the country, enabling robust surveillance and research in animal health. Together, these institutions underscore India's commitment to preserving equine heritage while advancing science and innovation in the service of animal health and productivity.

MANDATE OF NRCE

- Basic and strategic research on equine health and production
- To provide advisory and consultancy services and capacity development

OBJECTIVES OF NRCE

- Generation of demand-driven technologies for equine health and production management.
- Capacity building for competitive equine power utilization in agricultural operations to serve the underprivileged under changing environment & socio-economic scenario.

SALIENT ACHIEVEMENTS

During the past 39 years, ICAR-NRCE has contributed significantly in the area of diagnosis and control of equine infectious diseases by developing state-of-the-art diagnostics and biologicals. Some of the major achievements and accolades of the Centre are enlisted below:

Development of diagnostics

The Centre has been recognized as the National Referral Centre for diagnosis of important equine infectious diseases by the Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, Government of India. The Centre has developed and refined diagnostics against various equine diseases:

- HERP kit for field diagnosis of equine herpesvirus 1 (EHV1) infection.
- COFEB kit for diagnosis of *Theileria equi*.
- Monoclonal antibody-based diagnostic kit 'Equiherpes B-ELISA' for EHV1 antibody detection.
- A type-specific ELISA and real-time PCR for differentiation of EHV1 and EHV4 infections.
- Complement fixation and r-protein-based ELISA for diagnosis of glanders.
- A monoclonal antibody-based sandwich ELISA and RT-PCR for detection of equine rotavirus (ERV) from faecal samples.
- RT-PCR and real-time RT-PCR based assays for typing and diagnosis of equine influenza virus.
- A recombinant antigen based-ELISA for detection of antibodies to *Theileria equi*.
- An indirect ELISA using whole cell lysate antigen and PCR for detection of *Trypanosoma evansi*.
- ELISA and RT-PCR for diagnosis of Japanese encephalitis.
- A recombinant protein-based indirect ELISA for sero-diagnosis of glanders and equine infectious anemia.
- Lateral flow assay based rapid diagnostic for *Theileria equi* infection.
- Lateral flow assay kit for glanders.
- Lateral flow assay kit for equine infectious anemia.
- Standardization of a nested (gB-nPCR) and real-time PCR (gB- qPCR) targeting gB gene for detection of EHV1 latency.
- Recombinant protein based Indirect ELISA for detection of JEV specific antibodies in horse and pig.
- Standardization of Multiplex PCR to differentiate *Streptococcus equi* subsp. *equi* and *zooepidemicus*.
- Lateral flow assay for rapid diagnosis of trypanosomosis using different *T. evansi* antigens.
- ELISA for detection of *T. evansi* antibodies in multiple animal species.
- Monoclonal antibody-based ELISA kit for detection of equine influenza (H3N8) antigen.
- Development of Recombinant antigens based indirect ELISA kit for detection of anti-*Trypanosoma evansi* antibodies in animals
- Developed of recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines
- Standardized RT-RPA-CRISPR based LFA assay for detection of SARS-CoV-2
- Developed isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs
- Development of RPA-LFA assay for detection of EHV1 & EHV4 viruses

Development of vaccines and immuno-biologicals

- Inactivated EHV1 vaccine “Equiherpabort” using indigenous virus for prevention of abortions in mares.
- Updated equine influenza vaccine by incorporating recent virus strain {A/eq/Katra-Jammu.06/08 (H3N8)}.
- Bacterin and outer membrane protein-based vaccine for *Salmonella Abortus equi*.
- Monoclonal antibodies against EHV-1, equine rotavirus, equine influenza, Japanese encephalitis and *Trypanosoma evansi*.
- Inactivated EHV1 vaccine using montanide adjuvant (The modified vaccine is currently under trial in horses).
- Encapsulated phage formulation carrying *Salmonella* phages for therapeutic application in poultry.
- Developed of LSD vaccine (Lumpi-ProVaclnd) to prevent Lumpy skin disease (LSD) in animals
- Developed of SARS-CoV2 vaccine (Anocovax) for animals

Characterization of equine pathogens

- Nucleic acid sequencing of HA, M, M1 and M2 genes of equine influenza virus(EIV) isolates from 2008 outbreak (A/eq/Jammu- Katra/08, /eq/Mysore/08 and A/eq/ Ahmedabad /09) revealed clustering of Indian and Chinese isolates in a separate cluster designated as “Asian clade” and vaccine updated accordingly.
- Sequencing of VP7 gene of equine rotavirus isolates indicated circulation of G10, G3 and G6 serotypes in India.
- Whole genome sequence analysis of Japanese encephalitis virus isolated from an equine indicated virulent strain of genotype 3 is causing the disease in equine.
- The *in-vitro* cultivation of *Trypanosoma evansi* and *Theileria equi* was successfully established.
- Experimental mouse models for equine influenza and equine herpesvirus-1 infections developed.
- Complete genome sequencing of two EHV1 isolates was carried out using NGS. The primary NGS data obtained covered up to 90% of the genome.
- Sequence comparison of Indian EHV1 isolates with other published isolates revealed that Indian isolates are more closely related to EHV1 isolates (OH03 and VA02) from Japan (97.4- 98.8%).
- Phylogenetic analysis based on US segments classified our isolates into clade 5 along the reference isolates V592.
- Genotypic characterization of *Burkholderia mallei* isolates recovered from glanders outbreaks and currently circulating isolates are differing from the older Indian isolates.
- Whole genome sequencing of RVA/Horse-wt/IND/ERV3/2003, RVA/Horse-wt/IND/ERV2/2015, RVA/Horse-wt/IND/ERV4/2017, RVA/Horse-wt/IND/ERV6/2017 carried out.
- Development of multiplex taqman qPCR for *in vitro* detection and *Streptococcus equi* subsps. *equi* and other *Streptococcus* sps.

Surveillance and monitoring of equine diseases in India

- India has gained OIE disease-free status for African horse sickness (AHS) in 2014 based on sero-monitoring data generated by ICAR-NRCE.
- Control of EIA in India was possible due to timely diagnosis and implementing a package of practices formulated by ICAR-NRCE.

- Effective control of the equine influenza outbreak of 1987 (involving 83000 equines) was done by implementing bio-security and development of effective vaccines. Similarly, a major outbreak of equine influenza that spread in 13 different states of India during 2008-09 and caused huge mortality and economic losses was timely diagnosed and controlled in collaboration with state animal husbandry departments.
- The National Action plan for control and eradication of glanders in India was drafted by ICAR-NRCE and the same has been implemented by the Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India in June 2019.

Establishment of nucleus herd of equines and characterization of Indian equine breeds

- ICAR-NRCE has initiated *in-situ* conservation programme in the form of developing an equine sanctuary at EPC, Bikaner where nucleus herds of different Indian horse breeds are being maintained which include Marwari horses from Rajasthan; Kathiawari horses from Gujarat; Zanskari ponies from Zanskar valley (Jammu & Kashmir) and Manipuri ponies from Imphal (Manipur). In addition, Large white (Halari) donkeys for conservation and improvement of donkeys and exotic Poitou donkey herd for production of superior mules are being maintained in the campus.
- Seven equine breeds, namely, Marwari, Kathiawari, Kachchhi-Sindhi, Spiti, Zanskari, Bhutia and Manipuri, have been characterized on the basis of their biometric indices and coat colour.
- Microsatellite marker based genetic diversity analyzed for proposing effectual population breeding and management strategies for future.
- Recognition of the Bhimthadi horse as the 8th officially gazette-notified indigenous equine breed of India

Development of production and reproduction technologies for improving performance

- In order to conserve the germplasm of indigenous equine breeds, cryopreservation of semen of Marwari, Kathiawari, Zanskari and Manipur stallions and Halari & Poitou donkeys are being practiced.
- Artificial insemination using frozen semen has been perfected for production of superior quality horses, mules and donkeys.
- An eCG based sandwich ELISA has been developed for pregnancy diagnosis between days 35 to 120 of gestation in mares.
- Pregnancy diagnosis between days 14 and 18 post-insemination has been perfected using ultrasonography in donkeys and in mares.
- Donkey fibre has been used to produce carpets by mixing with sheep fibres (40:60).
- Studies on assessment of fertility related genes in stallions have been assessed. Expression of SPATA1, PLCz and CRISP3 fertility genes has been studied and established their correlation with DNA integrity and mitochondrial membrane potential of the stallion spermatozoa.
- Research initiated in the direction of treatment of Fibroblastic sarcoid, excessive growth of granulation tissue (proud flesh), Alopecia and Habronemiasis using herbal formulations.
- Developed fatigue cum fitness score card for working equines.
- Customized artificial vagina has been designed for collection and cryopreservation of equine semen and customised artificial vagina was also transferred to equine farmers/breeders.
- Developed donkey milk-based products (Bathing soap, Body butter and Lip balm)

- Estrus synchronization protocols were optimized and methods of embryo recovery from mares and successful transfer of embryos to the surrogates were optimized
- SNP markers associated with fertility in indigenous breeds of horse were studied
- Compositional changes in Halari donkey milk during Lactation were studied
- New creep feeders were designed and fabricated for optimum growth of the foals
- Development of protocols for embryo transfer in indigenous horses
- Development of non-egg yolk based alternative semen extender for stallion semen
- Development of customised novel cryodevices for vitrification of horse embryos
- Development methods for cryovial preservation of stallion semen and thawing protocols
- Production of Marwari breed horse foals through embryo transfer technology for the first time in the country
- Development of a standardized protocol for vermicomposting using equine dung
- Development of the first indigenous high-density SNP chip for Indian horses (Axiom_Ashwa)

Utilization of equine energy in agricultural activities

- Single animal drawn matching plough, seed drill (two furrows) and harness have been designed and developed for donkeys and mules for agricultural operations like ploughing and sowing.
- Draught ability studies conducted on adult donkeys using conventional pneumatic two-wheel cart.
- The technique of vermin composting of equine dung has been optimized for use in agricultural fields.

Services to farmers and equine breeders

- Disease diagnostic services for various infectious and non-infectious diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Health certification for movement of equines within and outside the country to promote export of horses.
- Clinical and diagnostic (including pregnancy diagnosis) services for equine diseases.
- Artificial insemination to augment the production of superior quality horses, mules and donkeys.
- Provision of quality jacks and jennies to various states, breeding societies and farmers, for production of superior quality mules and donkeys.
- On site and online consultancy in equine health and production, including toll-free telephonic advisory at Hisar and Bikaner campuses for farmers and stakeholders.
- Training and supply of educational materials for equine management, production and health.
- Organization of health camps, awareness campaigns and farmers meets in different areas of the country.
- During the COVID-19 pandemic ICAR-NRCE served as a COVID-19 testing facility amongst one of the four institutes of ICAR

Patents granted

- Nano-drug delivery for quinapyramine sulphate (Patent No.310429,Application, No.2560/DEL/2011, dated 06.09.2011).
- A method for preparation of a diagnostic kit for forecasting equine herpesvirus-1 disease (Patent No. 55E4-

1891278 dated 25.10.2003).

- A method for preparing complement fixation test based (COFEB) kit for diagnosis of *Babesia equi* infection of equines (Patent No. 196690 dated 31.07.2009).
- Recombinant *TssA* protein for detection of antibodies against *Burkholderia mallei* and uses thereof. Application No.3610/DEL/2015.
- A recombinant protein for diagnosis of glanders (Patent No: 296824, 2018).
- Polymeric metal nanocomposites and methods of synthesis thereof (Patent No. 411620, dated 16.11.2022).

Patents filed

- A highly sensitive kit for detection of antibodies against *Theileria equi* in serum of equids. Application No. 2763/DEL/2012 dated 06.09.2012
- Polynucleo-desequence, process, composition and methods thereof. Application No. 2560/DEL/2011, dated 06.09.2011.
- Polynucleo-desequence, processes, composition and methods thereof. Application No.1575/CHE/2010 and PCT/IB 2011/052475.
- A recombinant haemagglutinin domain containing protein for the detection and diagnosis of glanders and method of preparation thereof. Application No. 1328/DEL/2010 dated 08.06.2010.
- Recombinant *Hcp1* protein for detection of antibodies against *Burkholderia mallei* in Equines. Application No.4120/DEL/2015.
- *Aerva javanica* extract for the treatment of exuberant granulation tissue and tumors in horses. Application No. 201811048899, dated 24.12.2018. (Provisional).
- Modified vaccine construct for EHV 1 and methods of preparing the same. Application No 202111000312, dated 05.01.2021.
- Monoclonal antibody based immunoassay for detection of equine influenza (H3N8) antigen. Application No 202111004847, dated 04.02.2021.
- Mutated EHV-1(TOH Strain) genome based vaccine construct and method for preparation. Application No.202111057300, dated 09.12.2021.
- Recombinant nucleocapsid protein based indirect ELISA kit for detection of anti SARS-COV-2 antibodies in canines. Application No.202111057358, dated 09.12.2021
- Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulation. Application No.202111057698, dated 11.12.2021
- Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulations. Application No. PCT/IB2022/062019, dated 10.12.2022.
- Recombinant antigens based indirect ELISA kit for detection of anti *Trypanosoma evansi* antibodies in animals. Application No. 202211008619, dated 18.02.2022
- Development of a Novel Modified Attenuated Lumpy Skin Disease Virus (LSDV) For Use as Vaccine Application No. 202211013092, dated 10.03.2022
- A novel vaccine formulation (Ancovax) to prevent SARS-CoV-2 infection in animals. Application No. 202211026023, dated 04.05.2022

- A method for encapsulation of bacteriophage cocktail against Salmonella sp for oral delivery in poultry. Application No. 202211050633, dated 05.09.2022
- Development of a Novel test to differentiate the vaccine and field strains of LSDV. Application No. 202211074538, dated 22.12.2022.
- Chitosan-Alginate-Zinc Artificial Skin Construct. Application No. 202311086503 , dated 18.12.2023.
- miR-29a serves as a novel immunovirological marker to predict the functionality of immune response to Lumpy Skin Disease virus infection. Application No. 202311063758 , dated 22.09.2023
- Development of a phage endolysin-encapsulated antimicrobial formulation for effective amelioration of bacterial biofilms.
- Generation of HDAC6/Cav1 double knockout BHK21 cell line to enhance scalability and yield in Foot-and-Mouth Disease (FMD) vaccine production.
- Design of specific primers and guide RNA complexes for SARS-CoV-2 detection, and development of a rapid diagnostic kit based thereon.
- Development of a lateral flow assay (LFA) kit for isothermal detection of Porcine Circovirus 3 (PCV3) nucleic acids.
- Identification of a gain-of-function mutation in poxvirus-encoded 2 O-methyltransferase enhancing viral mRNA stability and translational efficiency.
- Discovery and characterization of diazo compounds exhibiting potent anti-trypanosomal activities.
- Formulation of a novel semen extender for improved preservation and viability of equine spermatozoa.



National Centre for Veterinary Type Cultures

National Centre for Veterinary Type Cultures (NCVTC) initiated its activities in 2005 for conservation of the microbial diversity of animal origin. The activities comprise acquisition, authentication, preservation, documentation, and repository database management system of animal microbes. A network programme is in operation with 15 network units located in 9 different states viz., Haryana, Rajasthan Uttar Pradesh, Himachal Pradesh, Assam, Tamil Nadu, Gujarat, Kerala and Karnataka. These network units are contributing in conservation of animal microbial diversity in three specialized areas: veterinary microbes at NRCE Hisar, dairy microbes at NDRI, Karnal and rumen microbes at NIANP, Bengaluru.

MANDATE OF NCVTC

- National repository of veterinary, dairy and rumen microorganisms and their identification, characterization and documentation.
- Distribution of microbes for teaching, research and development of new technologies

OBJECTIVES OF NCVTC

- Exploration and collection of microorganisms of animal origin/significance/relevance
- Central storage of animal microbes from existing culture collection centres, institutions and universities
- Characterization, documentation and digitization of microbial database of cultures of animal microbes
- Development of a National Microbial Gene Bank for conserving the biodiversity of animal microbes
- Conservation (both short-term and long-term) and utilization of microorganisms.

SALIENT ACHIEVEMENTS

- During the past few years, ICAR-NRCE has contributed significantly in the area of conservation of microbial diversity and characterization of microbial pathogens. Some of the major achievements of the Centre are enlisted below

Veterinary Microbes

- First laboratory confirmed camelpox virus zoonosis.
- First isolation of BoHV-5 from cattle, Swinepox virus from pigs and Lumpy skin disease virus from cattle.
- First confirmatory report equine pythiosis in India
- First isolation of bacteria such as *Bordetella bronchiseptica* from horse, *Actinobacillus equuli* from foal, *Staphylococcus hyicus* from pig, Methicillin-resistant coagulase negative *Staphylococcus sciuri* from goats, *Trueperella pyogenes*, *Exiguobacterium* spp. from pigs, *Nocardia otitidiscaviarum* from equine granulomatous pneumonia, *Moraxella (Branhamella) ovis* from ovine keratoconjunctivitis in sheep and *Mannheimia varigena* from buffalo.
- Whole genome sequencing of viruses such as SARS-CoV-2, BPXV, LSDV, NDV and Jaagsiekte sheep retrovirus, Avian nephritis virus, Chicken astrovirus and classical swine fever virus.
- Whole genome sequencing of bacteria such as *Pasteurella multocida* sub spp. *multocida* B:2 serotype, *Trueperella pyogenes*, *Bordetella bronchiseptica*, *Clostridium botulinum* isolate from horse, *Pasteurella multocida*, *Actinobacillus equuli* and *Salmonella Gallinarum*.
- Isolation of anaerobic bacterium viz., *Clostridium perfringens*, *Clostridium sordelli* and *Clostridium sporogenes*

isolated from disease outbreak in brick-kiln ponies; Isolation of strains of genera *Gemella*, *Sphingomonas*, *Ochrobactrum*, *Rodentibacter*, *Gallibacterius*, *Shewanella* and *Aggregatibacter*.

- Isolation of Novel thermo tolerant bacteriophage from Ganga river water against *Klebsiella pneumonia* and Isolation and characterization of bacteriophages against mastitis causing *Staphylococcus aureus*.
- Development of bacteriophage cocktail to ameliorate *Pseudomonas aeruginosa* infections in Biofilms
- Development of phage delivery system for safe oral delivery of bacteriophages in poultry gut.
- Characterization of bacteriophages against ESBL producing bacteria for targeting biofilms in bovines
- Methodologies developed to successfully purify a positive sense-RNA virus (FMDV) from a virus mixture containing a negative sense-RNA virus (PPRV).
- Adopted CRISPR/Cas9-mediated gene editing technology to generate knock out cell lines.
- First time demonstrated that MAPK interacting kinase I (MNK1, a host factor) regulates buffalopox virus (BPXV) replication at the level of protein translation initiation.
- Identified the role of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1, an RNA-binding protein) in regulating early translation to replication switch in SARS-CoV-2 life cycle.
- Evaluated the role of p38 mitogen-activated protein kinase (MAP Kinase) in buffalo pox virus replication
- Generated flexible Gateway ORF library of equine influenza virus to study protein-protein interactions.
- First time demonstrated *in vitro* and *in ovo* broad spectrum antiviral activity of emetine against RNA and DNA viruses (PPRV/NDV/BPX/BHV-1).
- Development of isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 2 (PCV2) and 3 (PCV3).
- Development of LSD vaccine (Lumpi-ProVac^{ind}) to prevent Lumpy skin disease (LSD) in animals.
- Development of SARS-CoV2 vaccine (Ancovax) for animals
- Developed recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines
- Development of a novel HRM-based gap-qRT-PCR for identification and quantitation of the vaccine and field strain(s) of lumpy skin disease virus.
- Development of point-of-care diagnostic for detection of SARS-CoV-2 nucleic acids employing RPA-CRISPR-LFA assay
- Development of Salmoquell, a bacteriophage-based therapy targeting Salmonella infections
- Development of Lysibact, an antimicrobial nanoemulsion effective against a broad range of Gram-negative and Gram-positive bacterial infections.

Rumen Microbes

- Isolation and characterization of tannin degrading bacteria such as *Streptococcus gallolyticus* from goat; fibre degrading bacteria *Ruminococcus flavefaciens*, *Prevotella* sp. and *Butyrivibrio* sp. from buffaloes and cattle; and nitrate reducing and cellulose degrading *E. coli* from buffalo.
- Isolation of rumen fungi such as *Anaeromyces* sp., *Orpinomyces intercalaris* and *Orpinomyces joyonii* from buffaloes; *Piromyces* sp. and *Neocallimastix* sp. from goats.
-

Dairy Microbes

- Preservation of dairy microbes, viz, *Lactobacillus* spp; *Lactococcus* spp; *Lactococcus lactis* ssp. *lactis*; *Lactococcus lactis*. ssp. *cremoris*; *Lactococcus lactis* ssp. *diacetylactis*; *Streptococcus thermophiles*; *Leuconostoc* sp; *Bifidobacterium* sp; *Bifidobacterium dentium*; *Bifidobacterium longum*; *Micrococcu* sp., *Kluyveromyces lactis* and *Saccharomyces bisporus*.
- Combination of *L. lactis* ssp *Lactis*-C12 and *Leuconostoc mesenteroides* subsp *mesenteroides* is very suitable for curd and butter milk preparation.
- Six *Lactobacillus* sp. having phytase degrading potential and strong antifungal activity have been isolated from milk-cereal fermented products (Rabadi samples).
- An amylytic strain of *Pediococcus acidolactici* isolated has potential as starter culture in preparation of milk cereal fermented products.

Landmark Achievements Since Inception

Year	Salient Achievements
1985	Foundation of NRCE, Hisar
1987	Detection of first outbreak of equine influenza in northern India
1989	Establishment of Equine Production Campus, Bikaner
1990	Import of Poitou donkey from France
1995	Cryopreservation of Jack semen for AI
1996	Establishment of a herd of Marwari horses
1996	Crystal structure of mare milk lactoferrin
1997	Release of inactivated equine influenza vaccine
2003	Award of Indian patent to HERP kit for diagnosis of EHV1 infection
2005	Establishment of National Centre for Veterinary Type Cultures (NCVTC)
2006	Collection and cryopreservation of stallion semen at farmers' door
2008	Release of 'Equiherpes B-ELISA' kit for EHV1 diagnosis
2008	Release of 'Pregmare kit' for pregnancy diagnosis in mares
2009	Establishment of a herd of Zanskari ponies
2010	Re-emergence of a case of Equine Infectious Anaemia (EIA)
2011	First report of Buffalo pox virus causing concurrent disease in cow, buffalo and human
2011	Whole genome sequencing of Japanese Encephalitis (JE) virus isolated from a horse
2011	Establishment of a herd of small grey and large white indigenous donkeys
2012	Organisation of SAARC trainings on equine piroplasmiasis under OIE twinning program
2012	Development of r-protein based ELISA for Equine Infectious Anaemia (EIA)
2012	Technique for Vermicomposting using equine dung optimized
2012	Quinapyramine sulfate nanoformulation developed against <i>Trypanosoma evansi</i>
2013	Establishment of ATIC and infoequine museum
2014	Development of r-protein based ELISA for diagnosis of <i>Burkholderia mallei</i>
2014	Development of r-HSP70 based ELISA for <i>Trypanosoma evansi</i> infection
2015	NRCE conferred Sardar Patel Outstanding ICAR institution award
2015	Release of 'Equiherpabort vaccine' for prevention of EHV1 abortions in mares
2015	Release of r-protein based <i>Theileria equi</i> antibody detection kit
2015	Whole genome sequencing of classical swine fever virus
2016	Organisation of SAARC trainings on equine influenza and glanders under OIE twinning program
2016	Methodology for isolation of RNA virus from mixed infection developed

Year	Salient Achievements
2017	Establishment of a herd of Kathiawari horses
2018	Ecotourism started at Equine Production Campus, Bikaner
2018	Release of ELISA kits for EHV1/4 and LFA for equine piroplasmosis
2020	Japanese Encephalitis (JE) virus antibody test kit was released
2021	Technology commercialization and transfer on semen collection and cryopreservation in Equines; commercialization of prototype of AV for semen collection from Stallions
2022	Development of Recombinant antigens based indirect ELISA kit for detection of anti-Trypanosoma evansi antibodies in animals
2022	Development of LSD vaccine (Lumpi-ProVac ^{Ind}) to prevent Lumpy skin disease (LSD) in animals
2022	Development of SARS-CoV2 vaccine (Ancovax) for animals
2022	Development of recombinant nucleoprotein based indirect ELISA for SARS-CoV-2 antibody detection in canines
2022	Developed isothermal "Recombinase Polymerase Amplification" (RPA) based assays for detection of Porcine circovirus 3 (PCV3) in pigs
2023	Development of point-of-care diagnostic for detection of SARS-CoV-2 nucleic acids employing RPA-CRISPR-LFA assay
2023	Developed of a novel HRM-based gap-qRT-PCR for identification and quantitation of the vaccine and field strain(s) of lumpy skin disease virus
2023	Development of RPA-LFA assay for detection of EHV1 & EHV4 viruses
2023	Production of Marwari breed horse foals through embryo transfer technology for the first time in the country
2023	Development methods for cryovial preservation of stallion semen and thawing protocols
2023	Development of customised novel cryodevices for vitrification of horse embryos
2023	Development of non-egg yolk based alternative semen extender for stallion semen
2023	Development of protocols for embryo transfer in indigenous horses
2024	Development of a standardized protocol for vermicomposting using equine dung
2024	Development of Salmoquell, a bacteriophage-based therapy targeting Salmonella infections
2024	Development of Lysibact, an antimicrobial nanoemulsion effective against a broad range of Gram-negative and Gram-positive bacterial infections.
2024	Development of the first indigenous high-density SNP chip for Indian horses (Axiom_Ashwa),
2024	Development of a lateral flow assay (LFA) kit for isothermal detection of Porcine Circovirus 3 (PCV3) nucleic acids.
2024	Generation of HDAC6/Cav1 double knockout BHK21 cell line to enhance scalability and yield in Foot-and-Mouth Disease (FMD) vaccine production
2024	Recognition of the Bhimthadi horse as the 8 th officially gazette-notified indigenous equine breed of India
2024	Raj-Sheetal: India's first horse foal produced through the transfer of vitrified/cryopreserved embryo

Summary of Expenditures under Unified budget and NCVTC including SCSP, NEH & TSP

Head-wise Details	2023-2024 (Rs in Lakhs)	2024-2025 (Rs in Lakhs)
Other charges including equipment's and recurring charges	1070.22	1322.89
Establishment charges including LSP/PF, wages, OTA	1261.04	1274.45
Travelling allowances and HRD	22.78	24.87
Works	57.12	119.75
Equipment	140.87	0.00
Loan and Advances	7.50	0.00
Disaster Emergency fund - General	69.56	0.00
Disaster Emergency fund - Capital	0.00	0.00
Non-Scheme	25.00	0.00
Total	2654.09	2741.96

Summary of Revenue Receipts

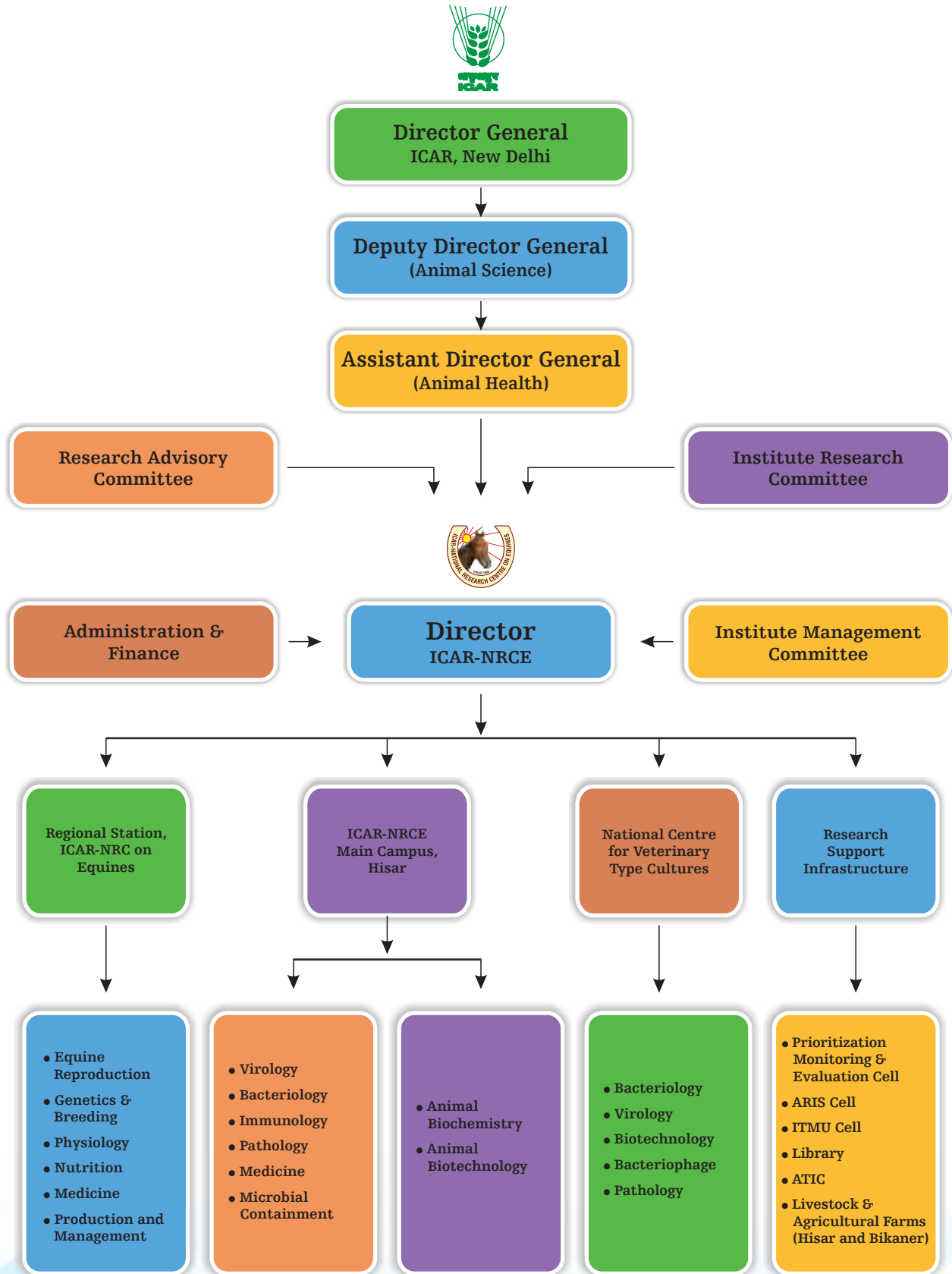
Head-wise Details	2023-2024 (Rs in Lakhs)	2024-2025 (Rs in Lakhs)
Leave Salary & Pension Contribution	0.00	0.00
Sale of Farm Produce	19.12	32.44
Sale of Livestock.	0.21	1.66
Eco Tourism	4.06	5.14
Rents (Charges & License Fee)	2.89	3.47
Contractual Diagnostic Services	66.68	105.00
Sale of cultures	0.78	70.63
Sale of Vaccine	67.48	
Candidates Tuition Fees, Diploma Charges/Training Fee etc.	2.33	0.00
Sale of machine tool/etc.	1.10	0.00
Interest on Short Term Deposit	4.84	6.39
Interest on short term deposit under DBT/DST Project/ Award Money	2.19	0.00
Recovery Loans & Advances	6.70	0.00
Interest on Loans & Advances	2.71	2.59
Other Miscellaneous Receipts	15.69	10.14
Total	196.7	237.46

STAFF POSITION AT ICAR-NRCE & NCVTC AS ON 31.12.2024

Post Cat.	Sanctioned	Filled	Vacant
Director	01	01	-
Scientific			
NRCE, Hisar	13	8	5
NCVTC, Hisar	10	5	5
EPC, Bikaner	10	4	6
Sub-Total	33	17	16
Technical	25	18	7
Administration	19	11	8
Skilled Support Staff	20	9	11
Sub-Total	64	38	26
Grand Total	98	58	40



Organizational SET-UP





Research Achievements

EQUINE HEALTH

Surveillance of emerging and existing disease of equines in India

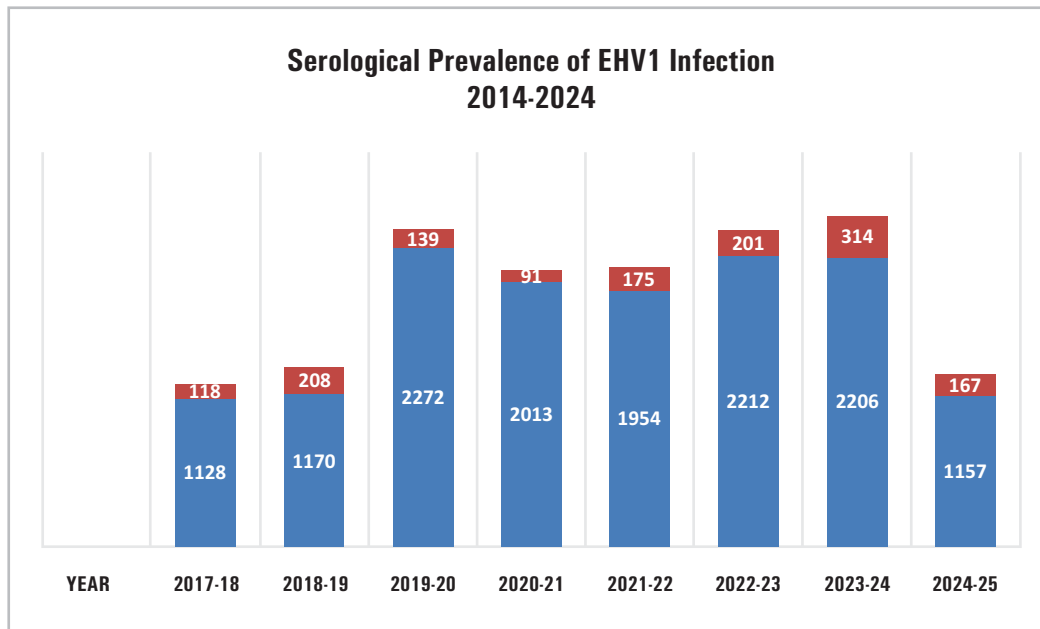
Comprehensive surveillance, diagnostic and consultancy activities were undertaken in the year 2024 to safeguard equine health across India. Emphasis was placed on viral, bacterial and protozoal pathogens of national and international importance, alongside antimicrobial resistance profiling and post-mortem investigations. The disease surveillance seamlessly integrated serological, microbiological and pathological approaches and data were generated through surveillance and disease investigation. Alongside, consultancy services were also provided to the stakeholders. A total of 1125 equine serum samples were collected from eleven states such as Haryana, Jammu & Kashmir, Uttar Pradesh, Uttarakhand, Rajasthan, Andhra Pradesh, Chhattisgarh, Himachal Pradesh, Tamil Nadu, Madhya Pradesh and Telangana and screened for antibodies against a panel of viral and bacterial pathogens, including equine infectious anemia (EIA), equine herpesvirus-1/4 (EHV-1/4), equine influenza (EI), equine viral arteritis (EVA), Japanese encephalitis/West Nile virus (JE/WNV), African horse sickness (AHS), rotavirus, glanders, contagious equine metritis (CEM), *Rhodococcus equi*, *Streptococcus abortus equi*, brucellosis, trypanosomosis and equine piroplasmosis. In parallel, nasal swabs and other clinical specimens underwent bacteriological isolation and antimicrobial-sensitivity testing.

The serological survey revealed active circulation of multiple pathogens: equine glanders, EHV-1, equine piroplasmosis, equine influenza, JE/WNV and trypanosomosis. Besides, EIA, EVA, AHS and CEM, *R. equi*, *S. Abortus equi* and brucellosis was also monitored and all tested samples were found to be negative for these pathogens. Real-time multiplex PCR assays complemented serology by detecting active infection in nasal swabs, further strengthening diagnostic accuracy.

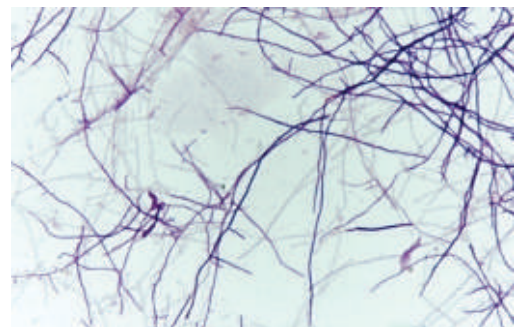
Details of testing under surveillance and monitoring and disease investigation for various diseases in 2024

Disease	Surveillance & monitoring		Disease investigation	
	Tested	Positive	Tested	Positive
Glanders	780	00	15942	34
Equine infectious anaemia	1196	00	34	0
Equine Influenza	1196	19	2420	30
Equine Herpesvirus 1	1152	153	175	14
Equine Viral Arteritis	74	00	01	00
Trypanosomosis	1547	47	106	01
Equine Piroplasmosis	1546	593	98	09
Japanese Encephalitis Virus	354	02	145	00
Brucellosis	1101	00	26	00
Salmonella Abortus equi	1101	00	21	00

Pathological investigations included seven post-mortem or biopsy examinations. Gross and microscopic analyses identified bronchopneumonia (n=2), enterocolitis (n = 1), hepatitis (n = 2), acute enteritis (n = 1) and non-suppurative encephalitis (n = 1), highlighting the spectrum of clinical presentations in affected equines.

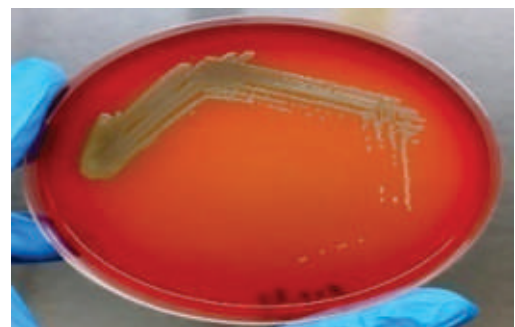


A total of 99 nasal swab samples of equine origin were received in the bacteriological laboratory for bacterial diagnosis and a total of 147 bacterial isolates were identified. Important pathogenic and commensal bacteria isolated and identified included *Streptococcus equi*ssp. *zooepidemicus*, *Streptococcus dysgalactiae* ssp. *Equisimilis* (n = 2 strains), *Staphylococcus aureus*, *Nocardia asteroides* (n = 3 strains), *Stenotrophomonas maltophilia* (n = 14 strains), *Rhodococcusequi*, *Aeromonas hydrophila*, *Sphingobacterium multivorum*, *Chryseobacterium indologenes*, *Brevundimona vesicularis*, *Enterobacter cloacae*, *Moraxella morgani*, *Candida albicans*, *Achromobacter denitrificans*, *Pasteurella* spp., *Acinetobacter*, *Ochrobactrum anthropi*, *Serratia marcescens*, *Ralstoniapicket tii*, *Corynebacterium*, *Pantoea* spp, etc. High incidence of *Stenotrophomonas maltophilia* needs to be investigated as it has been considered an important pathogen of horses in upper airway disease.



Nocardia asteroides isolated from nasal swab from animal with respiratory infection

Focused surveillance efforts screened 18, 766 equines for glanders, confirming 34 positive cases (0.18 %) and facilitated to implement prompt field containment measures.



Stenotrophomonas maltophilia isolated from nasal swab of horse.

Revenue generated through testing for various diseases in 2024

Under contractual diagnostic services, the centre tested 10866 samples for glanders (4879), EIA (n=3738), CEM (n=1044), AHS (n=294), EVA (n=317), dourine (n=355), trypanosomosis (30), equine piroplasmosis (n=75) and equine influenza (n = 25) and generated revenue of RS 97,31,050/-.

Sr. No.	Disease	Total Sample Tasted	Revenue
1.	Glanders	4879	41,47,150
2.	Equine Infectious Anemia	3738	24,29,700
3.	Equine Influenza	25	55,000
4.	EHV-1	34	37,400
5.	Trypanosoma	30	33,000
6.	Equine Piroplasmiasis (Theileriaequi)	75	82,500
7.	Japanese Encephallitis	4	8,800
8.	Sal. abortus equ.	2	1,300
9.	Brucellosis	1	650
10.	Dourine	355	5,32,500
11.	Equine Viral Arteritis	317	6,97,400
12.	CEM	1044	12,52,800
13.	Babesia caballi	50	55,000
14.	Surra	13	8,450
15.	African Horse Sickness	294	3,82,200
16.	Pregnancy	2	600
17.	West Nile Fever	3	6,600
	Total	10866	97,31,050

Overall, the 2024 surveillance program demonstrated robust methodological integration and stakeholder engagement, yielding actionable epidemiological data on equine pathogens and has provided a detailed snapshot of disease prevalence and supported evidence-based recommendations for controlling both endemic and emerging infections in India's equine populations.

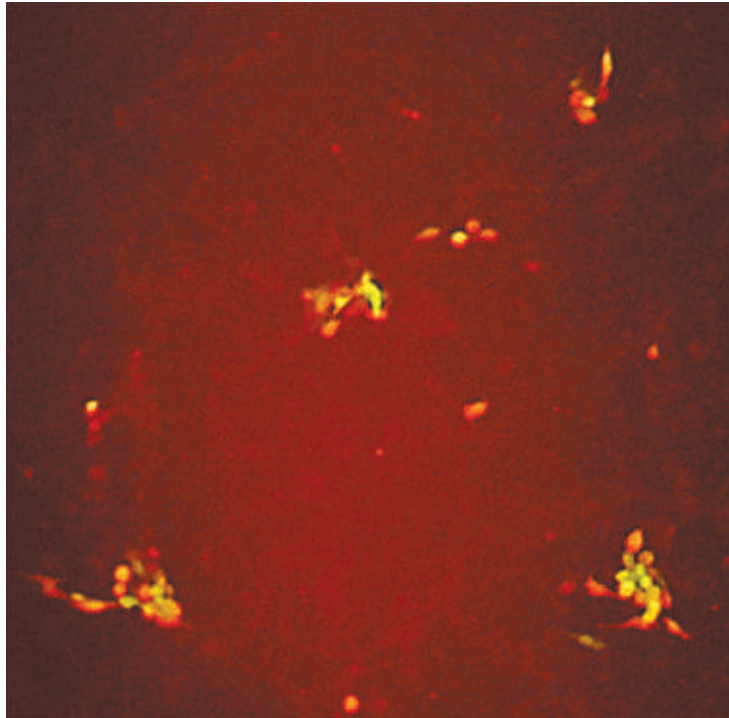
(Nitin Virmani, H. Singha, R. Kumar, S. Kumar, S. Barua, R.K. Vaid, R. Dedar, A. Manuja, Balvinder Kumar, K. Shanmugasundaram, Ana Raj and T. K. Bhattacharya)

Development of combined vaccine candidates for equine influenza and EHV1

Constructs were developed with attenuated EHV1 virus having HA gene of Clade 1 and Clade 2 equine influenza viruses. Viral growth kinetics and plaque size estimation of both recombinant EHV1 viruses were performed to determine the growth patterns and the time course of replication of mutant strains as compared to the parent virus. Indirect immunofluorescence assay test (IFAT) confirmed protein expression of the inserted gene, indicating its suitability as vaccine candidate. High OD values observed with positive serum in the ELISA indicate strong binding between antibodies in the hyperimmune serum and the EHV1 Δ IR6/gE-HA(FC2) viruses. This robust interaction confirms that the recombinant virus effectively presents relevant antigenic epitopes recognized by the immune system. After confirmation bulk propagation of recombinant viruses was done to purify the viruses.

Table: Percentage Plaque size reduction Recombinant EHV1 with Clade 2 EIV

Virus	EHV-1 wild virus	Attenuated EHV-1	Recombinant EHV1 with Clade 2 EIV
Mean \pm SD	74535 \pm 3900.08	48025 \pm 3700.01	19296.33 \pm 2639.06
Percentage	100	64.43	25.88
Reduction	0	35.57	74.12

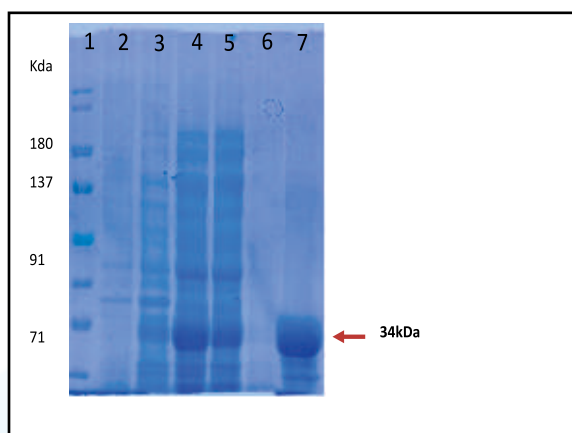


Protein expression studies of EHV1 with –Clade 2 EIV at 36hpi. PK-15 cells were infected with recombinant virus and Equine Influenza serum was used as primary antibody and secondary conjugate goat anti-horse TRITC was used. Exposure of fluorescent used GFP at 400ms and TRITC at 30ms. Magnified at 200X

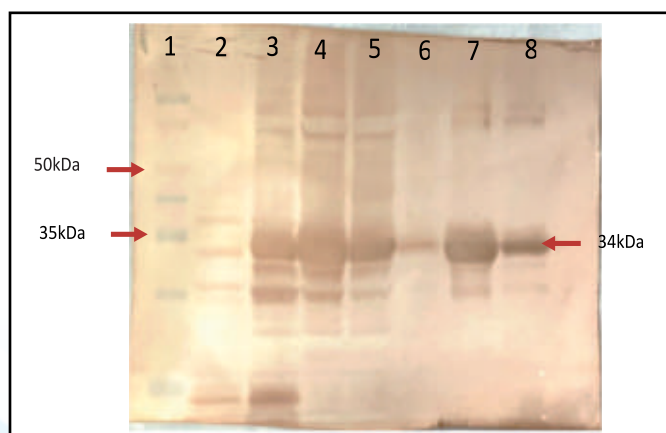
(Nitin Virmani, B.C.Bera and Taruna Anand)

Development of recombinant protein based indirect ELISA for studying seroprevalence of EHV2

The development and validation of a serological assay for Equine Herpesvirus-2 (EHV-2) began with the design, synthesis and optimization of the viral glycoprotein B (gB) gene. This gene was cloned into the pGEX4T1 vector and transformed into *E. coli* Rosetta cells, expressed and GST-tagged gB protein was purified by glutathione–Sepharose affinity chromatography purified. SDS-PAGE analysis revealed a dominant band at ~ 34.5 kDa, and Western blotting assay using a gB-specific primary antibody (1:2,000) and HRP-conjugated goat anti-mouse secondary (1:4 000) confirmed the identity and purity of the recombinant antigen.



SDS-PAGE analysis expressed recombinant protein glycoprotein B (gB)



Western blotting analysis of recombinant gB-protein.

An indirect ELISA was optimized using the purified gB protein. Serial coating dilutions (1:2,000 to 1:64,000) and an anti-horse-IgG-HRP secondary dilution (1:7,000) were evaluated to maximize assay sensitivity and specificity. Pre-colostrum foal serum served as a negative control ($OD_{450} \leq 0.03$). When testing three PCR-confirmed EHV-2-positive equine sera, one sample yielded a clear positive signal ($OD_{450} \geq 0.40$), establishing preliminary assay cut-offs. To assess field applicability, 288 equine sera were screened at an optimal antigen coating of 1:40,000 and 57 sera samples were test positive, corresponding to a seroprevalence of 19.8%. This demonstrates robust immunogenicity of the recombinant gB and the assay's potential for large-scale surveillance. Complementing serology, a real-time multiplex qPCR targeting the EHV-2 gB gene was applied to 33 nasal swabs. Five samples (15.2%) tested positive, delineating active viral circulation. Altogether, these results validate both the indirect ELISA and qPCR approaches for sensitive and specific detection of EHV-2 infection in equine populations.

(Nitin Virmani, B.C. Bera and Taruna Anand)

Japanese encephalitis viruses circulating in equines, swines and other species

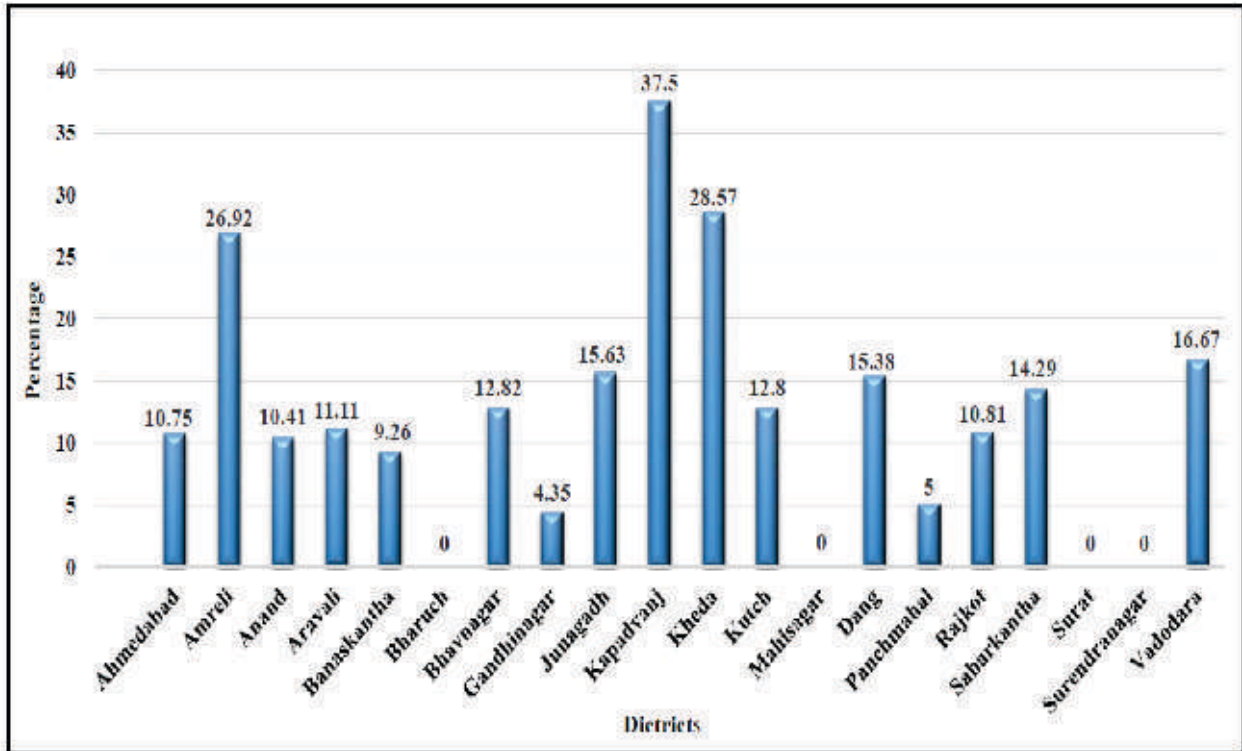
Japanese encephalitis (JE) is a mosquito-borne flavivirus that poses a significant public health threat across much of Asia. While clinical disease in horses is uncommon, equines serve as valuable sentinels for local viral activity, reflecting underlying transmission dynamics that may also affect humans. A study was performed under All Indian network project on One Health employing the hemagglutination-inhibition (HI) assay to assess the seroprevalence of JE virus (JEV) antibodies in 600 horses from districts across Gujarat. Overall, 75 of the 600 sera (12.5 %) tested positive, indicating widespread but variable exposure of the equine population to JEV.

District-level analysis revealed marked heterogeneity in seropositivity. The highest rates were observed in Kheda (30.6 %) and Amreli (26.9 %), suggesting focal hotspots of transmission. Several other districts—including Vadodara, Junagadh, Dang, Sabarkantha, Bhavnagar, and Kutch—showed moderate seroprevalence (ranging from approximately 12 % to 17 %), while a cluster of districts such as Aravali, Rajkot, Ahmedabad, Anand, and Banaskantha registered lower but still notable rates (around 9 % to 11 %). In contrast, Gandhinagar and Panchmahal exhibited minimal seropositivity (4 %–5 %). These geographic differences likely reflect variation in ecological factors conducive to mosquito breeding as well as in equine management and husbandry practices.

To contextualize equine findings within the broader animal enzootic cycle, we also screened serum samples from other domestic species: pigs ($n = 28$), donkeys ($n = 87$), and goats ($n = 65$). Seroprevalence among pigs was highest at 17.9 %, whereas both donkeys and goats displayed similar rates of approximately 9.2 %. The elevated pig seropositivity aligns with their role as amplifying hosts, while the lower—but still detectable—rates in donkeys and goats further underscore the multi-species circulation of JEV in the region.

Complementing serological surveillance, a total of 40 nasal swabs and blood samples collected from equines in Tripura were examined by a TaqMan real-time RT-PCR assay targeting the NS gene of JEV. One specimen tested positive by RT-PCR, providing molecular confirmation of active viral presence in that area and reinforcing the need for continued vigilance.

Taken together, these findings highlight ongoing JEV transmission in Gujarat, with distinct district-level hotspots and involvement of multiple host species. Regular sentinel monitoring in horses, targeted vector-control interventions in high-risk districts, and the integration of both serological and molecular diagnostic approaches are recommended to inform public health strategies and mitigate the risk of JE outbreaks.



Seroprevalence of Japanese Encephalitis in horses in various districts

(Nitin Virmani, B. C. Bera and Taruna Anand)

Glanders Surveillance Report: 2024

Progress, Shortfall and Recommendations

Glanders is a disease caused by bacteria called *Burkholderia mallei*. It primarily affects equid species like horses, donkeys, and mules. Humans can also get infected if they have direct or long-term contact with sick animals or the bacteria. In 2006, Glanders reappeared in seven states in India. Since then, it has continued to increase and spread to new areas. For control and prevention of glanders, a National Action Plan on Glanders was approved in 2019 by the Department of Animal Husbandry & Dairying, Govt of India.

Annual Glanders Surveillance Report (Jan 2024 to Dec 2024)

ICAR-NRCE is leading the Glanders monitoring program along with help from the State Animal Husbandry Departments. A two-step testing system—ELISA and CFT—was adopted for serological diagnosis of the disease. In addition, molecular methods (PCR, and qPCR) and culture isolation was conducted on biological samples collected from a glanders outbreaks. ICAR-NRCE has imparted training to lab staff on serological and molecular diagnosis of Glanders. This training helped to create a network of labs that can quickly and effectively carry out surveillance. Currently, state labs are using a commercially available glanders ELISA kit made by Genomix Diagnostic Pvt. Ltd. According to existing policy, the network lab first does an initial screening by ELISA test, and if the result is found positive, the sample is sent to NRCE for confirmation using CFT and molecular methods. Glanders positive case is declared on the basis of positive laboratory test and presence of clinical signs of the animals and previous epidemiological linkages.

In 2024, a total of 38,515 equine sera from 313 districts of 20 States/UTs were collected and tested for glanders. Out of these, 21774 equine samples were screened by commercial Hcp1-ELISA at 13 State Lab/RDDLs (Gujarat, Madhya Pradesh, Punjab, Maharashtra, Karnataka, Uttarakhand, Tamil Nadu, Chhattisgarh, West Bengal, Bihar, Himachal Pradesh, Jammu and Kashmir). Among these, Uttarakhand, Maharashtra/WRDDL and Kashmir have significantly

contributed by testing 7966, 2534 and 2456 equine samples, respectively.

In this year, 34 glanders positive cases were reported in 10 states. Glanders affected states include Uttarakhand (n=9), Haryana (n=5), Himachal Pradesh (n=5), Delhi (n=3), Gujarat (n=3), Uttar Pradesh (n=3), West Bengal (n=2), Karnataka (n=2), Rajasthan (n=1) and Chhattisgarh (n=1). Two cases were reported from West Bengal indicating spread of glanders to new geographical areas. State wise glanders surveillance data is shown below Table.

Surveillance of in-contact humans

In zoonotic point of view, 190 sera from occupationally exposed humans (Veterinary Officers, equine handlers, and laboratory workers) were tested and none of them were found positive.

Glanders surveillance data (Jan 2024 - Dec 2024)

Sr No.	State	No. of samples tested at NRCE	No. of samples tested at State Lab/RDDLs	No. of districts surveyed	Positive cases
1	Uttar Pradesh	13667	-	74	3
2	Haryana	739	-	10	5
3	Madhya Pradesh	16	309	14	-
4	Himachal Pradesh	572	127	7	5
5	Punjab	27	1384	25	-
6	Uttarakhand	38	7966	6	9
7	Gujarat	13	802	24	3
8	Maharashtra	-	2534	26	-
9	Chhattisgarh	76	335	17	1
10	Tamil Nadu	-	1803	32	-
11	Delhi	102	-	1	3
12	Rajasthan	133	-	8	1
13	Jammu	641	2003	8	-
14	Kashmir	-	2456	2	-
15	Andhra Pradesh	-	-	2	-
16	Karnataka	566	1754	20	2
17	Kerala	-	-	13	-
18	West Bengal	5	213	9	2
19	Bihar	125	88	14	-
20	Jharkhand	2	-	1	-
	Total	16722	21774	313	34
	Grand Total	38496		313	34

Glanders affected districts in India (Jan-Dec 2024)

Sr No.	State	Positive Case	Distt. / Place
1	Uttarakhand	9	Dehradun, Chamoli, Pauri
2	Haryana	5	Kaithal, Hisar, Rohtak, Jhajjar
3	Himachal Pradesh	5	Bilaspur, Mandi
4	Delhi	3	Azadpur
5	Gujarat	3	Ahmedabad, Kheda
6	Uttar Pradesh	3	Gautam Buddha Nagar, Mau,
7	West Bengal	2	Uttar Dinajpur, North 24 Parganas
8	Karnataka	2	Bangalore
9	Rajasthan	1	Churu
10	Chhattisgarh	1	Dhamtari
	Total	34	18-Districts of 10 States

Training of Field Veterinary Officer's

Mass awareness of veterinary officers and equine stake holders on glanders disease is one of the instrumental factors for effective implementation of physical, clinical and serological surveillance, control and containment of glanders outbreak. In 2024, five days training programme were organized at NRCE, Hisar on "Zoonotic Diseases Diagnosis Under One Health Approaches and Equine Husbandry Practices" from 18-22 March, 2024. A total of 20 Veterinary Officers from Tamil Nadu state participated in the training programs.

Shortfall and Recommendations

- **State participation:** Taking account of past six-year surveillance data, sample size has been significantly increased in this year. However, it was observed that only 12 states regularly participate in the glanders surveillance. On the other hand, irregular surveillance was observed in Southern and North-Eastern India. Therefore, pro-active participation of all State Animal Husbandry Department in the glanders surveillance programme is necessary to assess state wise sero-prevalence and to devise future strategies for control and eradication of glanders in India.
- **Compensation:** To support immediate economic losses and sustenance of poor equine keeper's profession, immediate release of compensation to beneficiaries is utmost importance. The action plan recommended revision of compensation every three years. The present rate has been continued since last six years. Therefore, monetary compensation may be revised to a reasonable amount, which will encourage equine keeper to test their equines for glanders and make them aware of the disease.
- **Funding provision:** Sufficient fund may be provided to State Animal Husbandry Department to carry out surveillance and glanders testing at net-work laboratory. To coordinate the national glanders surveillance funds should also to be provided to NRCE, Hisar.
- **Monitoring and evaluation of surveillance & response systems:** The surveillance plan include a detailed monitoring & evaluation plan so that recommendations resulting from monitoring and evaluation should be acted upon in a timely and appropriate way. There is an urgent need to form a central monitoring unit for overall monitoring and evaluation of the surveillance activities as mentioned in action plan.

- **Revision of National Action Plan:** National Action Plan on Glanders may be revised to include some changes for example restricted area after glanders outbreak may be reduced to 5 km radius and increasing surveillance coverage (30%).

(Harisankar Singha, K. Shanmugasundaram and T. K. Bhattacharya)

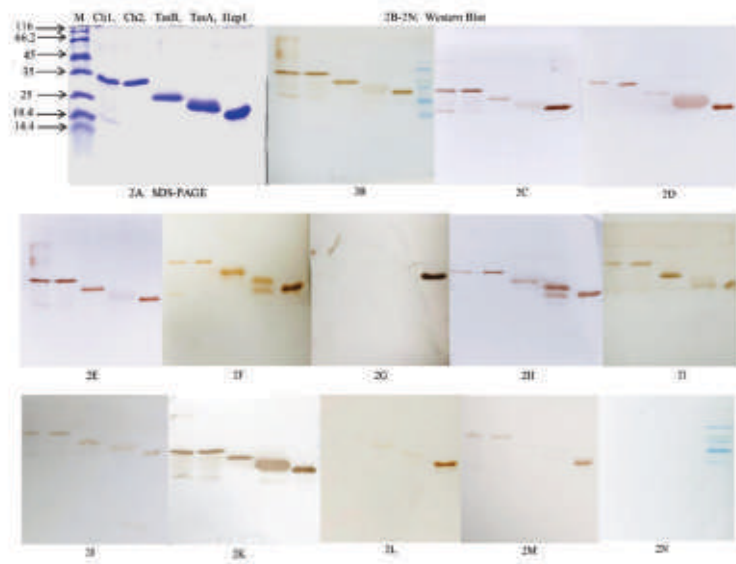
Immune responses and host-pathogen interaction analysis in *Burkholderia mallei* infected equines

Glanders is a fatal bacterial infection of equids caused by *Burkholderia mallei* (*B. mallei*), a Gram-negative, coccobacillus, non-motile and facultative intracellular bacterium. No vaccine and specific treatment regime are available against glanders. Recent progress in *B. mallei* research identified type 6 secretion system as a major virulence determinant required for pathogenesis. Some of the effector molecules of this secretion system have shown promising results as a potential vaccine at the pre-clinical stage.

In the last decade, research on host-pathogen interactions identified several virulence factors for the development of diagnostics and vaccines against *B. mallei* infection. Nevertheless, *B. mallei* infection in natural host was less extensively studied and restricted to experimental infection in mouse models and non-human primates.

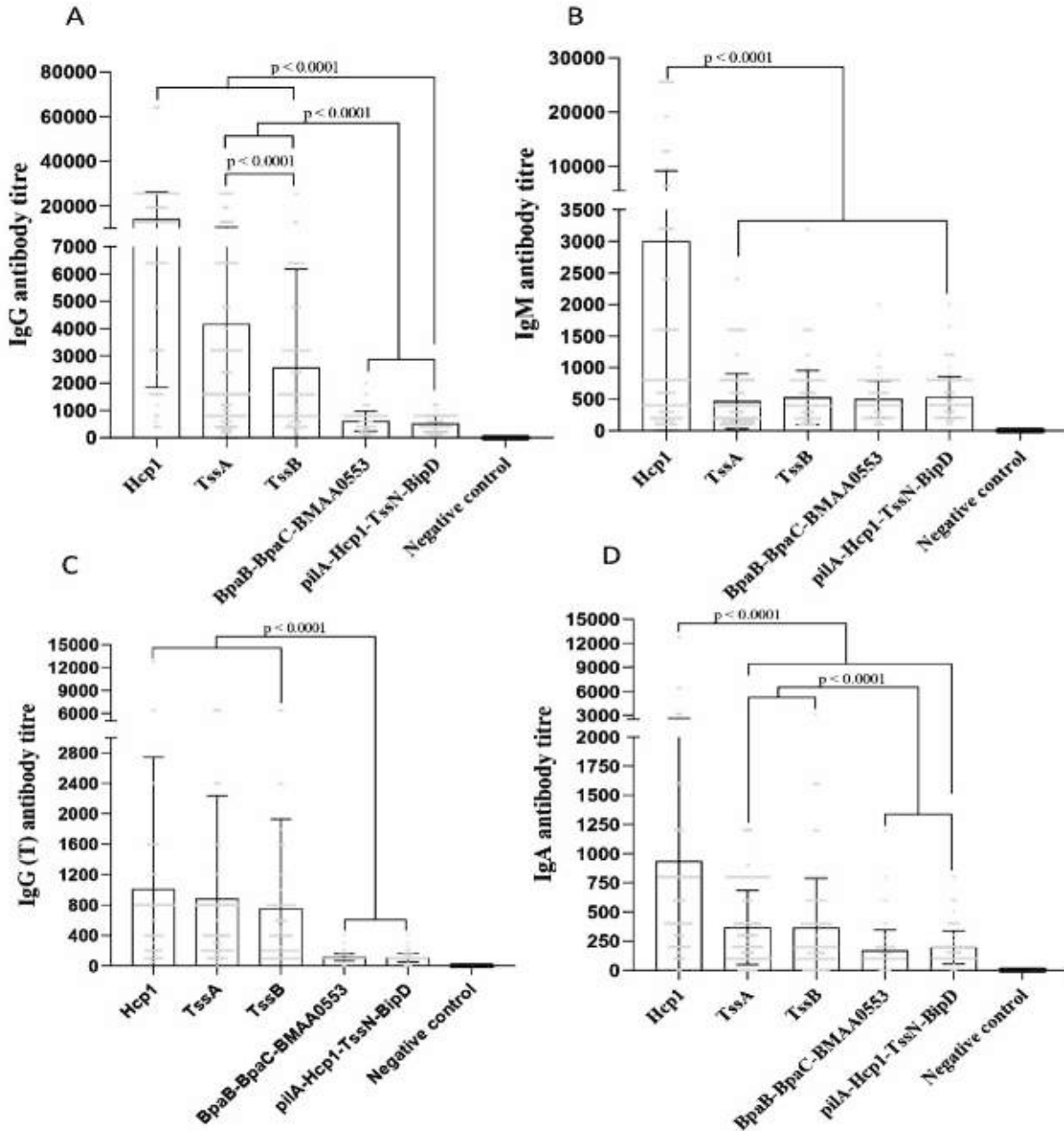
A recent study, conducted in our laboratory on natural cases of equine glanders (n = 24) showed presence of IgG, IgM and IgA antibodies against recombinant *B. mallei* Hcp1, TssA and TssB proteins. An elevated IFN- γ , IL-6, IL-12p35, TNF- α and IL-17 response were also observed in equine PBMCs. To gain further insights on immunology of *B. mallei* infection in equines, the present study was conducted on a large number of natural cases of equine glanders (n = 151). In addition to recombinant Hcp1, TssA and TssB proteins two chimeras PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553 were also included to determine antigen specific IgG, IgM, IgA and IgG (T) titre. Further, IL-1, IL-17, IL-6, TNF- α , MCP-1 and IFN- γ concentration were estimated in glanders positive equine serum samples.

Five recombinant proteins showed molecular weight of 21 kD, 25 kD, 26 kD and 32 kD for Hcp1, TssA, TssB, PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553, respectively. Purity of the recombinant proteins were visualized by running 12.5% SDS-PAGE and Coomassie brilliant blue staining. Specific reactivity of recombinant Hcp1, TssA, TssB, PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553 protein to *B. mallei* infected horse serum was determined by western blot. Among the five proteins, Hcp1 protein showed very quick and strong reactivity against glanders positive serums. These proteins were explored for IgG isotyping.



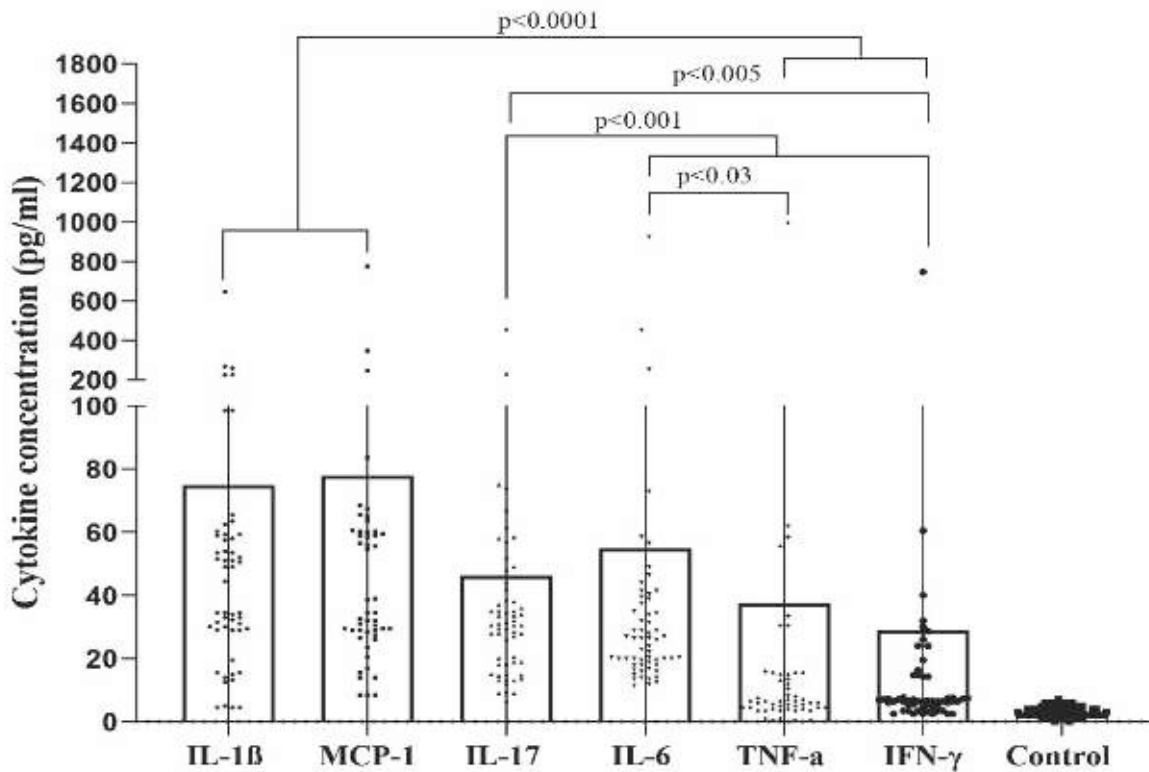
SDS-PAGE and wester blotting using recombinant *B. mallei* proteins. Lane 1 = PilA-Hcp1-TssN-BipD, Lane 2 = BpaB-BpaC-BMAA0553, Lane 3 = TssB, Lane 4 = TssA, Lane 5 = Hcp1

Immunoglobulin (Ig) responses (IgM, IgG, IgG (T) and IgA) to recombinant *B. mallei* proteins namely Hcp1, TssA, TssB, PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553 were assessed in 151 glanders positive equids by ELISA. The study showed that *B. mallei* infected equids generated strong IgG > IgM > IgG (T) and IgA antibody responses to recombinant Hcp1, TssB and TssA. Comparative analysis of immune response showed that Hcp1 was more potent immunogen in eliciting antibody response, while TssA, TssB were moderate responder PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553 showed minimal antibody responses.



Comparison of IgG (A), IgM (B), IgG(T) (C), and IgA (D) antibody titre in response to Hcp1, TssA, TssB, PilA-Hcp1-TssN-BipD and BpaB-BpaC-BMAA0553 proteins in 151 glanders positive serum samples.

Serum cytokine concentration of IL-1, IL-6, IL-17, MCP-1, TNF- α , and IFN- γ were measured in 60 glanders positive equines and 10 healthy equines. These 60 glanders positive serum were selectively chosen from the panel of 151 serum used for antibody isotyping. The study revealed that IL-1, IL-6, IL-17 and MCP-1 cytokines were significantly higher in glanders positive serum.



Estimation of IL-1, IL-17, IL-6, TNF- α , MCP-1 and IFN- γ in glanders positive serum samples (n = 60). Data are presented as means \pm SD, with the p-value indicating statistical significance

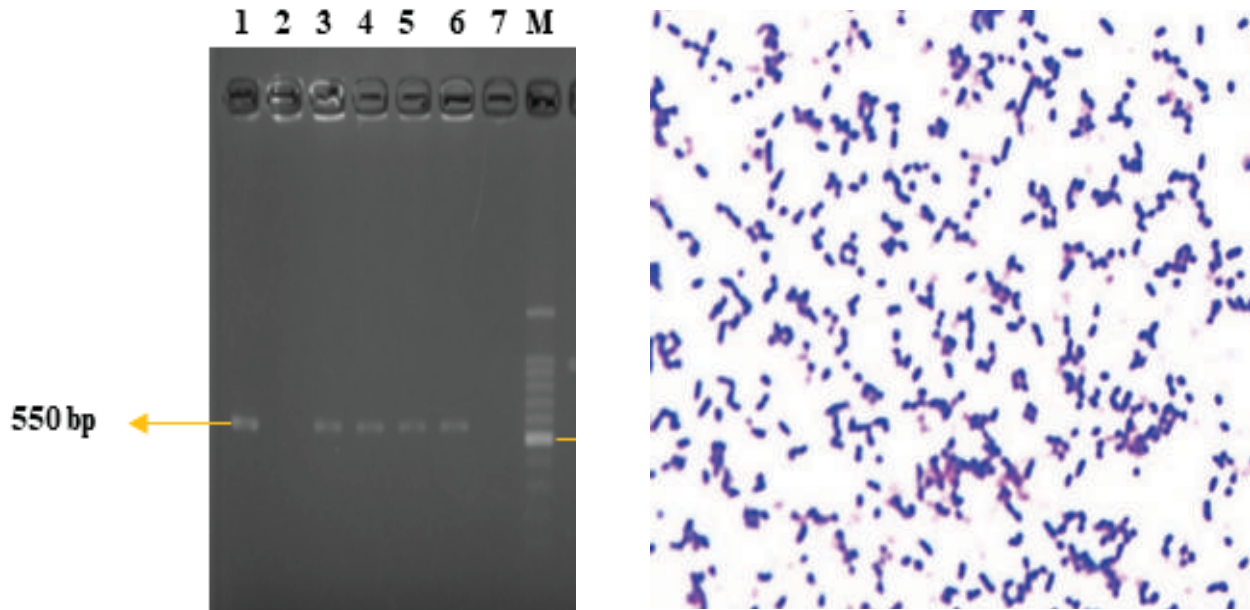
The present study revealed that *B. mallei* infection in equines induced a strong IgG responses followed by IgM, IgG (T) and IgA responses. Among the five proteins, Hcp1 was found to be most potent antibody inducers. Elevated levels of IL-1, MCP-1, IL-17, IL-6, IFN- γ and TNF- α were also observed in serum samples. Our findings were in accordance with previous observations in mice and other animal models of *B. mallei* infection. Further studies should be conducted to determine memory cell responses in natural cases of equine glanders using recombinant *B. mallei* proteins for identifying well-characterized immuno-protective vaccine candidates.

(Harisankar Singha, Pooja, Shanmugasundaram K, T. K. Bhattacharya)

Development and evaluation of immunotherapy and vaccine constructs against *Rhodococcus equi* infection to protect foals from pneumoniae

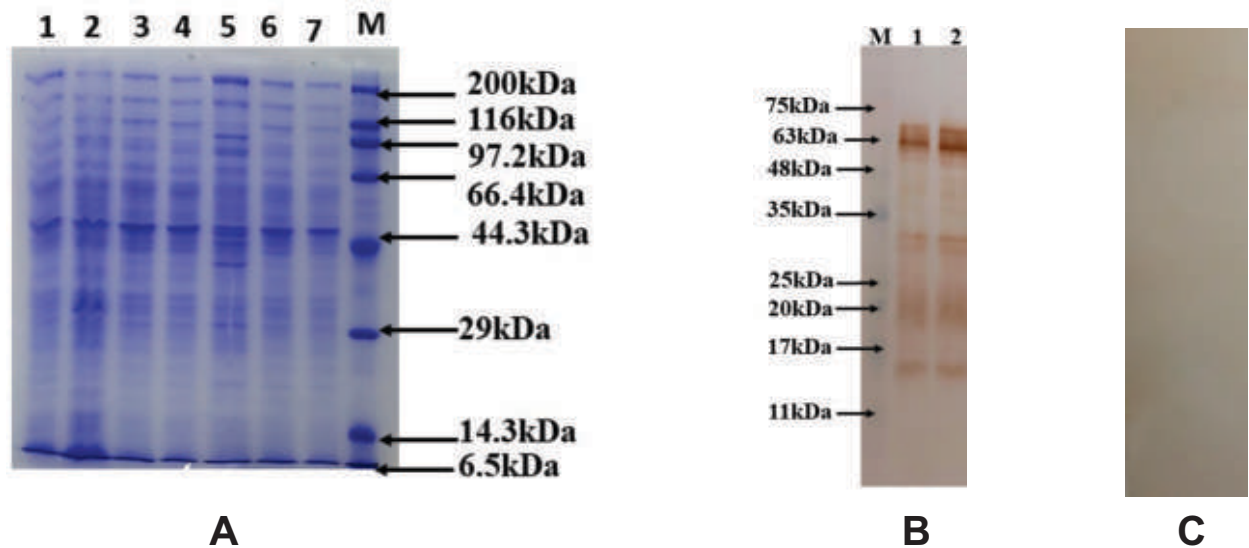
Rhodococcus pneumoniae, a severe and frequently fatal form of intracellular bacterial infection in foals, is caused by the Gram-positive *Rhodococcus equi*. This respiratory pathogen mainly affects foals of less than six months of age, resulting in suppurative bronchopneumonia and pyogranulomatous lesions in the lungs. Besides, invasion of *R. equi* in the colonic mucosa leads to severe diarrhoea in foals. Conventional vaccine strategies, like live-attenuated and killed vaccines were ineffective in providing reliable protection against *R. equi* infection. On the other hand, contemporary vaccine approaches, including DNA plasmid vaccines, genetically attenuated vaccines, subunit vaccines, and e-beam inactivated bacterial vaccines, have yielded mixed results in terms of safeguarding foals from the pathogen. Therefore, the project was undertaken to evaluate immunotherapy and vaccine constructs to protect foals from *R. equi* infection.

The isolation and identification of *R. equi* from clinical samples such as nasal swabs of infected foals were done on nutrient agar medium. Gram's staining confirmed the Gram-positive coccobacilli. Further confirmation was performed using PCR targeting the *VapA* gene.



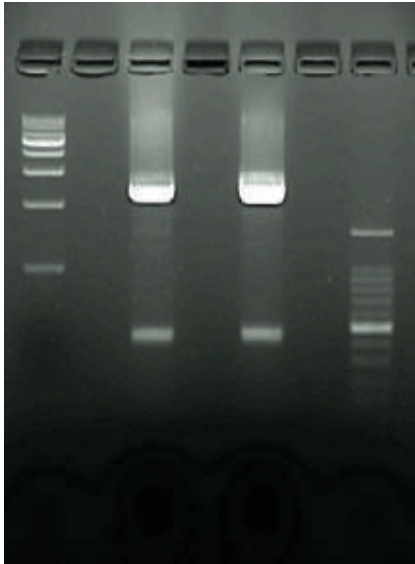
A **B**
 Agarose gel showing *VapA* (550 bp) positive *R. equi* (A). Gram positive coccobacilli (B)

The different protocols for preparation of whole cell lysate from *vapA* positive *R. equi* strain 5890 were standardized. SDS lysis buffer was found as the most effective method for the purpose as this protocol yielded the highest concentration of proteins. Dialysis was performed to remove salt from the protein solution, and protein concentration was estimated using the Bradford method.

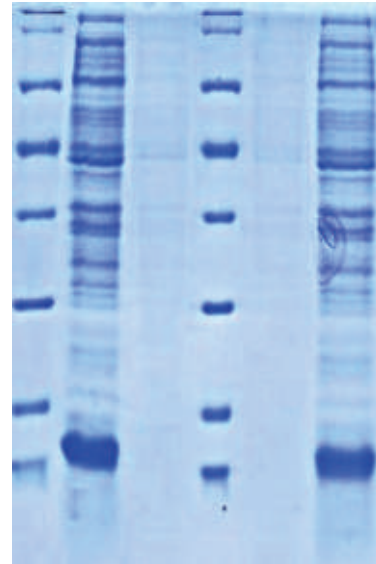


A **B** **C**
 SDS-PAGE analysis showing whole cell lysate protein of *R. equi* after dialysis (A) Western blot analyses of whole cell lysate protein of *R. equi* against *R. equi* positive serum (B) and negative serum (C).

Western blotting analysis was performed to validate the immunogenicity of the *R. equi* whole cell lysate using known positive and negative serum. *R. equi* cell lysate showed strong reactivity with positive control serum, confirming its immunogenic nature. Notably, no antigen-antibody reactions were observed against negative control serum.



A



B

Expression and purification of Horse and Mice Leptin

In conclusion, this study optimized the protocol for preparation of *R. equi* whole cell lysate and characterized its immunogenic proteins. Mice and horse leptin gene has been expressed and purified. The whole cell lysate, recombinant vapA and leptin proteins will be used for immunization study in mouse model and foals.

(Harisankar Singha, Sonia, Shanmugasundaram K and Ramesh Dedar)

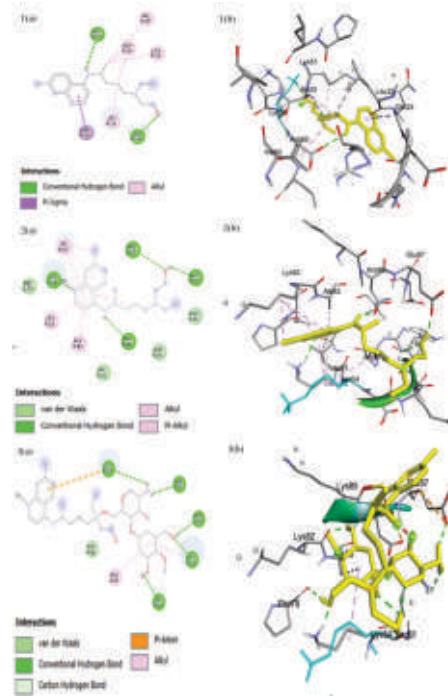
Chitosan-based nanocarriers for Zinc and Hydroxychloroquine delivery: synthesis, characterization, molecular docking against fibronectin protein, and antibacterial assessment against *Streptococcus equi*

Chitosan, a naturally derived polysaccharide from chitin, has garnered significant interest due to its dual function as a biocompatible drug delivery system and intrinsic antibacterial agent. In this study, chitosan was employed as a carrier for zinc (Zn) and hydroxychloroquine (HC), aiming to enhance therapeutic efficacy while minimizing potential cytotoxic effects. The biocompatibility, biodegradability, and muco-adhesive properties of chitosan make it a favorable platform for the controlled delivery of Zn and HC.

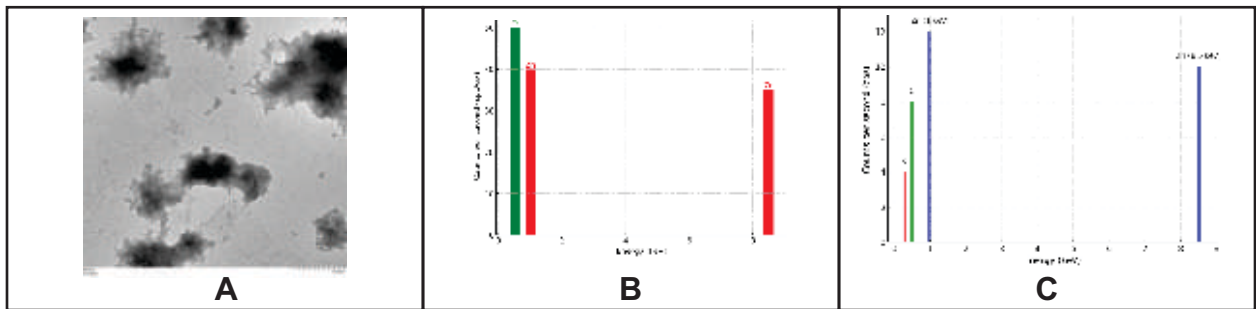
Fibronectin, an extracellular matrix glycoprotein abundantly present in various tissues, serves as a key adhesion target for microbial pathogens, including species of *Streptococcus*. Given the individually reported antibacterial properties of Zn, HC, and chitosan, we investigated their combined potential to inhibit fibronectin-binding proteins through molecular docking studies. The docking results indicated that Zn, HC, and chitosan exhibit inhibitory interactions with the fibronectin protein of *Streptococcus* species.

We successfully synthesized a novel chitosan-based nanoformulation incorporating zinc oxide and hydroxychloroquine. The formulation was extensively characterized using transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), elemental analysis, and dynamic light scattering (DLS) for particle size determination. Fluorescence microscopy with FluoZin-3 staining confirmed zinc deposition on the surface of the targeted bacterial cells.

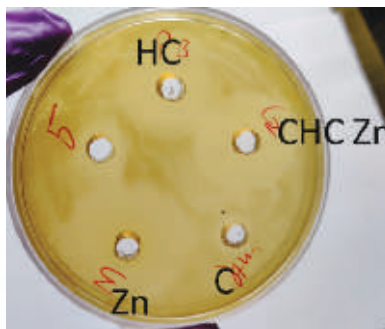
Antibacterial activity of the formulation was evaluated against *Streptococcus equi*, demonstrating significant inhibitory effects. This is the first study to report the antimicrobial potential of a chitosan-Zn-HC composite against *S. equi*, highlighting its promise as a safe, effective alternative to conventional antibiotics and paving the way for further development of targeted nano-therapeutics.



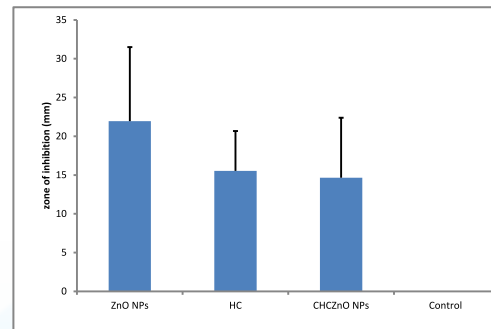
D interactions (a) and 2D diagram (b) of hydroxychloroquine(1); Zn-ligated hydroxychloroquine (2); Chitosan-Zn-ligated hydroxychloroquine (3); with Fibronectin protein



Morphology and elemental analysis of Chitosan hydroxychloroquine zinc oxide nanoparticles. a. Images observed under Transmission electron microscope; b. Elemental analysis. X-Axis (Energy in keV), represents the energy of the detected X-rays, ranging from 0 to 10 keV Y-Axis (Counts per second per eV) which represents the intensity of the detected X-rays, measured in counts per second per eV. Oxygen peak (O), caption for C is missing



A



B

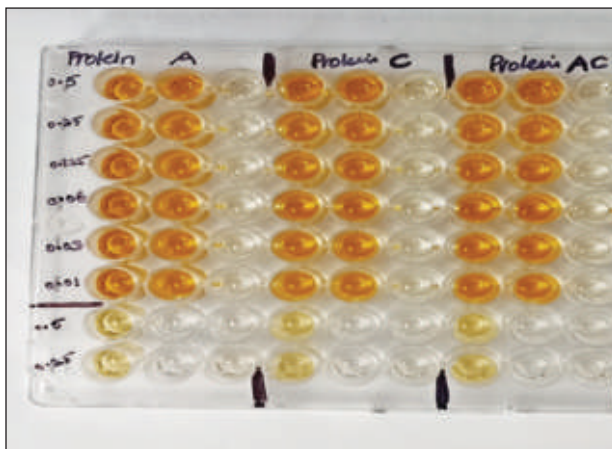
Anti-bacterial activity of ZnO, HC, Ch-ZnHC Complex against *Streptococci equi* azone of inhibition

(Anju Manuja, Balvinder Kumar & RK Vaid)

Development of recombinant protein-based immunoassay for detection of serum antibodies specific to *S. equi*.

Two genes coding for immunogenic proteins having molecular weight 22 KDa and 26 KDa respectively of *S. equi* have been amplified, purified and cloned in expression vector and recombinant proteins were expressed. The expressed proteins were purified by Ni-NTA sepharose affinity chromatography and quantified. Final protein products were checked on Coomassie stained SDS-PAGE and western blot analysis. The recombinant proteins expressed were used to develop immunoassay for detection of serum antibodies specific to *S. equi*.

Ninety-six well plates were coated with antigen A or C and kept overnight at 4°C. Plates were washed with phosphate buffered saline (PBS) and blocked with 3% Bovine serum albumin in PBS for 1hr at 37°C. Plates were washed with PBS containing 0.05% Tween 20 (PBST) and 100 µl of test or control serum diluted 1:200 in PBST was added to appropriate well for 1 hr at 37°C. Plates were washed with PBST and 100 µl of peroxidise conjugated goat anti-horse IgG diluted 1:2000 in blocking buffer was added to each well and incubated for 1 hr at 37°C. Plates were washed with PBST, 100 µl of OPD substrate was added and the plates were incubated for 10-15 min at 37°C, 100 µl of 1M sulphuric acid was added and optical density was measured at 450 nm. The percentage of sample OD at 450 nm relative to the positive control was calculated.



Recombinant antigen A, C & cocktail AC (at varying conc.) checkboard ELISA with positive and negative sera.



Cocktail of antigen A & C at 0.5µg/ml with varying sera and conjugate dilutions

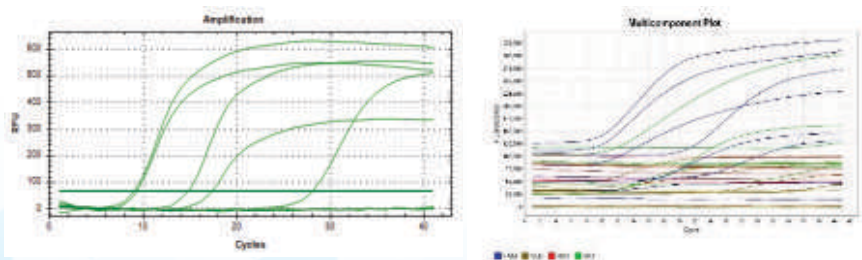
ELISA plates showing results of immunoassay using recombinant proteins as antigen

Antibody capture ELISA has been standardized using two recombinant proteins as coating antigens. The proteins have been used individually and as well as cocktail. The results are being further analyzed.

(Balvinder Kumar, RK Vaid, Anju Manuja, K Shanmugasundaram & Harisankar Singha)

Duplex qPCR for specific detection of *S. equi*

A duplex qPCR for detection *Streptococcus* sps and *S. equi* has been developed. The internal and external validation of the assay has been completed.



qPCR plots showing detection of *Streptococcus* sps and *S. equi*

Sensitivity and specificity of developed real-time PCR were calculated and are expressed as percentages: Sensitivity = $a/a+b$ where a stands for true positives; b stands for false negatives, Specificity = $d/c+d$ where d is negatives and c is false positives.

A total of 45 *Streptococcal* cultures demonstrated positive results for *Streptococcus* species (100%), which comprised 34 samples (75.5%) positive for *S. equi* with conventional PCR as well as with real time PCR. The method used taqman chemistry and VIC and FAM (reporter dyes) were used as the probes to amplify a fragment. FAM reporter dye detected the genus *Streptococcus*, while VIC indicated the species as *S. equi*. The duplex real-time PCR for 45 cultures for *Streptococcus* available in the lab demonstrated 100% sensitivity and 100% specificity compared to conventional PCR. The minimum detection limit for detection of *S. equi* was found as 0.35 picograms/ μ l of genomic DNA.

Ct values were interpreted as follows:

SAMPLE	FAM	VIC	Interpretation
Test sample	Ct valued"35	No amplification	<i>Streptococcus</i> genus.
Test sample	Ct valued"35	Ct valued"35	<i>S. equi</i>
Test sample	No amplification	No amplification	Negative for <i>Streptococcus</i> spp.

(Balvinder Kumar, RK Vaid, Anju Manuja, K Shanmugasundaram, Harisankar Singha)

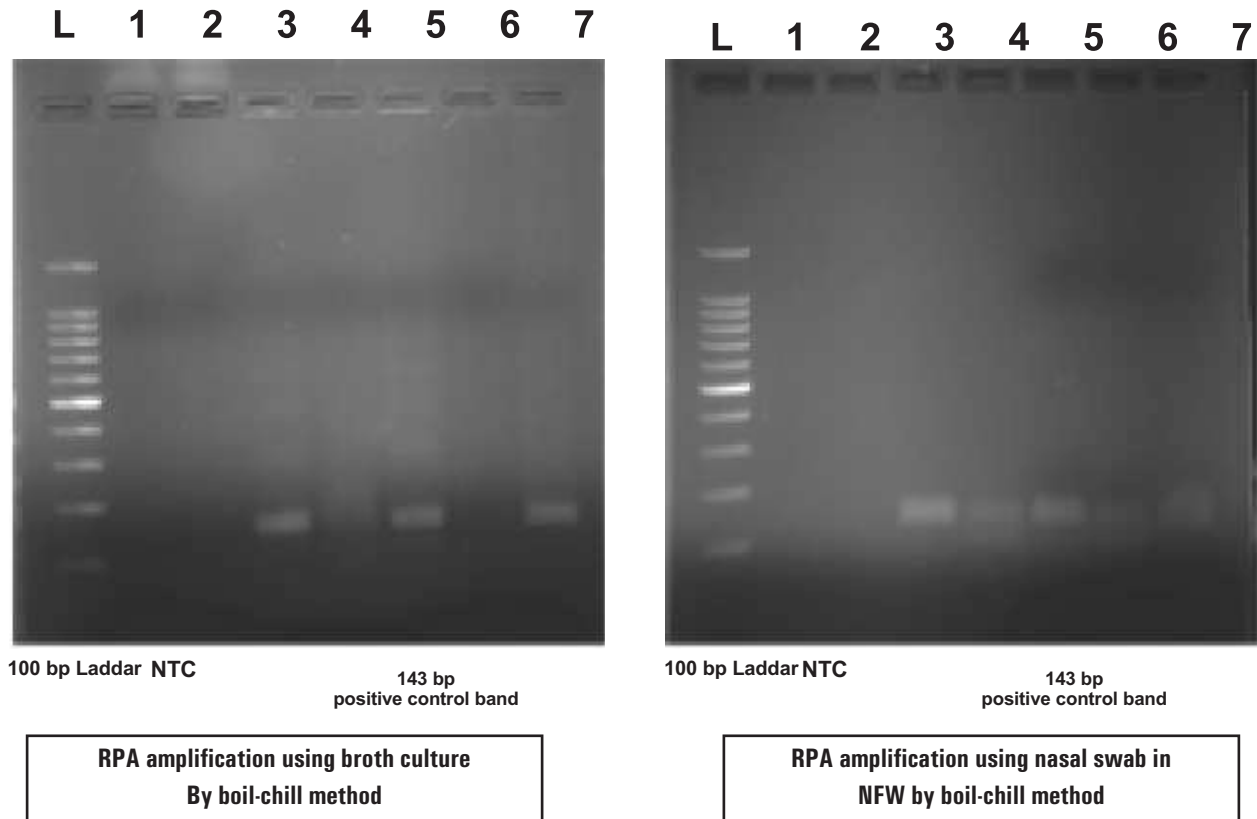
Recombinase Polymerase Amplification (RPA) assay-a pen side test for detection of *Streptococcus equi*

Highly sensitive and selective isothermal amplification technique, operating at 37–42°C, with minimal sample preparation and capable of amplifying as low as 1–10 DNA target copies in less than 20 min. Twist Dx-Twist AMP Basic kit used for the detection of *Streptococcus equi*.

Nasal swab samples from field sources were directly processed for DNA extraction by both boil-chill method and commercial nucleic acid extraction kit. Nasal swab sample for RPA testing was immersed in 100 μ l of nuclease free water (NFW) or normal saline (NS). A volume of 50 μ l of content solution was boiled at 95°C, followed by incubation at -20°C. The supernatant was used as template in RPA. The DNA extracted from remaining 50 μ l of solution using commercial DNA extraction kit (ZYMO Research) or from Todd Hewitt broth culture growth at 37°C was used in RPA.

Designing *S. equi* specific primers for RPA: The primers used were designed following TwistAmp® assay design manual guidelines (<https://www.twist dx.co.uk>). RPA primers were designed based on streptococcal ICE (Integrative conjugative element) called Equibactin gene. All equibactin gene sequences of *S. equi* available at the National Center for Biotechnology Information (NCBI) were utilized for primer design. The major characteristics such as primer length, amplicon size, and GC contents were taken into consideration. The site-specificity of the primers was verified by Primer-BLAST (<https://www.ncbi.nlm.nih.gov>). The expected product length for *S. equi* was 140 bp.

Recombinase polymerase amplification: RPA was done using newly designed primer pairs at a temperature range of 35–41 °C. The assay was carried out in a reaction volume of 50 μ l following the protocol as outlined in the TwistAmp® Basic kit (TwistDx, Cambridge, UK) with slight modifications. The RPA reaction system was 50 μ L in total: 29.5 μ L primer free rehydration buffer, 11.2 μ L sterile water, 2.4 μ L forward primer (10 mM), 2.4 μ L reverse primer (10 mM), 2 μ L template, lyophilized enzyme pellets, mixed thoroughly and added 2.5 μ L 280 mM Magnesium Acetate (MgOAc) to trigger the reaction. After 20 min of amplification at 37°C, RPA products were run on 2% agarose gel containing ethidium bromide dye and visualized under gel documentation system with 100bp Plus DNA Ladder.

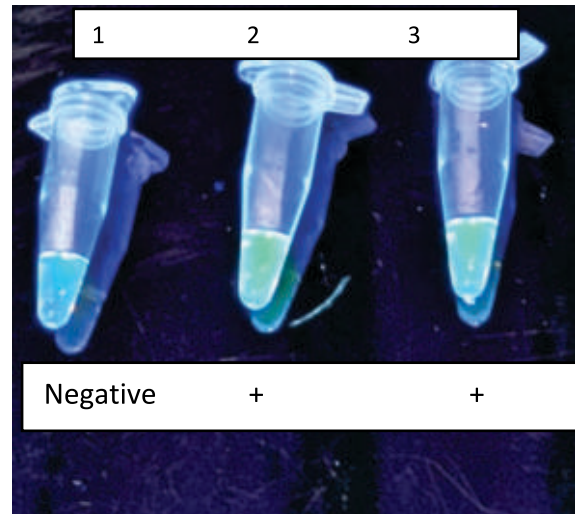
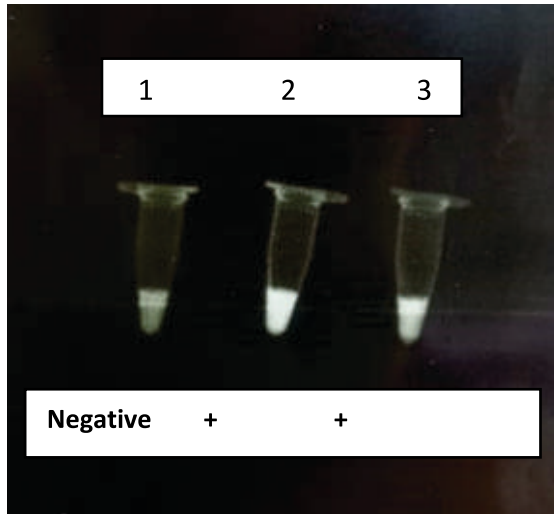


Amplification from broth culture and nasal swabs by boil-chill method

Field based end point detection: To eliminate the gel electrophoresis step used to visualize RPA results in the laboratory, different fluorescence and colorimetric methods were evaluated as follows. RPA was done as described above using extracted DNA of *S. equi* as positive control along with a negative control.

RPA in conjugation to gold nanoparticles (Colorimetric detection): In this method, specific sequence (*S. equi*) was conjugated to gold nanoparticles (GNPs). The conjugated gold nanoparticles were titrated with increasing concentration of sodium chloride. The maximum conc. of NaCl at which nanoparticles are not precipitated was determined. In presence of strangles positive DNA sample (target) and at defined conc. of NaCl, gold nanoparticles aggregated leading to colour change from red to purple.

RPA – SYBR Green I (Fluorescence detection): The RPA reaction was performed with the TwistAmp® Basic Kit (TwistDx, UK) as described earlier. After 20 min of amplification at 37°C, 10 µL of each amplified product and 20 µL ddH₂O were added to 2 µL SYBR Green I (1:100 dilution of 1000x stock solution), which was then mixed and observed for fluorescence intensity under the 365 nm UV transilluminator.



SYBR Green I dye fluoresces (bright) under 365 nm UV light when *S. equi* positive sample detected while negative sample shows no fluorescence. Tube 1 shows negative sample control with no fluorescence while Tube 2 and 3 shows bright fluorescence detecting positive samples.

(Balvinder Kumar, RK Vaid, Anju Manuja, K Shanmugasundaram, Harisankar Singha)

Sero-surveillance and monitoring of Strangles in equines

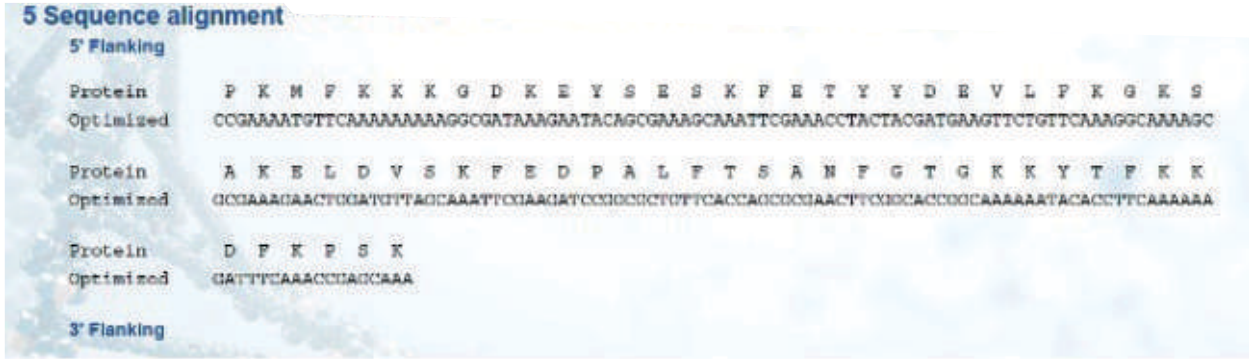
In this project, we investigated the sero-prevalence against *S. equi* using 179 serum samples collected from apparently healthy as well as clinically affected animals from northern India. The total prevalence rate of strangles in equine was 34% in field animals while it was 5.5 % in organized farms. Nasal swab samples (75) were also tested for the bacteria/bacterial DNA using qPCR, PCR and isolation and biochemical characterization.

(Balvinder Kumar, RK Vaid, Anju Manuja, K Shanmugasundaram, Harisankar Singha)

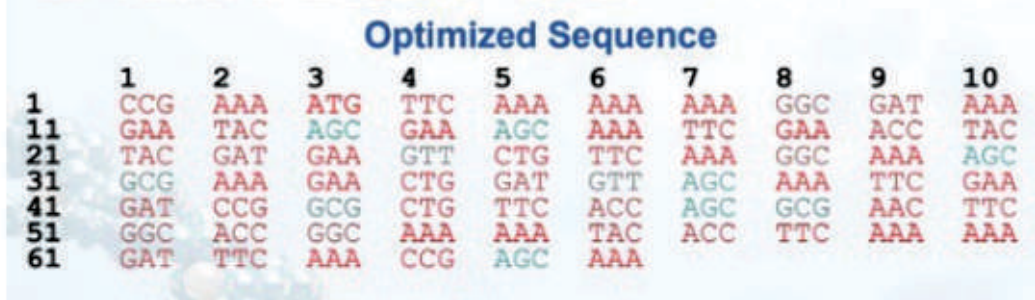
TAMS-1 a candidate vaccine for *Theileria annulata*

Theileria, an apicomplexan parasite, exhibits complex gene expression patterns at various developmental stages. Analysing these patterns can reveal stage-specific functions and potential vaccine targets. Cloning and expression of *Theileria* genes are common approaches for producing recombinant proteins for diagnostics and candidate vaccines.

In vitro cultivation of *Theileria annulata*, isolation, and purification of schizonts: The *Theileria annulata* (Hisar Strain)-infected bovine cell line was cultured in RPMI 1640 complete medium [RPMI 1640 supplemented with 10% heat-inactivated foetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 lg/ml streptomycin]. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂, 3% O₂, and 95% N. *Theileria annulata* schizonts were isolated from infected cultures. Cells were counted using a haemocytometer and adjusted to 4.0x10⁷/μl. The cells were pelleted by centrifugation at 8000x *g* for 20 min. The pellet was washed with PBS and resuspended in 100 μl of PBS. PCR primers were designed to amplify the full-length and truncated TAMS-1 genes. PCR-amplified products and the pGEX-4T-1 expression vector were digested with enzymes and processed for cloning. Competent *E. coli* was used as host cells for cloning and positive colonies were selected. Expression was induced using IPTG. The expressed protein was purified on Sepharose 4 B beads and validated using SDS-PAGE, and Western blot analysis.



TA-TAMS Truncated - 66AA



TA-TAMS truncated – 66AA (198 bp)

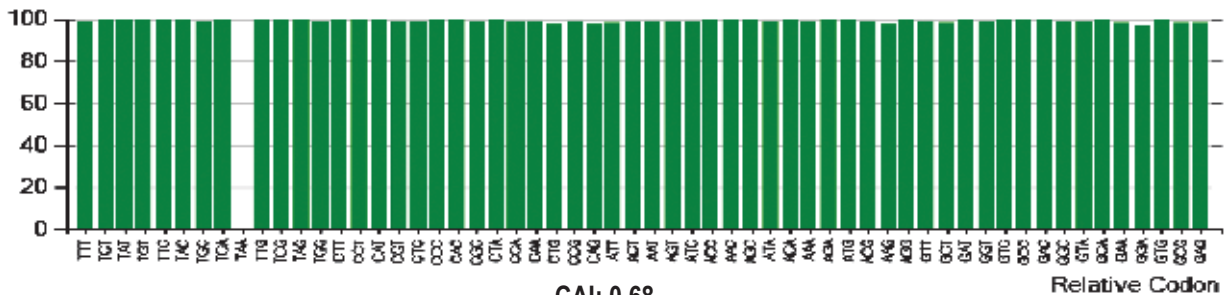
Cloning site : BamH1 and Xho1

Gene Name: TA_TAMS_1
 Expression System: *Escherichia coli*
 Gene Length: 846 (bp)

1. Codon Used Adjustment

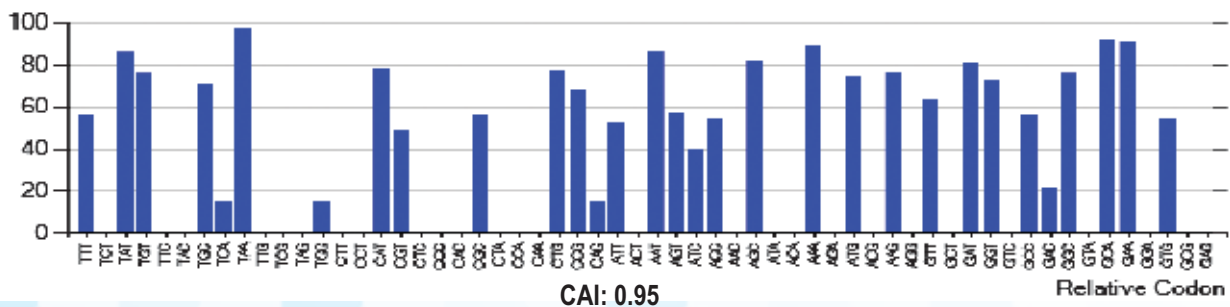
The best value is 1 for sequence optimization.

Codon Usage Adjust

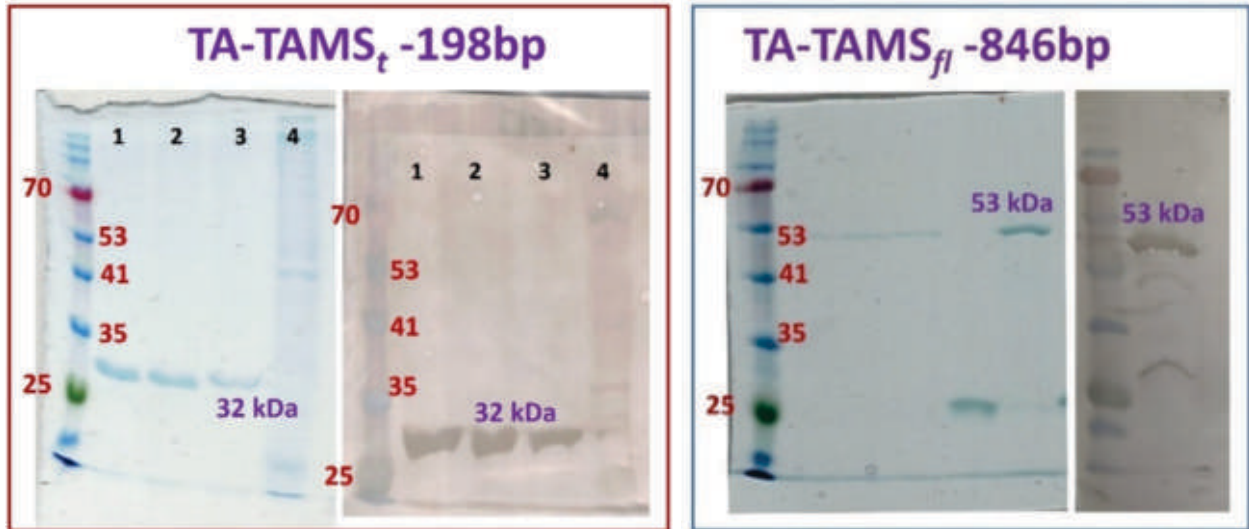


Before Codon Adjustment

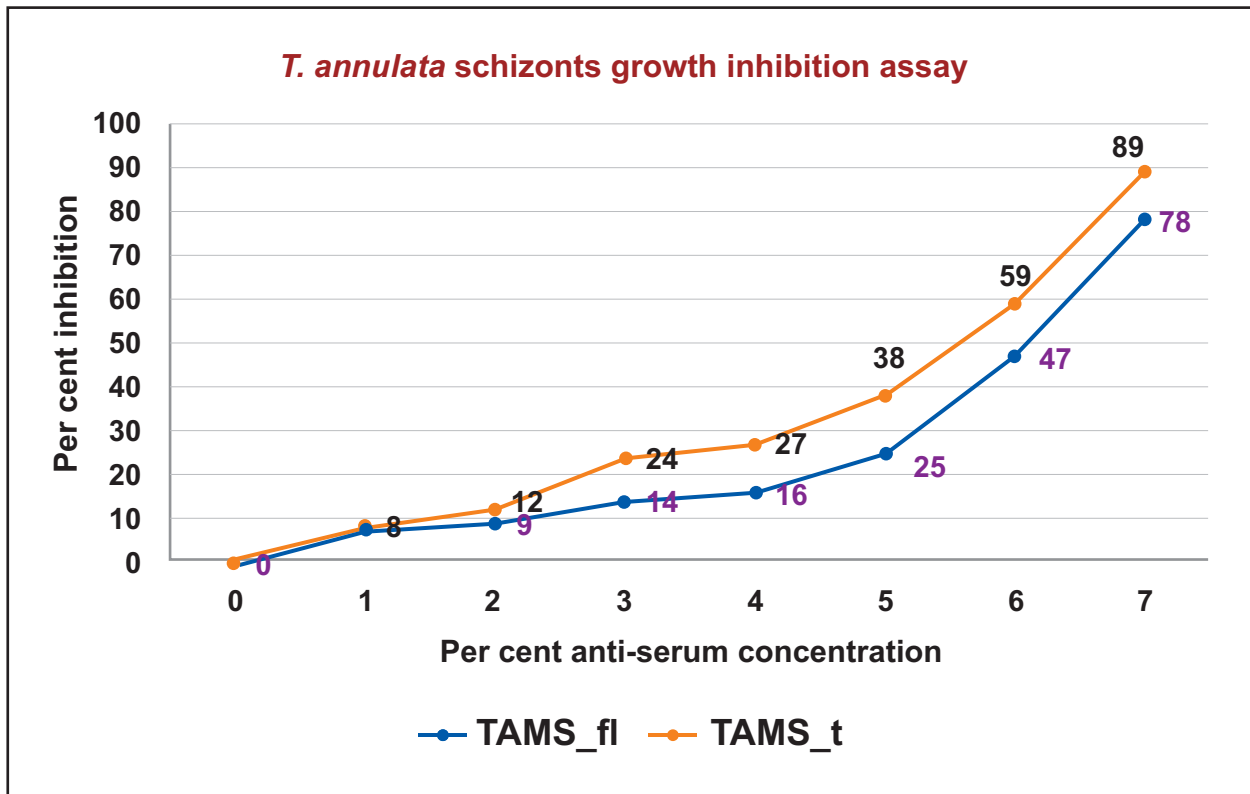
Codon Usage Adjust



TA-TAMS Full Length – 282AA (846 bp)



T. annulata expressed purified protein on SDS-PAGE and Western Blotting with *T. annulata* infected bovine serum



T. annulata schizonts neutralization assay using anti-TAMS-1 specific IgG

Theileria annulata schizonts neutralisation assay: Hyperimmune serum against TAMS-1 recombinant proteins has been raised in rabbits. Rabbits were immunised with 100 µg of recombinant protein, using Titre Max Gold as an adjuvant. Blood was withdrawn from the immunised rabbits at different intervals and the ELISA titre was monitored. Anti-TAMS-1 specific IgG was purified from hyperimmune serum and quantified. The specificity of these sera was confirmed by Western blot analysis using the recombinant proteins.

The *Theileria annulata* schizont neutralisation assay evaluates the ability of specific IgG to impede schizont growth. *T. annulata* cultures were exposed to various concentrations of anti-TAMS-1 IgG. Following incubation, the parasite

growth was quantified to determine the percentage of parasitised erythrocytes. The inhibitory effect was quantified by calculating the concentration required to inhibit the parasite growth by 50%. IgG specific to anti-TAMS-1 successfully inhibited the growth of *T. Annulata* schizonts in culture. This IgGs specifically restricted the growth of *T. Annulata* schizonts by 78 % - 89%. Similarly, anti-TAMS-1 IgG specifically neutralised the *T. annulata* schizont growth by 78 % - 89%. The results of these experiments indicate the potential of recombinant proteins of *T. annulata* (TAMS-1) as candidate vaccines.

(Sanjay Kumar, Rajender Kumar & Shanmugasundarm K)

Investigation of mRNA expression profiles of key metabolic genes of *Trypanosoma evansi* in response to Diminazene Aceturate and Isometamidium Chloride

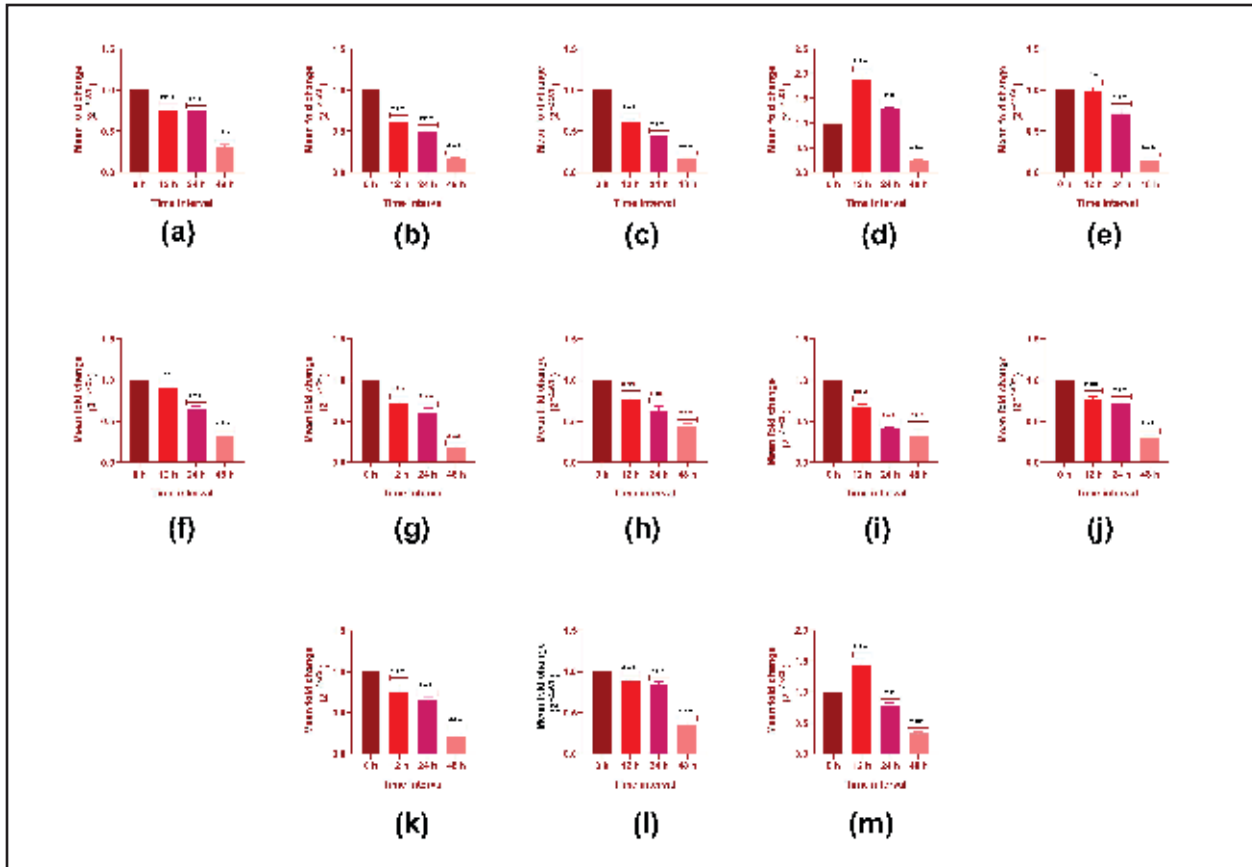
Trypanosoma evansi leads to a fatal emaciated condition in domesticated animals, which is popularly known as Surra disease. Currently, diminazene aceturate (DA) and isometamidium chloride (ISM) are the most commonly used chemotherapeutic agent for the treatment of Surra disease in animals. There is still little knowledge on DA and ISM anti-trypanosomal method of action more than 60 years after its discovery. Scattered biochemical evidences of mode of action are available, however; molecular evidence of alteration of cellular homeostasis in trypanosomes is still lacking. The goal of the current work was to fill this knowledge gap by analysing the gene expression of 13 key metabolically active genes viz., hexokinase, trans-sialidase, trypano thionereductase, expression site associated Gene, aurora kinase, oligopeptidase, casein kinase, arginine kinase, topoisomerase, calcium ATPase 1, ribonucleotide reductase 1, ribonucleotide reductase 2 and ornithine decarboxylase, to determine the inhibitory mechanism of DA and ISM against *T. evansi*.

For elucidation of trypanocidal mechanism of action, one step real-time PCR was used to examine the expression patterns of 13 genes in HMI-9 adapted *T. evansi* parasites before and after treatment with IC₅₀ of DA and ISM, respectively. The half maximal inhibitory concentration of DA and ISM for *T. evansi* pony isolate (T. ev-India-NRCE-Horse1/Hisar/Haryana) was estimated as 335.3 nM and 308.6 nM, respectively.

(Sanjay Kumar & Rajender Kumar)

Effect on mRNA expression profiles of *T. evansi* metabolic genes after exposure with diminazeneaceturate

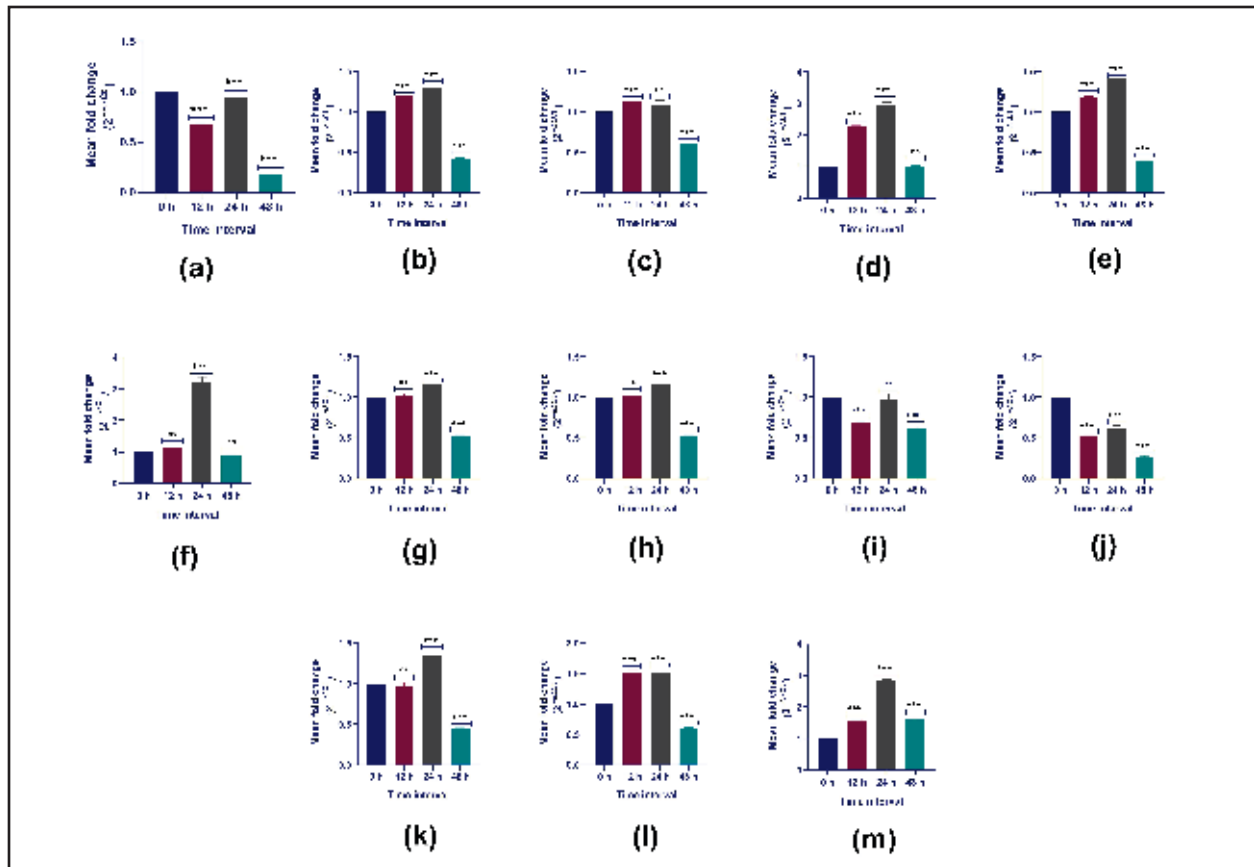
All the targeted genes except ESAG8 and ornithine decarboxylase showed a notable down-regulation of mRNA expression on exposure to DA. After a period of 24 h, a highly significant decline ($p < 0,0001$) in mRNA expression of hexokinase (1.33-folds), trans-sialidase (1.61-folds), trypanothione reductase (1.61-folds), oligopeptidase B (1.09-folds), casein kinase 1 (1.38-folds), arginine kinase (1.30-folds), topoisomerase II (1.46-folds), calcium ATPase 1 (1.29-folds), ribonucleotide reductase I (1.31-folds) and ribonucleotide reductase II (1.14-folds) was observed on exposure to DA. Further, the highly significant ($p < 0.001$) up-regulation of ornithine decarboxylase (1.44-folds) and ESAG8 (1.88-folds) was also observed. At 24 h, significant down-regulation ($p < 0.001$) of the mRNA expression of hexokinase (1.33-folds), trans-sialidase (1.98-folds), trypanothione reductase (2.28-folds), oligopeptidase B (1.54-folds), casein kinase 1 (1.66-folds), arginine kinase (1.60-folds), topoisomerase II (2.41-folds), calcium ATPase 1 (1.38-folds), ribonucleotide reductase I (1.54-folds) and ribonucleotide reductase II (1.18-folds) was observed on exposure to DA. Similarly, up-regulation of ESAG8 continued at 24 h of exposure with DA. However, functional genes such as aurora kinase (1.39-folds) and ornithine decarboxylase (1.26-folds) showed significant down-regulation at 24 h interval. After 48 h of exposure with DA, ESAG8 gene of *T. evansi* get significantly ($p < 0.001$) down-regulated (4.21-folds). In addition, highly significant ($p < 0.001$) down-regulation of the mRNA expression of hexokinase (3.23-folds), trans-sialidase (5.98-folds), trypanothione reductase (5.98-folds), oligopeptidase B (3.169-folds), casein kinase 1 (5.48-folds), arginine kinase (2.33-folds), topoisomerase II (3.08-folds), aurora kinase (6.55-folds) calcium ATPase 1 (3.23-folds), ribonucleotide reductase I (4.62-folds), ribonucleotide reductase II (2.79-folds) and ornithine decarboxylase (1.44-folds) was also observed after 48 h of exposure with DA.



Relative quantification of mRNA expression of selected metabolic gene of *T. evansi* (a: Hexokinase; b: Trans-sialidase; c: Trypanothione reductase; d: Expression site associated Gene 8; e: Aurora kinase; f: Oligopeptidase B; g: Casein kinase; h: Arginine kinase; i: Topoisomerase II; j: Calcium ATPase 1; k: Ribonucleotide reductase 1; l: Ribonucleotide reductase 2; m: Ornithine decarboxylase) using qPCR after exposure with IC_{50} concentration of diminazeneaceturate drug.

Effect on mRNA expression profiles of *T. evansi* metabolic genes after exposure with isometamidium chloride

After 12 h of exposure with ISM, significant ($p < 0.001$) decline in gene expression of hexokinase (4.21-folds), topoisomerase II (4.21-folds) and calcium ATPase 1 (4.21-folds), whereas, rise in mRNA expression of of trans-sialidase (4.21-folds), trypanothione reductase (4.21-folds), ESAG8 (4.21-folds), aurora kinase (4.21-folds), ornithine decarboxylase (4.21-folds) and ribonucleotide reductase II (4.21-folds) was recorded. In 24 h period of exposure with ISM, significant down-regulation ($p < 0.001$) of calcium ATPase 1 (1.64-folds) remain continued. Likewise, significant ($p < 0.01$) rise in mRNA expression of aurora kinase (1.40-folds), hexokinase (1.05-folds), trypanothione reductase (1.09-folds), arginine kinase 1 (1.06-fold), trans-sialidase (1.30-folds), ESAG8 (2.93-folds), ornithine decarboxylase (2.84-folds) and ribonucleotide reductase II (1.52-folds) was also observed. In addition, significant ($p < 0.001$) up-regulation of oligopeptidaseB (3.19-folds), casein kinase 1 (1.15-fos) and ribonucleotide reductase I (1.34-folds) was also noted. At 48 h, all targeted genes except ornithine decarboxylase I (1.60-folds) showed significant ($p < 0.001$) down-regulation. The various drug target gene such as hexokinase (5.414-folds), topoisomerase II (1.61-folds), trans-sialidase (2.33-folds), aurora kinase (2.54-folds), arginine kinase 1 (1.54-folds), calcium ATPase 1 (3.75-folds), trypanothione reductase (1.44-folds), ribonucleotide reductase I (2.21-folds), ribonucleotide reductase II (1.65-folds) and casein kinase 1 (1.94-folds) showed significant ($p < 0.001$) decline in mRNA expression after 48 h of exposure with ISM (Fig. 2).



Relative quantification of mRNA expression of selected metabolic gene of *T. evansi*: Hexokinase; b: Transsialidase; c: Trypanothione reductase; d: Expression site associated gene 8; e: Aurora kinase; f: Oligopeptidase B; g: Casein kinase; h: Arginine kinase; i: Topoisomerase II; j: Calcium ATPase 1; k: Ribonucleotide reductase 1; l: Ribonucleotide reductase 2; m: Ornithine decarboxylase) using qPCR after exposure with IC₅₀ concentration of isometamidium drug.

Transcript analysis of DA and ISM exposed *T. evansi* population showed its multi-modal effects on metabolic machinery of *T. evansi* by change in the mRNA expression of targeted genes and significantly affects the cellular homeostasis in *T. evansi*. However, ISM exposure did not affect mRNA expression of ESAG8, oligopeptidase B and ornithine decarboxylase genes. Further, comprehensive transcriptome and proteomic analysis could provide a deeper insight on precise molecular pathway of these medications against *T. evansi*.

(Snehl Gupta, Sukhdeep Vohra, Khushboo Sethi, Ruma Rani, Surbhi Gupta, Sanjay Kumar and Rajender Kumar)

National One Health Programme for Prevention and Control of Zoonoses (NOHP-PCZ)

ICAR-National Research Centre on Equines (NRCE), Hisar is one of the Regional Coordinators under "National One Health Program for Prevention and Control of Zoonotic Diseases (NOHPCZ)" funded by National Centre for Disease Control, Ministry of Health and Family Welfare, Govt. of India. In this context, NRCE is actively involved in capacity building and strengthening of laboratory diagnostic facilities and inter-sectoral coordination and creating awareness about zoonotic diseases of public health importance by organizing training, workshop and webinar on various zoonotic diseases. The overall objectives of the programmes are -

- Establish an inter-sectoral coordinating mechanism at National, State and District Level by utilizing the existing surveillance system (IDSP) to detect early warning signals of impending outbreaks for timely and effective public health actions.

ICAR-NRCE

- Facilitate sharing of relevant information within stakeholders for taking appropriate actions.
- Development of Laboratory capacity for diagnosis of Zoonotic diseases.
- Capacity building and creating awareness among health and veterinary professionals about Zoonotic Diseases of Public Health Importance (ZPHI).
- Activities such as Information, Education and Communication for spreading awareness among target population for all ZPHI.
- Mapping of different diagnostic laboratories available in the state and working on zoonotic diseases.

Training Programme on “One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases at ICAR-NRCE”

A five days training program on 'One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases' has been organized at ICAR-NRCE from 12- 16 February 2024. A total of 29 Medical, Veterinary and Wildlife Professionals from Haryana & Punjab and 3 technical staff from ICAR-NRCE had attended this training. The lectures and practical on the diagnosis of zoonotic bacterial, parasitic and viral diseases like glanders, brucellosis, tuberculosis, and important zoonotic parasitic infection had been included in the course to update the participants about these important zoonotic diseases.



A five days training program on 'One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases' has been organized at ICAR-NRCE from 19- 23 February 2024. A total of 31 Medical and Veterinary Professionals from Haryana & Punjab and 6 technical staff from ICAR-NRCE had attended this training. The lectures and practical on the diagnosis of zoonotic bacterial, parasitic and viral diseases like glanders, brucellosis, tuberculosis, and important zoonotic parasitic infection had been included in the course to update the participants about these important zoonotic diseases.



Training Programme on “Surveillance and Diagnosis of Zoonotic Diseases through One Health Approach at ICAR-NRCE”

A five days training program on 'Surveillance and Diagnosis of Zoonotic Diseases through One Health Approach' has been organized at ICAR-NRCE from 19-23 November, 2024. A total of 24 Medical Veterinary and Wildlife Professionals from Uttar Pradesh has attended this training. The goal of this training was to strengthen the knowledge of medical and veterinary professionals about Zoonotic diseases diagnosis and capacity building to carry out surveillance and timely reporting of zoonotic diseases. Hands on training were also imparted on laboratory diagnosis of glanders, rabies, tuberculosis, paratuberculosis brucellosis and parasitic diseases.



Workshop on Zoonotic diseases

An awareness workshop on Zoonotic Diseases has been organized at Krishi Vigyan Kendra, Bhiwani, Haryana, Sonipat, Haryana, by ICAR-NRCE team on 01 March, 2024. A total of 31 Medical and Veterinary Professionals and 45 animal attendants from Hisar district attended this workshop. The goal of this workshop was to make aware the animal attendants about common zoonotic diseases and diminish the gap between veterinary and medical profession to control zoonotic diseases.



ICE activities under National One Health Programme for Prevention and Control of Zoonoses

For information, communication and education, posters (glanders and JE) and leaflets (Rabies, brucellosis, glanders, anthrax, JE) were prepared in Hindi language and distributed to various stakeholders.

रेबीज

"रेबीज: एक के लिए सभी, सभी के लिए एक स्वास्थ्य"
रेबीज मानव और जानवरों के मरिहक का एक घातक वायरल संक्रमण है।

रेबीज

- भारत में प्रति वर्ष 18000-20000 मौतें रेबीज से होती हैं।
- विश्व में रेबीज से हर 10-15 मिनट में 1 मौत हो जाती है।
- 10 में से 4 मौतें बच्चों की होती हैं।
- 99% रेबीज के मामले कुत्ते के काटने से होते हैं।
- काटने से पहले और बाद के टीकाकरण से ही रेबीज को रोकना जा सकता है।
- एक बार रेबीज होने पर मौत होने की 100% संभावना है।

बलुएँ और जानवरों में रेबीज संक्रमण चक्र

रेबीज वायरस कई फलसू और जंगली जानवरों जैसे कुत्ते, बिल्ली, घमण्डर, नेकल, भेड़ और लोमड़ी आदि में पाया जा सकता है।

कुत्ते या जानवर के काटने के बाद करने योग्य बातें

काटने की जगह को ठीक साफ और खोले कपड़े से ढाँके या कम से कम 15 मिनट तक धोएं।

काटने की जगह को अस्पष्ट या अस्पष्ट के घोंघ से भीतराणित करें।

कुत्ते के काटने से कैसे बचे?

अपनी आँखें काटने से बचें।
पूरा हाथ काटने से बचें।
किसी भी चीज को अपने मुँह में न लें।
किसी भी चीज को अपने हाथों में न लें।

रेबीज की टीकाकरण 100% टीके से संभव है

"एक स्वास्थ्य अपनाओ: रेबीज रोकें"

एशियस

(बिजुलरिख या डिफ्टी रोग या क्यू रोग)
रेबीज एक घातक वायरल संक्रमण है जो जानवरों को मारता है।

एशियस का टीकाकरण

- एशियस-संक्रमित जानवरों की मृत्यु से बचने के लिए एशियस टीकाकरण करना आवश्यक है।
- एशियस-संक्रमित जानवरों को मारने से बचना चाहिए।
- एशियस-संक्रमित जानवरों को मारने से बचना चाहिए।

एशियस के लक्षण

एशियस-संक्रमित जानवरों में निम्नलिखित लक्षण दिखाई दे सकते हैं:

- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।
- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।
- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।

एशियस का टीकाकरण

एशियस-संक्रमित जानवरों को मारने से बचना चाहिए।

एशियस के लक्षण


एशियस-संक्रमित जानवरों में निम्नलिखित लक्षण दिखाई दे सकते हैं:

- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।
- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।
- चलावना, पर्याप्त, पर्याप्त, कठोर गति आदि।

गलैंडर्स

लक्षण, बचाव और उपाय

गलैंडर्स एक संक्रामक सूक्ष्मीय बीमारी है जो मुख्य तौर पर अश्व प्रजाति (घोड़े, खच्चर और गधे) में होती है। यह बीमारी बरसोलेरिया मैलियाई नामक जीवाणु से होती है जो जानवरों से मनुष्य में भी फैलती है।



(Harisankar Singha, K. Shanmugasundaram, Riyesh T, Punit Jhandai, Rajender Kumar, Shreya Prasher, Radha)

National One Health Mission (NOHM) component of Pradhan Mantri Ayushman Bharat Health Infrastructure Mission (PM-ABHIM)

Under the National One Health Mission (NOHM), a key component of the Pradhan Mantri Ayushman Bharat Health Infrastructure Mission (PM-ABHIM), the institute has actively contributed to strengthening India's preparedness against emerging and re-emerging infectious diseases. The primary objectives of the initiative include establishing a robust network of BSL-3 and BSL-4 laboratories across departments, ensuring functional interconnectivity for

seamless outbreak communication, facilitating rapid and coordinated responses during public health emergencies, and promoting cross-departmental collaboration for a holistic outbreak management approach. In alignment with these goals, the institute has maintained its BSL-3 facility in fully operational condition, with essential equipment being procured, repaired, and placed under annual maintenance contracts (AMC).

To enhance human resource capacity, scientific personnel involved in BSL-3 pathogen diagnostics and research were deputed for advanced trainings: (1) "Biosafety and Biosecurity for Handling High-Risk Pathogens in BSL-3 Laboratory" at NIV, Pune (April 8–12, 2024), (2) "Training-cum-Workshop on Biosafety for Handling and Diagnosis of High-Risk Animal Pathogens in ABSL-3 and BSL-3 Laboratories" at ICAR-NIHSAD, Bhopal (February 3–7, 2025), and (3) "Hands-On Training on Seasonal and Non-Seasonal Influenza Diagnosis Using Real-Time PCR" at the National Influenza Centre, NIV Pune (October 23–25, 2024). Additionally, ICAR-NRCE, Hisar organized two in-house training programs on "Introduction to BSL-3 Laboratory and Risk Management for Handling High-Risk Pathogens" to build awareness and strengthen internal capabilities.



Trainees at microbial containment facility of ICAR-NRCE, Hisar

(Harisankar Singha, S. Barua, Riyesh T, and Assim Verma)

Antibiotic associated colitis in horses and fecal metagenomics

Colitis in horses, marked by inflammation of the colon, often presents with diarrhea, abdominal pain, and rapid dehydration, and in severe cases may lead to complications such as toxemia and laminitis. Common causes include stress, dietary changes, and especially antibiotic use, with clinical signs such as acute diarrhea and colic guiding diagnosis. Treatment emphasizes supportive care and managing the underlying cause. Broad-spectrum antibiotics like penicillin, streptomycin, gentamicin, amikacin, ceftriaxone, cotrimoxazole, amoxicillin, azithromycin, and rifampicin are frequently implicated in antibiotic-associated colitis. This study investigated field cases of equine colitis, involving 20 horses from both organized farms and private owners in Rajasthan. Twelve necropsies revealed severe colonic or cecal congestion, confirming colitis. Fecal samples from affected and healthy horses were preserved in liquid nitrogen and analyzed via metagenomics to assess changes in gut microbiota. Most cases had a history of antibiotic use, with norfloxacin and ceftriaxone most often linked to colitis. In one ceftriaxone-associated case, there was a notable rise in *Clostridium* genus reads, suggesting disruption of gut flora, while a decrease in *Fibrobacter* indicated reduced fiber digestion capacity. Such microbiota shifts are consistent with mechanisms seen in humans, where antibiotics like cephalosporins and fluoroquinolones can lead to *Clostridium difficile* overgrowth. Notably, neither ceftriaxone nor norfloxacin targets *Clostridium*, potentially facilitating *C. difficile* proliferation in horses. Based on these findings, the use of ceftriaxone and norfloxacin in horses should be restricted to cases lacking safer alternatives, and further studies are warranted to better assess their safety in equine medicine.



Severely congested colon of a horse died of colitis.

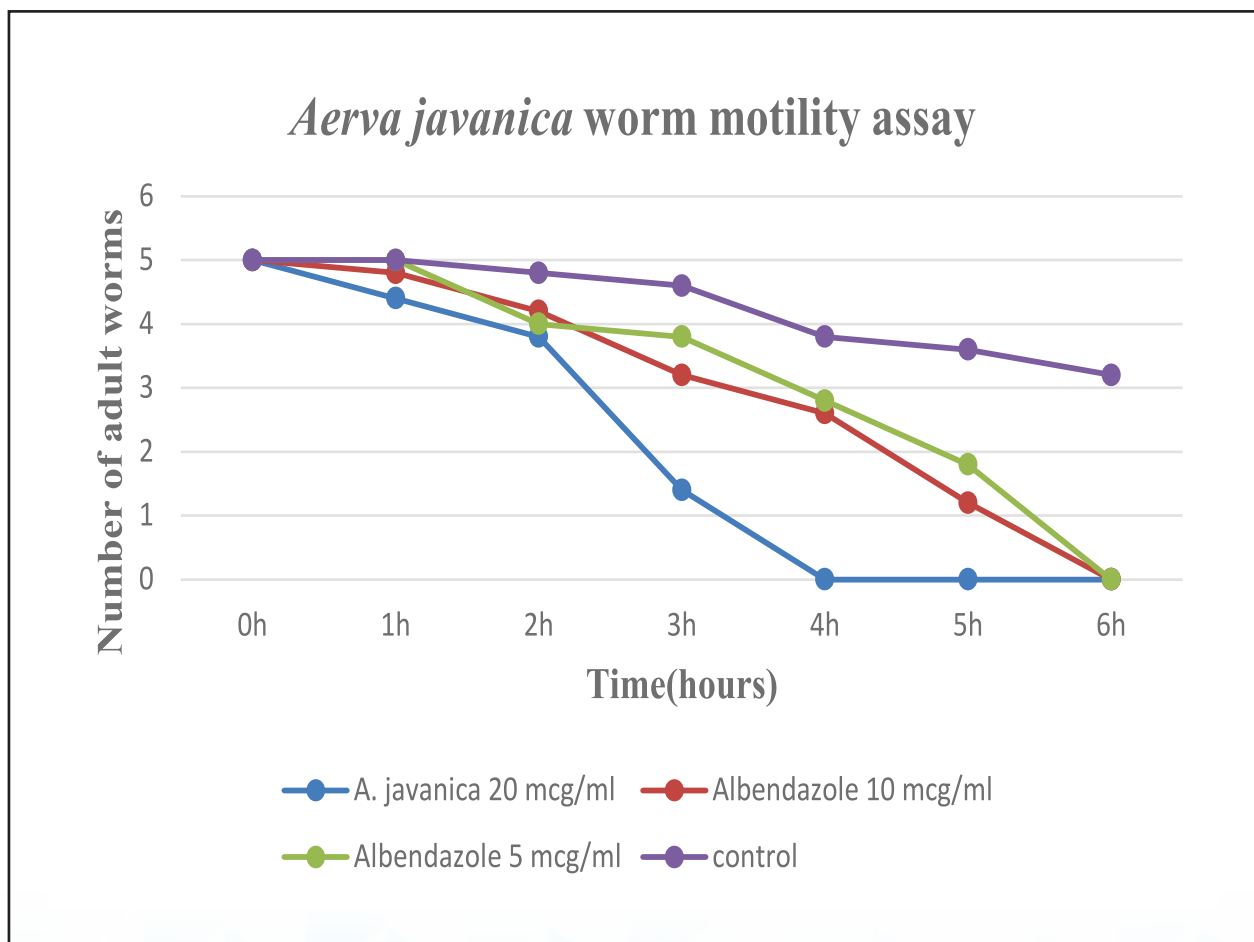
Table: Metagenomic OUT reads of some important genera in two cases of ceftriaxone associated colitis and in healthy horses.

Bacterial genus	Healthy horses (OTU reads) (n=3)	Horses with Ceftriaxone associated mild colitis (OTU reads) (n=1)	Horses with Ceftriaxone associated severe colitis (OTU reads) (n=1)
<i>Enterococcus</i>	9003 ± 529	346134	3208018
<i>Clostridium</i>	93625 ± 9368	212242	1494868
<i>Fibrinobacter</i>	49473 ± 2124	1105	39
<i>Lachnospira</i>	4632 ± 651	2120	50

(R.K. Dedar, T.R. Talluri, M. Kutty, S.C. Mehta and T.K. Bhattacharya)

Antiparasitic activity of desert plant extracts

Roundworms represent a major clinical challenge in livestock management due to their impact on animal health and productivity. Previous studies have demonstrated the therapeutic efficacy of the desert plant *Aerva javanica* in treating *Haemonchus* infections in equines and demodectic mange in dogs, as well as improving clinical cases of sarcoptic mange and showing tick-repellent properties in cattle. Building on this evidence, the current study investigated the in vitro antiparasitic potential of ethanolic extracts from *Aerva javanica* and other desert plants against gastrointestinal roundworms. *Haemonchus contortus* worms were collected from a slaughterhouse in Bikaner and subjected to standard in vitro assays, including the egg hatch assay (EHA) and adult worm motility inhibition assay (WMIA), to evaluate the efficacy of extracts from *Aerva javanica*, *Azadirachta indica*, *Capparis decidua*, *Leptadenia pyrotechnica*, and *Citrullus colocynthis*, using albendazole as the positive control. Worm viability was assessed by the pinch method, and cytotoxicity was determined on Vero cells using the MTT assay. *Aerva javanica* extract exhibited the highest efficacy, achieving 100% worm mortality at 20 $\mu\text{g}/\text{mL}$ within 4 hours, surpassing albendazole at 10 $\mu\text{g}/\text{mL}$. *Capparis decidua*, *Leptadenia pyrotechnica*, and *Citrullus colocynthis* also demonstrated complete mortality but at higher concentrations. While egg hatch inhibition was dose-dependent, no extract prevented egg hatching at non-cytotoxic concentrations. These findings indicate that certain desert plant extracts, particularly *Aerva javanica*, possess promising in vitro anthelmintic properties and may serve as effective alternatives to conventional antiparasitic treatments.



***Aerva javanica* antiparasitic activity against round worms.**

(R.K. Dedar, T.R. Talluri, Pawan Kumar Sharma, M. Kutty, S.C. Mehta, and T.K. Bhattacharya)

EQUINE PRODUCTION

Bhimthadi recognised as 8th indigenous horse breed

The characterisation and recognition of Bhimthadi horse as the 8th breed of indigenous horses has been the outcome of joint efforts of ICAR-NRCE and ADT, Baramati. In order to conserve, improve and promote the breed, on September 5, 2023 "All India Bhimthadi Horse Association" was formed at Baramati, Maharashtra and registered under the Companies Act, 2023(18 of 2013) with Corporate Identification Number (CIN) No. U94990PN2023NPL223782. The breed has been gazette notified on dated December 9, 2024 by the Government of India. The first show of Bhimthadi has also been organized with great success on January 20-21, 2024. The molecular characterization and genetic diversity analysis of Bhimthadi has also been carried out.

(S C Mehta and S D Sorate)

Conservation of Halari donkey

The Institute herd of Halari donkey has been strengthened by purchase / transfer of 5 breedable animals of the breed. The strength of Halari donkeys at Bikaner is now seventeen. Four ethnoveterinary practices have been submitted for sending to NITI Ayog. The ethnoveterinary practices were for (i) The Pest Management: Tick Infestation (ii) Disease Management: Vesicular Stomatitis (iii) Disease Management: Udder Fibrosis in Mastitis and (iv) Disease management: Mange. Contact has been established with Govt. Horse and Donkey Farm, Chanasma, Patan, Gujarat and the ICAR-NRCE project team is working in close contact with field unit of Halari donkey being run by Sahjeevan, NGO, Bhuj.

(S C Mehta, M Kutty and T K Bhattacharya)

Development of SNP chip for Indigenous Horses

Indigenous horse SNP chip has been developed by ICAR-NBAGR in collaboration with ICAR-NRCE: First SNP chip for indigenous horses: The development of a high-density Axiom_Ashwa SNP chip for Indian horses and ponies.

(Ahlawat S, Niranjana S K, Arora R, Viji R K, Kumar A, Sharma U, Raheja M, Popli K, Yadav S and Mehta S C)

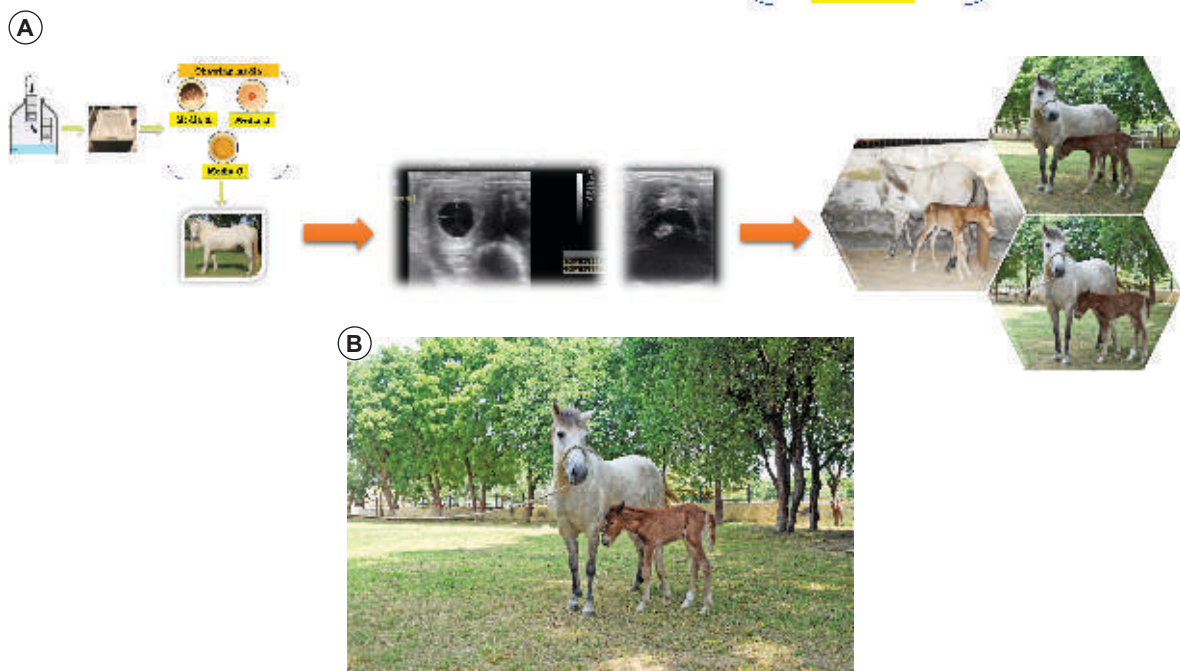
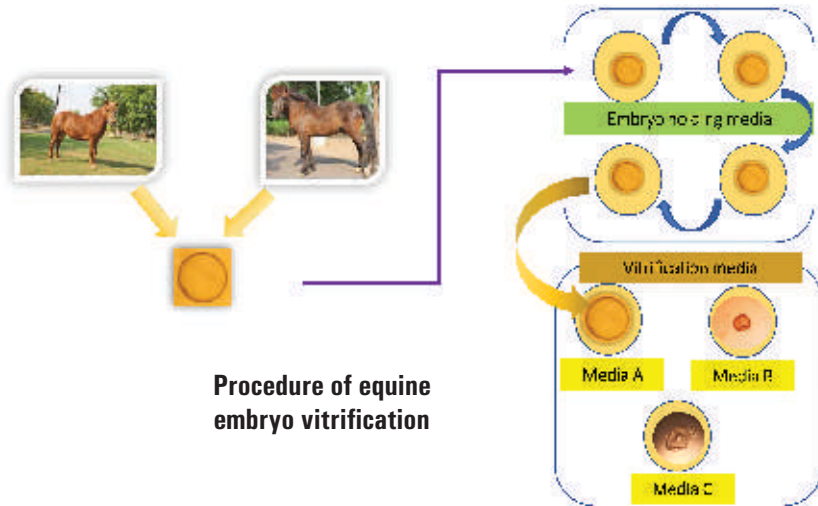
Development of a Panel of Genome-Wide Ancestry Informative Markers in Indian Horse and Pony Breeds

Forty (40) samples of true to the breed Marwari horses have been collected, DNA isolation and quality check has been performed and the samples have been sent for whole genome sequencing and further analysis.

(S C Mehta, T K Bhattacharya, S K Dixit, I. Ganguly, S Singh)

Raj-Sheetal: India's first horse foal born through vitrified embryo transfer

To produce the foals from vitrified embryo transfer in Zanskari horses, estrus of the mares was closely monitored through ultrasonography and at near ovulation, the donor mares were inseminated using frozen semen from proven fertile stallion. Four donor mares were flushed at day 7.5 after ovulation for recovery of embryos and the recovered embryos (4) were vitrified using the customized media and customized cryodevices such as cryoloop and cryotop. One of the vitrified embryos that was frozen in the liquid nitrogen and recovered by thawing the embryos after 2 months of storage in the liquid nitrogen. Recipient mares were carefully followed throughout their estrus cycle and for ovulation. The thawed vitrified embryo was transferred to surrogate mare on the seventh day of post ovulation. The recipient mare were checked for pregnancy on day 14 and found pregnant. The mare carried the foal until 324 days and delivered a healthy foal during unassisted foaling on 21 September 2024. The foal weighed 20 kgs and the foal was named as Raj-Sheetal. This is the first foal produced in India through vitrified embryo transfer.



Procedure of thawing of the vitrified embryo for the establishment of pregnancy in surrogate mare. b, Raj-Sheetal: Country's First horse foal produced through vitrified embryo transfer.



Raj-Sheetal: Country's First horse foal produced through vitrified embryo transfer.

(TR Talluri, Sajjan Kumar, RK Dedar, Mohd Kutty, SC Mehta, TK Bhattacharya)

Raj-zanskar: India's first Zanskari horse foal produced through embryo transfer technology

Zanskari, a native pony breed of Leh-Ladakh in trans-himalayan region of India is well adapted to high altitude regions. This breed of horses is known for their ruggedness, ability to withstand extreme cold climates, work tirelessly, and carry loads at high altitudes. A total of 4 Zanskari mares were synchronised for estrus and two mares were made donors and two mares were made recipients. Flushing of the donor mares were done to recover the embryos. Two embryos were obtained after flushing and one was transferred to synchronised recipient surrogate mare to establish the pregnancy. The mare was examine at day 10 after transfer and found pregnant. The mare carried the foal to full term and delivered a healthy female foal on 23.04.2024. The foal was weighing 28 kgs on birth.



Foal named 'Raj-Zanskar' produced through Embryo transfer technology in Zanskari breed of horse

(TR Talluri, Sajjan Kumar, RK Dedar, Mohd Kutty, SC Mehta, TK Bhattacharya)

Vitrification of horse embryos

This year during 2024-2025, a total of 16 flushings were done for recovery of embryos from the synchronised mares. Recovered 13 embryos with a success rate of 81.25% and out of the 13 embryos we have successfully, vitrified 9 embryos and rest of them were used for the recovery and survival and sexing studies of the embryos.

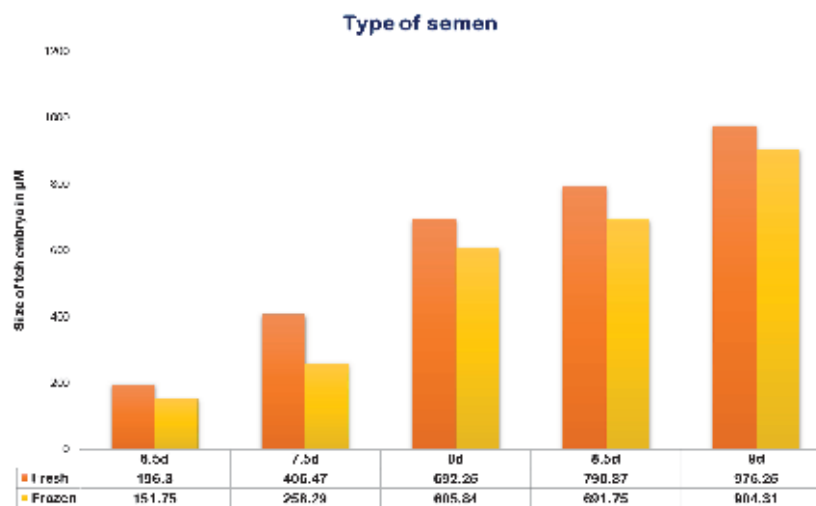
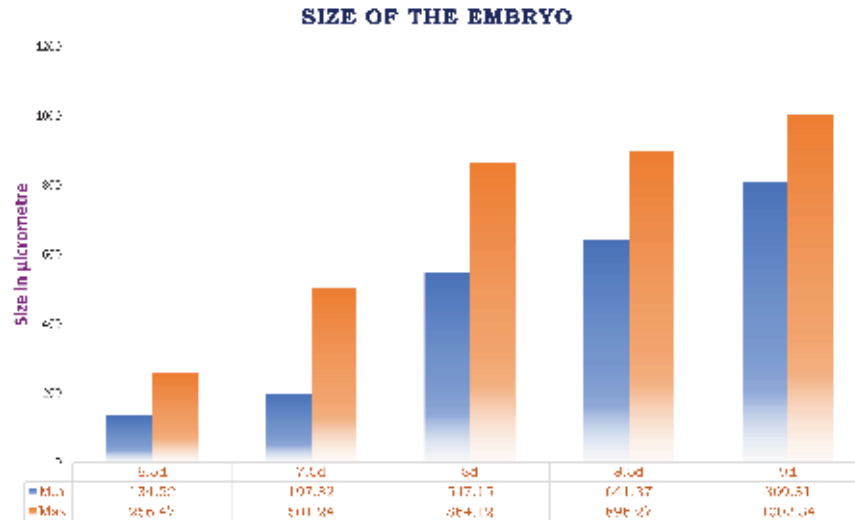
Day of Flushing	Total Flushings	Embryos recovered	Recovery rate	No of Embryos Vitrified
Flushings on Day 8 post -AI	10	8	80.00	6
Flushings on Day 9 Post-AI	6	5	83.33	3
	16	13	81.25	9

(TR Talluri, Sajjan Kumar, RK Dedar, Mohd Kutty, SC Mehta, TK Bhattacharya)

Effect of day of flushing and type of semen used on size of equine embryo

A study was conducted to see the effect of day of flushing and type of semen used for producing the embryos. A total of 18 mares were used for studying the type of semen and embryonic size out of them 9 mares were bred with fresh semen and other 9 were bred with frozen semen. It was observed that when we flushed the mares on day 7.5 post ovulation, the size of the embryo was found to be significantly different from the fresh inseminations in comparisons to

the frozen semen inseminations. The same observation was not observed in 6.5d, 8.5d and 9th day of flushings. Similarly, a total of 20 flushings data were recorded to note the differences in the size of the embryos recovered from various flushings done at different days (6.5,7.5,8.5 and 9th day) of post ovulation. We recorded a significant ($P \leq 0.01$) size differences of the embryos between all the days of flushing.



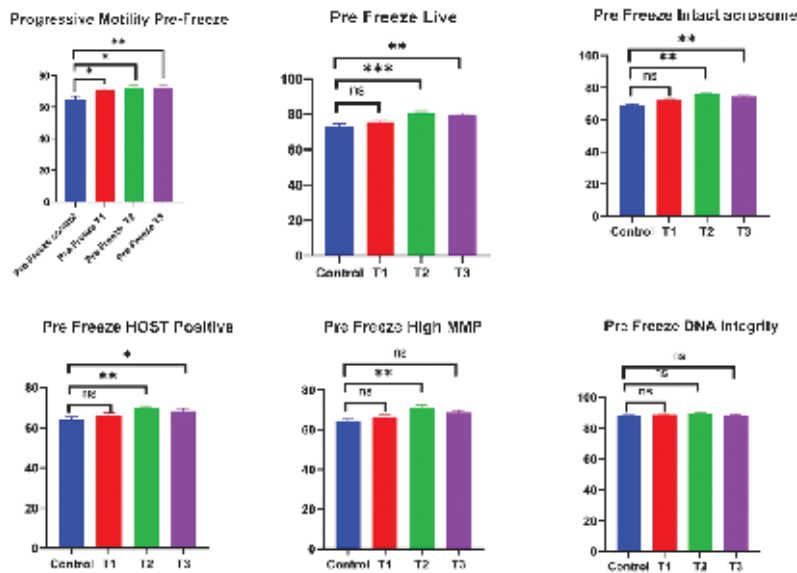
Factors affecting the embryonic size in mares during ET programme

(TR Talluri, Sajjan Kumar, RK Dedar, SC Mehta, TK Bhattacharya)

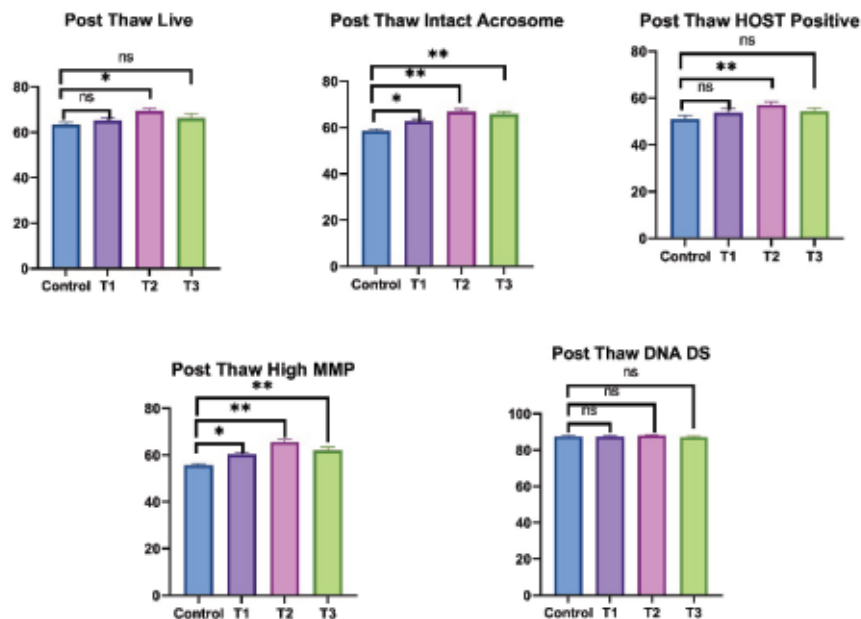
Effect of L-Carnitine supplementation to the semen extender on pre-freeze and post-thaw semen quality in Marwari breed stallions

A study was conducted to evaluate the effect of L-Carnitine supplementation to the semen extender on pre-freeze and post-thaw semen quality in Marwari breed stallions. For this purpose, thirty-six semen ejaculates (6 ejaculates from each stallion) were collected using an artificial vagina (AV) from six healthy Marwari stallions. Gel-free semen was diluted with a primary extender in equal volume (1:1) and after then with a secondary semen extender alone control (C) and supplemented with different concentrations of L-Carnitine i.e. 2.5 mM (T1), 5 mM (T2) and 10 mM (T3). Pre-freeze and post-thaw semen evaluations were conducted for various seminal characteristics, viz. total sperm motility, progressive sperm motility, sperm plasma membrane integrity, sperm viability, acrosome integrity, mitochondrial membrane potential and antioxidant enzyme levels including superoxide dismutase (SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GPX) and malondialdehyde (MDA). The results indicated that a 5 mM LC concentration

significantly ($P < 0.01$) increased, total sperm motility, progressive motility, acrosome integrity, and mitochondrial membrane potential at both the pre-freeze and post-thaw stages. Additionally, sperm viability and plasma membrane integrity were significantly improved at the pre-freeze stage ($P < 0.01$) and remained significantly higher at the post-thaw stage ($P < 0.05$). However, non-significant differences were observed in kinematic parameters (VAP, VCL, VSL, LIN, and STR) at both stages. Antioxidant enzyme levels exhibited significant improvements: SOD and T-AOC levels significantly increased ($P < 0.01$), GPX levels showed significantly increased ($P < 0.05$), and MDA levels decreased significantly ($P < 0.05$) at the post-thaw stage. In conclusion, supplementation of 5 mM L- carnitine significantly improved the cryosurvival rate of Marwari stallion spermatozoa.



Effect of L-Carnitine supplementation on stallion seminal characteristics at Pre-freeze stage of cryopreservation



Effect of L-Carnitine supplementation on stallion seminal characteristics at post-thaw stage of cryopreservation

(TR Talluri, Om Prakash Solanki, D Jhamb, Sajjan Kumar, Mohd Kutty, TK Bhattacharya)

Comparison of endometrial culture and cytology collection techniques in endometritis affected mares (*Equus caballus*) and intrauterine autologous platelet-rich plasma therapy (prpl)

Endometritis is one of the most important causes of infertility in the mare. Bacterial and fungal endometritis is the most major cause of mare infertility. The aim of the study was to compare diagnostic efficacy of three techniques for collection of sample for endometrial culture and cytology [double guarded cotton swab (DGS), double guarded uterine cytobrush (CB) and low volume lavage (LVL)] in endometritis affected mares. An ultrasonographic evaluation as well as cytological and bacteriological examination was performed in 36 endometritis affected mares for identification of the microorganisms involved and conformation of the endometritis. Among the three isolation techniques, LVL sampling technique was found to be effective in diagnosing the cytology positive (75%) and 97.2% culture positive (97.2%) mares. The most commonly isolated bacterial species was *E. coli* (12 isolates) *S. aureus* (10 isolates), *K. pneumoniae* (10 isolates) and fungus (17 isolates). In the present study intraluminal fluid accumulation was found through USG in 58.33% mares which were diagnosed as suffering from endometritis. In some mares (15) there was no intraluminal fluid accumulation was observed through USG and samples were negative for cytology, but found to be culture positive. LVL technique diagnosed higher number of cytology positive and culture positive mares than DGS and CB technique, though it was non-significant. From the current study, it may be suggested that to adopt LVL technique for diagnosis of endometritis as it found to be effective among the three techniques adopted in our study, further it is cheaper and can be performed at field. Both cytological and bacteriological examinations are recommended for endometritis diagnosis as USG alone provides false negative results in sub-clinical/clinical endometritis.

In second experiment, we investigated the efficiency of using alternative ways of treatment for endometritis by using selected antibiotics or autologous platelet-rich plasma or combinations of both. All bacterial isolates were susceptible to antibiotics like amikacin and gentamicin. Before the beginning of treatment, mares were subdivided into three groups: 12 mares were treated with selected antibiotic (T1), 12 mares were treated with autologous platelet-rich plasma (T2), and 12 mares were treated with a combination of antibiotics and autologous platelet rich plasma (T3). All mares received uterine lavage and ecobolic treatment along with specific treatment assigned. For antibiotic treatment of the mares, all the cultures were tested for ABST and most effective antibiotic found after ABST was adopted for treatment in T1 and T3 groups. The mares received assigned treatments during both pre-breeding and post-breeding stages of the same estrus. We found that the most effective result was detected in T2 (PRP treatment group), which recorded marked decrease in microbial positive and cytology positive post treatment which is also reflected in conception rate. Conception rate in T2 group (91.66%) was higher ($P < .02$) than T1 (41.66%). Conception rate in T3 (75%) group was higher than T1, though non-significantly.

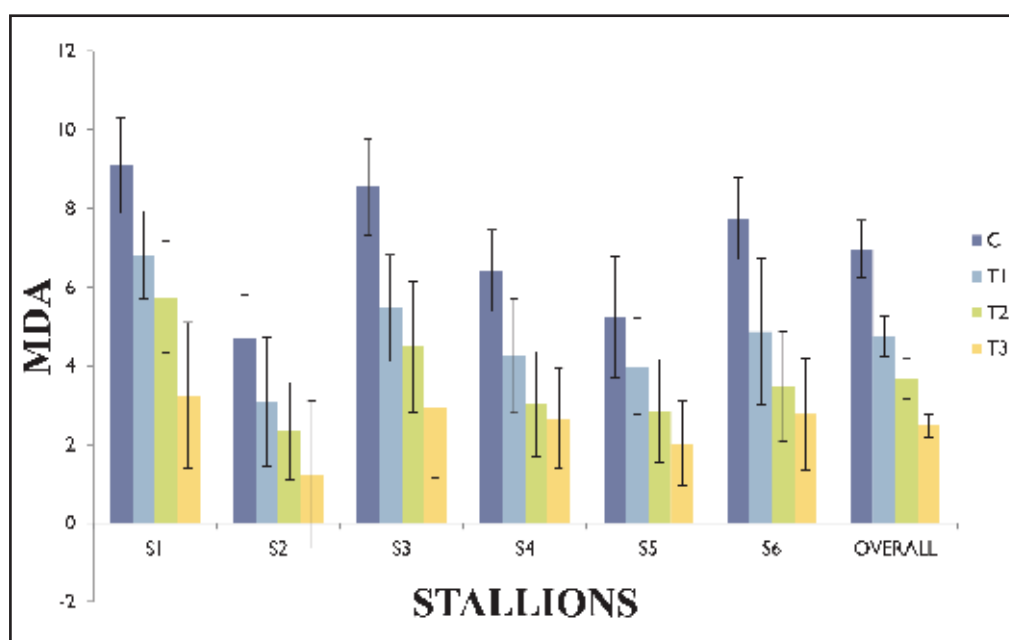
We concluded that using fresh autologous platelet-rich plasma along with uterine lavage and ecobolics found effective in the treatment of bacterial and fungal endometritis by decreasing the uterine infection and have a great role in improving immunological status and conception rate.

(TR Talluri, Madhu Meena, RK Vaid, Om Prakash Solanki, D Jhamb, TK Bhattacharya)

Melatonin supplementation to the semen freezing extender improved the quality of cryopreserved marwari stallion semen and reduces the oxidative stress

Successful cryopreservation of the spermatozoa with acceptable post-thaw quality measures paves the way for effective conservation and distribution of the outstanding genetic potentials in the equine breeding industry. Cryopreservation compromises the capacity of sperm fertilizing due to a series of alterations in the structure and physiology of the sperm. Reactive oxygen species production and lipid peroxidation during cryopreservation harm sperm membrane and as a result reduce the recovery of motile sperm. The use of antioxidants, such as melatonin, added to freezing media, may help to reduce sperm cryoinjury. This study was aimed to evaluate changes in post-thaw motility, viability, and Total anti-oxidant capacity (T-AOC) and malondialdehyde (MDA) in response to the addition of

melatonin to stallion sperm freezing extender. Semen from six adult fertile Marwari breed stallions was collected, analysed for fresh seminal parameters and further extended in citrate media for washing and removal of seminal plasma. The pellet was further extended with semen freezing extender (Lactose-Glucose- EDTA) either containing three different final concentrations: 1, 1.5 and 2 mM of melatonin or without Melatonin (Control). After 24 hrs of cryopreservation, semen was thawed at 37 degree Celsius for 30 sec and after thawing, semen was microscopically evaluated. Obtained results showed that cryopreservation significantly ($P < 0.05$) reduces viability and motility, but increases T-AOC and MDA of stallion sperm. The semen extender supplemented with 2 mM of melatonin significantly ($P < 0.05$) increased sperm motility, viability, plasma membrane integrity, acrosome integrity, DNA integrity and addition of Melatonin significantly increased T-AOC levels and significantly reduced MDA levels indicating increased in antioxidant levels and reduction in oxidative stress and lipid peroxidation levels of cryopreserved sperm after the thawing process, as compared with the control group. We also found that the most effective concentration of melatonin in protecting stallion spermatozoa from cryopreservation injuries was 2 mM. These findings suggest that melatonin exerts its cryoprotective effects on spermatozoa possibly by counteracting ROS, and thereby reduces MDA generation. This finally leads to increase of post-thaw viability and motility of cryopreserved stallion spermatozoa.

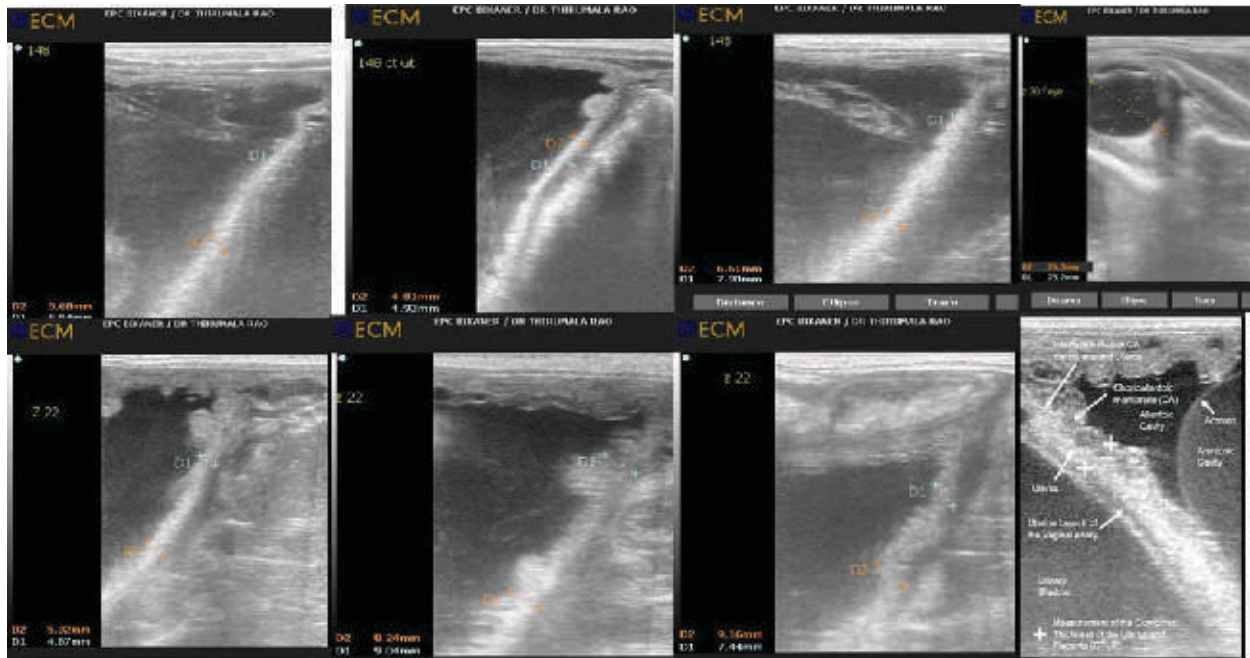


(TR Talluri, Naina Paswan, Alok Kumar, TK Bhattacharya)

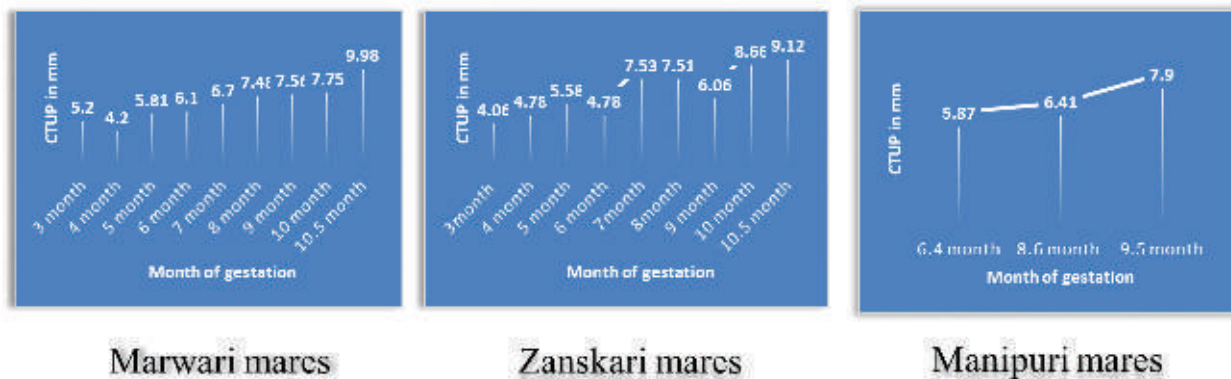
Combined thickness of the uterus and placenta in three indigenous horse breeds

Threatened abortions due to placentitis during late to advanced stages of gestation (> 5 months) represent a great colossal loss equine breeding industry. Transrectal ultrasonography of the equine placenta is a valuable diagnostic tool in detection of mares with placental failure. An abnormal combined thickness of the uterus and the placenta may serve as an early indicator of placental failure and impending abortion. The current study was aimed at finding the fetal wellbeing in pregnant mares of three indigenous horse breeds through the measurement of combined thickness of the uterus and placenta (CTUP) and to establish normal values of CTUP in these breeds at various stages of gestation. For measurement of CTUP values, we made 10, 14 and 3 observations in Marwari, Zanskari and Manipuri pregnant mares respectively. These observations were made using transrectal ultrasonography using 5 MHz recto-linear transducer, positioned at the cervical-placental junction. Measurements of the CTUP were obtained from the ventral aspect of the uterine body. CTUP values and blood parameters were measured from 100 days until 315 days every month and the mean values was recorded. In the current study we found that CTUP values in Marwari mares from 104 to 315 days

was observed to be ranging from 5.2-9.08 mm, in Zanskari mares from 156 to 319 days CTUP values ranged from 5.58-9.12 mm and in Manipuri mares of 194 to 287 days the values were ranging from 5.87-7.9 mm. The values were found to be increasing gradually as the gestation advanced. There was no significant difference observed for different breeds for CTUP values. At all examinations for all horses, the allantochorion and the uterus were indistinguishable from each other on the ultrasound examinations. No marked detachment was observed at any time. Only in one case we could detect marked detachment of the chorioallantoic membranes and physical signs of udder enlargement and lactation. Transrectal ultrasonography is an invaluable tool in diagnosing the fetal wellbeing and identifying mares with impending placentitis. This approach has shown promise in diagnosing ascending placentitis before the onset of overt clinical signs and placental compromise.



Normal ultrasonographic appearance of the CTUP, fluid and fetal presentation in a mare



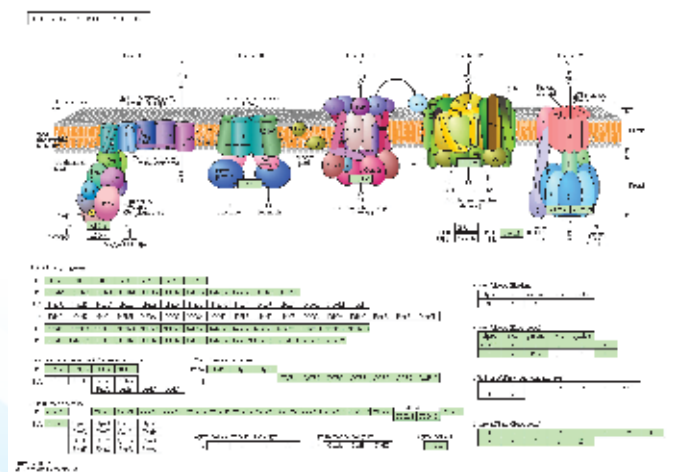
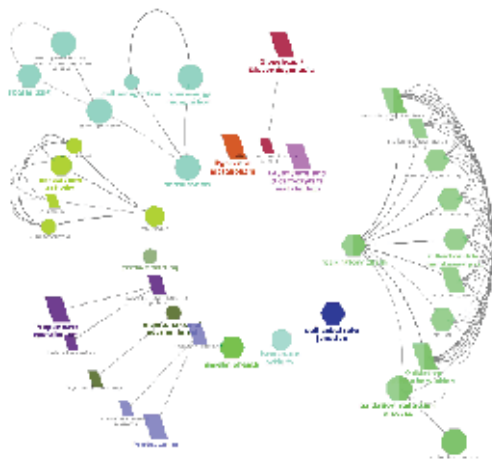
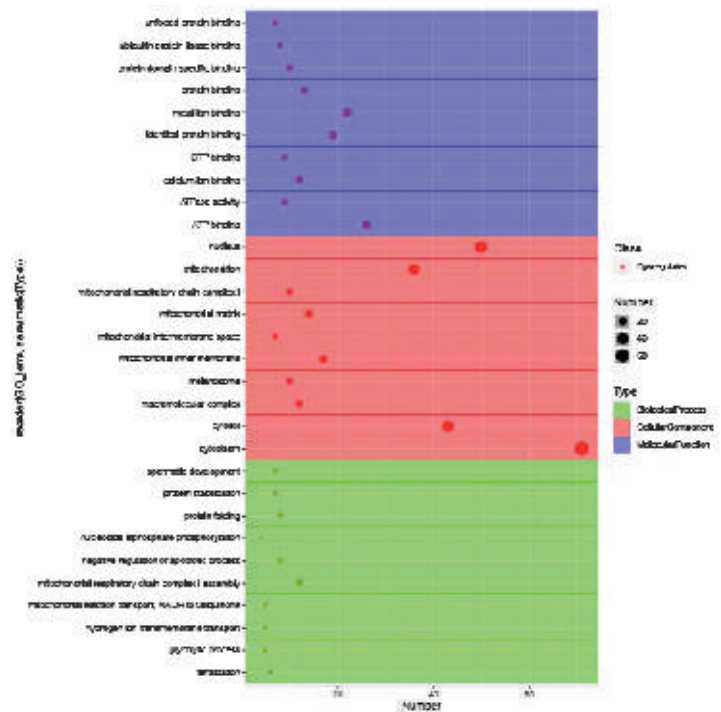
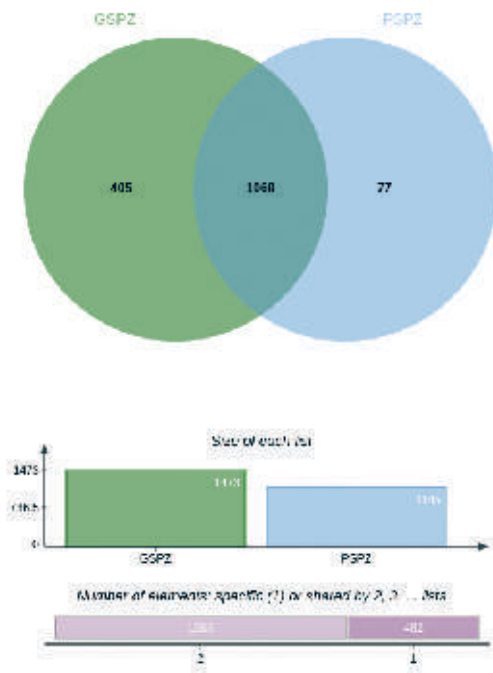
(TR Talluri, Om Prakash Solanki, Sajjan Kumar, TK Bhattacharya)

High-throughput proteomics analysis of stallion spermatozoa with contrasting semen motility

A study was conducted using highly sensitive LC/MS-MS technology and sequence database analysis to identify and characterize the proteome of percoll-isolated ejaculated equine spermatozoa with contrasting semen motility, with the

aim of furthering our understanding of this cell's complex biological machinery behind the differences in the sperm motility.

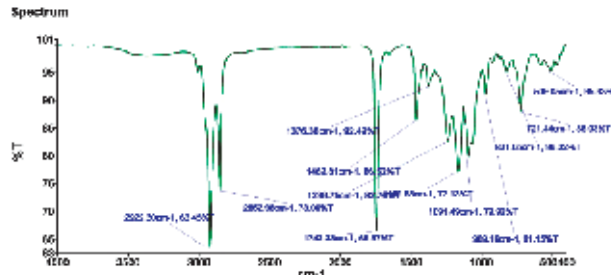
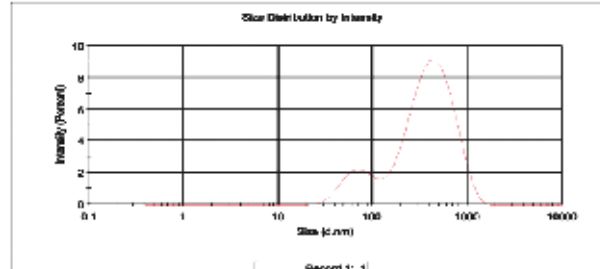
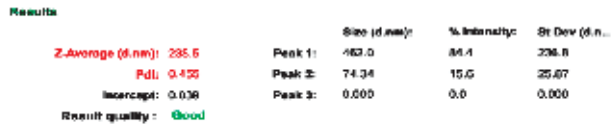
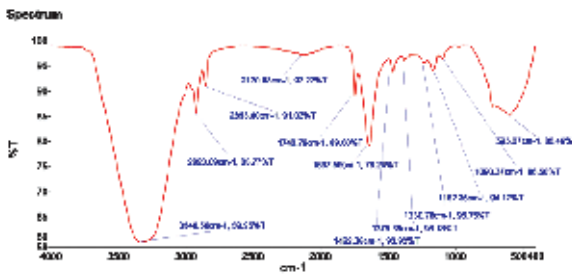
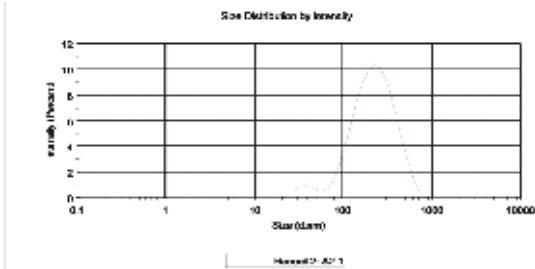
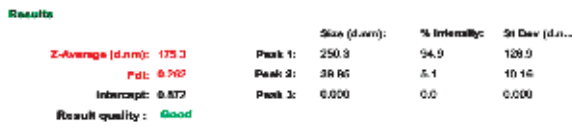
We were able to identify a total of 12627 peptides coding for 1550 proteins, out of which 77 and 405 are expressed exclusively for low and high sperm motile groups respectively. A total of 1068 proteins were commonly expressed between low and high motile sperm groups. 193 proteins were upregulated whereas 203 proteins were down regulated and 433 proteins were neutrally expressed. Gene ontology analysis for molecular and cellular processes revealed new information about the spermatid development, fertilization, glycolytic process, calcium ion binding, ATP binding and ATPase activity and receptors of stallion spermatozoa. Mitochondrial proteins and those involved in catabolic processes constituted dominant categories. Kegg pathway analysis revealed that the pathways involving oxidative phosphorylation, metabolic pathways were dysregulated in low motile stallion sperm group. Several enzymes specific to beta-oxidation of fatty acids, oxidative phosphorylation were identified, and further experiments performed to ascertain their functional significance.



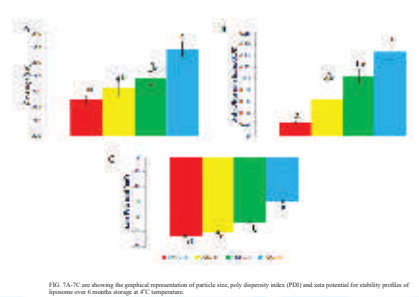
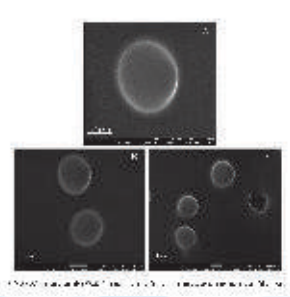
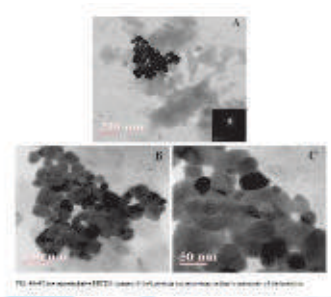
(TR Talluri, A Kumaresan, TK Bhattacharya, Mohd Kutty)

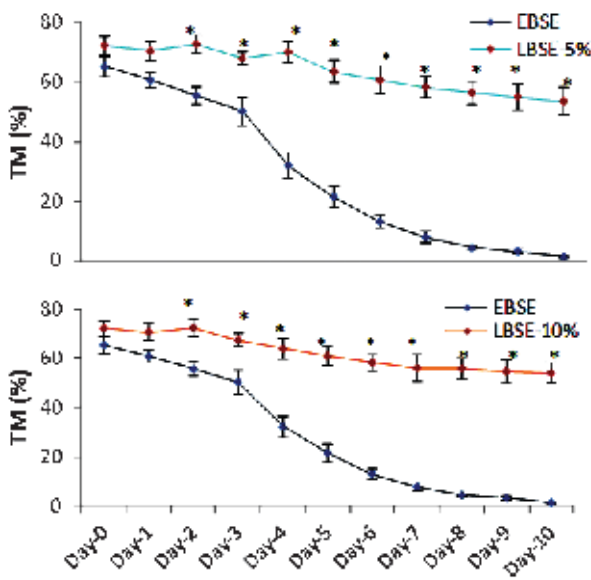
Development of a novel extender (non-egg yolk) for improving the shelf life of stallion sperm and enhancing the post thaw stallion sperm motility

To develop a new alternative semen extender, we explore the beneficial effect of modified antioxidant loaded nanoliposome derived from egg yolk phospholipids (lecithin) and cholesterol. The liposome based semen extender (LBSE) for stallion semen preservation at 4°C temperature provides better results of sperm motility and kinematics incompliance to egg yolk based semen extender. The liposome based semen extender maintains the stallion sperm motility over the ten days at 4°C temperature instead of egg yolk based semen extender (EBSE) which maintains the motility of stallion sperm only 2-3 days. The stability and purity of manufactured liposome by aforementioned protocol at 4°C temperature for 6 months is very good due to maintaining of low polydispersity index and higher amplitude of zeta potential of liposome particles. The efficiency of liquid preservation of stallion sperm with liposome based semen extender (LBSE) for 10 days at 4°C temperature is very good as compared to egg yolk based semen extender (EBSE).

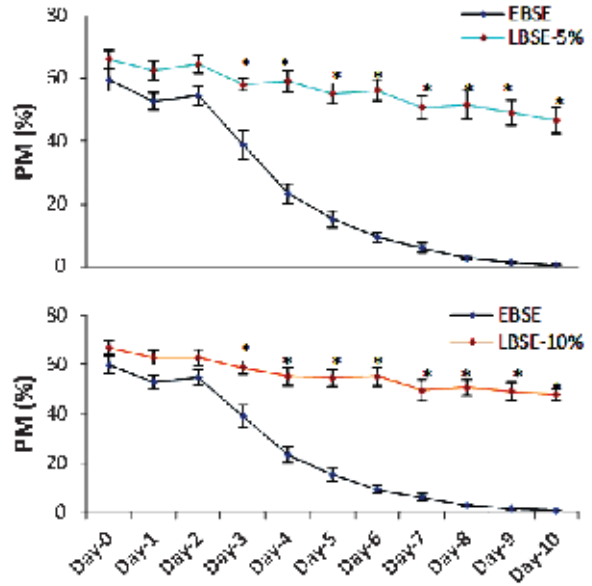


Stability profile of liposome for 6 months storage at 4 °C temperature				
Parameters	0 Month	1 Month	3 Month	6 Month
Z-average (nm)	217.31 ± 1.41 ^a	221.17 ± 2.56 ^b	224.71 ± 2.22 ^c	234.62 ± 2.37 ^c
Poly Dispersity Index (PDI)	0.024 ± 0.002 ^a	0.064 ± 0.031 ^{ab}	0.105 ± 0.012 ^{bc}	0.147 ± 0.015 ^c
Zetapotential (mV)	-52.94 ± 0.42 ^a	-50.42 ± 0.63 ^b	-43.97 ± 1.12 ^c	-29.74 ± 0.71 ^c
Stability	Stable	Stable	Stable	Stable





A



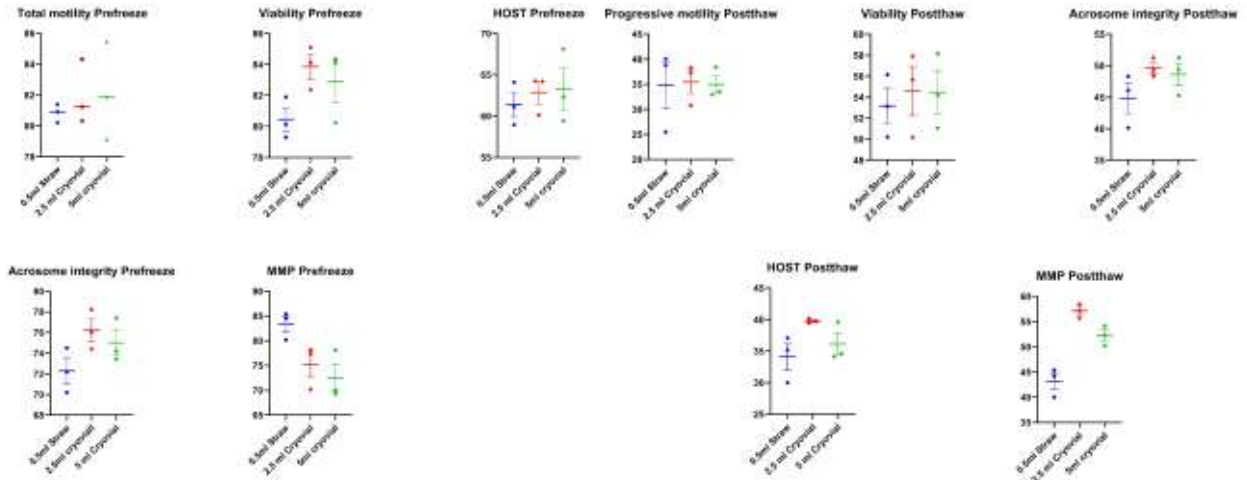
B

(TR Talluri, Pyarelal, NS Rathore, TK Bhattacharya)

Alternative methods of storage of equine semen

Routinely, we cryopreserve the stallion semen in 0.5 ml of medium French straws. For performing artificial insemination with frozen semen normally, we use 4-5 ml of frozen semen and we use 8-10 French medium straws at once. This procedure consumes much time in thawing as well as occupies more space in the cryotanks and it is not economical to use these many straws for single insemination. So in order to provide an alternative solution for the same we employed cryovials of 2.5 ml and 5 ml for effective storage of stallion semen. We studied various vital seminal parameters at pre-freeze and post thaw stage to find out if any difference occur due to variation in the storage devices. We have collected semen from 6 stallions and stored them in either 0.5 ml straws, 2.5 ml and 5 ml cryovials and studied the seminal parameters. After the analysis of vital seminal parameters we could not observe any significant differences in the sperm motility, acrosome integrity and sperm plasma membrane intact ness and integrity. This study encourages us to store the stallion semen in cryovials which will be much easier when performing the artificial insemination using frozen semen.



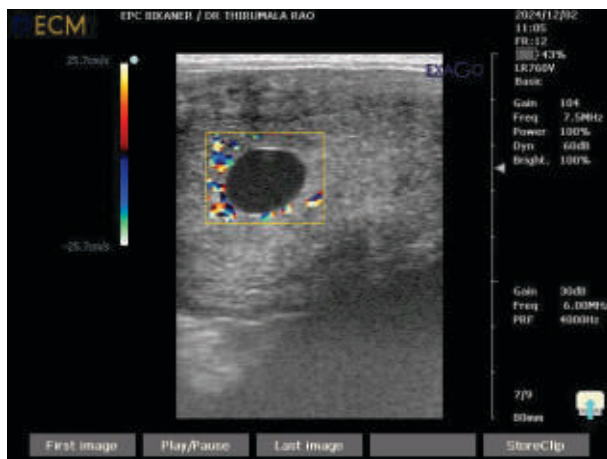


Seminal parameters at pre-freeze stage

Seminal parameters at post thaw stage

Standardization of deep horn intrauterine insemination with single straw in mares

In the current study, we selected 10 Marwari mares and closely monitored the estrus cycle of the mares. The mares were determined to inseminate with the elite stallion spermatozoa near to the ovulation using the single straw method with deep horn intrauterine insemination (DHI). All the 10 mares were inseminated with single straw having frozen thawed semen, while taking the inseminating catheter close to the ampullary isthmus junction and guided through placing another gloved hand in the rectum. Out of 10 mares, 8 mares got conceived successfully and are carrying the foals. One mare pregnancy got resorption during the study. Overall we could establish pregnancy in 8 mares with 80% success rate through DHI method of single straw insemination. This method of insemination can reduce the travel time of spermatozoa and effective use of germplasm of elite stallions.



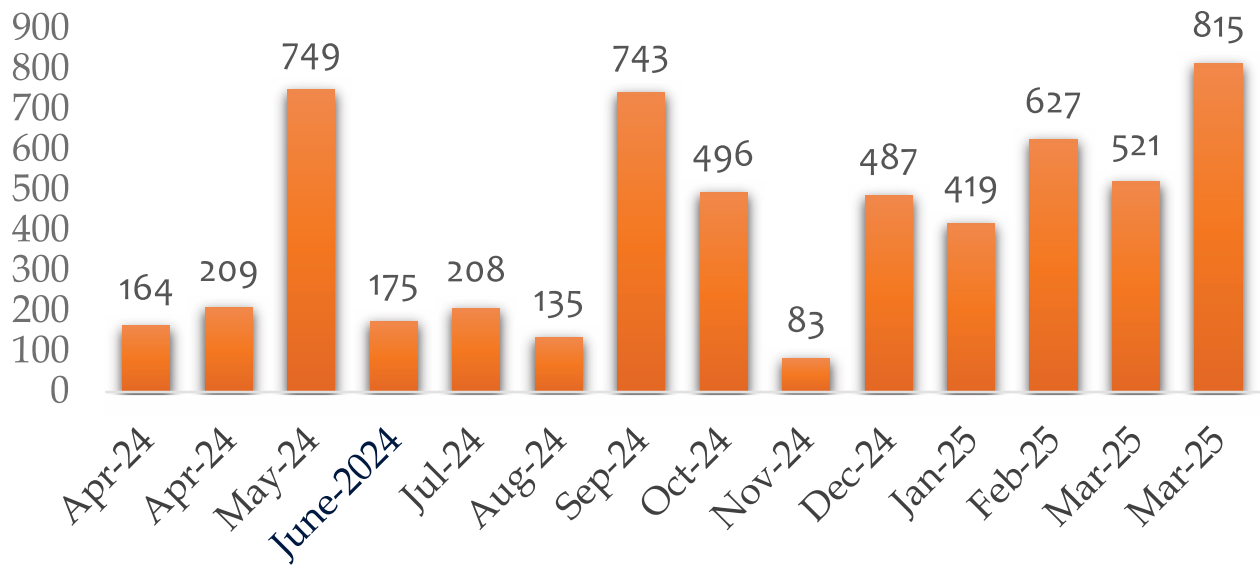
Ultra sonograms of pregnant mare uterus

(TR Talluri, RK Dedar, Mohd Kutty, SC Mehta, TK Bhattacharya)

Cryopreservation of germplasm/stallion semen and semen distribution

During the current year we have collected and cryopreserved a total of 5831 semen straws from Marwari, Zanskari, Manipuri and Halari donkeys. We have also distributed a sum of 540 semen straws to the paraveterinarians, veterinarians and stakeholders during the current year and generated a revenue of Rs. 54000/- through sale of germplasm.

ICAR-NRCE



■ No of straws cryopreserved

Semen Distributed and revenue generated 2024 – 2025

Month Year	Straw Number	Breed	Revenue generated
April 2024	30	Zanskari	3000
April 2024	300	Marwari	30000
May 2024	30	Zanskari	3000
July 2024	100	Marwari	10000
August 2024	20	Poitu (Donkey)	2000
October 2024	60	Marwari	6000
	540	Total revenue through sale of semen straws 2024-2025	54,000

(TR Talluri, Sajjan Kumar, SC Mehta, RK Dedar, TK Bhattacharya)

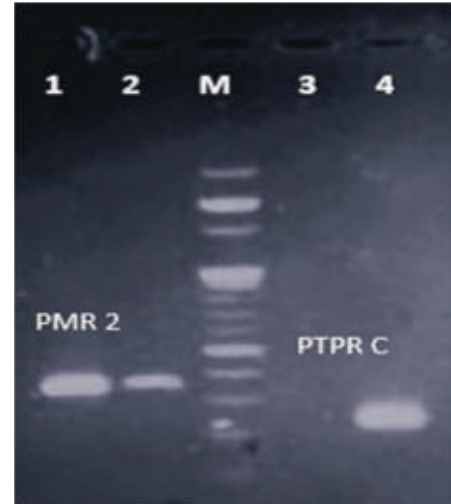
Technology transfer and outreach to the farmers/equine stakeholders

During the current year, we have visited Bengaluru, Khempur, Udaipur and adjacent areas to collect and cryopreserve the semen from the elite stallions. We have also established MoUs with equine entrepreneurs/stakeholders and assisted in establishing the private equine semen labs at the filed level. During the visits to various places, we have established the lab at filed level and collected the semen from elite stallions and cryopreserved. During the visit we facilitated th stakeholders in establishing the labs and cryopreservation of stallion semen. The techniques for preservation of stallion semen collection and cryopreservation were demonstrated at their doorstep and taught the procedures. The customized AV technology was also transferred simultaneously to the equine stake holders.

Analysis of freezability associated genes (*ProAKAP4*, *PLC zeta*, *SPATA1*, *INHBA* and *ZAM*) in fresh and frozen-thawed semen of equines for assessing freezability

Semen was collected from six stallions of Equine Production campus, Bikaner, following the SOPs. Expression studies

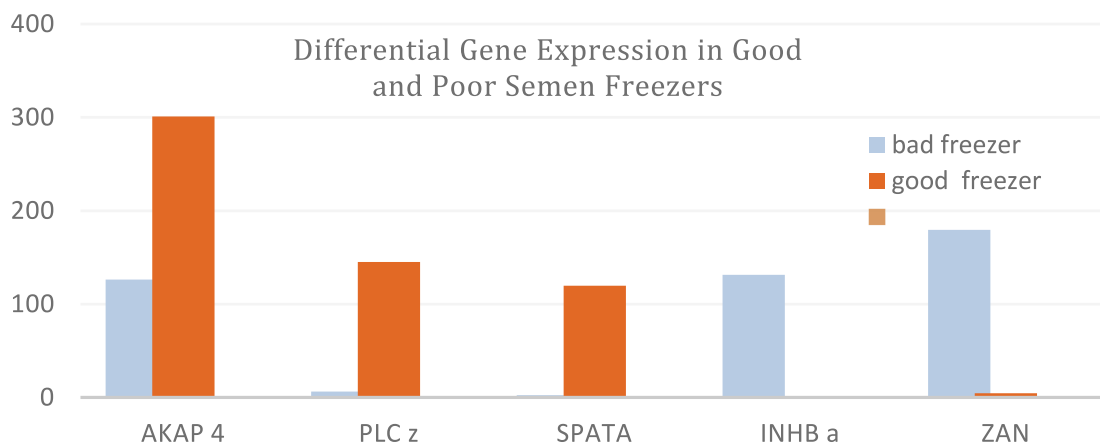
of AKAP 4, PLC zeta, INHB a, ZAN in fresh and frozen thawed semen carried using qPCR. The spermatozoa for total RNA isolation were collected through percoll gradient density separation from respective samples. Presence of gDNA and RNA from epithelial cells were tested using *PRM 2* and *PTCR C* genes. *PRM 2*, *PTCR C* are specific for spermatozoa and epithelial cells respectively. On analysis, it is found that genes *AKAP4*, *PLC zeta* and *SPATA* are more expressed in good freezers compared to bad freezers. Genes *INHBa* and *ZAN* found more expressed in bad freezers compared to good freezers.



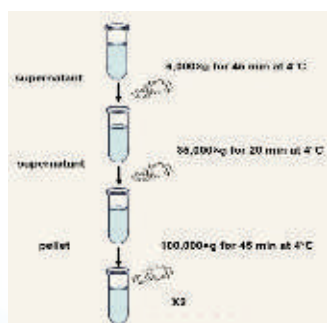
Confirmation of spermatozoal origin of RNA and absence of epithelial cell RNA and genomic DNA contamination: Lane 1&2 cDNA from spermatozoa RNA positive for *PRM 2* (167bp), Lane M 50bp marker, Lane 3 cDNA from spermatozoa RNA, Lane 4 cDNA from somatic cell RNA(147bp).

Isolation and characterisation of Exosomes from stallion seminal plasma

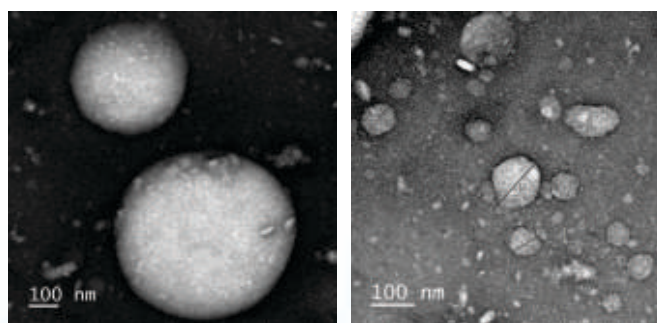
The protocol for isolation of exosomes from seminal plasma of equines were standardized. The seminal plasma was separated immediately after collection of semen by centrifugation at 6000Xg for 45 min at 4°C. Pellets were removed and supernatant subjected ultracentrifugation at 35000xg for 20 min at 4°C. The supernatant again centrifuged in ultracentrifuge for 100000xg 45 min at 4°C. the pellets were harvested and washed in ice cold PBS 100000xg 45 min at 4°C. Characterization of exosomes were carried using TEM analysis. Size of exosomes found less than 200 nm.



Graph 1: Fold change in expression of selected genes in fresh versus frozen-thawed semen



Protocol for isolation of Exosome vesicles (EV) from seminal plasma

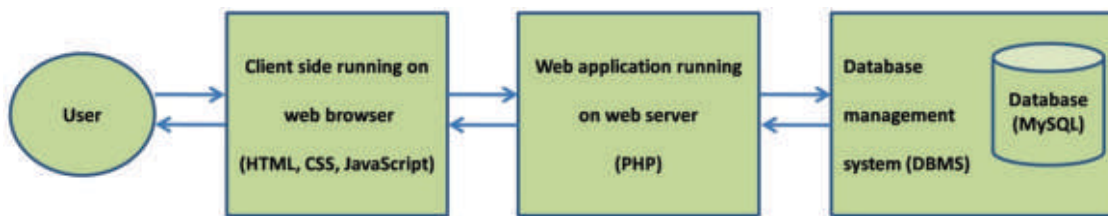


TEM characterization of EV from seminal plasma

(M. Kutty, TR Talluri, RK Dedar, SC Mehta, TK Bhattacharya)

Development of Equine CNVs database

The Equine CNVs database (EqCNVdb) is a web genomic resource that catalogues the identified CNVs in six horse breeds, namely, Manipuri, Zanskari, Bhutia, Spiti, Kathiawari and Marwari. The CNVRs and gene content of CNVRs are also included in the database. The EqCNVdb is based on a three-tier architectural database that houses detailed information on the horse CNVs, CNVRs and gene content within CNVRs. It includes details of 883 CNVs, 180 CNVRs and 434 genes, which were found within the CNVRs, from six horse breeds, namely, Manipuri, Zanskari, Bhutia, Spiti, Kathiawari and Marwari. It contains six tabs, that is, Home, CNVs, CNVRs, Gene Content, Analysis and Contacts. The *Home* page contains a brief description of the horse CNV database. Under the *CNV* page, the search for CNVRs can be made through CNV ID for chromosome-wise, location-wise and breed-wise CNVs. The *CNVR* page has been linked to find the CNVs and the associated genes where the user can again go for chromosome-wise, location-wise and breed-wise retrieval of CNVs. The CNVs were in the length range from 1kb to 4.3Mb nucleotides. The web interface for EqCNVdb and its user-friendly flexible search options for users are shown in figure. The EqCNVdb can be accessed at <http://backlin.cabgrid.res.in/eqcnvdb/>.

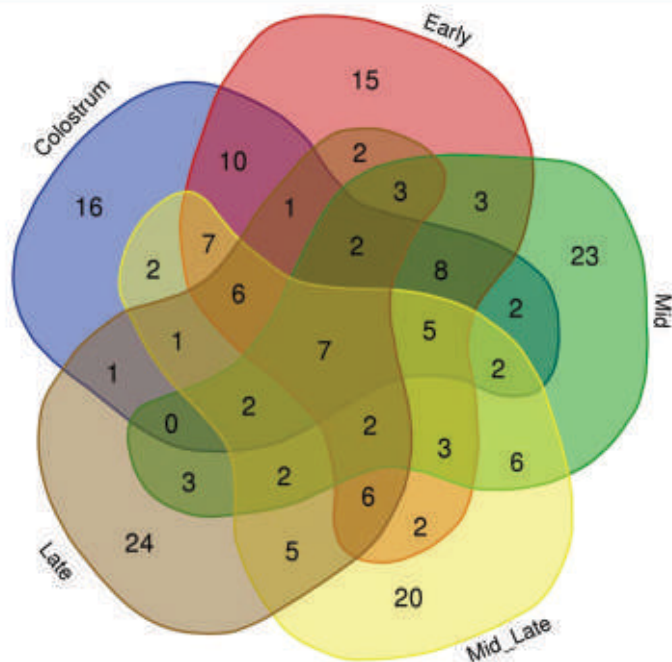


Three-tier architecture of horse CNV database (EqCNVdb).

(Anuradha Bhardwaj, Sarika, Yash Pal, M. A. Iquebal, Varij Nayan, U B Angadi, Shiv Kumar Giri, Anil Rai, Ram Avatar Legha, TR Taluri, Dinesh Kumar, TK Bhattacharya)

Milk Metabolome profiling in Indian Halari Jenny milk

Halari donkey jennies were milked for colostrum (0–5d postpartum) and mature milk (1-6 months of lactation) and samples were collected from Jenny Dairy Unit, NRCE Hisar, Haryana, and processed fresh for milk analyzer parameters or stored at -80 °C till further analysis for comprehensive metabolomics and proteome profiling throughout lactation stages. Also, the samples collected during both the morning and evening shifts were pooled. In the current study, milk samples were characterized for chemical composition, amino acids, and also through LC/MS for different metabolites and peptides in Colostrum, early lactation, mid lactation, mid to late lactation and late lactation stages. About 200 different metabolites were identified, 7 were common to all stages as shown in the venn diagram.



(Anuradha Bhardwaj, Varij Nayan, TR Talluri, RA Legha, Yash Pal, TK Bhattacharya, B N Tripathi)

Halari milk freeze dried powder

Samples of donkey milk obtained from nine healthy animals in mid lactation stage, were collected and stored in sterile HDPE bottles throughout the 21st to 30th week of lactation, during the months of August to October. The milk samples were pre-frozen at a temperature of -50°C for a duration of 12 hours. Following this, the samples were subjected to freeze-drying at a temperature of -80°C using the Christ Alpha 1–2 LDplus equipment. The dried donkey milk was crushed into a powder, sieved through a 100-mesh size, packaged, and kept under refrigerated conditions until further analysis. In current study, freeze-dried donkey milk powder (DMP) was analysed for its Biochemical, Dielectric, functional, Fourier Transform Infrared Spectroscopy and Surface Characteristics. DMP contains notably, high lactose content (62.09%), protein content was determined to be 21.49% with having relatively low-fat content (2.19%). Minerals, amino acid, and fatty acid profiles highlighted nutritional value of DMP. DMP had spherical shape particles with a smooth surface. The FTIR spectra analysis validated the existence of distinct functional groups in DMP. The potential of DMP as a leading candidate for the future of functional food is evident due to its exceptional functional and nutritive properties.

(Renu Garhwal, Anuradha Bhardwaj, Varij Nayan, Yash Pal, TK Bhattacharya)

Preparation of Yogurt from donkey milk

ICAR-NRCE and ICAR-NDRI in collaboration standardized the technology for the production Halari donkey milk-based yogurt. For this, investigations on the texture improvement of donkey milk yoghurt using ultrafiltration and hydrocolloids addition was carried out. The donkey milk was ultrafiltered to VRR 66% and retentate was heated to 85°C for 8 minutes, cooled to $37 \pm 1^{\circ}\text{C}$ and inoculated with desired concentration of starter. Among the various hydrocolloids investigated, only κ -carrageenan addition appeared promising in strengthening the gel structure. Different levels of κ -carrageenan (0.1-0.5%) were added along with fat replacers such as inulin and xanthan gum to provide the textural as well as sensorial attributes to donkey milk yoghurt. Based on findings, yoghurt with 0.3% κ -carrageenan and 0.3% inulin is considered the best product.

(Mananpreet Singh, Ashish Kumar Singh, Anuradha Bhardwaj, Varij Nayan, Yash Pal, TK Bhattacharya)

NATIONAL CENTRE FOR VETERINARY TYPE CULTURES

Authentication and accessioning of bacteria in NCVTC Repository

During the year 2024, a total of 134 bacterial cultures were processed at NCVTC, out of which 116 cultures were authenticated and accessioned into the bacterial repository. This has increased the total number of veterinary bacterial cultures preserved in the repository to 1,942. The cultures were mainly submitted by various collaborating institutions, including ICAR-IVRI (Izatnagar), College of Veterinary Sciences (Palampur), ICAR-CSWRI (Avikanagar), ICAR-NIVEDI (Bengaluru), Sri Venkateshwara Veterinary University (Tirupati), Bihar Animal Sciences University (Patna), RAJUVAS (Bikaner), SKUAST (Jammu), Christian Medical College (Vellore), SRDDL-IAH&VN (Bengaluru), College of Veterinary and Animal Sciences (Udgir and Navania, Udaipur), in addition to the NCVTC Bacteriology Laboratory. However, the majority of the cultures accessioned during this period were contributed by NCVTC, NRCE, Hisar. In addition to accessioned cultures, bacterial isolations were also carried out in-house using various clinical and post-mortem samples received at the NCVTC laboratory. These included nasal swabs, post-mortem tissues, organ samples, stomach contents, uterine swabs, tracheal lavage, skin scrapings, mastitic milk samples, and samples from canine post-mortems. A total of 295 isolates were cryopreserved in the repository under the general category of bacterial preservation.

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Molecular identification using 16S rRNA sequencing

Bacterial isolates, both from accessioned cultures and those received from network units or isolated at NCVTC, were identified through 16S rRNA gene amplification followed by sequence alignment and phylogenetic analysis. Some of the significant bacterial species identified by 16S rRNA sequencing include: *Staphylococcus canis* (Ch20), *Staphylococcus lloydii* (Ch21C), *Staphylococcus capitis* ssp. *urealyticus* (Ch21E), *Staphylococcus caledonicus* (Ch22; BAA218), *Staphylococcus aureus* (Eq639), *Staphylococcus coagulans* (BAA64; 614A), *Staphylococcus argenteus* (BAA887), *Corynebacterium flavescens* (Ch21B), *Vagococcus luciliae* (Eq561), *Arthrobacter gandavensis* (Eq581), *Mammaliococcus sciuri* (Eq584, Eq614), *Streptococcus equi* ssp. *equi* (Eq701), *Pseudomonas aeruginosa* (Eq570), *Brevundimonas faecalis* (Eq584), *Pantoea vagans* (Eq587), *Empedobacter brevis* (Eq613), *Pseudomonas hunanensis* (Eq613A), *Myroides odoratus* (Eq613B), *Branhamella ecdela* (Eq673), *Bacillus mediterraneensis* (BAA915), *Lysinibacillus fusiformis* (BAA952), *Moraxella tetradonis* (Eq4), and multiple isolates of *Brucella abortus* (BAA227, BAA228, BAA465, BAA466, BAA1072, BAA1074, RR/2024/25, RR/2024/156), *Salmonella enterica* ssp. *enterica* (BAA156), *Shigella flexneri* (BAA162), *Pasteurella multocida* ssp. *gallicida* (RR/2022/172), and *Comamonas terrigena* (RR/2025/23).

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Phenotypic and biochemical characterization

Several bacterial isolates were also identified based on phenotypic traits and standard biochemical assays. These included *Brevibacterium* spp. (Eq567), *Pseudomonas putida* (Eq568, Eq638B), *Pseudomonas stutzeri* (Eq645), *Pseudomonas aeruginosa* (Eq570, Eq624A, Eq641), *Pseudomonas fluorescens* (Eq613A, Eq711, Eq692), *Comamonas testosteroni* (Eq570A, Eq619A, Eq700A), *Achromobacter denitrificans* (Eq572), *Ochrobactrum anthropi* (Eq573), *Rhizobium radiobacter* (Eq574B, Eq710A), *Pasteurella* spp. (Eq585C), *Pantoea* spp. (Eq587), *Stenotrophomonas maltophilia* (Eq606, Eq608, Eq628, Eq631A, Eq632, Eq644A), *Streptococcus equi* ssp. *zoepidemicus* (Eq606A, Eq627), *Streptococcus dysgalactiae* ssp. *equisimilis* (Eq668A, Eq670, Eq676, Eq677B, Eq681, Eq682, Eq623A, Eq626), *Streptococcus* group L (Eq624B), *Enterobacter cloacae* (Eq621, Eq653B), *Staphylococcus aureus* (Eq622C), *Aeromonas hydrophila* (Eq629, Eq652), *Chryseobacterium indologenes* (Eq630, Eq632A, Eq635, Eq673A, Fo153), *Brevundimonas vesicularis* (Eq634), *Ralstonia pickettii* (Eq636B, Eq639A), *Weeksella virosa* (Eq637B), *Morganella*

morganii (Eq647), *Moraxella* spp. (Eq657A), *Alcaligenes faecalis* (Eq658C, Eq681A, Eq681B), *Sphingobacterium multivorum* (Eq662), *Rahnella aquatilis* (Eq673), *Enterococcus faecium* (RR|2024|188), *Trueperella pyogenes* (Bi55), *Rhodococcus* spp. (Eq697A), and *Staphylococcus lugdunensis* (Bi54).

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Expansion of genus-level diversity in the NCVTC Repository

As part of its ongoing exploration and biodiversity enhancement efforts, the NCVTC Bacteriology Laboratory continues to isolate, identify, and characterize bacterial strains from animal-origin and environmental samples. These efforts aim to expand genus-level diversity within the repository, thereby improving the overall taxonomic representation of veterinary microbes. During the reporting period, several bacterial genera previously unrepresented in the NCVTC repository were successfully isolated, identified, or acquired. These included: *Planococcus plakortidis*, *Kocuria polaris*, *Priestia flexa*, *Silvania confinis* (Oa70A), *Franconibacter helveticus* (Eq507), *Jeotgalibaca arthritidis* (Eq535), *Microbacterium esteraromaticum* (Eq535A), *Luteimonas aestuarii* (Eq535C), *Pseudarcobacter venerupis* (Eq537A), *Prescottella equi* (Eq539), and *Leclercia tamurae* (Oa70). These isolates were obtained from a variety of clinical and environmental sources and represent important additions to the genus-level diversity of the repository. Notable features of these genera include environmental and opportunistic pathogens, novel species, and reclassified taxa such as *Prescottella equi* (formerly *Rhodococcus equi*), a known cause of foal pneumonia, and *Franconibacter helveticus*, which may harbor resistance genes. The inclusion of these taxa strengthens the biodiversity representation and research utility of the NCVTC bacterial repository.

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Bacteriological profiling and identification of opportunistic pathogens from equine clinical samples

As part of routine diagnostic services at the NCVTC Bacteriology Laboratory, bacteriological investigations were carried out on nasal swabs and various clinical samples received from equines. Bacteria were isolated on standard culture media and identified using phenotypic and biochemical methods. In cases involving animals undergoing treatment, antimicrobial susceptibility reports were provided to optimize therapeutic protocols. During the year, a total of 126 bacterial isolates were identified from clinical samples. The predominant isolates included *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Pseudomonas* spp., *Corynebacterium* spp., *Nocardia* spp., *Escherichia coli*, *Ralstonia pickettii*, *Streptococcus dysgalactiae* ssp. *equisimilis*, *Pantoea* spp., *Chryseobacterium indologenes*, *Klebsiella pneumoniae*, *Acinetobacter* spp., and yeast species. A significant observation was the high frequency of isolation of *Stenotrophomonas maltophilia* and *Nocardia* spp. *Nocardia*, a Gram-positive, filamentous, branching bacterium belonging to actinomycetes, is an opportunistic pathogen commonly found in soil. It typically infects animals via inhalation or wound contamination and was previously reported by this laboratory from a case of granulomatous pneumonia in a Marwari horse (*Nocardia otitidiscaviarum*). Similarly, *Stenotrophomonas maltophilia* is gaining importance as an emerging respiratory pathogen in equines, particularly associated with lower airway diseases. Other opportunistic pathogens isolated included *Aeromonas hydrophila*, *Enterobacter cloacae*, *Streptococcus equi* ssp. *zooepidemicus*, *Achromobacter denitrificans*, *Staphylococcus lugdunensis*, *Weeksella virosa*, *Morganella morganii*, *Sphingobacterium multivorum*, and *Rahnella aquatilis*. These findings underscore the increasing diversity of bacterial pathogens in equine clinical infections and highlight the need for continued surveillance and precise identification for appropriate therapeutic management.

(R.K. Vaid, Sumanshu, Taruna Anand, Nitin Virmani)

Isolation of a *Moraxella* spp. from Zebra

A bacterial strain was isolated from a vaginal swab sample of a zebra received at the NCVTC Bacteriology Laboratory. The organism grew as small grey colonies within 24 hours on Chocolate Blood Agar. It was further sub-cultured on Nutrient Agar and MacConkey Lactose Agar, where it exhibited late lactose fermentation. The isolate was catalase-positive, oxidase-negative, and non-motile by hanging drop examination. Microscopically, the organism appeared as Gram-negative cocci, predominantly in pairs. A partial 16S rRNA gene sequence (1437 bp; 98.7% complete) of the isolate showed 96.69% identity with *Moraxella tetraodonis* strain PS-22, a novel species recently reported in 2022 from the skin of fresh water pufferfish (*Tetraodon cutcutia*). API 20NE biochemical profiling of the isolate showed a low identification probability (25.6%). Given the 16S rRNA identity of 96.69%, the isolate is likely to be a novel strain of *Moraxella* spp. However, further polyphasic taxonomic characterization is required for precise classification. The genus *Moraxella* comprises over 34 species within the family *Moraxellaceae*, several of which are of clinical importance. These include *Moraxella catarrhalis* in humans, *M. oculi* in cattle with infectious bovine keratoconjunctivitis, *M. nasovis* in sheep with respiratory illness, and *M. equi* from horses with conjunctivitis. The relatively low sequence identity with *M. tetraodonis* and distinct biochemical profile suggest that the zebra isolate may represent a novel strain or a closely related species within the genus *Moraxella*. However, further polyphasic taxonomic characterization is required to confirm its classification

(R.K. Vaid, Sumanshu, Taruna Anand, Nitin Virmani)

Authentication and accessioning of viruses of animal origin

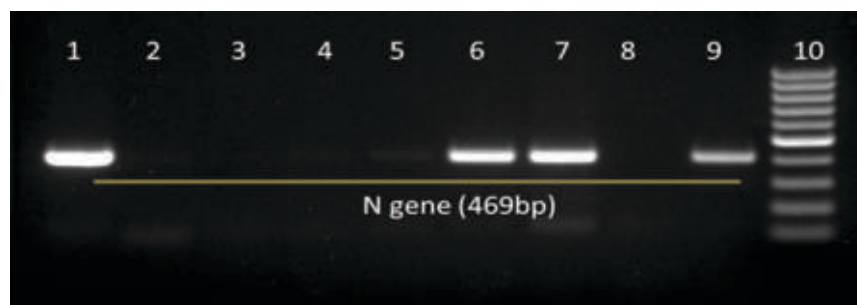
The NCVTC virus repository is being enhanced by incorporating viruses collected from various geographical regions across the country through the deposition and collection of isolates and clinical samples from different animal species and poultry. The primary objectives of this repository include the isolation and characterization of animal-origin viruses, accessioning, preservation, database development of the isolates, and the distribution of authenticated virus isolates and cell lines to relevant stakeholders. The collected samples were processed for virus isolation, with the details of virus authentication and isolation summarized as follows. A total of 28 viral isolates were received as deposits from NCVTC Network units. These deposits included Avian Infectious Bronchitis (n=4), Fowlpox Virus (n=2), Marek's Disease Virus (n=2), Fowl Adenovirus (n=2), *Peste des Petits Ruminants* (PPR) Virus (n=9), Bovine Rotavirus (n=7), and Equine Rotavirus (n=2). These samples and isolates were processed for authentication and accessioning of the respective viruses. A total of 18 viruses were accessioned (VTCC AVA 385 - 402) into the repository, comprising Avian Infectious Bronchitis Virus (IBV) (n=3), PPR Virus (n=6), Bovine Rotavirus (n=7), and Equine Rotavirus (n=2). The remaining viruses are currently undergoing processing to confirm their viability. Additionally, bulk production and preservation of 30 accessioned viruses (10 vials each) were conducted. These included Newcastle Disease Virus (NDV) (n=8), Fowlpox Virus (n=2), Infectious Bronchitis Virus (n=6), Avian Nephritis Virus (n=4), Chicken Astrovirus (n=4), Swinepox Virus (n=1), Pigeonpox Virus (n=1), Duck Plague Virus (n=1), and Infectious Bursal Disease Virus (n=3).

Furthermore, 45 previously preserved viruses were revived and evaluated for viability, including Newcastle Disease Virus (n=8), Swinepox Virus (n=1), Lumpy Skin Disease Virus (n=1), Bluetongue Virus (n=9), Infectious Bursal Disease Virus (n=3), Avian Nephritis Virus (n=4), Infectious Bronchitis Virus (n=6), Buffalopox Virus (n=10), African Swine Fever Virus (n=1), Classical Swine Fever Virus (n=1), and Foot and Mouth Disease Virus (n=1). All revived viruses were found to be viable. During this period, several viruses, including African Swine Fever Virus (distributed to M/s Biovet Pvt Ltd) and Lumpy Skin Disease Virus (distributed to the Institute of Life Sciences, Bhubaneswar, Orissa), were provided to different stakeholders. Additionally, various cell lines, including Vero and BHK-21 (from SKUAST, Jammu), NLBK and BHK-21 (from ICAR-NRCE), PK-15 (from LUVAS, Hisar), and BKH-21 (from M/s Biovet Ltd), were distributed to stakeholders.

(Riyesh T, Naveen Kumar, Taruna Anand, BC Bera and Sanjay Barua)

Identification of Bovine coronavirus (BCoV) and Bovine torovirus (BToV)

Biological samples, including nasal swabs and fecal samples, were collected from cattle exhibiting clinical signs of respiratory illness and diarrhea in the states of Haryana, Rajasthan, and Punjab. A total of 94 nasal swabs and 156 fecal samples were processed for the detection of viral pathogens. Nucleic acids were extracted and subjected to virus-specific RT-PCR/PCR assays targeting bovine coronavirus (BCoV) and bovine torovirus (BToV). Among the tested samples, 10 were found positive for BCoV and 7 for BToV. To confirm the viral identity, gene-specific PCR amplicons were sequenced. A 469 bp fragment of the nucleocapsid (N) gene of BCoV and a 741 bp fragment of the spike (S) gene of BToV were amplified and sequenced. Nucleotide sequence analysis using NCBI-BLAST revealed that the BCoV sequences shared 98.6–99.0% identity with reference BCoV strains, while the BToV sequences showed 99.8% identity to known BToV sequences. These findings confirm the circulation of both BCoV and BToV in cattle populations of northern India and highlight the importance of molecular surveillance in the diagnosis of enteric and respiratory diseases in livestock.



Detection of BCoV by PCR amplification of N gene (469bp)



Detection of BCoV by PCR amplification of S1 gene (741bp)

(B.C. Bera, Taruna Anand and Nitin Virmani)

Standardization of Isothermal RPA Assay for the Detection of ILTV and BCoV

An isothermal Recombinase Polymerase Amplification (RPA) assay was standardized for the rapid detection of Infectious Laryngotracheitis Virus (ILTV) and Bovine Coronavirus (BCoV). For ILTV, primers and probes targeting highly conserved regions of the viral genome were designed and synthesized. The isothermal amplification was successfully carried out at 39 °C for 20 minutes using a thermal block, and the expected amplicon was confirmed by gel electrophoresis. Similarly, multiple sets of primers and probes targeting conserved genomic regions of BCoV were designed and evaluated. The assay was optimized under the same isothermal conditions (39 °C for 20 minutes), resulting in successful amplification of the target regions, as evidenced by the presence of the expected bands in agarose gel electrophoresis.

Detection and isolation of Infectious Laryngotracheitis Virus (ILTV) from clinical cases

Clinical samples were collected from 7–10-day-old chicks exhibiting respiratory symptoms from poultry farms in Haryana. A total of 56 samples were processed for the detection of major respiratory viruses, including Infectious Laryngotracheitis Virus (ILTV), Infectious Bursal Disease Virus (IBDV), Infectious Bronchitis Virus (IBV), and Newcastle Disease Virus (NDV), using PCR, RT-PCR, qPCR, and qRT-PCR techniques. Among these, two samples tested positive for ILTV based on PCR amplification of the ICP4 gene. Tracheal tissues from the ILTV-positive birds were homogenized and inoculated into the chorioallantoic cavity of 10-day-old specific pathogen-free (SPF) embryonated chicken eggs. After 3 days of incubation, the embryos were harvested, frozen, and the allantoic fluids and whole embryos were collected. These materials were subjected to further inoculation into fresh embryonated eggs for three blind passages. The presence of ILTV was confirmed by the appearance of characteristic pock-like lesions on the chorioallantoic membrane and by PCR amplification of the ILTV-specific ICP4 gene (668 bp).



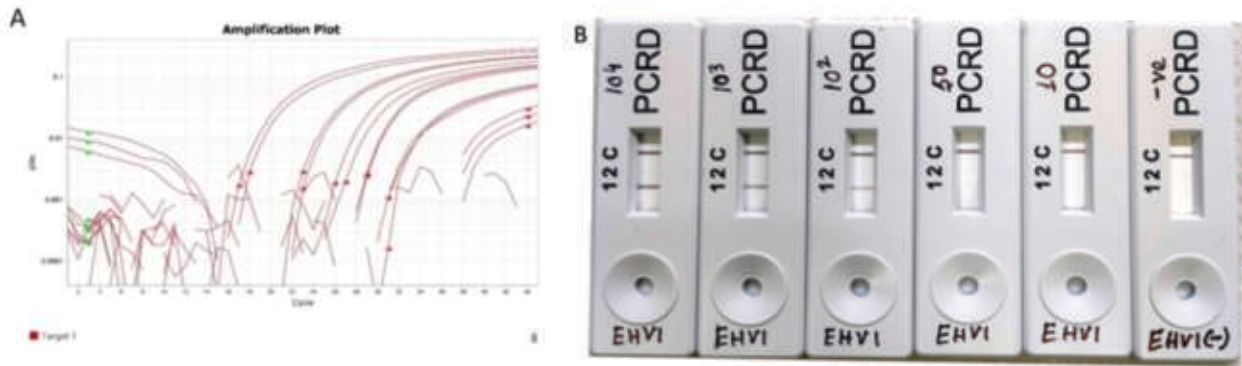
Detection of ILTV in clinical samples by PCR amplification of ICP4 gene (688bp)

Isolation of ILTV in chick's embryo showing ILTV-specific plaques in chorioallantoic membrane

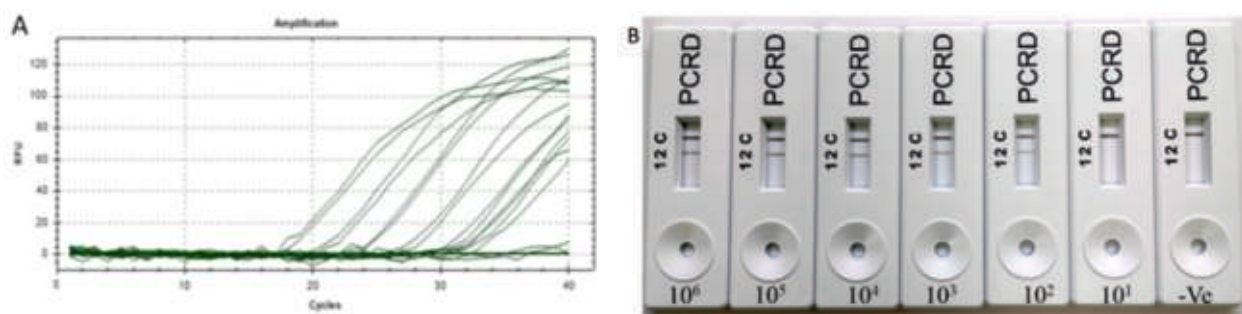
(BC Bera, Taruna Anand, MR Reddy & TK Bhattacharya)

Development of an *nfo* probe-based RPA-LFA assay for detection of EHV-1 and EHV-4 nucleic acids

Recombinase Polymerase Amplification combined with Lateral Flow Assay (RPA-LFA) was developed for the rapid detection of Equine Herpesvirus-1 (EHV-1) and Equine Herpesvirus-4 (EHV-4) nucleic acids using specific sets of primers and *nfo* probes. The sensitivity of the RPA assay was evaluated using cloned plasmids containing the target regions, and the detection limit was compared with that of quantitative PCR (qPCR). The RPA-LFA assay successfully detected as low as 100 copies of both EHV-1 and EHV-4, whereas the detection limit of the corresponding qPCR assays was 10 copies. The diagnostic performance of the developed RPA-LFA assay was further validated using 10 field samples previously confirmed as EHV-1 positive. All samples tested positive by the RPA-LFA assay, demonstrating its reliability and field applicability. To enhance point-of-care testing (POCT) utility, the RPA-LFA assay was further optimized for direct detection from crude lysates without the need for nucleic acid purification. For this, purified viruses were spiked into collection medium and treated with lysis buffer, followed by incubation at 98 °C for 5 minutes. The supernatant from the crude lysate was directly used in the RPA assay, yielding clear, specific bands on LFA strips. This optimized protocol allows for the rapid and sensitive detection of EHV-1 and EHV-4 directly from clinical samples, making it suitable for field-level diagnostics.



Evaluation of detection limit of RPA-LFA assay for detection of EHV1. A. qPCR assay for detection of EHV1. B. RPA-LFA assay for detection of EHV1.

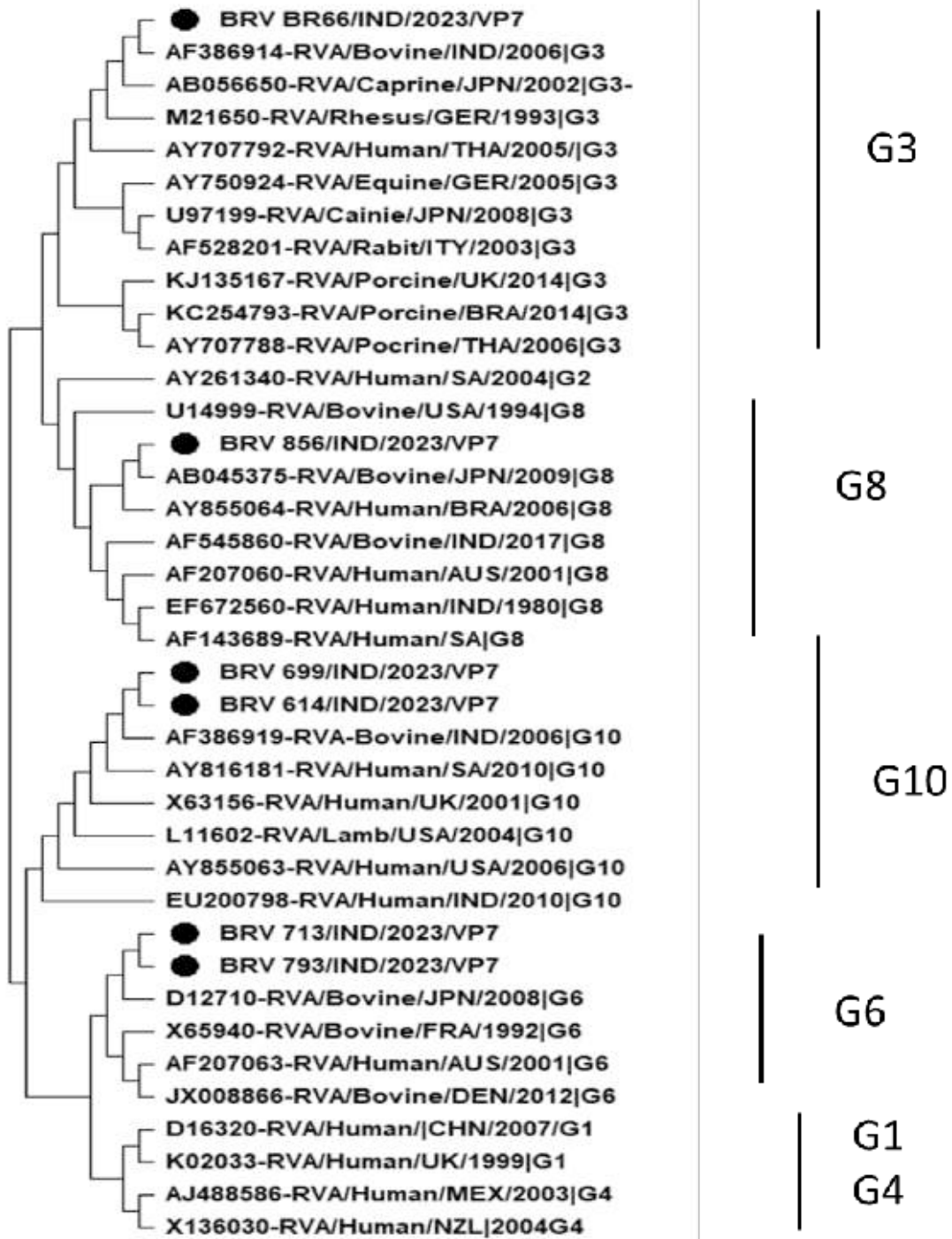


Evaluation of detection limit of RPA-LFA assay for detection of EHV4. A. qPCR assay for detection of EHV4. B. RPA-LFA assay for detection of EHV4.

(BC Bera, Nitin Virmani & Taruna Anand)

Isolation and phylogenetic analysis of rotaviruses from diarrheic calves

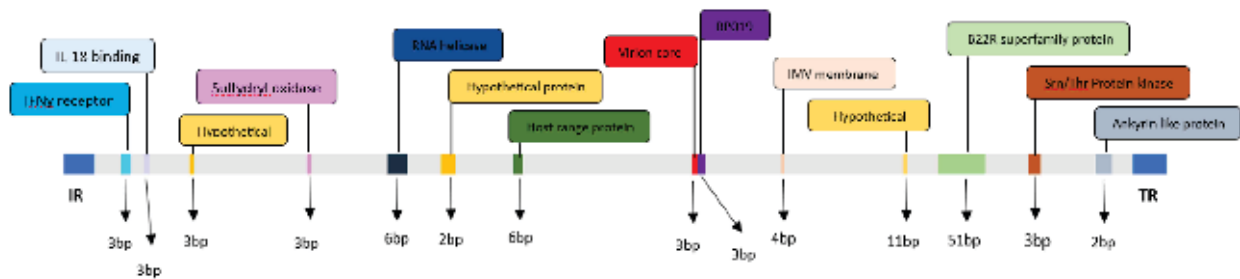
A total of 90 fecal samples were collected from diarrheic cattle ($n=82$) and buffaloes ($n=8$) from organized livestock farms, gaushalas, and equine farms across Haryana and Punjab. Viral RNA was extracted from each sample and subjected to TaqMan-based quantitative reverse transcription PCR (qRT-PCR) for the specific detection of rotaviruses. Out of 90 samples, 8 samples from cattle were found positive for rotavirus, while none of the samples from buffaloes or equines tested positive. The total RNA from qRT-PCR positive samples was analyzed by polyacrylamide gel electrophoresis (PAGE) to confirm the presence of rotavirus genomic segments. Five qRT-PCR-positive samples displayed the characteristic 11-segment RNA profile of group A rotaviruses (RVA), confirming rotavirus infection. For virus isolation, a total of 18 rotavirus-positive samples (including both newly collected and archived samples) were inoculated into MA104 cell monolayers maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS), and incubated at 37°C in a 5% CO_2 atmosphere. The cultures were monitored for cytopathic effect (CPE) and passaged up to five times. Four rotavirus isolates were successfully obtained and confirmed by qRT-PCR and RNA-PAGE analysis. For genotypic characterization, the VP7 gene sequences of the bovine rotavirus (BRV) isolates were analyzed. Nucleotide sequences were aligned using MEGA software, and a phylogenetic tree was constructed using the Maximum Likelihood method. The analysis revealed that the Indian BRV isolates clustered with G3, G6, G8, and G10 genotypes. Furthermore, the isolates showed genetic relatedness to rotavirus strains from bovine, caprine, canine, and human origins, suggesting interspecies transmission and genetic diversity among circulating BRV strains in India.



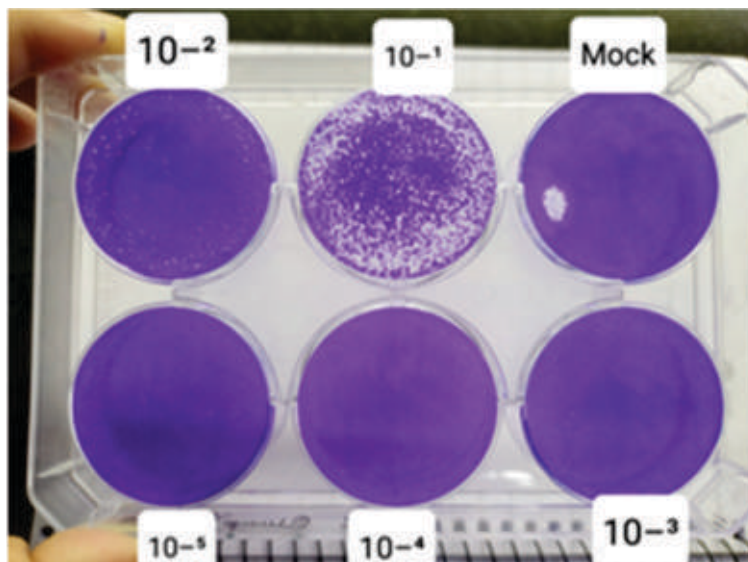
(B.C. Bera and Anubha Pathak)

Characterization of swinepox virus for attenuated candidate vaccine development

Genomic changes and viral quantification- To develop an attenuated candidate vaccine virus, the swinepox virus isolated at NCVTC was passaged in PK-15/primary cells. The virus was passaged up to the 30th passage, and whole genome sequencing (WGS) was conducted to analyze genomic changes in swinepox virus (SWPV) following sequential passage through cell lines. Sequences from passages P1, P10, and P30 were analyzed, with results obtained for P10 and P30, while sequencing for P1 did not yield reliable results. The WGS aimed to determine the nucleotide order and identify variations across passages. To assess genomic differences, the sequences of P10 and P30 were aligned using ClustalW, achieving over 85% coverage when compared to the reference sequence (MW036632.1). Compared to P10, the P30 virus exhibited significant nucleotide changes, including 95 transitions, 32 transversions, and nucleotide deletions in various genes and hypothetical genes. These changes are shown in the accompanying figure. Additionally, a plaque assay was standardized to improve viral quantification. Although the virulent virus typically does not form plaques, the assay was optimized by passaging the virus in cell lines and adjusting the agar concentration in the overlay. Microplaques began to appear by the 7th day post-infection (dpi), with viral quantification conducted on day 9, resulting in a calculated viral titer of 8.2×10^4 PFU/mL.



Schematic representation of the Swinepox Virus (SWPV) genome highlighting major genetic changes observed in the passaged virus (SWPV-P30) compared to the standard reference strain. Notable mutations, insertions, and deletions were identified in SWPV-P30, suggesting genomic alterations during serial passaging.

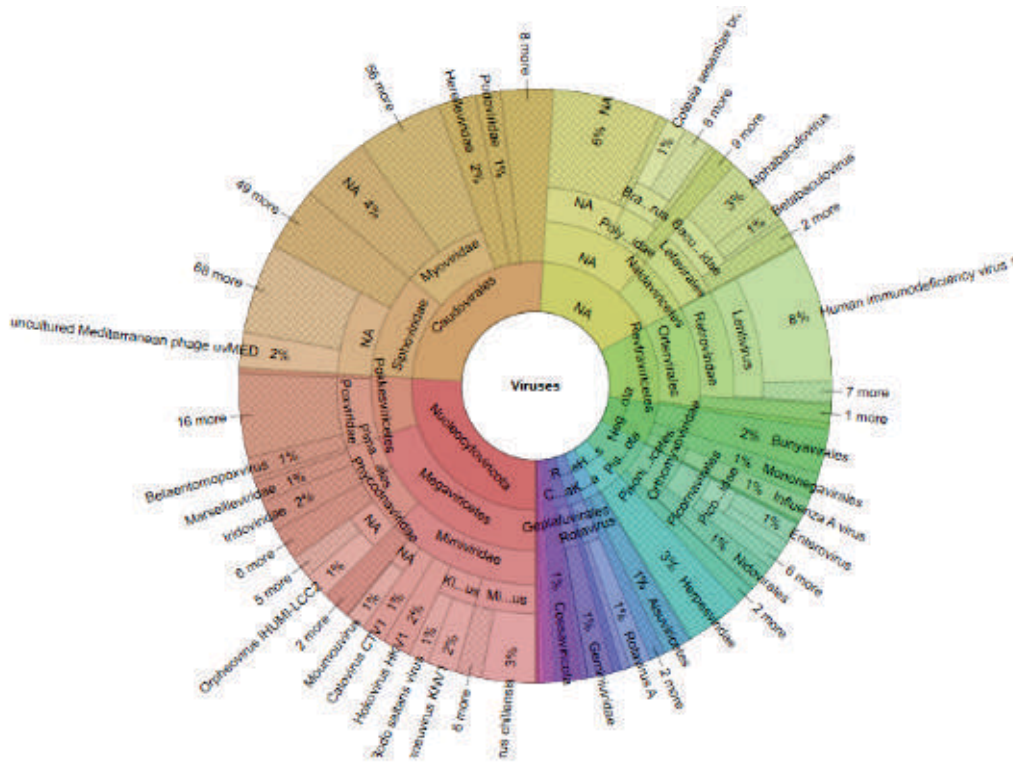


Standardization of plaque assay for Swinepox Virus (SWPV) in porcine stable cells. The virus produced distinct microplaques upon infection, indicating successful viral replication and cytopathic effect in the cell monolayer.

(Riyesh T, Shanmugasundaram K, Nitu Kharb, Naveen Kumar, Sanjay Barua, TK Bhattacharya)

Metagenomic analysis of the bat virome in Haryana

This study explored the viral diversity in bats from Haryana using metagenomic sequencing of guano samples collected non-invasively from four bat colonies—two frugivorous (Rohtak Lake, Bhiwani Zoo) and two insectivorous (Gujri Mahal, Hisar; Bikaner Fort, Rajasthan). A total of 200 samples (50 per site) were pooled and processed at the BSL-3 Microbial Biocontainment Facility, ICAR-NRCE, Hisar. DNA and RNA were extracted and used to prepare eight libraries (four DNA and four RNA), generating 34.44 million paired-end reads, of which 98.14% were high quality (Q > 30). Taxonomic analysis revealed that 40–50% of reads could be classified, including 15,221 viral reads (13,246 from DNA and 1,975 from RNA). Viral abundance was highest in insectivorous bat colonies (Gujri Mahal: 2.7%, Bikaner Fort: 0.3%) compared to frugivorous ones (Bhiwani Zoo: 0.2%, Rohtak Lake: 0.17%). Most viral reads were bacteriophages (e.g., Myoviridae, Siphoviridae), followed by insect, mammalian, and plant viruses. Detected mammalian virus families included *Poxviridae*, *Herpesviridae*, *Coronaviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Flaviviridae*, *Retroviridae*, and *Reoviridae*. This study highlights the virome complexity in bats and underscores their role as reservoirs of emerging viruses, emphasizing the need for continued surveillance to assess potential zoonotic threats.



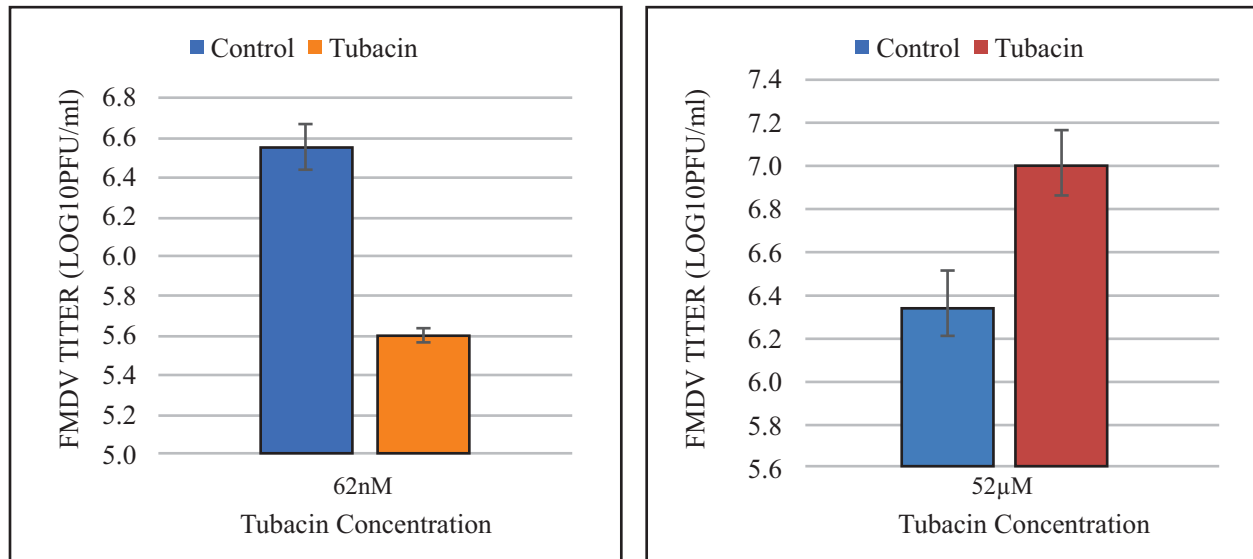
Krona plot illustrating the viral diversity identified in guano samples from the insectivorous bat colony at Gujri Mahal, Hisar. The plot represents the taxonomic distribution of the viral sequences, highlighting the relative abundance of different viral families detected through metagenomic sequencing.

(Riyesh T, Shanmugasundaram K, Garvit Kumar, Shreya Prasher, Naveen Kumar, Sanjay Barua, RK Vaid and TK Bhattacharya)

Tubacin modulates FMDV replication in a dose-dependent manner

Foot-and-mouth disease virus (FMDV) causes a highly contagious disease in cloven-hoofed animals. HDAC6, a cytoplasmic deacetylase, plays a key role in host cellular processes by targeting non-histone proteins such as HSP90 and α -tubulin. Tubacin, a selective HDAC6 inhibitor, was assessed for its impact on FMDV replication. The study

demonstrated a dual, dose-dependent effect of Tubacin on FMDV. At a lower concentration (62nM), Tubacin exhibited antiviral activity, significantly reducing viral replication. In contrast, a higher concentration (5 μ M) enhanced FMDV replication, suggesting a proviral effect, possibly due to off-target inhibition of other HDAC isoforms (e.g., HDAC5 and HDAC9). These findings indicate a complex regulatory role of HDAC6 in FMDV infection and underscore the need for careful dose optimization in exploring HDAC6 as a therapeutic target. Ongoing studies aim to elucidate the host-virus interaction pathways involved in this biphasic response.



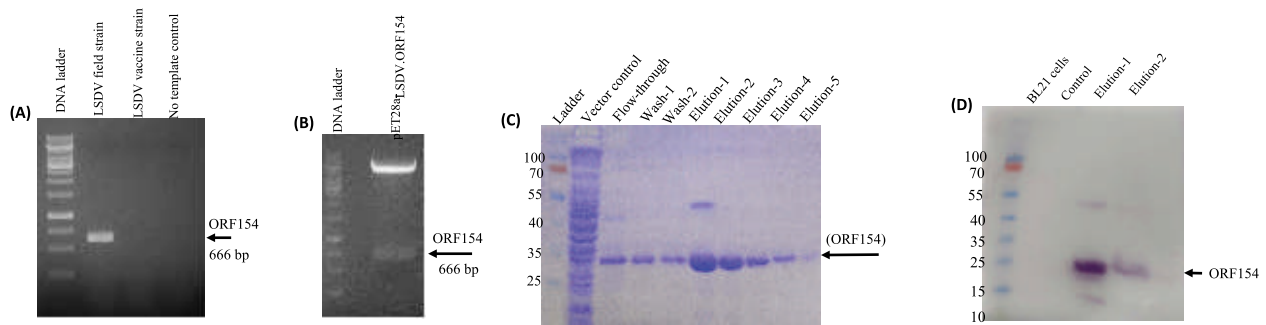
Antiviral and proviral activity of Tubacin against Foot-and Mouth Disease virus (FMDV) in BHK-21 cells. Cells were infected with FMDV at an MOI of 0.1, followed by treatment with DMSO or Tubacin. At 12 hours post-infection (hpi), supernatants were harvested, and virus titers were quantified using a plaque assay. (A) Antiviral effect of Tubacin at 62nM concentration showing a reduction in viral titers. (B) Proviral effect of Tubacin at 5 μ M concentration, indicating enhanced viral replication.

(Riyesh T, Jitender Rathee, Naveen Kumar and TK Bhattacharya)

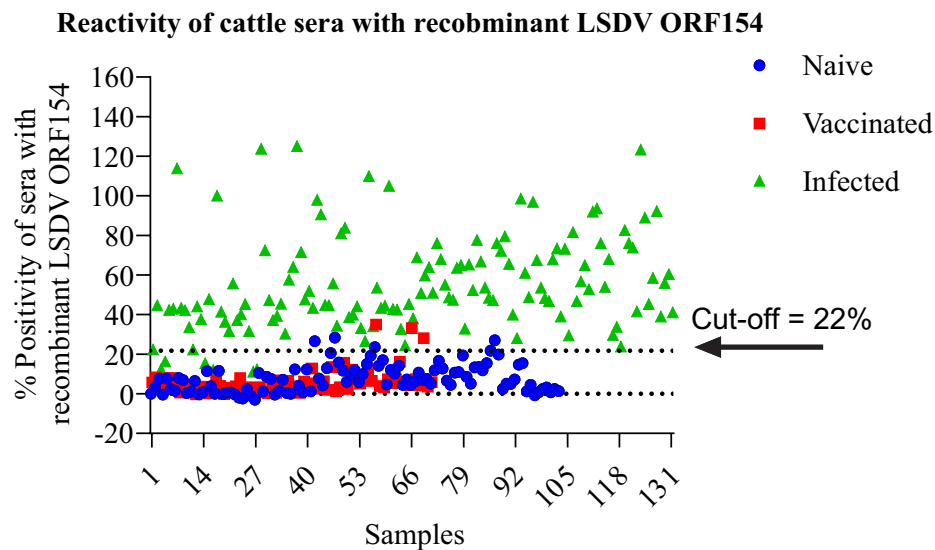
Development of an ORF 154-based DIVA ELISA for differentiating LSDV-infected and vaccinated animals

Mass vaccination to achieve disease-free status requires a serological test to differentiate infected and vaccinated animals (DIVA) towards the end of the control program. The lumpy skin disease (LSD) vaccines currently in use (licensed) are not DIVA-compatible. India recently approved a new live-attenuated LSD vaccine derived from the local Ranchi strain. Unlike field strains of the LSD virus (LSDV), the Ranchi strain has a distinct 801-nucleotide deletion in its inverted terminal repeat (ITR) region, affecting ORF003/ORF154. In this study, we successfully cloned and expressed LSDV ORF154 into pET28a and purified the His-tagged protein using Ni-NTA affinity chromatography. SDS-PAGE and Western blot analyses confirmed the presence of a ~28 kDa protein, consistent with the predicted molecular weight. An optimized antigen concentration of 500 ng/well and serum dilution of 1:50, and at a positivity cut-off of 22%, the assay showed high sensitivity (96.125%) and specificity (95.77%), effectively distinguishing infected from vaccinated cattle. These findings demonstrate the potential of an ORF 154-based ELISA as a reliable serological diagnostic tool for LSDV surveillance and disease control programs, making the Ranchi strain-based LSD vaccine the first DIVA-compatible vaccine.

Fig. 2



Cloning, expression and purification of recombinant LSDV ORF154: (A) **Amplification of ORF154 from field LSDV strain.** LSDV ORF154, lacking the signal peptide sequences, was amplified from wild-type LSDV (LSDV/2019/India/Ranchi) by PCR. (B) **Cloning.** The PCR product was digested with *NdeI* and *XhoI* and cloned into the pET-28a(+) vector. The recombinant plasmid (pET28a-LSDV.ORF154) was transformed into DH5 α *E. coli* and selected on LB agar containing kanamycin. Successful cloning was confirmed by restriction digestion and nucleotide sequencing. (C) **Expression and purification of recombinant protein.** The recombinant construct, pET28a-LSDV.ORF122 was transformed into BL21(DE3) cells and selected with kanamycin. A single transformed colony was inoculated into fresh LB medium containing kanamycin, grown to an OD₆₀₀ of 0.3–0.6 at 37°C with shaking, and induced with 1 mM IPTG. Following a 6-hour incubation at 37°C, cells were harvested by centrifugation at 4800 \times g for 10 min. Recombinant ORF154 was purified using a native His-tag affinity purification method. The target protein, ORF154, was eluted in up to five serial fractions using elution buffer (B). Protein purity was assessed using 8% SDS-PAGE. (D) **Confirmation by Western blot analysis.** The expression of His-tagged ORF154 was further confirmed using anti-6X His-tag monoclonal antibody in Western blot assay.

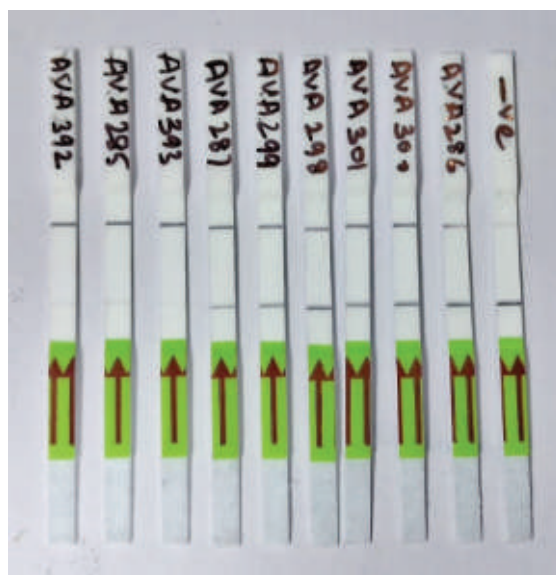


Development of DIVA ELISA: The optimum concentration of purified recombinant protein (500 ng/well) and serum dilution (1:50) was determined by checkerboard titration. For DIVA ELISA, the 96-well ELISA plates were coated with purified recombinant antigen and incubated overnight at 4°C. The plates were then washed with PBST and blocked with blocking buffer for 1 h. After another five washes with PBST, serum samples were added and incubated at 37°C for 1 h, followed by washing with PBST. Subsequently, rabbit anti-bovine IgG/HRP was added and incubated for 1 h before washing with PBST. The substrate was then added, and the plates were incubated at room temperature in the dark for 5 minutes to allow colour development. The reaction was stopped by adding 50 μ L/well of 1N H₂SO₄ ELISA stop solution, and OD₄₅₀ values were measured using a microplate absorbance reader. The OD₄₅₀ values of the serum samples were used to calculate percent positivity. The % positivity of naïve and infected cattle was compared to determine the sensitivity and specificity of the ELISA.

(Naveen Kumar, Alka Nohkhwal, Yogesh Chander, Azim Verma and Riyesh T)

CRISPR-Cas12a-RPA-LFA-based diagnostic assay for Newcastle disease virus

Newcastle disease virus (NDV), a virulent *Paramyxoviridae* family member, poses a significant threat to the poultry industry due to its high transmissibility and severe respiratory, enteric, and neurological effects. Early, accurate detection is critical for outbreak control. This study presents a novel diagnostic platform combining CRISPR-Cas12a, Recombinase Polymerase Amplification (RPA), and Lateral Flow Assay (LFA) for rapid NDV detection. A conserved region in the hemagglutinin-neuraminidase (HN) gene was identified from 72 full-genome alignments, guiding the design of specific RPA primers and CRISPR gRNA. RPA was performed at 40 °C, followed by Cas12a-mediated cleavage of a dual-labeled ssDNA reporter at 37 °C. Cleavage generated visible bands on LFA strips for positive samples. The assay showed high sensitivity and specificity, with potential for on-site use without sophisticated equipment. Its isothermal nature and visual readout make it ideal for low-resource settings. Further validation and RNA extraction-free protocols are in progress to streamline field deployment.

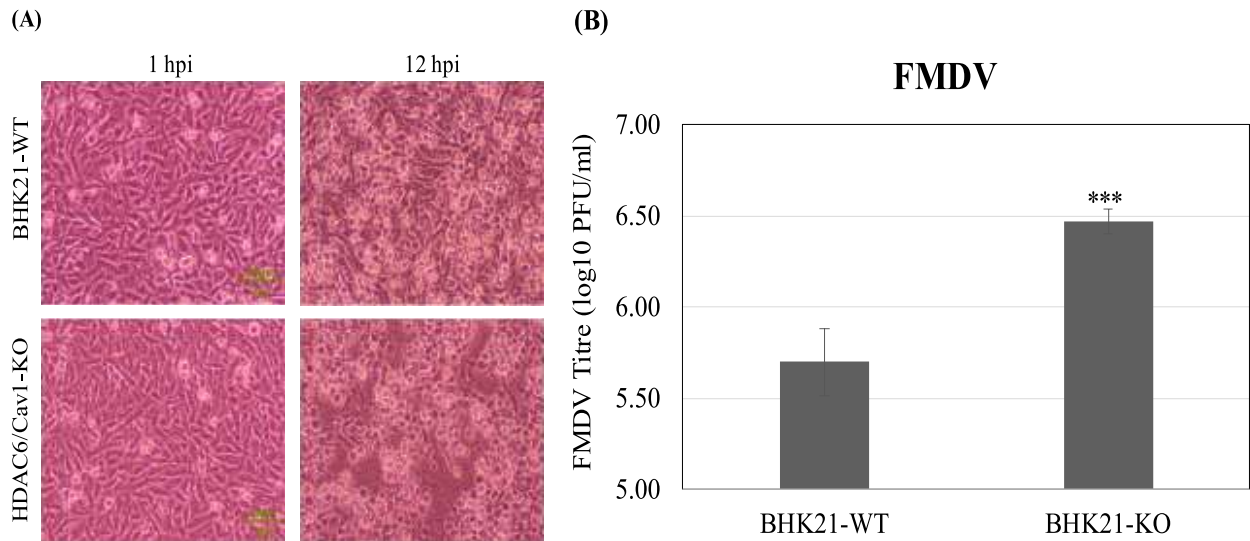


LFA based detection of Newcastle disease virus

(Riyesh T, Geetanjali Sheoran, Shanmugasundaram K and TK Bhattacharya)

Generation of a genetically engineered BHK-21 cell line for scale up of vaccine production

Foot-and-mouth disease (FMD) is a highly contagious disease of ruminants and pigs, caused by an Aphthovirus that belongs to the family Picornaviridae. It is currently endemic in India and country is running a FMD control program through mass vaccination campaign. In order to generate herd immunity, ~ 500 million animals need to be vaccinated twice a year as the protective immunity of FMD vaccine lasts only for 6 months. Production of 1000 million vaccine doses is a difficult task and the manufacturers are barely able to meet this requirement. Therefore, technological interventions are required to scale up vaccine production. For vaccine production, FMD virus (FMDV) is propagated in BHK-21 cells and then inactivated and mixed with adjuvant. The amount of vaccine doses produced depends of the amount of virus being produced. Recent progress using transcriptomics, genome-wide siRNA and CRISPR screens have enabled the identification of numerous host factors regulating virus replication. Some cellular proteins serve as host restriction factor and therefore interfere with the virus replication. In this invention, we generated genetically engineered BHK-21 cells lines deficient in two of the cellular proteins Cav1 (Caveolin-1) and HDAC6 (histone deacetylase 6) that serve as host restriction factor in FMDV life cycle. The knockout cells generated ~ 10-times higher virus titre as compared to the wild type (normal) BHK-21 cells, and therefore, it has high commercial interest to economize the cost of vaccine.

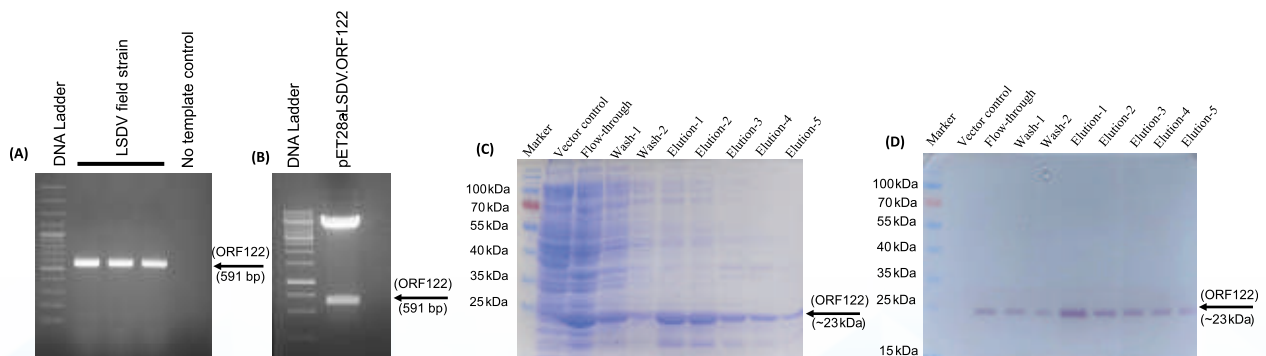


Growth of FMDV in HDAC6/Cav1-KO and WT BHK21 Cells: Confluent monolayers of *HDAC6/Cav1-KO* and WT BHK21 cells in triplicates, were infected with FMDV at MOI of 0.1. (A) The images showing cytopathic effects were taken at 1 hpi and 12 hpi (scale = 20 μm). The virus particles released in the infected cell culture supernatants at 12 hpi were qualified by plaque assay (B). Error bars indicate SD. Pair-wise statistical comparison's were performed using Student's t test. (NS = non-significant difference, : : : = P < 0.001). Value are means ± SD and representative of the result of at least 3 independent experiments.

(Naveen Kumar, Alka Nohkhwal, Yogesh Chander, Azim Verma and Riyesh T)

Development of an ELISA for serological detection of lumpy skin disease virus antibodies in cattle

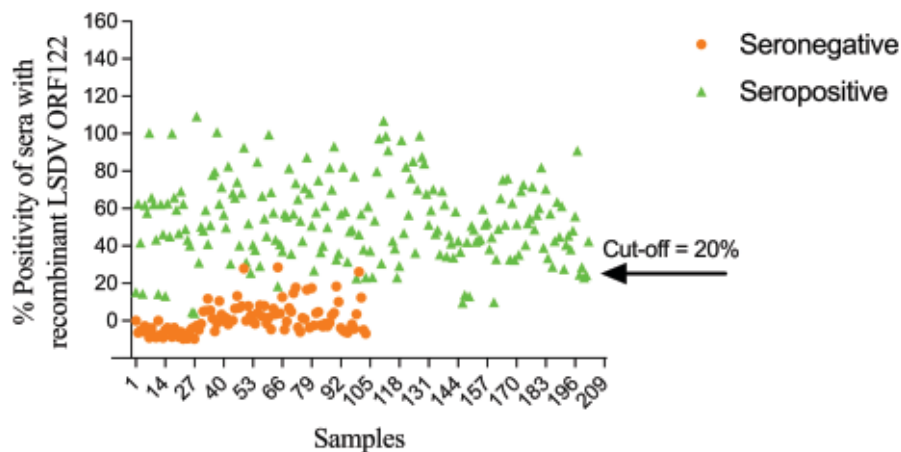
Lumpy Skin Disease Virus (LSDV), a *Capripoxvirus*, causes significant economic losses in cattle. Serological tools are critical for assessing immune responses, vaccine efficacy, and disease surveillance. A broad-spectrum ELISA capable of detecting antibodies against both vaccine and field strains is essential for effective monitoring. In this study, the conserved LSDV ORF122 gene (a homolog of vaccinia virus A33) was cloned, expressed in *E. coli*, and purified. The recombinant His-tagged protein (~23 kDa) was confirmed via SDS-PAGE and Western blot. The ELISA was optimized using 250 ng/well antigen and 1:50 serum dilution. The assay demonstrated high sensitivity (97.09%) and specificity (94.37%) and detected antibodies against both vaccine and field strains. When used alongside the ORF154-based DIVA ELISA, it offers a comprehensive serodiagnostic tool for LSDV control programs.



Cloning, expression and purification of recombinant LSDV ORF122: (A) **Amplification of ORF122 from field LSDV strain.** LSDV ORF122 was amplified from wild-type LSDV (LSDV/2019/India/Ranchi) by PCR. (B) **Cloning.** The PCR product was digested with *NdeI* and *XhoI* and cloned into the pET-28a(+) vector. The recombinant plasmid (pET28a-LSDV.ORF122) was

transformed into DH5 α *E. coli* and selected on LB agar containing kanamycin. Successful cloning was confirmed by restriction digestion and nucleotide sequencing. **(C) Expression and purification of recombinant protein.** The recombinant construct, pET28a-LSDV. ORF122 was transformed into BL21(DE3) cells and selected with kanamycin. A single transformed colony was inoculated into fresh LB medium containing kanamycin, grown to an OD₆₀₀ of 0.3–0.6 at 37°C with shaking, and induced with 1 mM IPTG. Following a 6-hour incubation at 37°C, cells were harvested by centrifugation at 4800 × g for 10 min. Recombinant ORF122 was purified using a native His-tag affinity purification method. The target protein, ORF122, was eluted in up to five serial fractions using elution buffer. Protein purity was assessed using 8% SDS-PAGE. **(D) Confirmation by Western blot analysis.** The expression of His-tagged ORF122 was further confirmed using anti-6X His-tag monoclonal antibody in Western blot assay.

Reactivity of cattle sera with recombinant LSDV ORF122



Cloning, expression and purification of recombinant LSDV ORF122: **(A) Amplification of ORF122 from field LSDV strain.** LSDV ORF122 was amplified from wild-type LSDV (LSDV/2019/India/Ranchi) by PCR. **(B) Cloning.** The PCR product was digested with *NdeI* and *XhoI* and cloned into the pET-28a(+) vector. The recombinant plasmid (pET28a-LSDV. ORF122) was transformed into DH5 α *E. coli* and selected on LB agar containing kanamycin. Successful cloning was confirmed by restriction digestion and nucleotide sequencing. **(C) Expression and purification of recombinant protein.** The recombinant construct, pET28a-LSDV. ORF122 was transformed into BL21(DE3) cells and selected with kanamycin. A single transformed colony was inoculated into fresh LB medium containing kanamycin, grown to an OD₆₀₀ of 0.3–0.6 at 37°C with shaking, and induced with 1 mM IPTG. Following a 6-hour incubation at 37°C, cells were harvested by centrifugation at 4800 × g for 10 min. Recombinant ORF122 was purified using a native His-tag affinity purification method. The target protein, ORF122, was eluted in up to five serial fractions using elution buffer. Protein purity was assessed using 8% SDS-PAGE. **(D) Confirmation by Western blot analysis.** The expression of His-tagged ORF122 was further confirmed using anti-6X His-tag monoclonal antibody in Western blot assay.

(Naveen Kumar, Alka Nohkhwai, Yogesh Chander, Azim Verma and Riyesh T)

CRISPR-Cas12a-RPA-LFA platform for visual detection of IBV in clinical samples

Infectious bronchitis virus (IBV), a contagious avian Coronaviridae member, causes significant economic losses in poultry. Rapid and accurate detection is essential for effective control. This study developed a CRISPR-Cas12a-based assay integrated with Recombinase Polymerase Amplification (RPA) and Lateral Flow Assay (LFA) for IBV detection. A conserved region in the N gene was identified by aligning 34 IBV genomes, guiding the design of specific RPA primers and gRNA. RPA was performed at 40 °C, followed by Cas12a-mediated cleavage of a dual-labeled ssDNA reporter at 37 °C for visual detection on LFA strips. Positive samples showed a test line, while negatives displayed only the control line. The assay demonstrated high sensitivity and specificity, with potential for point-of-care use. Ongoing work focuses on validating with more field samples and simplifying the assay by bypassing RNA extraction. This platform offers a rapid, sensitive, and field-deployable diagnostic tool for timely IBV detection and control.

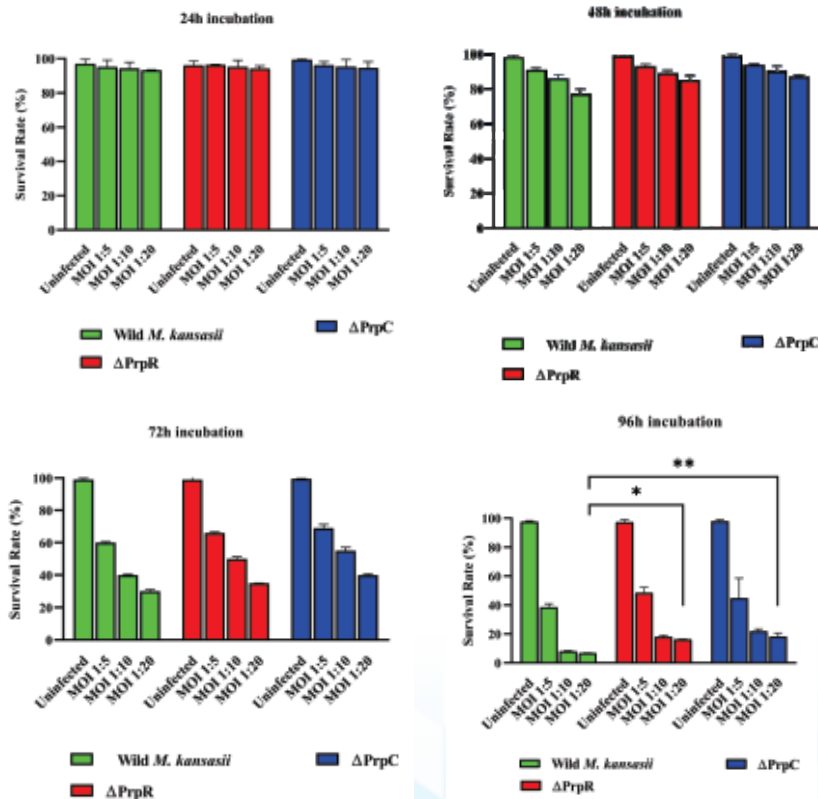


LFA based detection of Infectious bronchitis virus.

(Riyesh T, Geetanjali Sheoran, Shanmugasundaram K and TK Bhattacharya)

Role of methylcitrate cycle genes in the pathogenesis of *Mycobacterium kansasii*

Mycobacterium kansasii is a slow-growing, photochromogenic non-tuberculous mycobacterium that causes pulmonary infections, especially in immunocompromised individuals. Often misdiagnosed as tuberculosis, it poses significant public health concerns. As an intracellular pathogen, *M. kansasii* relies on macrophages for replication, utilizing host cholesterol as an energy source and generating toxic propionate as a byproduct. To detoxify propionate, it employs the methylcitrate cycle (MCC), yet the role of MCC genes in its virulence remains poorly understood. This study evaluated the function of MCC genes (*prpC* and *prpR*) by generating CRISPR-Cas9-mediated knockout mutants in *M. kansasii*



Assessment of cell viability of macrophages infected with *M. kansasii*, *prpC*, and *prpR* at different MOIs and time intervals- a) 24 hrs, b) 48 hrs, c) 72 hrs, and d) 96 hrs using MTT assay.

(ATCC 12478). J774A.1 murine macrophages were infected with wild-type and mutant strains at varying MOIs (5, 10, 20), and cell viability was assessed via MTT assay over 24–96 hours. Results showed reduced cytotoxicity in cells infected with MCC-deficient mutants, particularly at 96 hours with MOI 20, suggesting impaired intracellular adaptation. SEM analysis revealed no major differences in bacterial-macrophage interactions between wild-type and mutant strains. Overall, the study highlights the importance of MCC genes in the intracellular survival and virulence of *M. kansasii*, identifying them as potential therapeutic targets.

(Indu Rani, Rakesh Kumar, Shanmugasundaram K, Harisankar Singha, Riyesh T and TK Bhattacharya)

Profiling of socio-demographic and associated risk factors in acquiring zoonotic tuberculosis

A custom-designed questionnaire was developed and circulated among experts from the human health and animal husbandry departments to obtain their feedback and suggestions for modifications. After two rounds of expert discussions, the questionnaire was finalized. The final Knowledge, Attitude, and Practices (KAP) questionnaire consisted of six sections: socio-demographic information (n = 7), species of animals owned, purpose of rearing, and management practices (n = 13), animal product consumption, behaviours, and marketing practices (n = 8), knowledge on zoonotic tuberculosis and its transmission (n = 17), attitude towards tuberculosis (n = 9), and practices related to zoonotic tuberculosis (n = 16). A total of 155 questionnaires were collected from livestock keepers across different regions of Haryana. Regarding socio-demographic information, 109 respondents were male and 46 were female, with most respondents (n = 148) residing in rural areas. Analysis of data on animal ownership, purpose of rearing, and management practices revealed that most respondents rear buffaloes (n = 104) primarily for milk production, which they sell to neighbours and local milk vendors. They manage animals under either intensive (n = 77) or semi-intensive (n = 78) systems. Analysis of animal product consumption, behaviours, and marketing practices showed that over 51% (n = 80) of respondents consumed both raw and boiled milk. Approximately 8% (n = 12) of respondents reported consuming raw milk due to its taste, which was cited as the main reason. In contrast, 41% (n = 63) preferred boiled milk to avoid food-borne illness. With respect to awareness about bovine tuberculosis (bTB), only 30 respondents (19%) had heard of bTB, while the remaining 125 (81%) had not. None of the respondents were aware of the causative agent of bTB, its zoonotic potential, modes of transmission, or symptoms in animals. However, most respondents were well-informed about human tuberculosis, including its symptoms, modes of transmission, and treatment. The sources of information on human TB included radio, newspapers, social media, and healthcare personnel. Analysis of attitudes toward TB patients indicated that most respondents expressed sympathy and a willingness to help. Additionally, 149 respondents agreed that TB has a negative impact on the socioeconomic status of affected individuals.

(Shanmugasundaram K, Indu, Bhumeet, Soni, Harisankar Singha, Riyesh T)

Isolation, Characterization, and Generation of Repository of *Mycobacterium* Species

A total of 94 biological samples were processed for the isolation of *Mycobacterium* species. These included fecal samples from buffaloes (n = 35) and sheep (n = 49), buffalo milk (n = 4), thoracic fluid (n = 1), cattle lung tissue (n = 1), soil samples (n = 3), and a water sample (n = 1). All 94 samples were screened for the presence of acid-fast bacilli (AFB), of which 17 sheep fecal samples and 11 buffalo samples tested positive. Forty-four fecal samples were screened for the presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) by targeting the *IS900* gene, and all were found negative. However, one of the 35 buffalo fecal samples tested positive for MAP by PCR. From the 94 processed samples, a total of 14 *Mycobacterium* isolates were recovered from sheep fecal samples. These isolates were characterized by PCR targeting the *afb* and *hspX* genes, with 9 isolates testing positive for both. Further characterization of the isolates is in progress. All isolates were cryopreserved for repository development. Whole genome sequencing (WGS) of three *Mycobacterium* isolates has been completed. Initial analysis identified the isolate with sample ID CSWRI as *M. avium* subsp. *paratuberculosis*, sample ID Nilgai as *M. abscessus*, and sample ID 577 as *M. branderi*.

(Shanmugasundaram K, Indu, Soni, R K Vaid, Riyesh T)

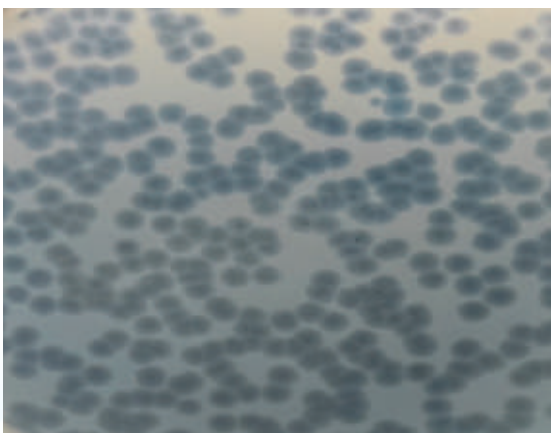
Coevolution of phages and XDR *Klebsiella pneumoniae*: Emergence of phage resistance and antibiotic resensitization

Extensively drug-resistant (XDR) *Klebsiella pneumoniae* is a critical nosocomial pathogen responsible for serious infections, including bloodstream and urinary tract infections, pneumonia, and liver abscesses, especially in immunocompromised patients. This study aimed to investigate the evolution of phage resistance in *K. pneumoniae* under selective pressure and evaluate the effectiveness of phage cocktails in controlling resistant strains. A total of 20 *K. pneumoniae* strains and nine specific lytic phages were tested to determine host range and lytic profiles. The XDR strain Fop185 was exposed to phages VTCCBPA194 and VTCCBPA205, resulting in the generation of 60 phage-resistant mutants using a time-kill curve approach. All mutants were also resistant to chloramphenicol (CL10) and ampicillin (AMP10). While most mutants were resistant to both individual phages and the cocktail, some— 185/194 (2.9, 3.5) and 185/205 (1.2, 3.2)—remained susceptible. The phage cocktail effectively lysed these specific mutants and eradicated their biofilms. Interestingly, a trade-off was observed where phage-resistant mutants showed partial restoration of antibiotic sensitivity. These findings highlight the dual potential of phage cocktails in both lysing resistant bacteria and re-sensitizing them to antibiotics, supporting their role in developing effective therapies against XDR *K. pneumoniae*.

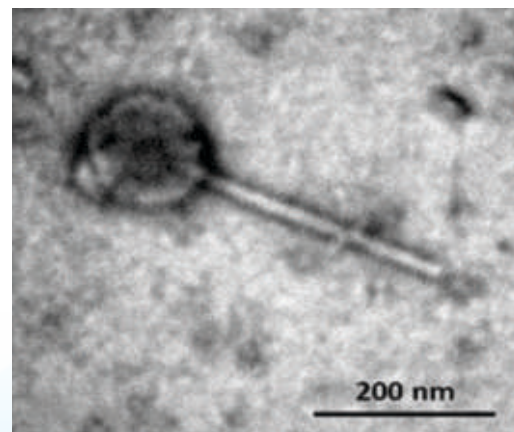
(Taruna Anand, BC Bera, RK Vaid & Nitin Virmani)

Isolation of a novel bacteriophage against MDR *Salmonella enterica* and its potential use in food biocontrol

Salmonella enterica is a major food borne pathogen, and the emergence of multidrug-resistant (MDR) strains poses a growing public health concern. As antibiotic efficacy declines, bacteriophages offer a promising alternative for controlling MDR bacterial infections, particularly in food safety applications. In this study, a broad-host-range lytic bacteriophage, VTCCBPA219, was isolated from pond water against MDR *Salmonella enterica* serotype Paratyphi. The phage exhibited strong lytic activity against 28 out of 30 tested *S. enterica* strains, indicating a wide host range. Notably, VTCCBPA219 remained active under acidic conditions (pH 3) and high temperatures (up to 70°C), suggesting its stability in harsh environments. Transmission electron microscopy (TEM) classified the phage within the class *Caudoviricetes*. Whole genome sequencing revealed a genome size of approximately 146 kb with 44.5% GC content, encoding 250 predicted coding sequences (CDSs) and 25 tRNAs. The absence of lysogeny-associated genes confirms its strictly lytic nature. Furthermore, the phage demonstrated the ability to eradicate *S. enterica* biofilms formed on borosilicate glass surfaces, highlighting its potential application as a biocontrol agent in food processing and sanitation.



Plaque characteristics of phage VTCCBPA19



TEM photograph of phage VTCCBPA19

(Taruna Anand, BC Bera, RK Vaid & Nitin Virmani)

Publication of NCVTC microbial repository Catalogue

The National Centre for Veterinary Type Cultures (NCVTC) at ICAR-NRCE, Hisar, has published its comprehensive *Microbial Repository Catalogue*, a pivotal resource aimed at the conservation, characterization, and utilization of microbial diversity associated with animals and their environments. Spanning 233 pages and comprising seven detailed sections, the catalogue includes over 4,000 authenticated entries of veterinary bacteria (including rumen and dairy bacteria), anaerobes, viruses, bacteriophages, rumen fungi, and recombinant clones. Each section is organized alphabetically and preceded by a user-friendly guide to entries, enabling researchers to efficiently locate microbial strains of interest. Essential metadata—such as genus, species, source, and media or cell culture requirements—accompany each entry, with additional protocols for media preparation and culturing methods included. The publication also outlines guidelines for culture submission and supply, safety considerations, and revival protocols, supporting both national and international exchange of microbial resources. This initiative not only facilitates advanced research in biotechnology, veterinary medicine, and industrial applications but also positions NCVTC for global recognition by paving the way for inclusion in the World Data Centre for Microorganisms (WDCM), thereby enhancing accessibility, interoperability, and collaborative potential in microbial science.

(T. K. Bhattacharya, R. K. Vaid, Taruna Anand, B. C. Bera, K. Shanmugasundaram, T. Riyesh, Naveen Kumar, S. Barua, Pradip Behare, A. K. Puniya, Yogita Sharma, D. Rajendran, Heena H. S., and Gopi M)

Total microbial accessions in the NCVTC repository

In the current year, a total of 294 microbes were accessioned into the NCVTC repository, raising the cumulative total to 5,400 microbial cultures as of December 2024. The bacterial repository grew to 1,942 isolates with the addition of 116 new bacterial cultures, including unique strains such as *Corynebacterium flavescens*, *Vagococcus luciliae*, *Arthrobacter gandavensis*, *Mammaliococcus sciuri*, *Streptococcus equi* ssp. *equi*, *Brevundimonas faecalis*, *Pantoea vagans*, *Empedobacter brevis*, *Pseudomonas hunanensis*, and *Myroides odoratus*. The virus repository processed 28 virus cultures, of which 18 were accessioned, bringing the total number of virus isolates to 415, including significant

Year	2009-18	2018-19	2019-20	2020-21	2021-22	2022**	2023*	2024*	Total
Veterinary Microbes									
Bacteria	1271	123	95	50	63	120	104	116	1942
Virus	227	31	44	28	31	26	10	18	415
Bacteriophage	129	8	8	48	92	44	44	28	401
Recombinant clone	557	16	8	0	0	0	0	0	581
Phage library	27	0	0	0	0	0	0	0	27
Genomic DNA	288	0	0	0	0	0	0	0	288
Total	2499	178	155	126	186	190	158	162	3654
Rumen microbes									
Anaerobic bacteria	290	49	46	62	80	61	51	78	717
Fungi/Yeast	107	0	0	0	0	0	0	0	107
Meth. Archae	8	0	0	0	0	0	0	0	8
Total	405	49	46	62	80	61	51	78	832
Dairy microbes									
Bacteria	577	36	44	20	48	70	65	54	914
Total	577	36	44	20	48	70	65	54	914
Grand total	3481	263	245	208	314	321	274	294	5400
* Calendar year data ; ** 9 month data (April-Dec 2022)									

additions like Avian infectious bronchitis virus, PPR virus, Bovine rotavirus, and Equine rotavirus. Furthermore, 28 bacteriophages were accessioned, increasing the total number of bacteriophages in the repository to 401. In addition, the rumen microbial repository at NIANP, Bengaluru, added 78 new isolates, raising its total to 832, while the dairy microbes repository at NDRI expanded to 914 cultures with the inclusion of 54 new bacterial strains.

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Sale of bacterial and virus cultures during 2023–24

During the year 2023–24, a total of 33 microbial cultures were supplied to eight universities and institutions for academic, research, and quality control purposes. Eight cultures were provided to Biovet Pvt. Ltd. on a sale basis, generating revenue of ₹4.5 lakh. Five cultures were supplied to the Deputy Commissioner of Animal Husbandry, Goregaon, Mumbai for quality control assessments. Four cultures were distributed to the Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur for academic use. The Division of Livestock Production and Management, SKUAST-Jammu requisitioned cultures of *Staphylococcus aureus* subsp. *aureus*. Additionally, three cultures were supplied to the Department of Veterinary Pathology, Nagpur Veterinary College, Nagpur, and five cultures were distributed to the Dairy Production Building, ICAR-NDRI, Adugodi, Bengaluru.

(R. K. Vaid, Taruna Anand, K. Shanmugasundaram, T. Riyesh, B. C. Bera)

Technology Development, Transfer and Commercialization

Since the establishment of ICAR-National Research Centre on Equines is relentlessly working for the upliftment of equine sector and refinement of existing technologies for the benefit of health and production of equine sector in the country. The institute has oriented its research, and focused on the development of farmer friendly technologies for the betterment of equine health, production and utilization in the country. Several diagnostic kits, modified and update vaccines and biologicals developed by the scientists of ICAR-NRCE are routinely used in the field. Several other technologies are under refinement, development, validation, transfer and commercialization.

I. List of Technologies developed by ICAR-NRCE, Hisar

A. Vaccines for Equines and other Livestock species

- i. Equiherpabort vaccine.
- ii. Updated Equine Influenza vaccine.
- iii. A modified vaccine construct for EHV1 and methods of preparing the same.
- iv. Corona virus SARS-CoV-2 vaccine for animals.
- v. Lumpy Skin Disease vaccine.
- vi. Reverse genetics based recombinant equine influenza virus as vaccine candidate.
- vii. LFA kit for SARS-CoV-2 nucleic acid detection.

B. Disease Diagnostics for Equines and other Livestock species

- i. Equiherpes B-ELISA Diagnostic Kit.
- ii. ELISA kit for differentiation of EHV1 & 4 Infections.
- iii. Equine Rotavirus Diagnostic kit.
- iv. Equine Infectious Anaemia (EIA) ELISA Diagnostic kit.
- v. Equine Japanese Encephalitis Virus Antibody Test Kit, iELISA.
- vi. Monoclonal antibody based immunoassay for detection of equine influenza (H3N8) antigen.
- vii. Canine SARS-CoV-2 Antibody detection ELISA Kit.
- viii. Glanders ELISA Kit.
- ix. Duplex Antigen Glanders ELISA Kit.
- x. *Theileria equi* ELISA Diagnostic kit.
- xi. Lateral flow assay for diagnosis of *Theileria equi* infection.
- xii. Surra (*Trypanosoma evansi*) ELISA Diagnostic Kit.
- xiii. RPA-LFA kit for point-of-care detection of Porcine circovirus 3.

C. Drug Development and delivery Technologies

- i. Polymeric metal nanocomposites and methods of synthesis thereof.

- ii. Poly ZnOxeno artificial skin construct.
- iii. Yasadbhasma, and hydroxychloroquine formulation as cytoprotective and antiviral effect.
- iv. Hydroxychloroquine/ chloroquine zinc oxide nanoparticle formulation.
- v. Nanodelivery of quinapyramine sulfate.
- vi. *Aerva javanica* extract for the treatment of exuberant granulation tissue and tumors in horses.
- vii. *Aerva javanica* extract for the treatment of seasonal dermatitis in horses.

D. Equine Reproduction and Production Technologies

- i. A pregnancy diagnostic kit for equine based on detection of eCG by ELISA (Pregmare Kit).
- ii. Customised AV (artificial vagina) for semen collection from Stallions.
- iii. Semen collection and cryopreservation in Indigenous horses.
- iv. Equine DNA Parentage Testing Kit.
- v. Development of protocol for embryo transfer in Marwari horses.
- vi. Modified vitrification protocols for cryopreservation of 7–8 day old large sized embryos in mares.
- vii. Modified cryoloop and Hemi-straw methods of vitrification of large sized horse embryos.
- viii. Improved methods of Cryovial preservation of stallion's semen and thawing protocols.
- ix. Development of non-egg yolk based (nano-encapsulated drug delivery based encapsulated systems) semen extender to increase the shelf life and post-thaw motility in stallion semen.

E. Equine Products Technologies

- i. Processes for formulation of donkey-milk based cosmetic products – bathing soap, body butter, and lip balm.

F. Therapeutics

- i. Antiviral activity of Apigenin against buffalopox.
- ii. Antiviral activity of Emetine against SARS-CoV-2 virus.
- iii. p38- α -a novel drug target against buffalopox virus.
- iv. ROCK1/MLC2- a novel drug target against buffalopox virus.
- v. Antiviral activity of DZNep against SARS-CoV-2 virus.

G. Methodologies

- i. Identification and quantitation of the vaccine and field strain(s) of lumpy skin disease virus.
- ii. Development of bacteriophage against drug-resistant (XDR) *Pseudomonas aeruginosa*.
- iii. Salmoquell™ – Encapsulated bacteriophage cocktail for treatment of Salmonella infections in poultry.
- iv. Development and treatment regimen using combination of phages and antibiotic to treat multidrug resistant bacterial infections.
- v. Construction of Bacterial artificial chromosome (BAC) of Equine herpesvirus 1.
- vi. Quadruple genes deletion mutant equine herpesvirus 1 as live vaccine candidate.
- vii. Double genes deletion mutant equine herpesvirus 1 as live vaccine candidate.

Technologies certified by ICAR

Sr No	Name of the Technology	Lead Developer
1.	Equi Heal	Dr RK Dedar
2.	Equi Skin	Dr RK Dedar
3.	Donkey Milk-Based cosmetic Products (Bathing Soap, Body Butter and Lip Balm)	Dr Anuradha Bhardwaj
4.	Semen cryopreservation in indigenous horses	Dr TR Talluri
5.	Customised AV for semen collection from Stallions	Dr TR Talluri

Technology development and assessment

Semen collection and cryopreservation in indigenous horses

Equine Production Campus (EPC), ICAR-NRCE has standardized the formulations and technology for collection and cryopreservation of indigenous stallion, exotic and indigenous jack semen. The population of indigenous equines is





MoU with Sh. Rajyavardhan Rathore, Fateh Stud Farm Kelwa, Udaipur. Semen collection and cryopreservation technology has been transferred

drastically declining at a rapid pace due to advancements in the mechanisation and hence necessary steps have to be taken to conserve this elite indigenous equine germplasm. In this endeavour, scientists at EPC campus have made efforts in standardizing the methodology of collection of the semen from the stallions, formulated various combinations of chemicals for successful cryopreservation of semen. These technologies are now made ready for transfer and distribution of this package of practices to the agripreneurs, stakeholders, equine owners and breeders. This year, this technology has been transferred to three equine farmers/ breeders and imparted training in stallion semen collection and cryopreservation.

Name of the technology	Technology transferred to	Revenue generated
Semen collection and cryopreservation in indigenous horses	1. Mr Raghavendra Singh, Dundlod, Jhunjhunu, Rajsthan	59,000/-
	2. Mr. Rajyavardhan Rathore, Fateh Stud Farm Kelwa, Udaipur	59,000/-
	3. Mr Navaneet Equine Breeding India Agrahara Main Road, Jakkur Post, Yelhanka, Bangalore -560064.	59,000/-
		1,77,000/-





Customised Artificial Vagina for semen collection from stallions

The currently available Artificial Vagina (AV) for semen collection in stallions is being imported from other countries and they are expensive. These AVs are not equipped with temperature monitoring. The maintenance of optimum temperature in AV is very much critical for successful collection of semen from stallions. In this direction, to address these issues in the existing AV, scientists at EPC have designed, assembled and developed inexpensive hand-made AV from locally available, easily obtainable, and cheaper materials and is suitable for collecting the semen from indigenous stallions. In the current year, this technology has been transferred to 3 equine farmers/breeders.

Name of the technology	Technology transferred to	Revenue generated
Customized AV for semen collection from Stallions	1. Mr Raghavendra Singh Dundlod, Jhunjhunu, Rajasthan	17,700/-
	2. Mr Jaykumar Desai , Gujarat	17,700/-
	3. Mr Navaneet , Equine Breeding India, Bangalore, Karnataka	17,700/-
		53,100/-



Prototype of customised Artificial Vagina for semen collection in horses



MoU between Equine Breeding India and ICAR-NRCE for the transfer of technology "Customized AV for semen collection from Stallions"

List of Technologies Developed

- 1. First Indigenous SNP Chip for Indian Horses (High-density *Axiom_Ashwa* SNP chip):** The unique horse and pony breeds of India are declining at an alarming rate. These horses have been integral to the Indian culture and customs for centuries and represent a valuable genetic resource. It is imperative to harness the potential of this equine genetic resource that urgently needs conservation. The study highlights the design and development of a high density SNP array, the *Axiom_Ashwa* to aid in the genetic analysis and conservation efforts for Indian horse and pony breeds. With 613,950 SNPs, this chip offers extensive genome coverage having an average inter-marker distance of 4 kb. The *Axiom_Ashwa* has been validated on a larger set of diverse indigenous samples as well as Thoroughbreds, demonstrating a high call rate of 99.4% and robustness for genotyping indigenous breeds.

Inventors: Ahlawat S, Niranjana S K, Arora R, Viji RK, Kumar A, Sharma U, Raheja M, Popli K, Yadav S, Mehta SC.
- 2. Salmoquell:** The invention relates to targeting Salmonella infections using bacteriophages which can be used as a feed additive and does not pose any side effects as antimicrobial application. Broad spectrum Salmonella phages have been encapsulated by using sodium alginate beads and other compounds and further coated to protect bacteriophage action at acidic pH. The preparation effectively kills the bacterial pathogen—*Salmonella* spp. in poultry birds.

Inventors: Taruna Anand, Nitin Virmani, B.C. Bera, R. K. Vaid, Manju Barnela, R.K. Singh, B.N. Tripathi
- 3. Lysibact:** Lysibact: An antimicrobial nanoemulsion targeting a broad range of gram negative as well as gram-positive bacterial infections prepared using broad-acting endolysin peptide which has been encapsulated along with lysozyme, naturally occurring monoterpenoids and lipid emulsifier for enhanced activity and stability. The preparation showed a broad spectrum of action and stability at room temperature and can be used for elimination of a broad range of bacterial infections by spray applications and those caused by biofilm formation.

Inventors: Taruna Anand, Nitin Virmani, B.C. Bera, R. K. Vaid, Medhavi Vashisth, T.K. Bhattacharya
- 4. Vermicomposting by utilizing Equine Dung:** Usually, equine dung (ED) is disposed-off as a waste by mostly equine farmers in nearby area. The current study was undertaken keeping in view the challenges in organic farming and lack of adequate information on proper disposal of ED. Moreover, there was neither any scientific document nor any report was available of making vermicompost utilizing equine dung (EDV). It is a scientific way of conversion of ED into compost by earthworms; *Eisenia foetida*. Earthworms are used to enhance the process of conversion of ED into a valuable bio-fertilizer. Earthworms consume ED and as an outcome produce EDV and no chemical element was used in entire process. This technology can be adopted by equine Farmers for generation of additional income by sale of EDV. The EQUI-COMPOST is being used by the farmers and other users as organic fertilizer for plants and crops. It can also be promoted for poverty eradication and women empowerment in rural area. It is a natural and scientific process for production of compost by using earthworms.

Inventors: Ajmer Singh, Dr. Sanjay Kumar, Dr. Rajender Kumar, Dr. T K Bhattacharya
- 5. Development of an ORF154-based DIVA ELISA for Differentiating LSDV-Infected and Vaccinated Animals:** Effective LSD control requires a DIVA (Differentiating Infected from Vaccinated Animals) test, especially during mass vaccination programs. Existing LSD vaccines are not DIVA-compatible. India recently approved a live-attenuated LSD vaccine (Ranchi strain) with an 801-nt deletion in the ITR region, affecting ORF003/ORF154, unlike field strains. In this study, ORF154 was cloned into pET28a, expressed, and purified using Ni-NTA chromatography. SDS-PAGE and Western blot confirmed a ~28 kDa His-tagged protein. The optimized ELISA (500 ng/well, serum dilution 1:50, 22%

positivity cut-off) showed 96.13% sensitivity and 95.77% specificity, effectively distinguishing infected from vaccinated animals. This makes the ORF154-based ELISA a promising DIVA tool and the Ranchi strain the first DIVA-compatible LSD vaccine

Inventors: Naveen Kumar, Alka Nohkhwai, Yogesh Chander, Azim Verma and Riyesh T

- 6. Generation of a Genetically Engineered BHK-21 Cell Line for Vaccine Scale-Up:** Foot-and-mouth disease (FMD) is endemic in India, requiring biannual vaccination of ~ 500 million animals. Vaccine production depends on virus yield from BHK-21 cells, but current capacity struggles to meet demand. To enhance virus replication, we developed genetically modified BHK-21 cell lines lacking Cav1 and HDAC6—host restriction factors that limit FMDV replication. These knockout cells produced ~ 10-fold higher virus titres than wild-type cells, offering a promising solution to boost vaccine output and reduce production costs.

Inventors: Naveen Kumar, Alka Nohkhwai, Yogesh Chander, Azim Verma and Riyesh T

Patents/ Copy right filed/granted

Patents filed

Sr.No.	Title of the Patent	Investigator and Team	Application No and Date
1.	Phage endolysin encapsulated antimicrobial formulation to ameliorate biofilms	Taruna Anand and Nitin Virmani, Bidhan Chandra Bera, Rajesh Kumar Vaid, Medhavi Vashisth & Tarun Kumar Bhattacharya	Application No. 02411043287 dated June 4, 2024
2.	Generation of HDAC6/Cav1 double knockout BHK21 cells for scale up FMD vaccine production	Naveen Kumar, Alka Nohkhwai, Yogesh Chander, Assim Verma and Riyesh T.	Application No. 202411057416, dated 29.07. 2024
3.	Primers and guide RNA complex for detection of SARSCov-2 virus, and a kit thereof	BC Bera , Taruna Anand and Nitin Virmani	Application No. 202411057595 dated July 30, 2024
4.	LFA kit for isothermal detection of porcine circovirus 3 nucleic acids.	BC Bera, Taruna Anand, Nitin Virmani and T K Bhattacharya	Application No. 02411043287 dated June 4, 2024
5.	A gain-of-function mutation in poxvirus encoded 2'O-methyltransferase: Enhancing stability and translational efficiency of viral mRNA	Naveen Kumar and Assim Verma	Application No. 202411057416, dated 29.07. 2024
6.	Diazo compounds with anti-trypanosomal activities	Anju Manuja and Team	Application No. 202411057595 dated July 30, 2024
7.	A semen extender formulation for preservation of equine spermatozoa and preparation thereof	TR Talluri and Team	Application No. 202411091668, dated 25-11-2024

Patent granted

- Hydroxychloroquine/chloroquine zinc oxide nanoparticle formulation (Patent No. 542925, dated 25.06.2024, Application No.202111057698, dated 11.12.2021) Name of inventors: Anju Manuja, Balvinder Kumar, Rajender Kumar, Yash Pal and Minakshi P

List of Copy rights granted

Sr.No.	Title of Copyright	Name of Scientist	Application No.	Institute
1.	EquiCare: All-in-One mobile companion App	Anju Manuja and Team	Diary No. 16483/2024-CO/SW dated 22.05.2024	NRCE, Hisar
2.	Mare-USG App: Application of Ultrasonography in mare reproduction	TR Talluri and Team	Diary No. 16950/2024-CO/SW dated 27.05.2024	NRCE, Hisar

Revenue generation and by commercialization of technology

1.	Polymeric metal nanocomposites and methods of synthesis thereof	Habitat Genome Improvement Primary Producer Company Ltd., Hansi, Haryana	Rs. 2.0 lakh towards technology fee.	06.05.2024
2.	Multi Recombinant Antigens-based ELISA kit for diagnosis of <i>Trypanosoma evansi</i> infection in animals	M/s AB Diagnopath Manufacturing Pvt. Ltd., G-1-500, RIICO, Industrial Area, Bhiwadi, Alwar, Rajasthan-301707	Rs. 9.0 lakh towards technology fee.	26.07.2024



Education and Trainings

Sr. No.	Name of the student	Guide (Major or Co-Guide)	Thesis title	Completed in 2024/ Under process
Ph.D. Students				
1.	Ms. Dharvi LPU, Jalandhar	Dr. Balvinder Kumar (Co-Major)	Epdemiological and molecular investigations of strangles in equine population of northern India	Ongoing
2.	Ms. Indu CCHAU, Haryana	Dr. Shanmugasundaram K. (Co-Major)	Targeted genome using CRISPR-Cas9 approach to decipher the functional role of predicted genes in Survival of <i>M.kansasii</i>	Ongoing
3.	Pyarelal RAJUVAS, Bikaner	Dr. TR Talluri (Co-Major)	Development of non-egg yolk extender for stallion semen	Ongoing
4.	Swati GJUS&T, Hisar	Dr. Anju Manuja (Co-Major)	Chloroquine/ Hydroxychloroquine for	Ongoing
5.	Dr. Diksha Khandpal LUVAS, Hisar	Dr. Nitin Virmani (Co-Major)	Genetic targeting of equine herpes virus 1 as a vector for developing vaccine and to explore its potential as a vaccine vector for targeting infectious bursal disease virus in poultry	Ongoing
6.	Priya Sharma GLA University, Mathura	Dr. Taruna Anand (Co-Major)	Isolation and characterization of bacteriophages against <i>Campylobacter jejuni</i> , MRSA and ESBL <i>E.coli</i>	Ongoing
7.	Alka Nokhwal CCS HAU, Hisar	Dr. Rajesh Kumar Vaid (Co-Major)	Isolation and characterization of bacteriophages against aeromonads from fish culture ponds	Completed

Sr. No.	Name of the student	Guide (Major or Co-Guide)	Thesis title	Completed in 2024/ Under process
8.	Swati Guru Jambheshwar University, Hisar	Dr. Nitin Virmani (Co-Major)	Studies on Development of Recombinant Porcine Circovirus Vaccine candidate(s) & their Immunological Response in Murine model / Piglets	Ongoing
9.	Khushbu Maharishi Dayanand University, Rohtak	Dr. Nitin Virmani (Co-Major)	Preparation and Evaluation of Nano-formulation of Equines Herpes Virus Type 1 Vaccine(s) for Immunogenicity in Murine Model	Ongoing
10.	Renu Amity University, Rajasthan	Dr. Anuradha Bhardwaj (Co-Major)	Characterization Of Physicochemical Qualities of Donkey Milk and Its Utilization in Value Added Dairy Products	Completed
11.	Diksha Sharma LUVAS, Hisar	Dr. Rajender Kumar (Co-Major)	In vitro & in vivo evaluation of chemotherapeutic potential of alkaloids compounds against <i>Trypanosoma evansi</i>	Completed
12.	Jitender Rathee MDU, Rohtak	Dr Riyesh T (Co-Major)	Studies on the antiviral activity of Tubacin against foot and mouth disease virus	Ongoing
13.	Mamta Tirdia CBLU, Bhiwani	Dr. Sanjay Kumar (Co-Major)	Anti-theilerial and anti-plasmodial activities of herbal based selected lead drug molecules and elucidation of their targets	Ongoing
MVSc /MSc. Students				
1.	Pawan Kumar Sharma RAJUVAS, Bikaner	Dr. Ramesh Kumar Dedar (Co-Major)	In Vitro Anthelmintic Activity of Some Desert Plants of Rajasthan Against Gastro-Intestinal Nematodes in Goats"	Completed

MVSc /MSc. Students				
2.	Saurabh Daria RAJUVAS, Bikaner	Dr. TR Talluri (Co-Major)	Studies on Ultrasonographic, Hematological and Immunological Markers Changes during Oestrus Cycle in Mares	Completed
3.	Vishal Yadav RAJUVAS, Bikaner	Dr. TR Talluri (Co-Major)	Effect of Addition of Spirulina platensis Extract to Semen Extender on Cooled and Post-Thaw Semen Quality of Marwari Stallion	Completed
4.	Naina Paswan Bihar Animal Sciences University	Dr. TR Talluri (Co-Major)	Effect of melatonin supplementation to the semen extender on cryopreserved stallion semen parameters	Completed
5.	Bhupender Kumawat CUH, Mahendragrh	Dr. Taruna Anand (Co-Major)	Studies on resistance development of <i>Klebsiella pneumoniae</i> against Bacteriophages as an external supervisor	Completed
6.	Vanshika Aggarwal GJUS&T, Hisar	Dr. Riyesh T (Co-Major)	A preliminary investigation into the involvement of HDAC2 in Buffalopox and Newcastle disease virus replication	Ongoing

Trainings Organised

Workshop on “Hands-on National workshop on translational research on bacteriophages as a promising antimicrobial alternative”

Hands-on workshop conducted on 18th October 2024 under Dr. Taruna Anad at NRC Equines, Hisar. Provided hands-on training to students and assistant professors on experimental demonstrations for isolation, and characterisation of bacteriophages, cocktail preparation, NGS analysis of bacteriophage genomes, genome assembly tools and techniques and basics of phage therapy in animal models (poultry, mice). A total of 35 people attended the workshop.

Training programme on “Polymeric metal nanocomposites and methods of synthesis thereof”

A training programme under Dr. Anju Manuja for the staff of HABITAT Genome Improvement Primary Producer Company, Hisar and National Research Centre on Equines, Hisar was conducted from 20th to 24th May 2024 at NRC Equines, Hisar.

Training programmes on “Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach”

A five-day training program under Dr. Riyesh T, on Rabies diagnosis by DFAT and real-time PCR was conducted at ICAR-NRCE, Hisar, for medical, veterinary, and wildlife professionals from Uttar Pradesh, held from November 19-23, 2024.

Five days hands on training program on "Zoonotic Diseases Diagnosis Under One Health Approaches and Equine Husbandry Practices" was organized at ICAR-NRCE, Hisar and EPC, Bikaner from 18 to 22 March 2024. 20 Veterinary officers of Tamil Nadu were participated in the program. The training program was organized by Dr Shanmugasundaram K, Dr Talluri TR, Dr H. Singha and Dr Riyesh T.

NRCE in Education and Technology

MoU for Cooperation in Research and Education

- An MoU signed between ICAR- National Research Centre on Equines and Bikaner Engineering College on 7th December, 2024 in the presence of Hon. Vice Chancellor Dr Ajay Kumar Sharma, Bikaner Technical University.
- MoU signed between ICAR- National Research Centre on Equines (NRCE), Hisar and Maharishi Dayanand University, Rohtak, Haryana.
- MoU signed between ICAR- National Research Centre on Equines (NRCE), Hisar and IG foundation Jhak, District- Barmer, Rajasthan.
- MoU signed between ICAR- National Research Centre on Equines (NRCE), Hisar and Himachal Pradesh Krishi Vishwavidyalaya
- MoU signed between ICAR- National Research Centre on Equines (NRCE), Hisar and Maharashtra Animal and Fisheries Sciences University



Workshop, Seminar and Institutional Activities

78th Independence Day Celebrations at ICAR-NRCE

78th Independence Day was celebrated with patriotic fervour at both the ICAR-NRCE, Hisar campus and the Regional Station, ICAR-NRCE, Bikaner. At the Hisar campus, Dr. TK Bhattacharya, Director, hoisted the National Flag and addressed the gathering. At the Regional Station, Dr. SC Mehta, Head of Station, performed the flag hoisting ceremony. On this occasion, the technology certificates were distributed to contributors in recognition to their valuable efforts and achievements.



Republic Day Celebrations

75th Republic Day was celebrated with great enthusiasm at the main campus Hisar and the Regional Station, Bikaner. At the Hisar campus, Dr. TK Bhattacharya, Director, hoisted the National Flag and addressed the gathering. Dr. S.C. Mehta hoisted the National Flag. In his address, he congratulated the staff for receiving the Breed Conservation Award 2023 and the official recognition of Bhimthadi as the eighth horse breed in the country. Dr. Thirumula Rao Talluri was felicitated for his achievements in embryo transfer technology, and Dr. Jitendra Singh, Shri Om Prakash, and Shri Gopal were honored for their exemplary institutional contributions. The major attraction of the day was the Rewal Chaal and Gallop Races that were organized in the campus.

40th Foundation Day of ICAR-NRCE, Hisar

The 40th Foundation Day of ICAR-NRCE, Hisar, was celebrated with traditional enthusiasm on 26th November 2024. The occasion was graced by Dr. Raghvendra Bhatta, Deputy Director General (Animal Science), ICAR, as the Chief Guest. Dr. TK Dutta, Director, ICAR-CIRB and Dr. Naveen Kumar, Director, ICMR-NIV, Pune, attended as Guests of Honour. The programme commenced with a welcome address by Dr. TK Bhattacharya, Director, ICAR-NRCE. On this occasion, the NCVTC Microbial Catalogue was released. Dr. Bhatta appreciated the Centre's significant contributions to research and development in the field of equine science. The event concluded with a vote of thanks proposed by Dr. RK Vaid, Principal Scientist and Organizing Secretary of the event. To mark the Foundation Day, a National Symposium on Companion Animals and Livestock under the One Health Paradigm was also organized. Distinguished invitees delivered expert lectures, and more than 50 research posters were presented. A Compendium on the symposium theme was released to commemorate the event.

36th Foundation Day – Regional Station, Bikaner

The 36th Foundation Day of the Regional Station, Bikaner, was celebrated on 28th September 2024 with great enthusiasm. Prof. Manoj Dixit, Hon'ble Vice-Chancellor, RAJUVAS, Bikaner, graced the occasion as the Chief Guest. The celebration featured various horse competitions and a stakeholders' meet, followed by the distribution of prizes to the winners and recognition of best-performing staff members.

Equine Health Camps and Farmers' Outreach Activities

Equine health camps and farmer interaction programmes were organized in Jammu & Kashmir in collaboration with the Animal Husbandry Department, Government of Jammu & Kashmir. These camps were conducted at the base camps of the Shri Amarnath Yatra. On 12th August 2024, a camp at Pahalgam witnessed participation from over 100



Organization of health camp by NRCE staff at Pahelgam and Baltal, J&K

beneficiaries along with their ponies, while a similar camp held on 13th August 2024 at Baltal, near Sonamarg, was attended by around 50 beneficiaries. During these camps, equines were examined for various health conditions, and samples were collected. Essential veterinary inputs, including dewormers, vitamins, ectoparasiticides, and mineral mixtures, were distributed among the participants.

In addition to these, several other health camps and Kisan Gosthies were conducted as part of outreach initiatives. Under the Scheduled Caste Sub-Plan (SCSP), a camp was held at Village Batla, Hisar, on 26th September 2024, benefiting approximately 65 SC farmers. Similar programmes were organized at Village Ludas, Hisar on 25th September 2024, and at Village Nagathala, Hisar on 27th December 2024, benefiting around 55 and 60 SC farmers, respectively. In all these camps, participants were provided with mineral mixtures, anthelmintics, and informative literature.



Organisation of equine health camp and Kisan Gosthi by NRCE staff at Batla and Ludas, Haryana

An equine health camp and Kisan Gosthi were organized during the Annual Livestock Mela at Tilwara, Rajasthan, from 3rd to 5th April 2024. A total of 35 equines were examined by a multidisciplinary team, and pregnancy diagnosis were carried out as well. Biological samples were collected, and veterinary kits were distributed among farmers. Approximately 200 farmers participated in the Kisan Gosthi conducted at the event. Furthermore, a centre-based Kisan Gosthi was organized at ICAR-NRCE, where around 25 farmers participated. The event was presided over by the Director, who interacted with the farmers and addressed their concerns.



Examination of horse by NRCE staff during health camp

Kisan Diwas Celebration

Kisan Diwas was celebrated on 23rd December 2024 at ICAR-NRCE with active participation from the farming community. Approximately 50 farmers attended the event. On this occasion, the Director, Dr. TK Bhattacharya, interacted with the farmers, listened to their concerns, and assured them of the Centre's continued support through appropriate scientific interventions and technical guidance.



NRCE staff interacting with equine stakeholders during Kisan Diwas

Motivational Talk for Staff

As part of staff well-being initiatives, a motivational lecture was delivered by Shri EV Girish from Brahma Kumaris on 28th October 2024 at ICAR-NRCE. The session focused on enhancing mental strength, managing stress effectively, and



Motivational talk at ICAR-NRCE by team from Brahma Kumaris

maintaining emotional balance in the workplace. Dr. TK Bhattacharya, Director, ICAR-NRCE, highlighted the importance of such sessions in fostering professional motivation, personal development, and overall mental well-being among staff members.

A meeting of the Official Language Implementation Committee was convened to assess the progress and implementation of Hindi in official communication. The fortnight-long campaign emphasized the promotion of Hindi as a medium for administrative and day-to-day functioning. Post-event reviews indicated a noticeable increase in the usage of Hindi among staff members, reflecting the success of the initiative.

19th Parthenium Awareness Week

The 19th Parthenium Awareness Week was observed from 16th to 22nd August 2024 at the Agricultural Farm of ICAR-NRCE, Hisar. The programme was chaired by Dr. TK Bhattacharya, Director, ICAR-NRCE. An expert lecture was delivered by Dr. Rajiv Bhatia, Agriculture Development Officer, Agroha Block, who highlighted the harmful effects of Parthenium on health and agriculture, and outlined effective methods for its eradication.



Parthenium week celebrations at ICAR-NRCE

Van Mahotsav

As part of the *Van Mahotsav* celebrations, a plantation drive was organized on 27th July 2024 in collaboration with the Department of Forest, Haryana. The event was presided over by Dr. TK Bhattacharya Director, ICAR-NRCE, and attended by Forest Ranger Ms. Jyotisana, Mr. Subash Birthal, and NCC cadets. The event emphasized the importance of afforestation and environmental conservation.



Celebration of *Van Mahotsav* programme at ICAR-NRCE



Plantation of trees during *Van Mahotsav* at ICAR-NRCE

International Yoga Day

International Yoga Day was celebrated at ICAR-NRCE on 21st June 2024. A yoga awareness and practice session was conducted in front of the main office building. Yogacharya Shri Deepak Solanki led the session, elaborating on the significance of yoga in daily life for physical and mental well-being. Scientists, officers, and staff members participated actively in the event.



Staff of ICAR-NRCE participating in International Yoga day

भा.कृ.अनु.प.-राष्ट्रीय अश्व अनुसंधान केन्द्र में हिन्दी पखवाड़ा का आयोजन

भा.कृ.अनु.प.-राष्ट्रीय अश्व अनुसंधान केन्द्र में हर साल सितम्बर माह में हिन्दी पखवाड़ा का आयोजन किया जा रहा है। इस पखवाड़े में 2 हिन्दी की कार्यशालाओं का आयोजन भी किया गया जिसमें हिन्दी भाषा पर व्याख्यान प्रस्तुत किए गए। आयोजित की गई कार्यशालाओं में हिन्दी भाषा का महत्व, उपयोगिता और इस भाषा को जन-जन की भाषा बनाने के रूप में प्रोत्साहित किया गया। संस्थान के सभी कर्मचारियों ने इस कार्यक्रम में भाग लिया और कार्यशाला से लाभान्वित हुए। हिन्दी पखवाड़ा के दौरान विभिन्न कार्यक्रम आयोजित किए गए जिनमें हिन्दी में किया गया सर्वाधिक कार्य, श्रुतलेख प्रतियोगिता, निबन्ध प्रतियोगिता, हिन्दी आशु भाषण, टंकण, अनुवाद, प्रश्नोत्तरी, प्रतियोगिता, वर्ग पहली प्रतियोगिता और कविता प्रतियोगिता शामिल थे। प्रतिभागियों को इनाम स्वरूप राशि भेंट भी प्रदान किया गया। हिन्दी के प्रोत्साहन हेतु संस्थान के सभी कर्मचारियों ने इस पखवाड़े में बढ़-चढ़कर भागीदारी की। तिमाही के दौरान हिन्दी कार्यान्वयन हिन्दी सलाहकार समिति की बैठक भी आयोजित की गई। हिन्दी का उपयोग बढ़ाने हेतु श्वेतपट्ट पर हिन्दी के नए शब्द प्रतिदिन एक लिखा जाता है। हिन्दी का प्रयोग का विस्तार करने हेतु समय-समय पर प्रशासनिक विभाग में अधीनस्थ अधिकारियों एवं संस्थान के विभागों में पत्र के माध्यम से सूचित किया जाता है। हिन्दी पखवाड़ा एवं कार्यशालाएं आयोजित करने के उपरान्त यह पाया गया कि अधिकारियों एवं कर्मचारियों में हिन्दी भाषा का अधिक उपयोग देखा गया। हिन्दी भाषा के पखवाड़े के आयोजन उपरान्त हिन्दी प्रचलन रोजमर्रा के कार्यों में अधिक से अधिक उपयोग में लाता हुआ देखा गया है। पुस्तकालय द्वारा भी भेजे जाने वाले पत्रों को अधिकतम हिन्दी भाषा में भेजा जाता है।



Annual Sports Meet

The Annual Sports Meet was successfully organized at ICAR-NRCE with enthusiastic participation from staff across all departments. A variety of events were conducted including chess, shot put, javelin throw, table tennis, slow cycling, carom, and tug-of-war, with active involvement from both men and women. The event was ceremoniously inaugurated by the Director, Dr. TK Bhattacharya, through the symbolic release of hydrogen balloons, setting an energetic tone for the competitions that followed. The meet fostered a spirit of camaraderie, fitness, and sportsmanship among all participants.

List of Prize Winners

Sl. No.	Name of event	Men			Women		
		1 st	2 nd	3 rd	1 st	2 nd	3 rd
1.	Chess	Sh. Amandeep	Sh. Lalit Chaudhary	Sh. Aman Godara	Dr. Sonia	Dr. Prexha	Ms. Monika
2.	Shot-put	Sh. Lalit Chaudhary	Sh. Man Singh	Sh. Man Godara	Dr. Prexha	Ms. Divya	Ms. Pooja
3.	Javelin Throw	Sh. Shilindra Kularia	Sh. Madan	Sh. Sunil Jain	Ms. Monika	Mrs. Jyoti	Ms. Divya
4.	Table Tennis	Dr. Riyesh	Sh. Sanjeev Kumar	Dr. Nitin Virmani	Smt. Jyoti	Ms. Priyanka	Ms. Shalini
5.	Carom Board	Sh. Deepak	Sh. Amandeep	Sh. Ashok	Ms. Sonia	Ms. Priya	Smt. Ruma
6.	Slow Cycling	Sh. Parveen	Sh. Rahul	Sh. Hanuman	Smt. Jyoti	Ms. Shalini	Ms. Swati
7.	Tug of war (Winner team)	Team A Captain : Sh. Aman Godara; Total Participants 15			Team B Captain : Dr. Taruna Anand; Total Participants 11		

Prizes were distributed among all winners.



Director, ICAR-NRCE releasing the balloons as a mark of commencement of the Sports day celebration



NRCE staff participating in the Sports Meet

Swachh Bharat Abhiyan Activities

ICAR-NRCE main campus Hisar, Haryana and Regional Centre, EPC, Bikaner conducted Swabhav Swachhata – Sanskar Swachhata activities between 17th September – 1st October 2024. Under Swachhata Hi Sewa drive at ICAR-NRCE, Hisar, the staff members took Swachhata Pledge. Swachhata Pakhwada was conducted main campus Hisar and Regional Centre, EPC, Bikaner between 16-31 Dec., 2024. During this period various activities such as swachhata awareness, cleanliness maintenance, sanitation and green drive, community Outreach and involvement, signature campaigns, and recycling the waste water were pertaining, plantation drive, nukkad natak Swachhata and cleaning of pond were carried at ICAR-NRCE. All the staff members including scientists, administrations, students, YPs, RAs, SRFs, students, and contractual staffs were actively participated in the events.



Swachhata activities in NRCE Hisar and EPC, Bikaner



IRC, RAC, QRT and IMC Meetings

27th Research Advisory Committee (RAC) meeting of ICAR- NRCE

The 27th RAC meeting of ICAR-National Research Centre on Equines was held under the Chairmanship of Dr. MC Sharma (Former Director, IVRI) on 15 Jan., 2024 to review the ongoing research activities at ICAR-NRCE for the year 2023-24. Dr. TK Bhattacharya, Director, ICAR-NRCE presented the action taken report on the 26th RAC recommendations. A total of 49 research projects were discussed in the meeting. The Chairman RAC appreciated the efforts of the scientists and emphasized that every scientist should make efforts to develop one product per year to set the pace for developing cutting edge technologies. The scientists should work in mission mode for vaccine development, patenting and commercialization of the technologies. Concerted efforts should be made towards out-reach programs.



RAC meeting of ICAR-NRCE in progress

The Chairman expressed his concern over steep decline in equine population in the country. He advised that the equine population is decreasing at a faster rate due to mechanization. The institute should prioritize efforts to improve the utility of animals and work for improving livelihood of equine farmers. The Chairman emphasized the need for transfer of the technologies to equine farmers and initiate extension activities in equine populated area of HP, J&K, Uttarakhand and NEH region etc.

Annual Institute Research Committee (IRC) Meeting

The annual IRC meeting (2023-24) of ICAR-NRCE, Hisar was held in the committee room (ICAR-NRCE) under the chairmanship of Dr. TK Battacharya, Director, ICAR-NRCE on 28th -29th May, 2024. A total of 24 institute research projects, 27 externally funded projects and two concept notes were discussed in the meeting. Dr. TK Battacharya, Director, ICAR-NRCE in appreciated the research activities and output from the scientists. He motivated the scientists to do research relevant to the stakeholders' needs. He also motivated the scientists to apply for the external funding in view of budget constraints. He also guided the scientists to work in collaborative mode and to share the manpower, resources and judicious utilization of the institute funds. One external expert (Prof. N K Kakkar , former Head of the department of Veterinary Microbiology at LUVAS, was also invited in the meeting for evaluation of the progress of ongoing research projects and suggestions.



Annual Institute Research Committee Meeting

Half Yearly Institute Research Committee (IRC) Meeting

The Half yearly IRC meeting (2024) of ICAR-NRCE, Hisar was held in the committee room under the chairmanship of Dr TK Bhattacharya, Director on 10th Dec., 2024. A total of 48 research projects, were reviewed and discussed in the meeting.

Institute Management Committee (IMC) Meeting

43rd Meeting of Institute Management Committee of ICAR-NRCE, Hisar, held online on 24.12.2024 at 11.00 AM under the Chairmanship of Dr. TK Bhattacharya, Director, ICAR-NRCE, Hisar. Dr. TK Bhattacharya briefed the house on the activities and achievements of the Centre. IMC appreciated the efforts and the achievements of the Centre. Shri Pawan Kumar, Administrative Officer & Member Secretary presented the agenda items and the following recommendations were given by the committee:

1. The proceeding 42nd meeting of IMC held on 31.07.2023 was confirmed.
- 2.. Appointment of AMA/Part Time Medical Officer at ICAR-NRCE, Hisar

IMC considered the proposal and accorded post-facto approval for extension of appointment of Dr. Inderjeet as Part Time Medical Officer/AMA from 01.05.2024 to 30.04.2025 on the prescribed rates and term & conditions.

FAO further informed the IMC that against the target of revenue generation of Rs. 94.25 lakhs during 2023-24, the actual revenue receipt generated was Rs. 190.08 lakhs.

8th Quinquennial Review Team (QRT)

The first meeting of 8th Quinquennial Review Team (QRT) of ICAR-National Research Centre on Equines (ICAR-NRCE), Hisar was conducted on 30th December 2024 at 10:00 AM. in the Committee Room of ICAR-NRCE, Hisar. The meeting was chaired by Dr. AK Srivastava, Vice Chancellor, DUVASU, Mathura.

The meeting started by welcome address by Director, NRCE, which was followed by address by Chairman QRT. The main proceedings started by presentation of an overview of NRCE by Director, NRCE. This was followed by ATR presentation by Member Secretary. Division wise presentation of achievements of NRCE Hisar for 2018-2023 was given by Dr. SC Mehta, Head, RS, EPC, Bikaner for Equine Production, Dr. N Virmani, Head, EHU, NRCE, Hisar and Dr. R K Vaid, I/C Head, NCVTC. QRT team also visited EHU and NCVTC Laboratory, & visited animal farm.



Director, ICAR-NRCE welcoming the QRT chairman

Visit of dignitaries

Dr. Raghavendra Bhatta DDG (Animal Sciences), ICAR reviews equine research and infrastructure at ICAR-NRCE, Bikaner campus

Dr. Raghavendra Bhatta, Deputy Director General (Animal Science), ICAR, visited the Regional Station, Bikaner, on April 7, 2024. He was accompanied by Dr. AK Tomar, Director, ICAR-CSWRI, Avikanagar, and Dr. A Sahoo, Director, ICAR-NRCC, Bikaner. During the visit, the delegation toured the equine museum, animal sheds, foaling line, embryo transfer (ET) laboratory, and other scientific facilities. Dr. Mehta, Head of the Regional Station, apprised the dignitaries of ongoing activities and developments.

Dr. Raghavendra Bhatta DDG (Animal Sciences), ICAR applauds research excellence and translational impact

Dr. Raghavendra Bhatta, Deputy Director General (Animal Science), ICAR, visited ICAR-NRCE, Hisar, on February 2, 2024, and was welcomed by Dr. TK Bhattacharya, Director. He visited key laboratories and evaluated the Centre's translational research activities. Dr. Bhatta praised the indigenous development of the LSDV vaccine and acknowledged ICAR-NRCE's impactful research ecosystem, highlighting its contributions to national livestock health initiatives and scientific leadership in equine health and production.



Dr. Abhijit Mitra, Animal Husbandry Commissioner, GOI applauds LSDV vaccine technology and equine health research

Dr. Abhijit Mitra, Animal Husbandry Commissioner, Government of India, visited ICAR-NRCE, Hisar, on February 2, 2024. He interacted with scientists and reviewed various ongoing research programs related to equine health and livestock disease management. Dr. Mishra appreciated the Centre's pivotal role in equine diagnostics and control strategies, and particularly commended the development of the Lumpy Skin Disease Virus (LSDV) vaccine, recognizing its significant potential in national disease control programs.



Dr. Vinod Kumar Verma, Vice Chancellor, LUVAS, Hisar highlights collaborative research and technological strength

On February 2, 2024, Dr. Vinod Kumar Verma, Vice Chancellor of LUVAS, visited ICAR-NRCE, Hisar. During his visit, he emphasized the importance of inter-institutional collaboration and admired the Centre's advanced infrastructure and research capabilities. He specifically appreciated the successful development of the LSDV vaccine, considering it a model for applied virology and vaccine innovation in veterinary sciences.





Dr. TK Dutta, Director, ICAR-CIRB appreciates scientific infrastructure and biosecurity focus

Dr. TK Dutta, Director of ICAR-CIRB, Hisar, visited ICAR-NRCE on February 2, 2024. He applauded the Centre's state-of-the-art laboratories, scientific rigor, and quality assurance systems.

Mrs. Geeta Bharti, IAS, Commissioner, Hisar praises work culture and equine research

On February 12, 2024, Mrs. Geeta Bharti, Commissioner of Hisar Division, visited ICAR-NRCE. She toured the laboratories and equine units and interacted with scientists. She expressed appreciation for the neat infrastructure, organized scientific environment, and inclusive work culture.



Prof. Narsi Ram Bishnoi, Vice-Chancellor, GJU, Hisar encourages academic–research collaboration

Prof. Narsi Ram Bishnoi, Vice Chancellor, GJU, Hisar, visited ICAR-NRCE on February 19, 2024. He was briefed on the Centre's advancements in virology, nanotechnology, and disease diagnostics. Prof. Bishnoi acknowledged the research quality and technical innovation at the institute and encouraged deeper collaboration between academia and research institutes.

Infrastructure, Developmental Activities and Herd Strength

Strengthening of laboratories and training infrastructure

During the year 2024, ICAR-National Research Centre on Equines (NRCE) implemented significant infrastructure development projects to enhance research capacity and training facilities. Notable among these were the strengthening of teaching and training facilities at the Hisar campus and the regional station in Bikaner, renovation of the Veterinary Medicine Laboratory (Nano-Tech). In addition, key improvements were made in the NCVTC building, including terrace repairs and installation of a modern lift system to support safe and efficient movement.

Upgradation of supporting facilities and Utilities

Road construction and farm fencing works were also undertaken to improve accessibility and security in the research farm area. The renovation of the AKMU Cell and infrastructure upgrades in the pathology lab enhanced digital operations and laboratory efficiency. These activities reflect ICAR-NRCE's commitment to maintaining state-of-the-art infrastructure, thereby enabling high-quality scientific research, technology dissemination, and capacity building.

WOAH Reference Laboratory for equine piroplasmosis

The WOAH Biological Standards Commission accepted to designate the ICAR-National Research Centre for Equines (NRCE) as a new WOAH Reference Laboratory for equine piroplasmosis, with Dr Sanjay Kumar as the designated expert.



ISO/IEC 17025 Accreditation

The bacteriology and equine pathology laboratories have achieved NABL-ISO/IEC 17025 accreditation for equine glanders and equine influenza testing respectively. This accreditation ensures compliance with international standards for testing quality and competence. It enhances the credibility and reliability of diagnostic results for equine influenza and glanders. The recognition marks a significant step in maintaining high standards in veterinary diagnostics.





National Accreditation Board for Testing and Calibration Laboratories			
SCOPE OF ACCREDITATION			
Laboratory Name:		EQUINE PATHOLOGY LABORATORY, NATIONAL RESEARCH CENTRE ON EQUINES, GURGAON	
Accreditation Standard:		ISO/IEC 17025:2017	
Certificate Number:		7C(L)303	
Validity:		23/11/2024 to 24/11/2028	
Page No:		1 of 1	
Last Amended on:			
S.No.	Description / Group	Method(s) or Products tested	Equipment, parameter or characteristic tested / Specific Test Performance / Tests or type of tests performed
Permanent Testing			
L	BIOLOGICAL / VETERINARY TESTING	Infectious / Susceptible Animals (Equine, Horse, Mule, Donkey, Dobra) (Horse / Parasitological) Serology	Bacterial, Viral Detection of Equine Infectious Virus
F	BIOLOGICAL / VETERINARY TESTING	Infectious / Susceptible Animals (Equine, Horse, Mule, Donkey, Dobra) Serology	Antigenic Detection of Equine Infectious Virus (EIV)

Restoration pond area

Pond area of the centre has been developed for Equine-Ecotourism activity. A beautiful lawn and herbal park have also been developed at that area. Miyawaki plantation has been done near pond area for the purpose of deep foresting in co-ordination of Forest Department of Haryana. Construction of brick road is under progress. Pond is being developed for fish culture activities.

Equine Eco-Tourism activities

Equine Eco-Tourism activities have been initiated for common man and are in progress. For this purpose; a buggy and tonga have been purchased. Construction of huts and washroom are in progress.

Reclamation of Land

After water stagnation in whole farm area has become abundant and wild grass and bushes have grown up. The land has been reclaimed and is under cultivation. Fencing work is in progress for purpose of security. Approximately 600 plants of different varieties have been planted in farm area. Water channels and kucha roads have been restored.



Equine herd strength at EPC, Bikaner during the period 01/01/2024 – 31/12/2024

	MARWARI		KATHIAWARI		ZANSKARI		MANIPURI		POITOU		HALARI		MULE		NUKRA		TOTAL
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Stock as on 01.01.2024	18	27	01	04	03	06	03	02	06	19	05	06	01	00	02	00	103
Birth	04	01	00	00	02	02	00	01	01	01	01	01	00	00	00	00	14
Purchased	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Transferred from NRCE, Hisar	00	00	00	00	00	00	00	00	00	00	02	03	00	00	00	00	05
Death	01	01	00	00	00	00	00	00	00	01	00	00	00	00	00	00	03
Auctioned / Sold	00	00	00	00	00	00	00	00	01	00	00	00	00	00	00	00	01
Balance as on 30.12.2024	21	27	01	04	05	08	03	03	06	19	08	10	01	00	02	00	118
Grand Total	48		05		13		06		25		18		01		02		118

Equine herd strength at Hisar campus during the period 01/01/2024 – 31/12/2024

Sr. No.	Kind of equine	OB 01.01.2024	Addition		Disposal		CB 31.12.2024
			Birth	From EPC	Death	To EPC	
Horses							
1.	Mares	07	nil	nil	01	nil	06
2.	Fillies	01	nil	nil	01	nil	nil
3.	Colts	02	nil	nil	01	nil	01
4.	Stallion	01	nil	nil	nil	nil	01
	Total	11	nil	nil	03	nil	08
Ponies							
1.	Mares	05	nil	nil	nil	nil	05
2.	Fillies	01	nil	nil	nil	nil	01
3.	Colts	01	nil	nil	nil	nil	01
	Total	07	nil	nil	nil	nil	07
Donkeys							
1.	Jennies	12	nil	nil	nil	01	12
2.	Fillies	07	02	nil	nil	02	05
3.	Colts	06	05	nil	nil	02	09
4.	Jacks	01	nil	nil	nil	nil	01
	Total	26	07	nil	01	05	27
	Grand Total	44	07	nil	04	05	42



Awards, Recognitions, Personal Milestones, New Joinings & Promotions



- Dr. Thirumala Rao Talluri selected as associate fellow of NAVS.
- First position for the best Poster Presentation was awarded to Dr. TR Talluri and team for the paper titled "*Raj-sheetal: country's first foal produced through vitrified embryo transfer*" authored by Sajjan Kumar, Thirumala Rao Talluri, Omprakash Solanki, RK Dedar, and TK Bhattacharya at 39th Annual Convention and National Symposium of ISSAR on "Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective" from November 29th to December 1st, 2024 organised by GADVASU, Ludhiana.
- Dr. Madhu Meena, secured 'Young Scientist' award for the paper titled '*Mitigation of endometritis in mares with intrauterine infusion of autologous platelet rich plasma*' authored by Madhu Meena, Dinesh Kumar Jhamb, Thirumala Rao Talluri, Rajesh Kumar Vaid, Om Prakash Solanki, Sajjan Kumar, Sumanshu Nawal, Kalpesh Pargi at 39th Annual Convention and National Symposium of ISSAR on "Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective" from November 29th to December 1st, 2024 organised by GADVASU, Ludhiana.





- Secured best oral presentation third position award for the paper titled “Combined thickness of the uterus and placenta in three indigenous horse breeds” authored by Omprakash Solanki, TR Talluri, Dinesh Jhamb, Sajjan Kumar, Neelam Kalasua, Kalpesh Pargi, Madhu Meena at 39th Annual Convention and National Symposium of ISSAR on “Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective” from November 29th to December 1st, 2024 organised by GADVASU, Ludhiana

- Dr. Anju Manuja awarded as 'Outstanding Scientist' for research contribution in 2024 by ICAR-National Research Centre on Equines, Hisar.
- Dr. Anju Manuja was awarded by OM Sterling University for 'excellence in research in health' on International Women's Day, 2024.
- Ms. Nisha Devi, JRF of Dr. Anju Manuja DST project was awarded 'best oral presentation' (in International Conference on Nanotechnology Addressing the Convergence of Materials Science, Biotechnology, and Medical Science at DY Patil University, Kohlapur, Maharashtra in February 2024, (Presented Online).
- Ms. Swati Rani, student of Dr. Anju Manuja was awarded 'First Prize' in a poster session in a seminar on "Companion animals and livestock diseases in one health paradigm" at ICAR-NRCE, Hisar on 26th November, 2024.



- Mr. Karan, secured second position in poster presentation in the Foundation Day National Seminar on 'Companion Animals and Livestock Diseases in One Health Paradigm' to poster titled “Evolution of antimicrobial activity of phage-encoded endolysin protein and assessment of its combination with colistin against MDR Salmonella sp. strain” authored Karan Bhutani, R. Rani, P Kapoor, P Sharma, B.C. Bera, R.K.Vaid, N. Virmani, T. Anand organized on 26th Nov, 2024 at ICAR- NRCE, Hisar, Haryana.
- Regional Station, ICAR-NRCE, Bikaner received the Breed Conservation Award 2024 for conservation of Halari Donkey in the Institute category on December 23, 2024, at ICAR-NBAGR, Karnal.
- Dr. Taruna Anand secured AMI-Springer Nature 2024 award for Best poster at Guru Jambheshwar University of Science and Technology, on Nov 14-17, 2024 organised by Springer Nature, during the 65th Annual International conference of Association of Microbiologists of India, 2024.

Personal milestones

- Dr RK Vaid, Principal Scientist, was assigned the responsibility of Head, NCVTC, NRCE, Hisar, with the approval of the Council from 30th August, 2024.
- Dr. Rajender Kumar served as Co-Chairman for the Keynote Address Session at the 33rd National Congress of Veterinary Parasitology and the National Symposium on “Innovations in Parasite Control Strategies for the Upliftment of Animal & Human Health,” organized by the Department of Veterinary Parasitology, College of Veterinary Science, PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India, from December 17 to 19, 2024.

Promotions

Sr. No.	Name	Designation	Date of Promotion
Promotion			
1	Sh. DD Sharma	Assistant to AAO	21.10.2024
2.	Sh. Hanuman Singh	MTS to Technician	11.09.2024
3.	Sh. Ishwar Singh	MTS to Technician	11.09.2024
4.	Sh. Sant Ram	MTS to Technician	11.12.2024



Research Publications

1. Ahlawat S, Niranjana SK, Arora R, Viji RK, Kumar A, Sharma U, Raheja M, Popli K, Yadav S, Mehta SC. Advancing equine genomics: the development of a high density Axiom_Ashwa SNP chip for Indian horses and ponies. *Functional & Integrative Genomics*. 2024 Dec;24(6):195.
2. Ahlawat, Sonika, Upasna Sharma, S. K. Niranjana, Pooja Chhabra, Reena Arora, Rekha Sharma, Karan Veer Singh, R. K. Viji, and S. C. Mehta. "Unraveling the maternal heritage: identifying the complex origins of indigenous Indian horse and pony breeds through mitochondrial genome analysis." *Mammalian Genome* 36, no. 1 (2025): 118-128.
3. Yadav V, Dholpuria S, Talluri TR, Joshi A, Khandelwal S, Kumar A. Study on Sexual Behaviour Parameters of Marwari Stallions during Breeding Season. *Journal of Advances in Biology & Biotechnology*. 2024 Jan 24;27(1):1-6.
4. Bhardwaj A, Tandon G, Pal Y, Sharma NK, Nayan V, Soni S, Iqbal MA, Jaiswal S, Legha RA, Talluri TR, Bhattacharya TK. Genome-Wide Single-Nucleotide Polymorphism-Based Genomic Diversity and Runs of Homozygosity for Selection Signatures in Equine Breeds. *Genes*. 2023 Aug 14;14(8):1623.
5. Chhabra D, Nagra J, Manuja A, Singha HS, Vaid RK, Goutam U, Kumar B. Phenotypic, Biochemical and Molecular Characterization of *Streptococcus equi* Isolates in Northern India. *Indian Journal of Microbiology*. 2024 Nov 26:1-7.
6. Damle MN, Chaudhari L, Tardalkar K, Bhamare N, Jagdale S, Gaikwad V, Chhabra D, Kumar B, Manuja A, Joshi MG. A biologically functional bioink based on extracellular matrix derived collagen for 3D printing of skin. *International Journal of Biological Macromolecules*. 2024 Feb 1;258:128851.
7. Dedar RK, Kumar N, Narnaware SD, Singh J, Legha RA, Pal Y. Management of seasonal dermatitis in horses by using leaf extract of *Aerva javanica*. *Indian Journal of Traditional Knowledge (IJTK)*. 2024 Feb 22;23(2):154-61.
8. Gupta S, Vohra S, Sethi K, Rani R, Gupta S, Kumar R, Kumar S. Study of mRNA expression of thirteen genes of *Trypanosoma evansi* in response to diminazene aceturate and isometamidium chloride. *The Indian Journal of Animal Sciences*. 2024 Oct 1;94(10):837-43.
9. Jaglan AB, Vashisth M, Sharma P, Verma R, Virmani N, Bera BC, Vaid RK, Singh RK, Anand T. Phage mediated biocontrol: a promising green solution for sustainable agriculture. *Indian Journal of Microbiology*. 2024 Jun;64(2):318-27.
10. Jaglan AB, Verma R, Vashisth M, Virmani N, Bera BC, Vaid RK, Anand T. A novel lytic phage infecting MDR *Salmonella enterica* and its application as effective food biocontrol. *Frontiers in Microbiology*. 2024 Aug 15;15:1387830.
11. Jangir M, Rathore NS, Lal P, Sareen M, Moolchandani A, Kumar A, Kumar V and Talluri TR. (2024). Spectrophotometric method for the rapid determination of sperm concentration in magra ram. *Haryana Vet*.

- 63(1): 26-31.
12. Jhandai P, Shanmugasundaram K, Bhattacharya TK, Singha H. Advances in understanding immunity to *Rhodococcus equi* infection and Vaccine Development Strategies: A Comprehensive Review. *The Microbe*. 2024 May 4;100077.
 13. Raval K, Kumaresan A, Sinha MK, Elango K, King JP, Nag P, Paul N, Talluri TR, Patil S. Sperm proteomic landscape is altered in breeding bulls with greater sperm DNA fragmentation index. *Theriogenology*. 2024 Mar 1;216:82-92.
 14. Kumar R, Bera BC, Anand T, Pavulraj S, Kurian Mathew M, Gupta RP, Tripathi BN, Virmani N. Evaluation of immunogenicity and protective efficacy of bacteriophage conjugated haemagglutinin based subunit vaccine against equine influenza virus in a murine model. *Veterinary Research Communications*. 2024 Jun;48(3):1707-26.
 15. Kumar S, Singh J, Dedar RK, Talluri TR. Management of Dystocia Due to Posterior Presentation and Umbilical Torsion in a Jenny. *Indian Journal of Animal Reproduction*. 2024 Jun 1;45(1).
 16. Manuja A, Kumar B, Chhabra D, Brar B, Riyesh T, Pal Y, Bhattacharya TK, Prasad M. Antiviral and Cytoprotective Effect of Zinc (Yasad Bhasma) Based Nanoformulations Against Bovine Coronavirus. *Indian Journal of Microbiology*. 2024 Sep;64(3):1123-31.
 17. Manuja A, Nagra J, Chhabra D, Manuja K, Sihag M, Prasad M, Kumar B. Harnessing chitosan for zinc and hydroxychloroquine delivery, interactions with fibronectin, and antibacterial activity. *Carbohydrate Polymer Technologies and Applications*. 2025 Mar 1;9:100647.
 18. Manuja A, Rani R, Devi N, Sihag M, Rani S, Prasad M, Kumar R, Bhattacharya TK, Kumar B. Chitosan-Zinc-Ligated Hydroxychloroquine: Molecular Docking, Synthesis, Characterization, and Trypanocidal Activity against *Trypanosoma evansi*. *Polymers*. 2024 Sep 30;16(19):2777.
 19. Mehta SC, Singh J, Bhattacharya TK. Mathematical models to define growth patterns in indigenous horses of India. *The Indian Journal of Animal Sciences*. 2024 Apr 1;94(4):386-92.
 20. Paul N, Kumaresan A, Talluri TR, Raval K, Elango K, Pradeep Nag BS, Duraisamy R, Manimaran A. Lectin Functionalised Iron Magnetic Nanoparticle-Based Sperm Selection: A Potential Technique to Improve Bull Sperm Quality In Vitro. *Reproduction in Domestic Animals*. 2024 Oct;59(10):e14733.
 21. Nokhwal A, Vaid RK, Anand T, Verma R, Gulati R. *Aeromonas* Species Diversity, Virulence Characteristics, and Antimicrobial Susceptibility Patterns in Village Freshwater Aquaculture Ponds in North India. *Antibiotics*. 2025 Mar 12;14(3):294.
 22. Paul N, Talluri TR, Kumaresan A. Sperm microencapsulation in bovine: An overview. *Journal of Reproductive Healthcare and Medicine*. 2024;5(7):1.
 23. Thapa N, Rani R, Shanmugasundaram K, Jhandai P, Bhattacharya TK, Singha H. Antibody isotyping and cytokine profiling in natural cases of *Burkholderia mallei* infection (glanders) in equines. *Cytokine*. 2025 Jan 1;185:156799.
 24. Pradhan SS, Balena V, Bera BC, Anand T, Khetmalis R, Madhwal A, Kandasamy S, Pavulraj S, Bernela M, Mor P, Tripathi BN. Multiple Gene Deletion Mutants of Equine Herpesvirus 1 Exhibit Strong Protective Efficacy Against Wild Virus Challenge in a Murine Model. *Vaccines*. 2025 Jan 8;13(1):45.
 25. Raj A, Pathak A, Shanmugasundaram K, Tripathi BN, Tripathi H, Singha H. Knowledge, awareness and

- perception about equine glanders among veterinarians and medical professionals in India. *Frontiers in Veterinary Science*. 2024 Mar 14;11:1334485.
26. Ranveer SA, Dasriya V, Ahmad MF, Dhillon HS, Samtiya M, Shama E, Anand T, Dhewa T, Chaudhary V, Chaudhary P, Behare P. Positive and negative aspects of bacteriophages and their immense role in the food chain. *npj Science of Food*. 2024 Jan 3;8(1):1.
 27. Rani S, Devi J, Kumar B, Manuja A. Organometallic analogs of chloroquine: Challenges and perspectives as anti-malarial agents. *Applied Organometallic Chemistry*. 2024 Sep;38(9):e7613.
 28. Thachamvally R, Chander Y, Kumar R, Kumar G, Khandelwal N, Manuja A, Kumar Vaid R, Kumar N, Barua S, Pal Y, Tripathi BN. First Isolation and Genetic Characterization of Avian Nephritis Virus 4 from Commercial Poultry in India. *Avian Diseases*. 2024 Sep 1;68(3):202-8.
 29. Sihag M, Manuja A, Rani S, Soni R, Rani N, Malik S, Bhardwaj K, Kumar B, Kingar M, Miglani M, Aneja DK. Synthesis of pyrazole and pyrazoline derivatives of α -ionone: Exploring anti-inflammatory potential, cytotoxicity, and molecular docking insights. *European Journal of Medicinal Chemistry Reports*. 2024 Dec 1;12:100204.
 30. Sultan T, Chaudhary AK, Mehta JS, Mehra R, Manuja A, Talluri TR, Bhattacharya TK. Cryoprotective Effect of Zinc and Gold Nanoparticles During Cooling and Freeze-thawing on Marwari Stallion Sperm Parameters and Reactive Oxygen Species Production. *CryoLetters*. 2024 Nov 1;45(6):361-9.
 31. Talluri TR, Kumar S, Dedar RK, Mehta SC, Bhattacharya TK. Raj-Sheetal: India's first horse foal born through vitrified embryo transfer. *Current Science (00113891)*. 2025 Feb 10;128(3).
 32. Talluri TR, Kumar S, Pal Y, Legha RA, Dedar RK, Bhattacharya TK. Raj-Prathama: India's first Marwari filly born through embryo transfer technology. *Current Science (00113891)*. 2024 Feb 10;126(3).
 33. Ram V, Jhamb D, Gaur M, Kumar N, Talluri TR, Solanki O, Juneja R, PARGI KK, Nandan D, Arora S, Vyas H. Effect of lyophilized heterologous seminal plasma supplementation on Marwari stallion epididymal spermatozoa during 24 hours cool storage. *Indian Journal of Animal Sciences*. 94 (9): 786–791
 34. Verma A, Kamboj H, Khandelwal N, Kumar R, Riyesh T, Kumar N. Genome sequence of bovine herpesvirus 5 (BoHV-5) isolate from India. *Microbiology Resource Announcements*. 2025 Feb 11;14(2):e00950-24.
 35. Yadav V, Dholpuria S, Talluri TR, Joshi A, Khandelwal S, Kumar A. Study on Sexual Behaviour Parameters of Marwari Stallions during Breeding Season. *Journal of Advances in Biology & Biotechnology*. 2024 Jan 24;27(1):1-6.
 36. Yadav V, Talluri TR, Dholpuria S, Joshi A, Daria S and Sharma PK. (2024) Correlation analysis of age and sexual behaviour parameters of Marwari stallions. *International Journal of Veterinary Sciences and Animal Husbandry*; SP-9(1): 71-73.
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 38. Yadav V, Talluri TR, Dholpuria S, Joshi A, Kumar P, & Kumar A. Fresh Seminal Characteristics of Marwari Stallions in Breeding Season. *Journal of Scientific Research and Reports* 2024. 30(2), 29–38.
 39. Bhardwaj A, Nayan V, Goutam U, Legha RA, Pal Y, Kumar J, Giri SK, Tripathi BN. Mitochondrial DNA Variation and Genetic Relationships in Indian Halari Donkey Breed using D-Loop Region. *Indian Journal of Animal Research*. 2024;58(9):1480-4.

40. Garhwal R, Bhardwaj A, Kumar H, Sangwan K, Kumari A, Bhavya, Singal M, Nayan V, Pal Y, Bhattacharya TK, Tripathi BN. Biochemical, dielectric and surface characteristics of freeze-dried donkey milk powder. *Food and Agricultural Immunology*. 2024 Dec 31;36(1):2442358.
41. Hemlata, Rani M, Kumar A, Dhiman SS, Priya K, Bhardwaj A, Singh G, Saini A, Giri SK. Investigating the relationship between pesticide exposure, GSTM1 and GSTT1 polymorphisms, and oxidative stress biomarkers in affected farmers. *Toxicology and Industrial Health*. 2025 Apr;41(4):220-33.
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43. Sangwan K, Garhwal R, Sorout S, Mehra R, Bhardwaj A, Pal Y, Nayan V, Kumar H. Physico-chemical, Functional, Thermal, Dielectric and Surface Characteristics of Freeze-dried Watermelon Rind Powder. *Annals of Agri-Bio Research* 29 (1): 136-145, 2024
44. Sharma NK, Singh P, Saha B, Bhardwaj A, Iquebal MA, Pal Y, Nayan V, Jaiswal S, Giri SK, Legha RA, Bhattacharya TK. Genome wide landscaping of copy number variations for horse inter-breed variability. *Animal Biotechnology*. 2024 Dec 31;36(1):2446251.

Abstracts published in conferences/symposia

1. Bakshi J, Kumar S, Sumanshu, Ritu rani, Taruna Anand & Vaid RK. (2024). Study of the prevalence of antimicrobial resistant *Escherichia coli* and *Staphylococcus* spp. from poultry and livestock in Haryana, India. In, Compendium of Research abstract and institutional Insights, 26th November, 2024, NRCE, Hisar, pp.30.
2. Bera B C, Bhandok Muskan, Anand Taruna and Virmani Nitin. (2024). Advancements in Animal Infectious Disease Diagnosis: The Promise of Point-of-Care Molecular Diagnostics; In compendium of International Conference of Indian Association of Veterinary Pathologists and National Symposium on "Exploring Veterinary Pathology and Diagnostic Innovations in Animal and Poultry Diseases Amidst Climatic Challenges organized at Sher-e-Kashmir University of Agricultural Sciences & Technology- Jammu, Union Territory of Jammu & Kashmir, from November 28-30, 2024.
3. Chhabra D, Nagra J., Rani S., Goutam U., Manuja A., Kumar B 2024. Molecular detection of *Streptococcus equi/zoepidemicus* in Indian isolates using multiplex real-time PCR, In National Conference on "Recent trends in Basic and Applied Science" & National Science Day -2024 Celebration on February 28, 2024 organized by Department of Sciences Jagannath University, Bahadurgarh (Haryana) (P-16).
4. Chhabra D, Nagra J., Rani S., Goutam U., Manuja A., Kumar B. "Differentiation of *Streptococcus equi/zoepidemicus* in Indian isolates using multiplex real-time PCR". In the "International Conference on Global Challenges in Environment, Food and Biotechnology for Sustainable Development" on February 21-23, 2024 organized by Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India
5. Chhabra D, Nagra J., Rani S., Goutam U., Manuja A., Kumar B. 2024. Molecular detection of *Streptococcus equi/zoepidemicus* in Indian isolates using multiplex real-time PCR, In National Conference on "Recent trends in Basic and Applied Science" & National Science Day -2024 Celebration on February 28, 2024 organized by Department of Sciences Jagannath University, Bahadurgarh (Haryana) (P-16).
6. Chhabra, D., Nagra, J., Manuja, A., Singha, H.S., Vaid, R.K., Goutam, U. and Kumar, B. 2024. "Seroprevalence of strangles in equines in northern parts of India". In the National Seminar on Companion animals and livestock Diseases in One Health Paradigm on 26th November 2024, organized by ICAR – National Research Center on

- Equines, Hisar (Haryana).
7. Devi N., Nagra J., Chhabra D., Rani S., Kumar R., Kumar B., Manuja A. 2024. Trypanocidal efficacy of quinapyramine sulfate chitosan nano formulations against *Trypanosoma evansi*. In the International Conference on "Nanotechnology Addressing the Convergence of Material Science, Biotechnology, and Medical Science" (IC-NACMBM-2024) on 12 -14th Feb., 2024, Organized by Centre for Interdisciplinary Research (CIR), D.Y. Patil Education Society, Kolhapur, Maharashtra, India.
 8. Gupta S., Sethi K., Rani R., Gupta S., Vohra S., Kumar S., and Kumar R. 2024. Parasitological and molecular investigation of *Trypanosomalewisi* and *Trypanosoma evansi* in *Rattus rattus* population distributed in urban areas of Hisar, Haryana. Compendium: 33rd National Congress of Veterinary Parasitology and National symposium on "Innovations in Parasite control strategies for the upliftment of Animal & Human health" organized by the Department of Veterinary Parasitology, College of Veterinary Science, PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India from 17th to 19th December, 2024, pp.28.
 9. Kishan Kumar Fataniya, A. Kumaresan, Anand Kumar Yadav, Ebenezer Samuel King, M.Sivaram, Thirumala Rao Talluri, G. V. Vedamurthy, A. Manimaran, S. Jeyakumar, Sakshi Payasi. 2024. Sperm quality attributes associated with fertility with fertility may be breed specific. Proceedings of the compendium of 39th Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and National Symposium and National Symposium on Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective. November 29 to December 1, 2024. Pg no 383
 10. Kumar B., Nagra J., Kadiyan P., Chhabra D., Dahiya R., Gautam U., Manuja A. Alginate and Gum acacia-based Zinc nanocomposites show promising effect against *Streptococcus equi*. In the International Conference on "Nanotechnology Addressing the Convergence of Material Science, Biotechnology, and Medical Science" (IC-NACMBM-2024) on 12th- 14th Feb., 2024, Organized by Centre for Interdisciplinary Research (CIR), D.Y. Patil Education Society, Kolhapur, Maharashtra, India.
 11. Kumar R., Rani R., Sethi K. and Kumar S.2024. Identification and evaluation of selected alkaloids for anti-trypanosomal efficacy against *Trypanosoma evansi*. Compendium: 33rd National Congress of Veterinary Parasitology and National symposium on "Innovations in Parasite control strategies for the upliftment of Animal & Human health" organized by the Department of Veterinary Parasitology, College of Veterinary Science, PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India from 17th to 19th December, 2024, pp208.
 12. Madhu Meena, Dinesh Kumar Jhamb, Thirumala Rao Talluri, Rajesh Kumar Vaid, Om Prakash Solanki, Sajjan Kumar, Sumanshu Nawal, Kalpesh Pargi.2024. Mitigation of endometritis in mares with intrauterine infusion of autologous platelet rich plasma. Proceedings of the compendium of 39th Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and National Symposium and National Symposium on Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective. November 29 to December 1, 2024. Pg no 432.
 13. Mamta Tirdia, Kapil Kumar Gupta, S. Dey, Lalita Gupta, Geetanjali Sharma, Rajender Kumar and Sanjay Kumar. 2024. Artemisinin and dihydroartemisinin interfere with *Theileria equi* metabolic pathway by targeting the dihydroorotate dehydrogenase (DHODH) enzyme. Presented during 33rd National Congress of Veterinary Parasitology and National Symposium On "Innovations in parasite control strategies for the upliftment of animal & human health" 17th - 19th December, 2024, organized by Department of Veterinary Parasitology,

- College of Veterinary Science, P.V.Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana.
14. Nagra, J., Chhabra, D., Dahiya, R., Goutam, U., Manuja, A and Kumar, B. 2024. "*Streptococcus equi* infection in Indian equines: Phenotypic, biochemical, molecular identification and differentiation from other Streptococcal infections using multiplex qPCR". In the National Seminar on Companion animals and livestock Diseases in One Health Paradigm on 26th November 2024, organized by ICAR – National Research Center on Equines, Hisar (Haryana).
 15. Omprakash Solanki, TR Talluri, Dinesh Jhamb, Sajjan Kumar, Neelam Kalasua, Kalpesh Pargi, Madhu Meena. 2024. Combined thickness of the uteruses and placenta in three indigenous horse breeds. Proceedings of the compendium of 39th Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and National Symposium and National Symposium on Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective. November 29 to December 1, 2024. Pg no 429.
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 17. Rani R, Kumar S, Sumanshu, Bakshi J, Bera B C, Taruna Anand & R. K. Vaid (2024). Simultaneous identification of virulent and avirulent *Bacillus anthracis* by PCR assay. In, Compendium of Research abstract and institutional Insights, 26th November, 2024, NRCE, Hisar pp.26.
 18. Rani S., Devi J., Chhabra D., Devi N., Kumar B., Manuja A. 2024. Zinc Oxide Hydroxychloroquine Chitosan Nanocomposites to Inhibit *Plasmodium falciparum* Parasite. In International conference on "Nanotechnology Addressing the convergence of material science, Biotechnology, and Medical Science" (IC - NACMBM -2024) on 12th-14th Feb., 2024, organized by Centre for Interdisciplinary research (CIR), D.Y. Patil Education Society, Kolhapur, Maharashtra, India.
 19. Sajjan Kumar, Thirumala Rao Talluri, Omprakash Solanki, RK Dedar, and TK Bhattacharyya. 2024. Raj-Sheetal: Country's First Foal Produced Through Vitrified Embryo Transfer. Proceedings of the compendium of 39th Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and National Symposium and National Symposium on Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective. November 29 to December 1, 2024. Pg no 449.
 20. Sharma D., Vohra S., Rani R., Sethi K., Gupta S., Kumar S., and Kumar R. 2024. Evaluating the growth inhibitory potential of artemisinin against *Trypanosoma evansi*. Compendium: 33rd National Congress of Veterinary Parasitology and National symposium on "Innovations in Parasite control strategies for the upliftment of Animal & Human health" organized by the Department of Veterinary Parasitology, College of Veterinary Science, PV Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana, India from 17th to 19th December, 2024, pp.212.
 21. Sharma D., Vohra S., Rani R., Sethi K., Gupta S., Kumar S., and Kumar R. 2024. Evaluating the growth inhibitory potential of conessine against *Trypanosoma evansi*. Compendium: National Seminar entitled "Companion Animals and Livestock Diseases in one Health Paradigm" organized by ICAR-National Research Centre on Equines, Hisar on 26-11-2024 at Hisar, pp.8.
 22. Thirumala Rao Talluri and Tarun Sutaria. Optimisation of reproductive potential in Horses. Proceedings of the

compendium of 39th Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and National Symposium and National Symposium on Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective. November 29 to December 1, 2024. Pg no 408-424.

Training Manual Chapter

1. H. Singha, Shanmugasundaram K, Punit Jhandai and TK Bhattacharya. Equine glanders: present scenario in India. In training program manual on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices. Organized in ICAR-NRCE, Hisar from 18-22 March, 2024. Pp 3-9.
2. H. Singha and Shanmugasundaram K. Diagnosis of glanders: culture isolation, serological and molecular methods. In training program manual on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices. Organized in ICAR-NRCE, Hisar from 18-22 March, 2024. Pp25-32.
3. H. Singha, Shanmugasundaram K, Punit Jhandai and TK Bhattacharya. Equine glanders: present scenario in India, diagnosis and control. In training program manual on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases. Organized in ICAR-NRCE, Hisar from 12-16 February, 2024 and 19-23 February, 2024. Pp 26-34.
4. H. Singha, Shanmugasundaram K, Pooja and Rakshita. Diagnosis of glanders. In training program manual on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases. Organized in ICAR-NRCE, Hisar from 12-16 February, 2024 and 19-23 February, 2024. Pp 114-122.
5. H. Singha, Shanmugasundaram K, Punit Jhandai. Equine Glanders: Present Scenario in India with Special Reference to Uttar Pradesh, Diagnosis and Control. In training program manual on Surveillance and diagnosis of zoonotic diseases through One Health Approach Organized in ICAR-NRCE, Hisar from 19-23 November, 2024. Pp 16-24.
6. H. Singha, Shanmugasundaram K, Radha Rani, Divya, Punit Jhandai. Diagnosis of Glanders. In training program manual on Surveillance and diagnosis of zoonotic diseases through One Health Approach Organized in ICAR-NRCE, Hisar from 19-23 November, 2024. Pp 79-86.
7. Jitendar Singh, TR Talluri, SC Mehta & TK Bhattacharya. 2024. Basic health management of donkey. Compendium of the training program conducted on Zoonotic Diseases Diagnosis under One Health Approaches and equine husbandry practices'. 18-22 March. 79-84.
8. Jitendar Singh, TR Talluri, SC Mehta & TK Bhattacharya. 2024. Care of pregnant mare and new born foal. Compendium of the training program conducted on Zoonotic Diseases Diagnosis under One Health Approaches and equine husbandry practices'. 18-22 March. 85-90.
9. Riyesh T published a chapter on "Diagnostic approaches for rabies: Ante-mortem and post-mortem testing" In the compendium of a five-day training program on "Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach" at ICAR-NRCE, Hisar, for medical, veterinary, and wildlife professionals from Uttar Pradesh, held from November 19-23, 2024.
10. Riyesh T, Shreya Prasher, Shanmugasundaram K and H. Singha. DFAT: A Reliable Technique for Detecting Rabies Antigens. In training program manual on Surveillance and diagnosis of zoonotic diseases through One Health Approach Organized in ICAR-NRCE, Hisar from 19-23 November, 2024. Pp 100-106.
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- Health approaches and equine husbandry practices. Organized in ICAR-NRCE, Hisar from 18-22 March, 2024. Pp 135-141.
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 17. TR Talluri and TK Bhattacharya. 2024. Clinically relevant peculiarities/exceptions of equine reproduction. Compendium of the training program conducted on Zoonotic Diseases Diagnosis under One Health Approaches and equine husbandry practices'. 18-22 March, 2024. 75-78.
 18. TR Talluri, J Singh, and TK Bhattacharya. 2024. Pregnancy diagnosis in equines. Approaches to the mare reproductive system. Compendium of the training program conducted on Zoonotic Diseases Diagnosis under One Health Approaches and equine husbandry practices'. 18-22 March, 2024. 107-114.
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 20. TR Talluri, Sajjan Kumar, Mohd. Kutty, RK Dedar, S C Mehta, J Singh and TK Bhattacharya. 2024. Collection and cryopreservation of stallion semen. Compendium of the training program conducted on Zoonotic Diseases Diagnosis under One Health Approaches and equine husbandry practices'. 18-22 March, 2024. 101-106.
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25. Shanmugasundaram K, H. Singha, Indu Rani and Soni. Diagnosis of tuberculosis and paratuberculosis by Ziehl-Neelesen staining technique. In training program manual on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices. Organized in ICAR-NRCE, Hisar from 18-22 March, 2024. Pp128-134.
26. Shanmugasundaram K, Indu Rani, and H. Singha. Zoonotic Mycobacterial infections, their diagnosis and control in animals. In training program manual on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases. Organized in ICAR-NRCE, Hisar from 12-16 February, 2024 and 19-23 February, 2024.Pp 10-17.
27. Shanmugasundaram K, H. Singha, Indu Rani and Punit Jhandai. Sample collection, packing and dispatch for Laboratory diagnosis of important zoonotic diseases. In training program manual on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases. Organized in ICAR-NRCE, Hisar from 12-16 February, 2024 and 19-23 February, 2024.Pp 18-25.
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32. Shanmugasundaram K and H. Singha. Sample collection, processing and dispatch for glanders diagnosis. In training program manual on Surveillance and diagnosis of zoonotic diseases through One Health Approach Organized in ICAR-NRCE, Hisar from 19-23 November, 2024. Pp 8-15.
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Book chapter published

1. Goswami P, Rai V., Chauhan P., Kutty VH, Rudrappa G, Rajak KK, and Mahendran K. (2024). Vaccine-driven sustainable animal husbandry in a climate-changing scenario: Challenges and opportunities. In Ch. S. Rao, A. V. Lingam, & B. S. Yashwanth (Eds.), *Research and technological advances for resilient agriculture* (p. 445). ICAR-NAARM. ISBN 978-93-340-3914-6.
2. Mehta SC (2024). Hindi as official language of India. In: *Ashvraj (Raj bhasha* Publication). ICAR-National Research Centre on Equine, Regional Station- equine Production Campus, Bikaner 334001, Rajasthan. ISBN 978-81-959087-4-5
3. Riyesh T. African horse sickness. Standard Veterinary Treatment Guidelines book published by Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Department of Animal Husbandry and Dairying, Government of India., pp: 292-293.
4. Riyesh T. Equine infectious anemia. Standard Veterinary Treatment Guidelines book published by Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Department of Animal Husbandry and Dairying, Government of India., pp: 293-294.
5. Riyesh T. Equine viral arteritis. Standard Veterinary Treatment Guidelines book published by Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Department of Animal Husbandry and Dairying, Government of India., pp: 291-292.
6. Riyesh T. Japanese encephalitis. Standard Veterinary Treatment Guidelines book published by Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Department of Animal Husbandry and Dairying, Government of India., pp: 289-290.

Books published

1. Mehta SC (2024). *Ashvraj (Raj bhasha* Publication). ICAR-National Research Centre on Equine, Regional Station- equine Production Campus, Bikaner 334001, Rajasthan. ISBN 978-81-959087-4-5
2. Mehta SC, Kumar R and Bhattacharya T K (2024). Standards for Identification of Indigenous Horse breeds. ICAR-NRC on Equines, Sirsa Road, Hisar 125001 (Haryana). ISBN: 978-81-959087-1-4.
3. Taruna Anand, Nitin Virmani, BC Bera RK Vaid, Medhavi Bashisth and TK Bhattacharya (2024). *Bacteriophage: Comprehensive Guide & laboratory Protocols for Phage Researchers*. Blue Duck Publications, Srinagar, J&K.

Technical Bulletin

1. TR Talluri, Sajjan Kumar, Yash Pal, RA Legha, RK Dedar, SC Mehta, TK Bhattacharya (2024). Practical guide to embryo transfer technology. Published by Director, ICAR-NRCE, Hisar

Training Manuals Published

1. Singha HS , Shanmugasundaram K, Punit Jindai, Riyesh T and Bhattachrya TK. 2024. "Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach" at ICAR-NRCE, Hisar. Feb 19-23. 1-163.
2. Singha HS , Shanmugasundaram K, Riyesh T, Punit Jindai, Shreya Parashar and Bhattachrya TK. 2024. "Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach" at ICAR-NRCE, Hisar. Nov. 19-23.1-138.
3. Singha HS , Talluri TR, Shanmugasundaram K, Punit Jindai, and Bhattachrya TK. 2024. Zoonotic Diseases Diagnosis Under One Health Approaches and Equine Husbandry Practices at ICAR-NRCE, Hisar. Mar. 18-22. 1-158.
4. Taruna Anand, RK Vaid, BC Bera and N Virmani. 2024. Training manual published for Hands-on National workshop on Translational Research On Bacteriophages As Promising Antimicrobial Alternative on October 18, 2024 under ICAR-National Fellow Project. Pg 1-50.

Policy paper

1. Talluri TR, Bhattacharya TK and Mehta SC (2024). SOPs for stallion semen collection and minimum standards for stallion semen quality. Policy paper-1, ICAR-National Research Centre on Equines, Hisar-125001.



Participation and Presentation in Conferences, Seminars, Symposia and Workshop

1. Dr. Anju Manuja participated in the 65th International Conference of the Association of Microbiologists of India (AMI 2024) on "Perspectives of Microbes for Human Welfare" held at Guru Jambheshwar University of Science and Technology, held at Hisar, Haryana during November 14–17, 2024.
2. Dr. Anju Manuja attended the International Conference on "Climate-Smart Nutri-Sensitive Integrated Farming System for Gender-Equitable Sustainable Agriculture: Prospects and Challenges (ICNSFS-2024)" organized by ICAR-CIWA at Bhubaneswar during November 06–08, 2024.
3. Dr. Anju Manuja attended the International Conference on "Nanotechnology Addressing the Convergence of Materials Science, Biotechnology, and Medical Science" organized by DY Patil University, Kolhapur, Maharashtra during February 2024.
4. Dr. BC Bera attended the International Conference of Indian Association of Veterinary Pathologists and National Symposium on "Exploring Veterinary Pathology and Diagnostic Innovations in Animal and Poultry Diseases Amidst Climatic Challenges" at Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu, UT of Jammu & Kashmir, during November 28–30, 2024.
5. Dr. Balvinder Kumar attended the National Seminar on Companion Animals and Livestock Diseases in One Health Paradigm at ICAR – National Research Centre on Equines, Hisar, Haryana during November 26, 2024.
6. Dr. H Singha attended the "National Consultation for Legal Environment Assessment for One Health Activities in India" organized by the Centre for One Health (COH), National Centre for Diseases Control (NCDC), Ministry of Health and Family Welfare (MoHFW), Government of India, in collaboration with the United Nations Development Program (UNDP), at Delhi during June 27–28, 2024.
7. Dr. Nitin Virmani participated in IAVPCON-2024 organized by Indian Association of Veterinary Pathologists and presented a lead paper entitled "Next Generation Vaccine Platforms for Livestock and Poultry" held at SKUAST, Jammu during November 28–30, 2024.
8. Dr. RK Vaid attended the "FAO Assessment Tool for Laboratories and Antimicrobial Surveillance Systems (FAO ATLASS)" held at ICAR-CIFT, Kochi during January 20–22, 2025.
9. Dr. RK Vaid attended the Use of "FAO Assessment Tool for Laboratories and AMR Surveillance Systems" (FAO-ATLASS) organized by FAO, India during July 19, 2024.
10. Dr. Riyesh T participated and presented at the International Conference on Emerging Viruses: Pandemic & Perspectives organized by the Indian Virological Society at DRDE, Gwalior, Madhya Pradesh during November 11–13, 2024.
11. Dr. Riyesh T participated in the 65th International Conference of the Association of Microbiologists of India (AMI 2024) on "Perspectives of Microbes for Human Welfare" held at Guru Jambheshwar University of Science and Technology, Hisar, Haryana during November 14–17, 2024.
12. Dr. Riyesh T participated in the "International Conference on One Health and Emerging Infections" organized by the Institute of Advanced Virology, Thiruvananthapuram, Kerala during November 20, 2024.

13. Dr. Riyesh T participated in the Workshop on “Development of Standard Veterinary Treatment Guidelines” organized by the Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Department of Animal Husbandry and Dairying, Government of India, held at New Delhi during August 08–09, 2024.
14. Dr. Riyesh T participated in the Regional Workshop on “State Action Plan for Dog-Mediated Rabies Elimination by 2030” for North Region States, organized by the Centre for One Health, NCDC, in collaboration with the World Health Organization, held at Delhi during September 27–28, 2024.
15. Dr. Riyesh T participated in the National Workshop on Public-Private Partnership to Strengthen National Veterinary Services organized by the Department of Animal Husbandry and Dairying (AH&D), Ministry of Fisheries, Animal Husbandry and Dairying in collaboration with the World Organisation for Animal Health (WOAH), held at New Delhi, India during February 11–13, 2025.
16. Dr. Shanmugasundaram K attended the National Consultation for Legal Environment Assessment for One Health Activities in India organized by the Centre for One Health (COH), National Centre for Diseases Control (NCDC), Ministry of Health and Family Welfare (MoHFW), Government of India, in collaboration with the United Nations Development Program (UNDP), held at Delhi during June 27–28, 2024.
17. Dr. Shanmugasundaram K participated as National Facilitator in the Animal Infectious Disease Prioritization Workshop in India organized by the Department of Animal Husbandry and Dairying (DAHD) in close collaboration with the Food and Agriculture Organization of the United Nations (FAO) in India, held at New Delhi during August 27–30, 2024.
18. Dr. SC Mehta attended the National Symposium on “Animal Production Systems and Its Role in Sustainable Use of AnGR” & XXI Annual Convention of Society for Conservation of Domestic Animal Biodiversity (SOCDAB) at NTR College of Veterinary Science, Gannavaram during February 15–16, 2024.
19. Dr. TR Talluri participated in the 39th Annual Convention of ISSAR and National Symposium on “Challenges in Enhancing Reproductive Efficiency of Livestock: An Indian Perspective” under the aegis of ISSAR, held at GADVASU, Ludhiana, Punjab during November 29 – December 01, 2024.
20. Dr. TR Talluri participated in the 4th Annual Convention of Animal Physiologists Association and National Conference on “Advanced Physiological Strategies for Sustainable Livestock Production and Reproduction” held at ICAR-CSWRI, Avikanagar during March 01–02, 2024.
21. Dr. TR Talluri participated in the ICAR Foundation Day celebrations held at NASC Complex, New Delhi during July 15–16, 2024.
22. Dr. TR Talluri participated in the Interactive Meet on 'Developing Roadmap for Ensuring Quality and Safety of Foods of Animal Origin' under the aegis of SoFTeL-FSSAI held at ICAR – National Meat Research Institute, Hyderabad during February 16, 2024.
23. Dr. TR Talluri participated in the National Conference and XI Annual Convention of the Society for Veterinary Sciences and Biotechnology (SVSBT-2024) at College of Veterinary Science, Proddatur, Andhra Pradesh during October 23–25, 2024.
24. Dr. TR Talluri participated in the National Symposium and XXI Convention of SOCDAB held at CVSc, Gannavaram, Andhra Pradesh during February 15–16, 2024.

Participation in Training Programmes

1. Dr. Riyesh T attended the training on Biosafety and Biosecurity for handling high-risk pathogens in a BSL-3 laboratory organized by the Maximum Containment Facility, ICMR-National Institute of Virology (NIV), Pune during April 08–12, 2024.

2. Dr. Riyesh T attended the training-cum-workshop on Biosafety for the handling and diagnosis of high-risk animal pathogens in ABSL-3 and BSL-3 laboratories organized by the ICAR-National Institute of High Security Animal Diseases (NIHSAD), Bhopal during August 19–23, 2024.
3. Dr. Riyesh T attended a four-day training on Laboratory Quality Management System and Internal Audit as per ISO/IEC 17025:2017 organized by Infinity Consultancy, Bhopal, Madhya Pradesh during January 21–24, 2025.
4. Dr. TR Talluri attended a ten-day training programme on Intracytoplasmic Sperm Injection (ICSI) at Life Fertility, Advanced IVF, Research and Training Centre, Visakhapatnam, Andhra Pradesh during August 20–30, 2024.
5. Dr. Muhammed Kutty attended a three days training programme on "Application of flowcytometry in semen analysis" held at SRS-NDRI, Bengaluru during 18-20 November 2024.
6. Dr. Muhammed Kutty attended professional attachment training at SRS-NDRI, Bengaluru from 28 November-27 February, 2024.

Lectures delivered

1. Dr. Anju Manuja delivered an invited talk at the 3rd International Conference (ICNSFS-2024) organized by ICAR-CIWA, Bhubaneswar, India, from November 06–08, 2024.
2. Dr. Anju Manuja delivered an invited talk in the 65th International Conference of the Association of Microbiologists of India (AMI 2024) on "Perspectives of Microbes for Human Welfare" held at Guru Jambheshwar University of Science and Technology, Hisar, Haryana during November 14–17, 2024.
3. Dr. Anju Manuja delivered an invited talk at the International Conference on Nanotechnology Addressing the Convergence of Materials Science, Biotechnology, and Medical Science organized at DY Patil University, Kolhapur, Maharashtra, during February 2024.
4. Dr. BC Bera delivered a lead lecture on "Advancements in Animal Infectious Disease Diagnosis: The Promise of Point-of-Care Molecular Diagnostics" at the International Conference of Indian Association of Veterinary Pathologists and National Symposium on "Exploring Veterinary Pathology and Diagnostic Innovations in Animal and Poultry Diseases Amidst Climatic Challenges" held at Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu, UT of Jammu & Kashmir, from November 28–30, 2024.
5. Dr. H Singha delivered an expert lecture on "Equine Glanders: Present Scenario in India" to field veterinarians from Tamil Nadu in a five-day training program on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices, organized at ICAR-NRCE, Hisar, from March 18–22, 2024.
6. Dr. H Singha delivered an expert lecture on "Diagnosis of Glanders: Culture Isolation, Serological and Molecular Methods" to field veterinarians from Tamil Nadu in a five-day training program on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices, organized at ICAR-NRCE, Hisar, from March 18–22, 2024.
7. Dr. H Singha delivered an expert lecture on "Equine Glanders: Present Scenario in India, Diagnosis and Control" to medical, veterinary, and wildlife professionals from Punjab and Haryana in a five-day (two-batch) training program on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases, organized at ICAR-NRCE, Hisar, from February 12–16, 2024 and February 19–23, 2024.
8. Dr. H Singha delivered an expert lecture on "Equine Glanders: Present Scenario in India with Special Reference to Uttar Pradesh, Diagnosis and Control" to medical, veterinary, and wildlife professionals from Uttar Pradesh in a five-day training program on "Surveillance and diagnosis of zoonotic diseases through One Health Approach", organized at ICAR-NRCE, Hisar, from November 19–23, 2024.

9. Dr. Nitin Virmani delivered a lead lecture on “Next Generation Vaccine Platforms for Livestock and Poultry” during the IAVPCON-2024 conference organized by Indian Association of Veterinary Pathologists at SKUAST, Jammu, from November 28–30, 2024.
10. Dr. Riyesh T. delivered an expert lecture on “Ante-mortem and Post-mortem Diagnosis of Rabies” during the five-day training program on “Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach” at ICAR-NRCE, Hisar, for professionals from Uttar Pradesh, from November 19–23, 2024.
11. Dr. Riyesh T. delivered an expert lecture on “Reliable Detection of Rabies Antigens Using DFAT and Real-Time PCR-Based Techniques” during the five-day training program on “Surveillance and Diagnosis of Zoonotic Diseases through the One Health Approach” at ICAR-NRCE, Hisar, for professionals from Uttar Pradesh and Uttarakhand, from March 03–07, 2025.
12. Dr. Riyesh T. delivered an expert lecture on “Status of Rabies in Haryana and the Role of One Health in Strengthening Surveillance and Control” in the Workshop on Zoonotic Diseases held at ICAR-NRCE, Hisar, Haryana, on January 29, 2025.
13. Dr. Riyesh T. delivered an invited lecture on poxvirus vector-based vaccine platforms during the National Training Program on “CRISPR/Cas9 and BAC-Based Viral Genome Editing,” organized under the ICAR-NPGET Project, SCSP Sub-plan, from March 17–19, 2025.
14. Dr. Riyesh T. delivered an invited lecture on swinepox virus-based vectored vaccine design using the CRISPR/Cas9 technique during the training program on “Strategies in Vaccine Design Using Genome Editing Technologies,” organized at ICAR-NRCE, Hisar, from February 17–21, 2025.
15. Dr. SC Mehta delivered an expert lecture on “Status and Conservation of Equine Genetic Resources of India” during the National Symposium on “Animal Production Systems and its Role in Sustainable Use of Animal Genetic Resources (AnGR)” and XXI Annual Convention of the Society for Conservation of Domestic Animal Biodiversity (SOCDAB), held at NTR College of Veterinary Science, Gannavaram, from February 15–16, 2024.
16. Dr. SC Mehta participated as a panel speaker in the Brainstorming Session during the National Symposium on “Animal Production Systems and its Role in Sustainable Use of Animal Genetic Resources (AnGR)” and XXI Annual Convention of the Society for Conservation of Domestic Animal Biodiversity (SOCDAB), organized at NTR College of Veterinary Science, Gannavaram, from February 15–16, 2024.
17. Dr. Shanmugasundaram K delivered an expert lecture on “Sample Collection, Packing and Dispatch for Laboratory Diagnosis of Important Zoonotic Diseases” to field veterinarians from Tamil Nadu in a five-day training program on “Zoonotic diseases diagnosis under One Health approaches and equine husbandry practices”, organized at ICAR-NRCE, Hisar, from March 18–22, 2024.
18. Dr. Shanmugasundaram K delivered an expert lecture on “Sample Collection, Packing and Dispatch for Laboratory Diagnosis of Equine and Human Glanders and Other Zoonotic Diseases” to field veterinarians from Tamil Nadu in a five-day training program on zoonotic diseases diagnosis under One Health approaches and equine husbandry practices, organized at ICAR-NRCE, Hisar, from March 18–22, 2024.
19. Dr. Shanmugasundaram K delivered an expert lecture on “Threat from Zoonotic Mycobacterial Infections, Their Diagnosis and Control in Animals” to field veterinarians from Tamil Nadu in a five-day training program on “Zoonotic diseases diagnosis under One Health approaches and equine husbandry practices”, organized at ICAR-NRCE, Hisar, from March 18–22, 2024.
20. Dr. Shanmugasundaram K delivered an expert lecture on “Zoonotic Mycobacterial Infections, Their Diagnosis and Control in Animals” to medical, veterinary, and wildlife professionals from Punjab and Haryana in a five-day (two-batch) training program on One Health Approach for Surveillance and Diagnosis of Priority Zoonotic

- Diseases, organized at ICAR-NRCE, Hisar, from February 12–16, 2024 and February 19–23, 2024.
21. Dr. Shanmugasundaram K delivered an expert lecture on “Sample Collection, Packing and Dispatch for Laboratory Diagnosis of Important Zoonotic Diseases” to medical, veterinary, and wildlife professionals from Punjab and Haryana in a five-day (two-batch) training program on “One Health Approach for Surveillance and Diagnosis of Priority Zoonotic Diseases”, organized at ICAR-NRCE, Hisar, from February 12–16, 2024 and February 19–23, 2024.
 22. Dr. Shanmugasundaram K delivered an expert lecture on “Zoonotic Mycobacterial Infections, Their Diagnosis and Control in Animals” to medical, veterinary, and wildlife professionals from Uttar Pradesh in a five-day training program on “Surveillance and diagnosis of zoonotic diseases” through One Health Approach, organized at ICAR-NRCE, Hisar, from November 19–23, 2024.
 23. Dr. Shanmugasundaram K delivered an expert lecture on “Sample Collection, Packing and Dispatch for Laboratory Diagnosis of Important Zoonotic Diseases” to medical, veterinary, and wildlife professionals from Uttar Pradesh in a five-day training program on “Surveillance and diagnosis of zoonotic diseases” through One Health Approach, organized at ICAR-NRCE, Hisar, from November 19–23, 2024.
 24. Dr. TR Talluri delivered an expert lecture on “Phenomics and Genomics in the Fertility Prediction in Male Livestock” during the ICAR-sponsored short course on “Recent Developments in Livestock Phenome Data Recording, Analysis and Interpretation in the Era of Genomics,” conducted at ICAR-National Research Centre on Camel, Bikaner, from January 03–12, 2024.
 25. Dr. TR Talluri delivered a lead lecture on “Application and Prospects of ARTs for Sustainable Livestock Production” at the National Conference and XI Annual Convention of Society for Veterinary Sciences and Biotechnology (SVSBT-2024), held at College of Veterinary Science, Proddatur, Andhra Pradesh, from October 23–25, 2024.
 26. Dr. TR Talluri delivered a lead lecture on “Embryo Transfer in Equines” at the 4th Annual Convention of Animal Physiologists Association and National Conference on “Advanced Physiological Strategies for Sustainable Livestock Production and Reproduction” held at ICAR-CSWRI, Avikanagar, from March 01–02, 2024.
 27. Dr. TR Talluri delivered a lead lecture on “Optimization of Reproductive Potential in Equines” at the ISSAR Conference held at GADVASU, Ludhiana, from November 29–December 01, 2024.
 28. Dr. Taruna Anand delivered an invited lecture on “Bacteriophage Bank – A Source of Phage-Based Treatments Addressing Biofilm-Related and Drug-Resistant Infection in Animals” and chaired a session at the 5th International Conference on Bacteriophage Research and Antimicrobial Resistance, held at SPJIMR Auditorium, Bhavan's College, Mumbai, from November 08–09, 2025.



On-going Research Projects

A. EQUINE HEALTH

Sr. No.	Title	Team	From	To
1.	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	Nitin Virmani*, H Singha, R Kumar, S Kumar, S Barua, RK Vaid, RK Dedar, A Manuja, Balvinder Kumar, K Shanmugasundaram, Ana Raj and TK Bhattacharya	April, 1997	Continuous Service Project
2.	Immune responses and host-pathogen interaction analysis in <i>Burkholderia mallei</i> infected equines	H Singha* and K Shanmugasundaram,	Sep, 2022	Aug, 2025
3.	Bio-evaluation of target specific potential drug candidates against <i>Trypanosoma evansi</i> and analysis of conventional trypanocides drug-resistance	Rajender Kumar*, Sanjay Kumar and BC Bera	May, 2023	April, 2026
4.	Preparation and evaluation of zinc based polymeric composites as joint supplements for equines	Anju Manuja* and Balvinder Kumar	Oct, 2023	Sept, 2026
6.	Molecular analysis of <i>Theileria equi</i> genotypes among Indian equines and its impact on diagnostic sensitivity	Sanjay Kumar*	April, 2024	March, 2027

B. EQUINE PRODUCTION

Sr. No.	Title	Team	From	To
1.	Characterization and recognition of Bhimthadi horse	SC Mehta* and Sachin D Sorate	July, 2021	June, 2023 extended till March 2025
2.	Analysis of quantitative traits for genetic improvement of indigenous equines	SC Mehta*, RA Legha and J Singh	April, 2021	March, 2026
3.	Conservation of Marwari Indigenous breed of Horse through AI	SC Mehta*, TR Talluri and J Singh	July, 2022	March, 2024
4.	Cryopreservation of semen from indigenous horses and donkeys	TR Talluri*, TK Bhattacharya, SC Mehta, RK Vaid, RK Dedar and M Kutty	July, 2023	June, 2026
5.	Standardization of protocols for preparation of low dose frozen semen for deep horn intrauterine insemination in mares and jennies	TR Talluri*, TK Bhattacharya, SC Mehta, RK Dedar, M Kutty and J Singh	Aug, 2023	April, 2026
6.	Analysis of fertility associated genes (ProAKAP4, PLCzeta, SPATA1, CRISP3, INHBA, ZAN) in fresh and frozen thawed semen of Equines	Muhammed Kutty*, TR Talluri, RK Dedar and SC Mehta	Dec, 2023	Dec, 2026
7.	Diagnostic and therapeutic Studies on incidences of colitis in horses and horses clinically suffering from hind quarter weakness/ataxia leading to recumbency	RK Dedar*, TR Talluri and RK Vaid	Dec, 2023	Dec, 2026
8.	Establishing a model precision Donkey Farming and exploration of therapeutic and cosmetic values of donkey milk to enhance farmers' income	Muhammed Kutty*, TR Talluri, RK Dedar and SC Mehta	Dec, 2023	Dec, 2026
9.	Development of Lateral Flow for ovulation detection in Mares	Muhammed Kutty*, TR Talluri, RK Dedar and SC Mehta	June, 2024	May, 2027

C. NATIONAL CENTRE FOR VETERINARY TYPE CULTURE

Sr. No.	Title	Team	From	To
1.	Authentication and accessioning of viruses of animal origin (Service Project)	Sanjay Barua (till June 2024),* Naveen Kumar (till March 2024), Riyesh T, BC Bera, and Taruna Anand	May, 2015	Service Project
2.	Phenotypic and genotypic authentication and preservation of network bacterial isolates	RK Vaid*, Taruna Anand, BC Bera, Riyesh T and K Shanmugasundaram	June, 2015	Service Project
3.	Isolation, characterization and generation of repository of Mycobacterium species	Shanmugasundaram K*, RK Vaid, BC Bera and Riyesh T	Oct, 2017	Service Project
4.	Development of respository of respiratory viruses of livestock and isothermal based diagnostics for rapid identification.	BC Bera*, Nitin Virmani, Taruna Anand and Riyesh T	July 2023 extended till March 2024	Service Project
5.	Indian network for fisheries and animal antimicrobial resistance (INFAAR)	RK Vaid*, Taruna Anand, and HS Singha	March, 2025	Service Project
6.	Isolation and characterization of bacteriophages against important biofilm forming bacteria (Service Project)	Taruna Anand*, Nitin Virmani, BC Bera, and RK Vaid	Dec, 2023	Dec, 2026
7.	A study on bat virome for unravelling the viral diversity in India	NCVTC: Riyesh T*, Naveen Kumar (till March 2024), Shanmugasundaram K, RK Vaid and Sanjay Barua (till June 2024)	April, 2021	March, 2024 extended till Dec,2024
8.	Adaptation of Lumpy skin disease virus in Vero cells	Riyesh T*	Jan., 2021	March 2025
9.	Isolation, characterization and development of repository of Infectious Laryngotracheitis virus of Poultry	BC Bera*, TK Bhattacharya, Nitin Virmani, Taruna Anand & Riyesh T	Aug., 2023	July, 2025

Sr. No.	Title	Team	From	To
10.	Genetic characterization and evaluation of the immunogenicity of swinepox virus in mice model	Riyesh T*, Shanmugasundaram Naveen Kumar (till 20 th March, 2024), Sanjay Barua (till June 2024) and TK Bhattacharya	Jan, 2024	Dec, 2026
11.	Evaluation of pathogenicity of accessioned bacterial strains by small animal experimental studies-Service Project	RK Vaid*	-	-

D. EXTERNALLY FUNDED PROJECTS

Sr. No.	Title	Team	From	To
1.	National One Health Program on Prevention and Control of Zoonotic Diseases (NOHPPCZ) Project: Regional Coordination center under program for Inter-Sectoral Coordination for prevention and control of Zoonotic Diseases	Bacterial Diseases: Harisankar Singha, Shanmugasundaram K, Viral Diseases: Riyesh T Parasitic Diseases: Rajender Kumar	June, 2019	March, 2025
2.	Development of ML and ANN-based breed and individual identification system for equine population differentiation	Anuradha Bhardwaj*, TK Bhattacharya and TR Talluri	July, 2020	June, 2025
3.	DBT Network Programme on Anthrax Diagnosis and Control in India	RK Vaid*, BC Bera, K Shanmugasundaram	Sept, 2021	Sept, 2024
4.	Surveillance of Rotavirus a Genotypes in bovine and Equines of India for Identification of Potential vaccine candidates	BC Bera*	April, 2022	March, 2025
5.	Optimisation of procedures for non-surgical recovery and bio-banking of Marwari breed horse embryos	TR Talluri*, TK Bhattacharya and RK Dedar	April, 2022	March, 2025
6.	Utilization of desert plants for the treatment of skin diseases of Horses	RK Dedar* and TR Talluri	April, 2022	March, 2025

Sr. No.	Title	Team	From	To
7.	Translation of Nano based quinapyramine sulphate formulation into product and its evaluation against Trypanosoma evansi in animals	Anju Manuja*, Rajender Kumar and Balvinder Kumar	April, 2022	March, 2025
8.	Development of vaccine against animal's haemoprotozoan parasites for mitigating biotic stress	Sanjay Kumar*, Rajender Kumar and K Shanmugasundaram	Oct, 2022	May, 2025
9.	Development and evaluation of genetically engineered vaccine candidates for African swine fever, Equine Herpes virus-1 and Equine Influenza (clade 1 & 2)	Nitin Virmani* Sanjay Barua, BC Bera and Taruna Anand	June, 2022	May, 2025
10.	CRP on Vaccine and Diagnostics: "Development and validation of multiplex assays for laboratory diagnosis of emerging equine herpesviruses (EHV2 & EHV5)"	Nitin Virmani*, BC Bera and Taruna Anand	Jan, 2021	March, 2025
11.	CRP on Vaccine and Diagnostics "Development of antigen detection point-of-care diagnostics for haemoprotozoan diseases of equines"	Sanjay Kumar* and Rajender Kumar	Jan, 2021	March, 2025
12.	CRP on Vaccine and Diagnostics: Development of point of care diagnostics for strangles in equines	Balvinder Kumar*, RK Vaid, Anju Manuja, K Shanmugasundaram and Harisankar Singha	Jan, 2021	March, 2025
13.	CRP on vaccine & Diagnostic project: Development of RPA-LFA based point-of-care diagnostic assay for rapid detection and differentiation of equine herpes viruses 1&4	BC Bera* and Nitin Virmani	Jan, 2021	March, 2025
14.	Developing novel therapeutic strategies for mitigating antimicrobial resistance	Taruna Anand*, Nitin Virmani, BC Bera and RK Vaid	June, 2022	May, 2025

Sr. No.	Title	Team	From	To
15.	National Fellow Project: "Bacteriophage based interventions for therapy and prophylaxis against" Campylobacteriosis, Colibacillosis, Salmonellosis and other diseases as an alternative to antibiotics in poultry.	Taruna Anand*	Oct, 2022	Oct, 2027
16.	Development of novel semen extender for the enhancement of post thaw semen quality in equines	TR Talluri*, RK Dedar, Anuradha Bhardwaj and TK Bhattacharya	Feb, 2023	Feb, 2025
17.	Integrated analysis for the ultra-deep compositional characteristics of donkey colostrum and mature milk	Anuradha Bhardwaj*, TR Talluri and TK Bhattacharya	Feb, 2023	Jan, 2025
18.	Development and evaluation of immunotherapy and vaccine constructs against <i>Rhodococcus equi</i> infection to protect foals from pneumonia	H Singha* and K Shanmugasundaram	Sept, 2023	Aug, 2026
19.	Histone acetylation/ deacetylation: Potential target for therapeutic intervention and vaccine development	Riyesh T*, and Naveen Kumar	Sept, 2023	Aug, 2026
20.	One-Health approaches to understand the burden and threat of zoonotic tuberculosis, melioidosis and glanders in Uttar Pradesh, Karnataka and Haryana states.	Shanmugasundara K*, Harisankar Singha and Riyesh T	March 2024	March 2027
21.	Network Project on AnGR : Conservation of Halari Donkey	SC Mehta*	-	-
22.	Targeting host restriction factors that inhibit Lumpy skin disease virus replication: Generating CRISPR/Cas9 knockout cells to scale up vaccine production	Riyesh T* BC Bera, K Shanmugasundram	June 2024	March 2026

Sr. No.	Title	Team	From	To
23.	Generation of recombinant swinepox virus vector expressing immunogenic proteins of classical swine fever virus by CRISPR/Cas9 mediated genome editing	Riyesh T*, BC Bera, Shanmugasundaram K, TK Bhattacharya	June, 2024	March 2026
24.	Establishment of a herpes virus vector platform and identification of vaccine candidates by gene editing for emerging and exotic diseases of livestock and poultry	ICAR-NISHAD: S Nagarajan * C Tosh, Manoj Kumar, Senthilkumar Naveen Kumar, K Rajukumar, G Venkatesh, Fateh Singh ICAR-NRCE: Nitin Virmani (CCPI), BC Bera, Rajender Kumar, Harishankar Singha, RK Vaid, Taruna Anand, Balwinder Kumar	June, 2024	March 2026
25.	Recombinant Lumpy Skin Disease (LSD) viral-vectored multivalent vaccine to control and combat foot-and-mouth disease in livestock	ICAR-NIFMD: JK Biswal * RP Singh, JK Mohapatra, Saravanan S, Rajeev Ranjan ICAR-NRCE: BC Bera (CCPI), Riyesh T, TK Bhattacharya	June, 2024	March 2026

E. INSTITUTE CORPUS FUNDED PROJECT

Sr. No.	Title	Team	From	To
1.	Development of recombinant antibody-based point-of-care for diagnosis of surra in animals	Rajender Kumar* and Sanjay Kumar	March, 2024	March, 2025
2.	Development of field-deployable diagnostics for detection of Infectious bronchitis virus and Newcastle disease virus using CRISPR-Cas13	Riyesh T, Shanmugasundaram K and TK Bhattacharya	March, 2024	March, 2025
3.	Development of ready-to-use extender for enhancement of shelf life of stallion semen	TR Talluri*, TK Bhattacharya, RK Dedar, Mohd Kutty and SC Mehta	March, 2024	March, 2025
4.	Designing of Mineral Mixture for horses of subtropical desert climate	RK Dedar, M. Kutty, TR Talluri and TK Bhattacharya	March, 2024	March, 2025



Staff at ICAR-NRCE (as on 31.12.2024)

Sr. No.	Scientific Staff	Designation
ICAR-NRCE Main Campus, Hisar		
1	Dr. TARUN KUMAR BHATTACHARYA	DIRECTOR
2	Dr. NITIN VIRMANI	Head, EHD
3	Dr. RAJENDER KUMAR	PRINCIPAL SCIENTIST
4	Dr. SANJAY KUMAR	PRINCIPAL SCIENTIST
5	Dr. ANJU MANUJA	PRINCIPAL SCIENTIST
6	Dr. BALVINDER KUMAR	PRINCIPAL SCIENTIST
7	Dr. HARISANKAR SINGHA	PRINCIPAL SCIENTIST
8	Dr. ANURADHA BHARDWAJ	PRINCIPAL SCIENTIST
9	Dr. ANA RAJ. J	SCIENTIST
NCVTC		
10	Dr. RAJESH KUMAR VAID	Head, NCVTC
11	Dr. TARUNA ANAND	National Fellow
12	Dr. BIDHAN CHANDRA BERA	PRINCIPAL SCIENTIST
13	Dr. K SHANMUGASUNDARAM	SENIOR SCIENTIST
14	Dr. RIYESH THACHAMVALLY	SENIOR SCIENTIST
Regional Station, ICAR-NRCE, Equine Production Campus, Bikaner, Rajasthan		
15	Dr. SHARAT CHANDRA MEHTA	Head, RS, EPC
16	Dr. RAMESH KUMAR DEDAR	PRINCIPAL SCIENTIST
17	Dr. THIRUMALA RAO TALLURI	SENIOR SCIENTIST
18	Dr. MUHAMMED KUTTY V.H	SCIENTIST
Technical Staff, ICAR-NRCE Main Campus, Hisar		
1	Sh. AJMER SINGH	ACTO
2	Sh. SANJEEV KUMAR	ACTO
3	Sh. JOGINDER SINGH	Sr. TECHNICAL OFFICER
4	Sh. MUKESH CHAND	Sr. TECHNICAL OFFICER

ICAR-NRCE

5	Sh. RAJKUMAR DAYAL	Sr. TECHNICAL OFFICER
6	Sh. BRIJLAL	TECHNICAL OFFICER
7	Sh. SAJJAN KUMAR	TECHNICAL OFFICER
8	Sh. SURESH KUMAR	TECHNICAL OFFICER
9	Sh. RAGHBIR SINGH	STA (DRIVER)
10	Sh. HANUMAN SINGH	TECHNICIAN
11	Sh. ISHWAR SINGH	TECHNICIAN
12	Sh. SANTRAM	TECHNICIAN

Sr. No.	Technical Staff	Designation
RS, ICAR-NRCE, Equine Production Campus, Bikaner, Rajasthan		
1	Dr. JITENDER SINGH	CTO
2	Sh. NARENDER CHAUHAN	ACTO
3	Sh. OMPARKASH	TECHNICAL OFFICER
4	Sh. SN PASWAN	TECHNICAL OFFICER
5	Sh. RAJENDER SINGH	TECHNICAL ASSISTANT
6	Sh. GOPALNATH	TECHNICAL ASSISTANT (DRIVER)
Administrative Staff, ICAR-NRCE Main Campus, Hisar		
1	Sh. BRAHM PARKASH	SAO
2	Ms. RITU	FAO
3	Sh. SUNIL	AAO
4	Sh. DINESH DATT SHARMA	AAO
5	Sh. ASHOK ARORA	PRIVATE SECRETARY
6	Sh. OMPARKASH	ASSISTANT
7	Ms. JYOTI	ASSISTANT
8	Sh. ANIL	ASSISTANT
9	Sh. AMANDEEP	ASSISTANT
10	Sh. AMAN GODARA	ASSISTANT
11	Sh. DEEPAK KUMAR	UDC
12	Sh. GURU DATT SHARMA	LDC
13	Sh. ISHWAR CHANDER	LDC

RS, ICAR-NRCE, Equine Production Campus, Bikaner, Rajasthan		
14	Sh. MAHINDER SINGH	UDC
MTS (Multi Tasking Staff), ICAR-NRCE Main Campus, Hisar		
1	Sh. JAI SINGH	MTS
2	Sh. SUBHASH CHANDER	MTS
3	Sh. RAM SINGH	MTS
4	Sh. LILURAM	MTS
5	Smt. SOMADEVI	MTS
6	Smt. SANTRA	MTS
MTS (Multi Tasking Staff), RS, ICAR-NRCE, Equine Production Campus, Bikaner, Rajasthan		
7	Sh. MP MEENA	MTS
8	Sh. RAJURAM	MTS
9	Sh. ASHOK KUMAR	MTS

Sr. No.	Name	Designation	Date of Retirement
Details of Retirement			
1.	Dr. RA Pachori	CTO	31.03.2024
2.	Sh. Subhash Chander	AAO	30.06.2024
3.	Sh. Sita Ram	ACTO	31.07.2024
4.	Sh. KK Singh	ACTO	31.10.2024

Sr. No.	Name	Designation	Date of Transfer
Transfer			
1.	Dr. Naveen Kumar	Head, NCVTC	21.03.2024 as Director at National Institute of Virology (NIV), Pune.
2.	Dr. Anubha Prashant Pathak	Scientist	19.01.2024 (ICAR-NIHSAD, Bhopal)





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