

ICAR-NRCE

ANNUAL REPORT
2019



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



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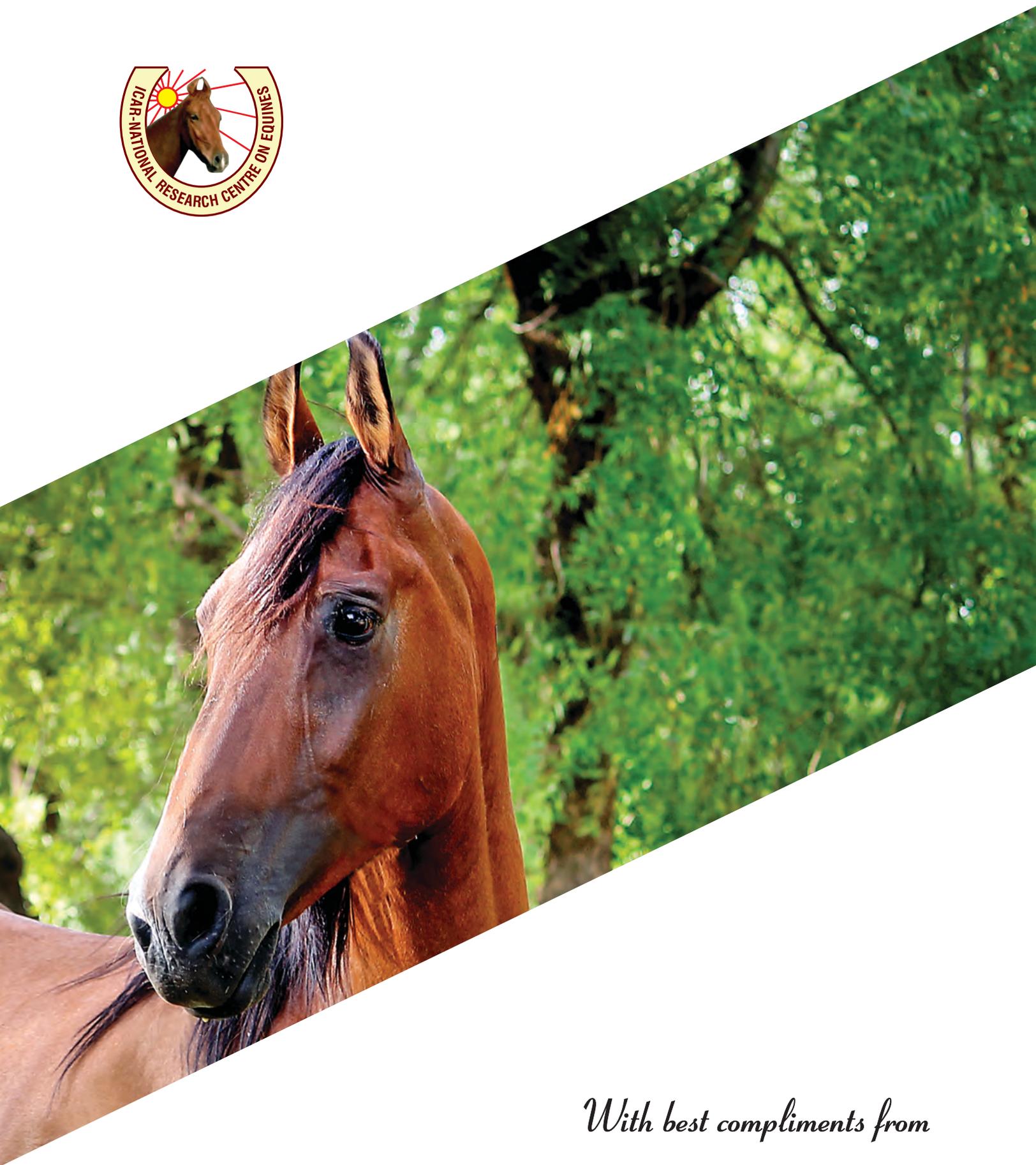
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With best compliments from

Dr. B. N. Tripathi

Director

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About the Cover



“The Stallion **Prince** is of Marwari horse breed reared at NRCE-EPC campus Bikaner. Marwari or Malani is a breed of horse from the Marwar region of India. These horses have majestic look with high-carried head, peculiar character of inward curved and touching ears. The breeding tract of this breed is mainly Jodhpur, Jalore, Sirohi, Jaisalmer, Barmer, Rajsamand and Udaipur area of Rajasthan and in some pockets of Gujarat. ”

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Marwari Mare at ICAR-NRCE,
EPC, Bikaner





Director's Foreword



The ICAR-National Research Centre on Equines (ICAR-NRCE) was established on 26 November 1985 at Hisar (Haryana) and is a premier research institute of the Indian Council of Agriculture Research (ICAR) in Animal Sciences, which is a component of National Agricultural Research System (NARS). Since its inception, the Centre is aiming to boost the lives of the landless and marginal equine farmers by providing diagnostic, advisory and consultancy services for augmenting equine health, productivity and utility in agriculture and transport through which the Centre has earned its recognition as a premier institution of international stature among the ICAR institutions. The institute is relentlessly making its emphasis and remarkable achievements since its establishment by pursuing basic, applied, and translational research, training and extension activities that have resulted in the improvement of equine health and production in India. The Centre is working on the projects related to vaccinology, diagnostics, therapeutics, equine reproduction and also concentrating at the emerging areas of research which include the refinement in production of new generation vaccines, development of rapid diagnostics techniques using nanotechnology, studying the host-pathogen interactions, development of therapeutics by applying ethno-veterinary medicine, creating the repository for microbes and bacteriophages, genetic studies on equine production and augmentation of reproductive efficiencies etc. The research activities at the Centre continue to bridge the gap between basic biology and clinical applications thereby providing cutting edge translational research for the amelioration of equine health and welfare in the country. The annual report of the current year displays the various achievements and attempts were made to produce viable technologies and efforts for generation of commercially viable technologies and demand-driven research for the benefit of the equine farmers.

The research and development activities of ICAR-NRCE are achieved through well-structured research programmes comprising 22 institute funded and 11 externally funded research projects, which also include inter-institutional collaborative research projects. ICAR-NRCE has been successful in getting external funding from almost all leading national funding agencies in the field of agricultural and biological sciences.

ICAR-NRCE is actively involved in surveillance of different equine diseases in the country and is of paramount importance for policy making purposes and to implement effective control measures. In this direction, during current report of the year 2019, a total of 2169 equine serum samples from 12 states were tested for various diseases like equine infectious anaemia (EIA), equine influenza (EI), EHV-1, Japanese encephalitis (JE), trypanosomosis, equine piroplasmiasis, *Salmonella Abortus equi* and brucellosis. None of the equines were found positive for EI, EIA, brucellosis and *Salmonella Abortus equi*. Total 219 human cases living in contact with equines were examined for glanders and no clinical case of glanders was detected. In addition, under disease investigation 22116 equine sera was tested for glanders and 210 glanders positive cases were reported. A total 157 equines were tested for JE antibodies and 13 were detected positive.

Equine herpesvirus-1 infection is a major problem in equines mostly associated with abortion and respiratory problems. EHV-1 viruses were isolated in RK13 cells from seven positive samples and all isolates were non-neuropathogenic. Partial genome sequences of two EHV-1 isolates (EHV-1/14 and EHV-1/Meerut) were generated using NGS platform covering more than 90% of genome. A refined EHV-1 vaccine was also developed by the



researchers of the centre which is showing better efficacy than earlier developed vaccine and at the same time research for the development of a combined vaccine for both EIV and EHV is going on. In order to diagnose JEV, a Taqman-based real-time PCR was developed for detection of JEV infection. The assay was specific for JEV and did not cross-react with WNV. For rapid and efficient execution of surveillance activities glanders ELISA developed by NRCE has been provided to 11 state diagnostic laboratories/RDDLs. This year, 12206 equine samples were screened by ELISA at eight State Lab/RDDLs (Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, Jammu & Kashmir).

ICAR-NRCE is continuously working on development of drugs against various pathogens. The target specific novel drug molecules against *Trypanosoma evansi* infection have been evaluated using nanotechnology approach. In another study, promising results were observed with 50 mg/kg fraction of the herbal plant for development of herbal drug against the *Theileria equi*. A Multiplex PCR was also optimized for simultaneous diagnosis of *T. equi* and *B. caballi* infection in a single PCR reaction. In another study, herbal drug against *Rhodococcus equi* gave promising results. *Aervajavanica* plant extract has been evaluated in treatment of various equine disease conditions like habronemiasis, proud flesh condition and alopecia and has shown very good efficacy in treating all the above-mentioned disease conditions. A patent for this endeavour has also been successfully filed.

Studies aiming at the improvement of equine production are also going on at the sub-campus of the centre at Bikaner. A breeding plan for the indigenous horses was also prepared with an aim to improve the average height of the herd. A new protocol for isolation of DNA from stallion spermatozoa was developed and good quality DNA was obtained. Studies on genetic characterisation of Marwari and Kacchi-Sindhi horse are in progress. Studies for identifying the various fertility related genes in Marwari stallions were also underway.

During the year 2019, a total of 200 microbes were accessioned in the repository thereby the cumulative strength of NCVTC reaching to 3842. The important virus isolates accessioned in the NCVTC repository during the year include, New castle disease virus ($n=06$), Fowl adenovirus ($n=15$) and Bluetongue virus ($n=19$). First report on isolation of BoHV5 from India was also reported from NCVTC. PCV3 was reported to be an important pig pathogen for the circulation of porcine picorna virus in the country. A triple knockout clone (tkO) HeLa cell line was generated using CRISPR/Cas9-mediated genome editing. Three cellular genes (CBX5, HOXA10 and NR3C2) reported to have antiviral function were knocked out from these cells. A bacteriophage (VTCCBPA118) which is having better stability at 37°C was isolated against FOP185A-K. *pneumonia* strain at NCVTC repository (from animal farm soil). Assessment of anti-biofilm activity of a bacteriophage (VTCCBPA139) isolated against MDR *Proteus mirabilis* strain was also carried out during the period. The surveillance of anti-microbial resistance in bacteria from cattle, buffalo, sheep, goat, pig and poultry was carried out by collection of milk samples from cattle/buffalo, cloacal swabs from chicken and rectal swabs from sheep and pig from Hisar and Hansi blocks of Haryana state. Researchers at NCVTC are also focussing on the development of a repository of mycobacterial species.

During the year, scientists published 58 high impact research papers in international and national peer-reviewed journals. In addition, 8 popular articles, 3 book chapters, 18 extension leaflets and 24 research abstracts were also published. Vision, guidance and technical support provided from time-to-time by Hon'ble chairmen and members of QRT, RAC and experts of IRC has immensely helped NRCE to be in right direction and be much focused.

I would like to take this opportunity to record my sincere thanks to the Chairman and the Members of the Publication Committee for bringing out this excellent annual report of the Centre with a new look and substantial improvement in the quality of its publication. I gratefully acknowledge the whole-hearted support extended to this institute by Dr. Trilochan Mohapatra, Secretary Department of Agricultural Research and Education, and Director General, ICAR and Dr Joykrushna Jena, Deputy Director General (Animal Science). My thanks are also due to the Assistant Director Generals Dr Ashok Kumar (Animal Health) and Dr Rajan Gupta, Dr Vineet Bhasin, Dr Jyoti Misri, Principal Scientists at ICAR Headquarters for their continuous support to NRCE. The whole NRCE team deserves my sincere gratitude for their wholehearted support and cooperation in completing yet another successful year.

B. N. Tripathi
(B.N. TRIPATHI)



Executive Summary

कार्यकारी सारांश

ICAR-NRCE is actively involved in surveillance of different equine diseases in the country and frontline research works to combat against major equine diseases. The information on epidemiology of various equine diseases is of paramount importance for policy making purposes and to implement effective control measures. In this direction, during current report of the year 2019, a total of 2169 equine serum samples from 12 states were tested for various diseases like equine infectious anaemia (EIA), equine influenza (EI), Equine Herpesvirus 1 (EHV-1), Japanese Encephalitis Virus (JEV), trypanosomosis, equine piroplasmosis, *Salmonella Abortus equi* and brucellosis. Highest seroprevalence of 41.40 % were observed for equine piroplasmosis (*Theileria equi*) followed by 6.52 % for EHV-1, 5.44 % for JE/WNV and 1.98 % for *Trypanosoma evansi*. None of the equines were found positive for equine influenza, equine infectious anaemia, brucellosis and *Salmonella Abortus equi*. Under disease investigation 22116 equine sera was tested for glanders and 210 glanders positive cases were reported. In public health point of view, 219 human serum samples from in-contact equine handlers were tested and all of them were negative for glanders. In addition, 414 samples tested for equine influenza were negative. The 56 random samples from 4 states tested for African Horse Sickness (AHS) and were found negative. Microbiological analysis of 473 clinical samples including nasal swab, tissue, abscess, aborted fetus etc., yielded 111 bacterial isolates including *Burkholderia mallei*, *Klebsiella pneumoniae*, *E. coli*, *Rhodococcus equi*, *Streptococcus equi* and *Streptococcus zooepidemicus*.

भा.कृ.अनु.प.-राष्ट्रीय अश्व अनुसंधान केन्द्र देश में विभिन्न अश्व रोगों की निगरानी में सक्रिय रूप से कार्यरत है और प्रमुख अश्व रोगों के विरुद्ध लड़ने के लिए अग्रणी शोध कार्य करता है। नीति बनाने के उद्देश्यों और प्रभावी नियंत्रण उपायों को लागू करने के लिए विभिन्न अश्व रोगों के महामारी विज्ञान की जानकारी सबसे महत्वपूर्ण है। इस दिशा में वर्ष 2019 की वर्तमान रिपोर्ट के दौरान, 12 राज्यों के कुल 2169 अश्व सीरम के नमूनों को विभिन्न रोगों जैसे कि अश्व संक्रामक एनीमिया (ईआईए), ईएचवी-1, जेईवी, ट्रिपैनोसोमोसिस, इक्वाइन पायरोप्लास्मोसिस, साल्मोनेल्ला एबॉर्टस इक्वाई और ब्रुसेलोसिस के लिए परीक्षण किया गया। अश्व पायरोप्लास्मोसिस, ईएचवी-1, जे.ई.वी. एवं ट्रिपैनोसोमोसिस रोगों की व्यापकता क्रमशः 41.40, 6.52, 5.44 एवं 1.98 प्रतिशत पाई गई। अश्व फ्लू, अश्व संक्रामक एनीमिया, ब्रुसेलोसिस और साल्मोनेला एबॉर्टस इक्वाई रोग किसी भी घोड़े में नहीं पाया गया। रोग जांच के तहत ग्लैंडर्स के लिए 22116 अश्व सीरा का परीक्षण किया गया और 210 घोड़ों में ग्लैंडर्स पाया गया। ग्लैंडर्स रोग से पीड़ित अश्वों के सम्पर्क में आए 219 व्यक्तियों के सीरम नमूनों की जांच की गई और इन सभी में ग्लैंडर्स रोग नहीं पाया गया। इसके अतिरिक्त अश्व फ्लू के लिए जांचे गए 414 नमूनों में यह रोग नहीं पाया गया। परीक्षण के लिए चार राज्यों से प्राप्त 56 रक्त नमूनों में अफ्रिकन होर्स रोग (ए.एच.एस.) नहीं पाया गया। 473 नैदानिक नमूनों (जिसमें नाक का फाहा, उक्तक, फोड़ा, गर्भस्थभ्रूण आदि शामिल हैं) के सूक्ष्मजीव विज्ञानी विश्लेषण से 111 बैक्टीरियल आइसोलेट्स (बी. मेलियाई, क्लेबसिएला न्यूमोनिया, ई. कोलाई, रोडोकोक्कस इक्वाई, स्ट्रेप्टोकोक्कस इक्वाई और स्ट्रेप्टोकोक्कस जूएपिडेमिकस) मिले।

अश्वों में इक्वाइन हरपीज वायरस-1 संक्रमण एक बड़ी समस्या है जो अधिकतर गर्भपात और सांस की समस्याओं का कारण है। देश में पाए जाने वाली ई.एच.वी.-1 की आनुवंशिक विविधता को कलमबध करने के लिए ई.एच.वी.-1 संक्रमण के लिए 218



EHV-1 infection is a major problem in equines and mostly associated with abortion and respiratory problems. For documentation of genetic diversity of EHV-1 circulating in the country, 218 clinical samples from equines were tested for EHV-1 infection by qPCR and 43 were detected positive. EHV-1 viruses were isolated in RK13 cells from seven positive samples and all isolates were non-neuropathogenic. Partial genome sequences of two EHV-1 isolates (EHV-1/14 and EHV-1/Meerut) were generated using NGS platform covering more than 90 % of genome. Phylogentic analysis was carried out based on US and UL segments of both the isolates for their genetic comparison.

To study the sero-prevalence of EHV-1 infections, a peptide ELISA (MAP-ELISA) has been developed for detection of EHV-1 specific antibodies. This assay increased the signal-to-noise ratio and specificity. The relative analytical sensitivity and specificity of this assay was 96.77 % and 97.78 %, respectively. The diagnostic sensitivity of the assay was further validated with 1136 field equine serum samples and 116 (10.21 %) were detected positive for EHV-1 antibodies. There was 99.1% agreement between virus neutralization and MAP-ELISA, with Cohen's K (0.952).

The Centre is continuously working on development of vaccines against major equine pathogens. ICAR-NRCE has earlier developed inactivated vaccine against EHV-1 (*Equiherpabort*) which is being utilized at the field. This vaccine was further refined by replacing the adjuvant with Montanide-Pet-Gel. This updated vaccine showed generation of good humoral and cell mediated immunity in murine model. This vaccine was further tested in natural host (pregnant and non-pregnant mares) at EPC, Bikaner. The immune responses were assayed by serum neutralization assay and result showed increasing SNT titres in the sera samples at various intervals. At 90 and 120 days of vaccination, animals showed better antibody titre in pregnant (74.66 ± 48.88 & 96 ± 55.42) and non-pregnant mares (56 ± 16 & 88 ± 48), respectively.

ICAR-NRCE is working toward development of combined vaccine employing earlier generated

नैदानिक नमूनों का परीक्षण क्यू.पी.सी.आर. द्वारा किया गया और इनमें से 43 नमूनों में ई.एच.वी.-1 संक्रमण पाया गया। ई.एच.वी.-1 विषाणु सात पॉजिटिव नमूनों से आर.के.-13 कोशिकाओं में आइसोलेट किया गया और सभी आइसोलेट्स गैर-न्यूरोपैथोजेनिक थे। एन.जी.एस. प्लेटफार्म का उपयोग करके दो ई.एच.वी.-1 आइसोलेट्स (ई.एच.वी.-1/14 एवं ई.एच.वी.-1/मेरठ) के आंशिक जीनोम अनुक्रम का निर्माण किया गया। दोनों आइसोलेट्स की आनुवंशिक तुलना के लिए इनके यू.एस. एवं यू.एल. हिस्सों के आधार पर वंशावली विश्लेषण किया गया।

ईएचवी-1 संक्रमण के सीरो-प्रसार का अध्ययन करने के लिए, ईएचवी-1 विशिष्ट एंटीबॉडी का पता लगाने के लिए एक पेप्टाइड एलिसा विकसित किया गया है। एस्से का सिग्नल-टू-शोर अनुपात और विशिष्टता को बढ़ाने के लिए, एक मल्टीमेरिक एंटीजेनिक पेप्टाइड (एमएपी) आधारित एस्से वर्ग के दौरान विकसित किया गया था। इस एस्से की सापेक्ष विश्लेषणात्मक संवेदनशीलता और विशिष्टता क्रमशः 96.77 प्रतिशत और 97.78 प्रतिशत थी। एस्से की नैदानिक संवेदनशीलता को 1136 सीरम नमूनों के साथ सत्यापित किया गया था और 116 (10.21 प्रतिशत) नमूनों में ईएचवी-1 एंटीबॉडी पाई गई। वायरस न्यूट्रलाइजेशन और एमएपी-एलिसा के बीच 99.1 प्रतिशत समानता देखी गई। एमएपी-एलिसा व वी.एन.टी. की नैदानिक संवेदनशीलता और विशिष्टता क्रमशः 96.73 प्रतिशत और 99.51 प्रतिशत थी।

केन्द्र अश्वों के प्रमुख रोगजनकों के विरुद्ध टीकों के विकास पर लगातार काम कर रहा है। आईसीएआर-एनआरसीई ने पहले ईएचवी-1 (इक्विहर्पवोर्ट) के विरुद्ध निष्क्रिय टीका विकसित किया है जो फिल्ड में उपयोग किया जा रहा है। वैक्सीन में सुधार के लिए, एडजुवेंट को मॉटेनाइड-पेट-जैल के साथ बदल दिया गया था। इस टीके ने म्युराइन मॉडल में अच्छे ह्युमोरल और कौशिका मध्यस्थता प्रतिरक्षा को दिखाया। इस टीके का परीक्षण अश्व उत्पादन परिसर, बीकानेर की गर्भवती और गैर-गर्भवती घोड़ियों में किया गया। प्रतिरक्षा प्रतिक्रियाओं को सीरम न्यूट्रलाइजेशन एस्से द्वारा किया गया और विभिन्न अंतरालों पर सीरम के नमूनों में एसएनटी टाइट्र में वृद्धि देखी गई। ई.एच.वी.-1 टीकाकरण के 90 और 120 दिनों बाद में क्रमशः गर्भवती (74.66 ± 48.88 और 96 ± 55.42) और गैर-गर्भवती (56 ± 16 और 88 ± 98) घोड़ियों में एंटीबॉडी टाइट्र देखा गया। महत्वपूर्ण श्वसन रोगजनकों जैसे कि अश्व हरपीज वायरस ईएचवी-1 और इक्वाइन इन्फ्लुएंजा वायरस (इआईवी) से निपटने के लिए भा.कृ.अनुं.प.-रा.अ.अनुं.केन्द्र संयुक्त टीके के विकास की दिशा में काम कर रहा है जो पहले से विकसित



mutant EHV-1-BAC based vectored vaccine using an immunodominant gene of EIV by red recombination technology. The immunodominant gene is inserted into the backbone of mutant EHV-1 for creating a combination vaccine holding protection against EHV-1 and EIV. This bivalent vaccine will save the time and labour of the field farmers and caretakers in the horse industry.

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus which causes significant epidemics of encephalitis in India. To understand the epidemiology of JE in animal and mosquito vectors, seroprevalence of JEV in different districts of Assam was carried out. The 947 serum samples from pigs from eight districts of Assam were tested for JEV antibodies by ELISA & HI. A total 87 porcine samples (9.18 %) were found positive for JEV antibodies. Further, JEV seroconversion in sentinel pigs were also evaluated in Kamrup rural village and in ICAR-NRC-Pig Farm, Rani. JEV sero conversion was recorded in eight sentinel pigs starting from the month of June with a peak in July and August month. Prevalence of JEV in vectors was also studied. Mosquito samples were collected from Jorhat, Lakhimpur and Kamrup districts and most prevalent vectors identified were *Culex tritaeniorhynchus*, *Cx. Gelidus* and *Mansonia* spp. and found significantly higher during summer season (April-September) than in winter months (December-February).

In order to diagnose JEV, a Taqman-based real-time PCR was developed for detection of JEV infection. The sensitivity of the assay was 23 copies of JEV cDNAs. The assay was specific for JEV and did not cross-react with WNV.

For Glanders surveillance, 34322 equine sera were collected from 15 states including Northern, Western, Central, Southern and Eastern India under glanders surveillance programme. A total of 210 glanders positive cases were reported from Uttar Pradesh (135), Uttarakhand (7), Haryana (5), Delhi (9), Jammu & Kashmir (5), Himachal Pradesh (3), Rajasthan (8), Gujarat (7), Maharashtra (9), Chhattisgarh (4), Madhya Pradesh (11), Karnataka (4) and Andhra Pradesh (3). This year surveillance data revealed that

उच्चपरिवर्ती ईएचवी-1-बीएसी पर आधारित वैक्टर युक्त वैक्सीन है जो लाल पुनर्संयोजन प्रौद्योगिकी द्वारा ईआईवी के एक इम्यूनोडोमिनेंट जीन का उपयोग करता है। ईएचवी-1 और ईआईवी के विरुद्ध सुरक्षा देने वाली एक संयोजन वैक्सीन बनाने के लिए प्रतिरक्षात्मक जीन को उच्चपरिवर्ती ईएचवी-1 बीएसी में डाला जाता है। यह टीका अश्व उद्योग में लगे किसानों और अश्व पालकों के समय और श्रम को बचाएगा।

जापानी एन्सेफलाइटिस वायरस (जेईवी) एक मच्छर जनित फ्लेविवायरस है जो भारत में एक महत्वपूर्ण एन्सेफलाइटिस महामारी का कारण बनता है। पशु और मच्छर वैक्टर में जेई के महामारी विज्ञान को समझने के लिए, असम के विभिन्न जिलों में जेईवी की व्यापकता का अध्ययन किया गया। असम के 8 जिलों में सूअरों के 947 सीरम नमूनों को जेईवी एंटीबॉडीज के लिए एलिसा और एचआई द्वारा परीक्षण किया गया। जेईवी एंटीबॉडीज के लिए 87 सूअरों के नमूने (9.18%) पॉजिटिव पाए गए थे। इसके अतिरिक्त सेंटिनल सुअरों में जेईवी सीरोकोनवर्जन का मूल्यांकन भी कामरूप ग्रामीण गांव और एनआरसी-सुअर फार्म, रानी में किया गया। जेईवी सीरो रूपांतरण जून के महीने में शुरू हुआ और जुलाई अगस्त महीने में शिखर पर पहुंच गया। विभिन्न वैक्टर में जेईवी की व्यापकता का अध्ययन किया गया। जोरहाट, लखीमपुर और कामरूप जिलों से मच्छर के नमूने एकत्र किए गए। सबसे अधिक प्रचलित वैक्टर क्यूलेक्स ट्राइटेनियोरिन्चस, सीएक्स गेलिडस और मैनुसानिया जाति के मच्छर पाए गए और सर्दियों के महीनों (दिसंबर-फरवरी) की तुलना में गर्मी के मौसम (अप्रैल-सितम्बर) के दौरान काफी अधिक पाए गए।

जेईवी का निदान करने के लिए एक तापमान-आधारित आरटी पीसीआर जेईवी संक्रमण का पता लगाने के लिए विकसित किया गया था। एस्से की संवेदनशीलता जेईवी सीडीएनए की 23 प्रतियां थी। एस्से जेईवी के लिए विशिष्ट था और डब्ल्यूएनवी के साथ क्रॉस-रिएक्शन नहीं करता था।

ग्लैंडर्स निगरानी कार्यक्रम के तहत उत्तरी, पश्चिमी, मध्य, दक्षिणी और पूर्वी भारत के 15 राज्यों से एकत्र किए गए 34322 इक्वाइन सीरा पर ग्लैंडर्स निगरानी की गई। उत्तर प्रदेश (135), उत्तराखण्ड (7), हरियाणा (5), दिल्ली (9), जम्मू और कश्मीर (5), हिमाचल प्रदेश (3), राजस्थान (8), गुजरात (7), महाराष्ट्र (9), छत्तीसगढ़ (4), मध्यप्रदेश (11), कर्नाटक (4) और आंध्रप्रदेश (3) से कुल 210 ग्लैंडर्स पॉजिटिव मामले सामने आए। इस साल निगरानी डेटा से पता चला कि यह बीमारी उत्तरी, मध्य, पश्चिमी और दक्षिणी भारत के 12 राज्यों में फैल गई है। ग्लैंडर्स के मामले 68 जिलों में दर्ज किए



disease has spread across 12 states in Northern, Central, Western and Southern India. District wise glanders cases were reported in 68 districts and Uttar Pradesh had maximum number of glanders reported districts (40). In public health aspect, 219 sera from occupationally exposed humans (Veterinary Officers, equine handlers, laboratory workers) were tested and all of them were found negative for glanders.

ICAR-NRCE developed ELISA for rapid and efficient execution of glanders surveillance activities. This ELISA technique has been provided to 11 state diagnostic laboratories/RDDLs namely Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, Karnataka, Jammu & Kashmir, Bihar, Chhattisgarh and Madhya Pradesh at different time point during last three years. In 2019, 12206 equine samples were screened by ELISA at seven State Lab/RDDLs (Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, Jammu & Kashmir).

For identification of diagnostic antigen(s) and development of multiple antigens based serodiagnosis of glanders, 24 secretory proteins belonged to T2SS, T3SS, T5SS and T6SS secretory systems of *B. mallei* were selected and six chimeric fusion gene(s) were commercially synthesized. Four fusion proteins were produced in *E. coli*, purified and recombinant proteins showed specific reactions to glanders positive sera by Western blot. Two chimeric proteins were evaluated by indirect ELISA. The sensitivity and specificity of chimera-1 was 92.14 % and 93.69 %, respectively. For chimera-2, sensitivity and specificity were 81.48 % and 91.22 %, respectively.

Strangles is the most contagious and infectious disease of horses, mules and donkeys. The disease is caused by *Streptococcus equi*. Multi-locus sequence typing (MLST) is a molecular technique that allows genetic comparison of bacterial strains. An MLST scheme has been developed for the α -hemolytic, Lancefield group C streptococcal bacterium *S. equi* subspecies *zooepidemicus*. A total of 45 isolates of *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* isolated from field cases were analyzed by sequencing *SeM* gene. Thirteen sequences having novel *SeM* allele were submitted to

गए थे और उत्तर प्रदेश में सबसे अधिक 40 जिलों में ग्लैंडर्स के मामले दर्ज किए गए। सार्वजनिक स्वास्थ्य पहलू में, व्यावसायिक रूप से सम्पर्क में आए मनुष्यों (पशु चिकित्सा अधिकारी, अश्व संचालकों, प्रयोगशाला श्रमिकों) से लिए गए 219 सीरा का परीक्षण किया गया और किसी में भी ग्लैंडर्स रोग नहीं पाया गया।

रा.अ.अनु.केन्द्र ने ग्लैंडर्स की निगरानी में तेजी लाने और कुशल निष्पादन के लिए एलिसा विकसित किया। यह एलिसा तकनीक गुजरात, हरियाणा, हिमाचल प्रदेश, पंजाब, राजस्थान, महाराष्ट्र, कर्नाटक, जम्मू और कश्मीर, बिहार, छत्तीसगढ़ और मध्य प्रदेश जैसे 11 राज्यों की नैदानिक प्रयोगशालाओं/आरडीडीएलएस को पिछले तीन वर्षों के दौरान अलग-अलग समय बिंदु पर प्रदान की गई है। 2019 में आठ राज्य प्रयोगशालाओं/आरडीडीएलएस (गुजरात, हरियाणा, हिमाचल प्रदेश, पंजाब, राजस्थान, महाराष्ट्र, जम्मू और कश्मीर) में एलिसा द्वारा 12206 इक्वाइन नमूनों की जांच की गई।

डायग्नोस्टिक एंटीजन (एस) की पहचान के लिए और ग्लैंडर्स के कई एंटीजन आधारित सिरोडायग्नोसिस के विकास के लिए बी0 मेलाई की स्त्रावी प्रणालियों से सम्बंधित 24 स्त्रावी प्रोटीन 1255, टी2एसएस, टी5एसएस और टी6एसएस का चयन किया गया था और छह काइमेरिक फ्यूजन जीन व्यावसायिक रूप से संश्लेषित किए गए थे। ई0 कोलाई से चार संलयन प्रोटीन का उत्पादन किया गया, शुद्ध और पुनर्संयोजित प्रोटीनों ने ग्लैंडर्स पॉजिटिव सीरा के लिए विशेष प्रतिक्रिया दिखाई। अप्रत्यक्ष एलिसा द्वारा दो काइमेरिक प्रोटीन का मूल्यांकन किया गया था। काइमेरा-1 की संवेदनशीलता और विशिष्टता क्रमशः 92.14 प्रतिशत और 93.69 प्रतिशत थी। काइमेरा-2 के लिए, संवेदनशीलता और विशिष्टता क्रमशः 81.48 प्रतिशत और 91.22 प्रतिशत थी।

स्ट्रैगल्स घोड़ों, खच्चरों और गधों की सबसे संक्रामक बीमारी है। यह बीमारी स्ट्रेप्टोकोकस इक्वाई के कारण होती है। मल्टी-लोकस अनुक्रम टाइपिंग (एमएलएसटी) एक आणविक तकनीक है जो बैक्टीरिया के उपभेदों की आनुवांशिक तुलना की अनुमति देती है। बीटा-हिमोलिटिक, लांसफील्ड समूह सी स्ट्रेप्टोकोकल जीवाणु एस इक्वाई उप-प्रजाति जूएपीडेमिकस के लिए एक एमएलएसटी योजना विकसित की गई है। फिल्ड से एकत्र एस इक्वाई सब संयोजित इक्वाई और एस. इक्वाई उप-प्रजाति जूएपीडेमिकस के 45 आइसोलेट्स का विश्लेषण एस.इ.एम. जीन के अनुक्रमण द्वारा किया गया था। नवीन एस.इ.एम. वाले एलील तेहर सीक्वेंस को बीआईजीएसडीबी को प्रस्तुत किया गया और भारतीय एस इक्वाई आइसोलेट्स को नवीन एलील नंबर 190 दिया गया। सात संरक्षित जीनों के अनुक्रमों का



BIGSdb and assigned novel allele no 190 from Indian *S. equi* isolates. The sequences of seven conserved genes have been analyzed and indicated the presence of unique *S. equi* isolates circulating in Indian equine population. In prevalence studies, seventy serum samples were tested for *S. equi* antibodies from three organized farms along with ten field samples. Seventy percent field samples were positive for antibodies while only one sample from organized farms (1/60) was positive.

The Centre is actively involved in surveillance on zoonotic diseases in India. A total 137 human cases living in contact with equines were examined for Glanders and no clinical case of Glanders was detected. In addition, 13696 equines were screened for Glanders and 94 positive cases were detected. For Japanese encephalitis seroprevalence, 157 equines were tested for Japanese encephalitis antibodies and 13 were detected positive.

NRCE is continuously working on development of drugs against various pathogens. The target specific novel drug molecules against *Trypanosoma evansi* infection have been evaluated using nanotechnology approach. Two bioactive compounds - piperine and emetine were selected. Both, piperine and emetine are alkaloids and isolated from the fruits of *Piper nigrum* and from root of *Carapichea ipecacuanha*, respectively. Based on inhibitory drug concentration, piperine was selected for nanoformulation. Piperine-loaded nanocapsules (NCs) were prepared by using emulsion-diffusion method and characterised. The NCs were then evaluated for growth inhibition assay and IC_{50} was found 5.04 μM , which was approximately one-third of the IC_{50} of pure piperine. It was concluded that piperine-loaded NCs have more significant inhibition of parasite growth as compared to pure piperine. Furthermore, the cytotoxicity profile for both, piperine as well as its NCs was dose dependent, but when compared in-between, then piperine showed more cytotoxicity as compared to piperine-loaded NCs.

The herbal plant extracts have been evaluated for development of safe herbal drug against *Theileria equi* which is responsible for

विश्लेषण किया गया है और यह पाया गया है कि विशिष्ट एस0 इक्वाई आइसोलेट्स की उपस्थिति भारतीय अश्वों में घूम रही है। रोग की व्यापकता का अध्ययन करने के लिए, 70 सीरम नमूने तीन संगठित अश्व शालाओं व 10 नमूने फिल्ड से लिए गए और उनमें एंटीबॉडी परीक्षण किया गया। फिल्ड से लिए गए नमूनों में से 70 प्रतिशत एंटीबॉडी पॉजिटिव पाए गए जबकि संगठित अश्व फार्मों का केवल एक (1/60) नमूना पॉजिटिव पाया गया।

भारत में पशुजन्य (जूनोटिक) रोगों पर निगरानी के लिए केन्द्र सक्रिय है। ग्लैंडर्स पीड़ित अश्वों के संपर्क में रहने वाले 137 मानव मामलों की जांच ग्लैंडर्स के लिए की गई और किसी में भी ग्लैंडर्स नहीं पाया गया। इसके अतिरिक्त ग्लैंडर्स के लिए 13696 अश्वों की जांच की गई और 94 ग्लैंडर्स के पीड़ित पाए गए। जापानी इंसेफेलाइटिस के लिए 157 अश्वों का परीक्षण किया गया और 13 में जापानी इंसेफेलाइटिस की एंटीबॉडीज पाई गई।

रा.अ.अनु.केन्द्र विभिन्न रोगजनकों के विरुद्ध दवाओं के विकास पर लगातार काम कर रहा है। नैनो टेक्नोलॉजी दृष्टिकोण को उपयोग करके ट्रिपैनोसोमा इवांशी संक्रमण के विरुद्ध लक्षित विशिष्ट नवीन दवा अणुओं का मूल्यांकन किया गया है। दो बायोएक्टिव यौगिकों पिपेरिन और एमेटिन का चयन किया गया था। पिपेरिन और एमेटिन दोनों ही एल्कालॉइड हैं और क्रमशः पाइपर नाइग्रम के फलों से और कैरापिशा आइपेकुआन्हा की जड़ से अलग किए जाते हैं। निरोधात्मक दवा सान्द्रता के आधार पर, पिपेरिन को नैनोफॉर्मूलेशन के लिए चुना गया था। पिपेरिन-लोडेड नैनोकैप्सूल (एनसी) इमल्शन-डिफ्यूजन विधि द्वारा तैयार किए गए और उनका चरित्र चित्रण किया गया। विकास अवरोध एस्से के लिए नैनो कैप्सूल का मूल्यांकन किया गया और आईसी50 की 5.04 μM पाया गया, जो शुद्ध पिपेरिन के आईसी50 का लगभग एक तिहाई था। यह निष्कर्ष निकाला गया कि शुद्ध पिपेरिन की तुलना में पिपेरिन-लोडेड नैनो कैप्सूल में परजीवी वृद्धि कम देखी गई। इसके अतिरिक्त पिपेरिन और नैनो कैप्सूल की साइटोटॉक्सिसिटी प्रोफाइल खुराक पर निर्भर थी। लेकिन जब इनके बीच में तुलना की गई तो पिपेरिन ने अधिक साइटोटॉक्सिसिटी की पिपेरिन-लोडेड एनसी की तुलना में दिखाया।

हर्बल पौधे के अर्क का मूल्यांकन थेलेरिया इक्वाई के विरुद्ध सुरक्षित हर्बल दवा के विकास के लिए किया गया है जो कि अश्व पायरैप्लाजमोसिस का प्रमुख कारण है। इससे पहले, मास्प इन-विट्रो कल्चर सिस्टम में बबूल एनिलोटिका के ए.एन.एम.ई.ए. अंश के एंटी-थेलेरिया इक्वाई गुणों का मूल्यांकन किया गया था। वर्तमान अध्ययन में, एलसी/एमएस स्पेक्ट्रोस्कोपी द्वारा उस अंश से प्रमुख अणुओं के पहचान की गई



causing equine piroplasmiasis. Previously, anti-*T. equi* properties of ANMEA fraction of *Acacia nilotica* was evaluated in MASP *in-vitro* culture system. In present study, the lead molecules have been identified by LC/MS spectroscopy and different major peaks were identified. The important lead molecules were identified as – catechol, procatechuric acid, gallic acid, epicatechin, quercetin, ellagic acid etc. Further organ toxicity of this fraction was also accessed in *in-vivo* studies in mouse model. The therapeutic dose for ANMEA in this study was calculated as 50 mg/kg of body weight. Organ toxicity trial in mouse model was conducted. There was no mortality or signs of toxicity recorded in the study period in any of the three groups. The promising results were observed with this fraction of the herbal plant for development of herbal drug.

NRCE has developed diagnostics for *Theileria equi* and *Babesia caballi* as per OIE guidelines. Equine piroplasmiasis is a tick transmitted haemoprotozoan disease caused by *Theileria equi* and/or *Babesia caballi* and poses a serious threat in international movement of the infected horses. An ELISA assay was optimized with recombinant BC48 protein and samples were tested for diagnosis of *B. caballi*. Multiplex PCR was also optimized for simultaneous diagnosis of *T. equi* and *B. caballi* infection in a single PCR reaction. Species-specific primers were designed and PCR conditions were optimized. A PCR product of size 392 bp and 540 bp were amplified specific for *Theileria equi* and *Babesia caballi*, respectively.

Centre has initiated development of herbal drug against *Rhodococcus equi* using Indian herbal medicinal plant extracts. Extracts of three plants viz NRCE-AN-CHL, NRCE-AZI-CHL and NRCE-TRC-MTL were having the highest inhibition potential against *R. equi*. Minimum inhibitory concentration (MIC) of the three plants was recorded upto 625 µg/ml in resazurin microtiter assay. These plants were found to be highly effective against *R. equi* in *in-vitro* studies.

Study was carried out to find an herbal disinfectant for farm soil against *R. equi*. Aqueous extracts of leaves of *Tamarindus indica* and *Eucalyptus globules* were fractioned on silica column by using ethanol, methanol and water

है और विभिन्न प्रमुख शिखरों की पहचान की गई है। कैटेसोल, प्रोक्टेच्यूरिक एसिड, गैलिक एसिड, एपिक्विटिन, क्वेरसेटिन, एलाजिक एसिड आदि प्रमुख अणुओं की पहचान की गई। इससे आगे, अंग विशाक्तता को चूहों के मॉडल में इन-विट्रो अध्ययन में भी प्रवेश किया गया। इस अध्ययन में एनएमईए के लिए चिकित्सीय खुराक की गणना शरीर के वजन के 50 मिलीग्राम/किलोग्राम के रूप में की गई थी। चूहों के मॉडल में अंग विशाक्तता का परीक्षण किया गया था। अध्ययन अवधि में तीनों समूहों में से किसी भी मृत्यु दर या विशाक्तता के संकेत दर्ज नहीं किए गए थे। हर्बल दवा के विकास के लिए हर्बल पौधे के इस अंश के साथ आशाजनक परिणाम देखे गए।

रा.अ.अनु.केन्द्र ने ओआईई दिशानिर्देशों के अनुसार थेलेरिया इक्वाई और बैबेसिया कबाली के लिए निदान विकसित किया है। अश्व पायरोप्लाज्मोसिस एक चिचड़ जनित हिमोप्रोटोजॉन बीमारी है। जो थेलेरिया इक्वाई और/या बैबेसिया कबाली के कारण होती है और संक्रमित घोड़े के अंतरराष्ट्रीय आवाजाही में एक गंभीर खतरा बन जाती है। एक एलिसा एस्से को पुनःसंयोजक बीसी 48 प्रोटीन के साथ अनुकूलित किया गया था और बी0 कबाली के निदान के लिए नमूनों का परीक्षण किया गया था। मल्टीप्लेक्स पीसीआर को एकल पीसीआर प्रतिक्रिया में टी0 इक्वाई और बी0 कबाली संक्रमण के एक साथ निदान के लिए भी अनुकूलित किया गया था। प्रजाति विशिष्ट प्राइमर डिजाइन किए गए थे और पीसीआर स्थितियों को अनुकूलित किया गया था। 392 बीपी और 540 बीपी के आकार का एक पीसीआर उत्पाद थेलेरिया इक्वाई और बैबेसिया कबाली के लिए विशिष्ट रूप से प्रवर्धित किया गया था।

केन्द्र ने भारतीय हर्बल औषधीय पौधे के अर्क का उपयोग करके रोडोकोकस के विरुद्ध हर्बल दवा का विकास शुरू किया है। एनआरसीई-एन-सीएचएल, एनआसीई-एजडआई-सीएचएल और एनआरसीई-टीआरसी-एमटीएल जैसे तीन पौधों के अर्क में रोडोकोकस इक्वाई के खिलाफ उच्चतम अवरोधक क्षमता थी। रेजाजुरिन माइक्रोट्राइट्टर एस्से में तीनों पौधों की एमआईसी को 625.माइक्रो ग्राम/एमएल तक दर्ज किया गया था। ये पौधे इन-विट्रो अध्ययनों में रोडोकोकस इक्वाई के खिलाफ अत्यधिक प्रभावी पाए गए।

रोडोकोकस इक्वाई के खिलाफ अश्वशाला की मिट्टी के लिए एक हर्बल कीटाणुनाशक खोजने के लिए अध्ययन किया गया था। ताम्रिन्दस इंडिका और नीलगिरी ग्लोब्यूलस की पत्तियों के जलीय अर्क को इथेनॉल, मेथेनॉल और पानी का उपयोग करके सिलिका स्तंभ पर विभक्त किया गया था। सक्रिय सिद्धान्त का पता लगाने के लिए इन-विट्रो में आर इक्वाई के विरुद्ध



serially. Different fractions were tested against *R. equi in-vitro* so as to find out active principal. The leaves of *T. indica* and *E. globules* have potential *in-vitro* antibacterial activity against *R. equi*. Both the plants found widely so leaves of these plants will be easy source for preparation of disinfectant against *R. equi* present in farm soil.

The incidences of colic cases in equines during last 5 yrs from 2014 to 2019 were analysed at an organized horse farm situated at sub-tropical desert climate of Rajasthan. In five years, total 243 incidences of colic occurred at farm. Chances of colic cases per animals per year was 0.77. Case fatality rate (CFR) for colic was 1.67 % (excluding colitis). Ponies were having higher chances of colic than horses per year. Incidences of spasmodic colic were highest (88 %). Incidence of impactive colic was 5.76 %, obstructive colic (intussceptions) was 0.82 %. Colitis was observed in 1.65 % of colic cases with CFR of 75 %.

In the direction of development of herbal drug against different equine diseases, *Aerva javanica* plant extract has been evaluated in treatment of equine diseases. Wound healing in horses often complicated by the excessive growth of granulation tissue, commonly known as proud flesh and is similar to keloids in human beings. At present there is no satisfactory treatment for proud flesh in horses. The leaf extract of *Aerva javanica* suppresses excessive growth of granulation tissue in horses. Another disease - Habronemiasis caused by invasion of *Habronema species* larvae in moist skin tissues and wounds, characterized by proliferative, moist and granulomatous wounds below eyes, over face and extremities. Total 20 cases of Habronemiasis in donkeys were found clinical recovery by using leaf extract of *Aerva javanica*. This herbal extract has also been used for treatment of Alopecia. On topical application of the extract, one case of confirmed *Alopecia areata* (AA) and three cases of alopecia in horses were treated. While with pure kaempferol isolated from this extract we treated one confirmed cases of AA in a horse.

A total of 83 samples from indigenous horses and 15 from Thoroughbred horses were subjected for sequencing for the assessment of the genetic variants through SNP mining. From the

अलग-अलग अंशों का परीक्षण किया गया था। इमली और यूकलिप्टस ग्लोब्यूलस की पत्तियों में आर.इक्वाई के खिलाफ इन-विट्रो जीवाणुरोधी गतिविधि होने की संभावना है। दोनों पौधों को व्यापक रूप से पाया जाता है, इसलिए इन पौधों की पत्तियां खेत की मिट्टी में मौजूद आर.इक्वाई के खिलाफ कीटाणुनाशक तैयार करने का आसान स्रोत होगी।

पिछले 5 वर्ष 2014 से 2019 के दौरान अश्वों में पेट दर्द के मामलों की घटनाओं का विश्लेषण राजस्थान के उपोष्णकटिबंधीय रेगिस्तान जलवायु पर स्थित एक संगठित घोड़े के फार्म में किया गया था। पांच वर्षों में कुल पेट दर्द की 243 घटनाएं फार्म में हुईं। प्रति वर्ष प्रति पशु पेट दर्द के मामलों की संभावना 0.77 थी। कॉलिक के लिए केस फेटलिटी रेट (सीएफआर) 1.67 प्रतिशत (कोलाइटिस को छोड़कर) था। पोनीज में प्रति वर्ष घोड़ों की तुलना में कॉलिक के मामलों की अधिक संभावना थी। स्पाज्मोडिक कॉलिक की घटनाएं उच्चतम (88 प्रतिशत) थी। स्पाज्मोडिक शूल के लिए सीएफआर (14 प्रतिशत) थी। 75 प्रतिशत के सीएफआर के साथ पेट के मामलों में (1.65 प्रतिशत) कोलाइटिस देखा गया।

विभिन्न अश्व रोगों के विरुद्ध हर्बल दवा के विकास की दिशा में अर्वा जवनिका पौधों के अर्क का मूल्यांकन अश्व रोगों के उपचार में किया गया है। घोड़ों में होने वाले घावों को अक्सर दानेदान ऊतक के अत्यधिक विकास से जटिल किया जाता है, जिसे आमतौर पर प्राउड फ्लेश के रूप में जाना जाता है और मनुष्य में पाए जाने वाले केलोइड के समान है। वर्तमान में घोड़ों में प्राउड फ्लेश के लिए कोई संतोषजनक उपचार नहीं है। सफेद बूई अर्वा जवनिका के पत्तों का अर्क घोड़ों में दानेदार ऊतक के अत्यधिक विकास को दबा देता है। नम त्वचा के ऊतकों और घावों में हब्रोनेमा प्रजाति के लार्वा के आक्रमण के कारण होने वाली हैब्रानेमासिस एक अन्य बीमारी है; जिसकी प्रमुख पहचान आखों और चेहरे के नीचे प्रोलिफरेटिव नम और गैनुलोमेटस घावों का होना है। गर्धों में हैब्रोनेमियासिस के 20 मामलों में सफेद बूई के पत्तों के अर्क का उपयोग करके नैदानिक सुधार पाया गया। इस हर्बल अर्क का उपयोग एलोपेसिया के उपचार के लिए भी किया जाता है। अर्क के सामयिक अनुप्रयोग पर, एलोपेसिया एरेटा (एए) के एक मामले और घोड़ों में गंजापन के तीन मामलों का इलाज किया गया था। जबकि इस अर्क से शुद्ध kaempferol निकालकर एक घोड़े में एलोपेसिया एरेटा का इलाज किया गया।

एसएनपी खनन के माध्यम से आनुवांशिक वेरिएंट के मूल्यांकन के लिए स्वदेशी घोड़ों से कुल 83 नमूनें और थोरोब्रेड घोड़ों से 15 नमूनों का अनुक्रमण किया गया। अनुक्रमण से कुल 1789642



sequencing, a total of 1789642 SNiPS and 1698013 biallelic SNPs were found and a total number of 108367 effects of which 0.006% were of high impact effects and majorities were identified as classifiers.

The genetic diversity in Kachchhi Sindhi horses was evaluated at 30 microsatellite loci using unrelated animals. A high level of genetic diversity (10.1000 ± 3.1878) was obtained with 303 alleles. Very low inbreeding coefficient (-0.17497 ± 0.0102) was observed for this breed which indicates that the population is distinct and not identical.

A trial was conducted on indigenous mules to develop a fatigue-cum-fitness scorecard. Three mules with an average body weight of 399 kg were used for carrying 10%, 20% and 30% of their body weight and the physiological indices were measured at intervals. It was inferred that the speed of the mules was reduced after 2 hrs of load carrying and no sweating and panting were noted. The score was observed to be 7 out of 28 which indicates that the mules can be used safely for 2 hrs with 30% of their body weight but they cannot be used continuously for 4 hours with 30% of their body weight as the fatigue score reached to 14 out of 28. However, for using the mules in continuous and regular type work the fatigue score should be less than 7 for keeping the animal healthy and productive. In another study, energy efficient nutritionally balanced feed was standardised for equines in which there was no negative effect on haematological parameters.

An inventory database pertaining to biometry, growth and breeding was prepared from the year 1989 to 2019. A breeding plan for the indigenous horses was also prepared with an aim to improve the average height of the herd. A new protocol for isolation of DNA from stallion spermatozoa was developed and good quality and quantity of DNA was obtained.

In an attempt to find the markers for fertility in stallions. Indigenous breeds were evaluated for various physical and seminal quality parameters, Scrotal circumference and seminal parameters were observed to vary between the stallions of different age groups. The morphometry

एसएनआईपीएस और 1698013 द्विअलीलिक एसएनपी पाए गए और कुल 108362 प्रभाव जिनमें से 0.006 प्रतिशत उच्च प्रभाव और प्रमुखता के रूप में वर्गीकृत किए गए।

कच्छी सिंधी घोड़ों में आनुवांशिक विविधता का मूल्यांकन असंबंधित जानवरों का उपयोग करके 30 माइक्रोसेटेलाइट लोसाइड में किया गया था। इस नस्ल में इनब्रीडिंग कोफिसियंट (-0.17497 ± 0.0102) पाया गया, जो दर्शाता है कि पापुलेशन अलग है और समान नहीं है।

एक थकान सह फिटनेस स्कोरकार्ड को विकसित करने के लिए स्वदेशी खच्चरों पर एक प्रयोग किया गया था। तीन खच्चरों जिनका औसत शरीर भार 399 किलोग्राम था, का उपयोग उनके शरीर के वजन के 10, 20 और 30 प्रतिशत भार को खींचकर ले जाने के लिए किया गया था, और शारीरिक प्रतिक्रियाओं को अंतराल पर मापा गया था। यह पाया गया था कि भार ढोने के 2 घंटे बाद खच्चरों की गति में कमी आ गई थी, लेकिन पसीना और हांफना नहीं देखा गया। खच्चरों द्वारा उनके शरीर भार का 30 प्रतिशत भार दो घण्टों तक आसानी से खींच लिया गया, क्योंकि इस दौरान थकान स्कोर मात्र सात ही देखा गया। लेकिन खच्चर उनके शरीर के भार के 30 प्रतिशत के साथ 4 घंटे तक लगातार भार खींचने के लिए उपयोग नहीं किए जा सकते, क्योंकि इस दौरान थकान स्कोर 14 पहुंच गया था। कुल थकान स्कोर 28 में से 14 थकान स्कोर का होना खच्चर का अधिक थकना दर्शाता है। खच्चरों से नियमित एवं निरन्तर प्रकार का काम लेने के लिए तथा उन्हें स्वस्थ और उत्पादक बनाए रखने के लिए थकान स्कोर सात से कम रहना चाहिए। एक अन्य अध्ययन में ऊर्जा कुशल पौष्टिक संतुलित फीड को अश्वों के लिए मानकीकृत किया गया जिसमें हेमेटोलॉजिकल मापदंडों पर कोई नकारात्मक प्रभाव नहीं देखा गया था।

बायोमेट्री, विकास और प्रजनन से संबंधित एक इन्वेंट्री डेटाबेस 1989 से 2019 तक तैयार किया गया था। अश्वों की औसत ऊंचाई में सुधार लाने के उद्देश्य से स्वदेशी घोड़ों के लिए एक प्रजनन योजना भी तैयार की गई थी। घोड़ों के शुक्राणुओं से डीएनए के अलगाव के लिए एक नया प्रोटोकॉल विकसित किया गया था जिसके माध्यम से डीएनए की अच्छी गुणवत्ता और मात्रा प्राप्त की जा सकती है।

घोड़ों में प्रजनन क्षमता के लिए मार्करों को खोजने के प्रयास में विभिन्न शारीरिक और वीर्य गुणवत्ता मापदंडों के लिए स्वदेशी नस्लों का मूल्यांकन किया गया था। अलग-अलग आयु वर्ग के घोड़ों के बीच भिन्नता के लिए अंडकोशीय परिधि और सेमाइनल मापदंडों का अध्ययन किया गया। विभिन्न नस्लों के घोड़ों के



parameters also varied significantly between stallions of different breeds. The expression of fertility related genes was also positively correlated with DNA integrity and acrosome integrity of the stallion spermatozoa and the genes expression was not changed between the seasons.

For effective reproductive management in equines estrus detection and pregnancy diagnosis are very much crucial. In this endeavour ICAR-NRCE has standardised the techniques of estrus detection and ovulation through ultrasonography. Ovarian follicular dynamics relating to the pre-ovulatory follicle and size of the embryonic vesicle at early pregnancy were measured in mares of four different indigenous equines breeds and it was observed to be significantly differ from each breed.

The activities of NCVTC comprise acquisition, authentication, preservation, documentation, and repository database management system of animal microbes. During the year 2019, a total of 200 microbes were accessioned in the repository thereby the cumulative strength of NCVTC reaching to 3842. In the bacterial repository (veterinary microbe component), a total of 69 bacteria were accessioned during the year, making cumulative culture collection to 1427 bacteria of veterinary importance. Similarly, a total of 40 viruses were accessioned during the year making the total strength to 300 virus isolates comprising 30 different species. In addition, six bacteriophages were also isolated from sewage, soil, and sludge and farm yard slurry. A total 49 rumen bacteria were accessioned in the rumen microbe repository at NIANP, Bengaluru making a total strength of 454 rumen microbes and 36 bacteria were added in the dairy microbe's repository at NDRI, Karnal increasing the strength to 613 dairy microbes.

The important virus isolates accessioned in the NCVTC repository during the year include, Newcastle disease virus ($n = 06$), Fowl adenovirus ($n = 15$) and Bluetongue virus ($n = 19$). Partial genetic characterization of two newly isolated avian nephritis viruses was carried and it was found that the viruses belonged to genotype-2 (ANV-2) and is closely related to an isolate (GA-

बीच मॉर्फोमेट्री पैरामीटर भी काफी भिन्न थे। प्रजनन संबंधी जीनों की अभिव्यक्ति को भी सकारात्मक रूप से डीएनए अखंडता और स्टेलियन शुक्राणुओं की एक्रोसोम अखंडता के साथ सहसंबंध) किया गया था और मौसमों के बीच जीन की अभिव्यक्ति में कोई बदलाव नहीं देखा गया।

मद का पता लगाना और गर्भावस्था निदान, अश्वों में प्रभावी प्रजनन प्रबंधन के लिए बहुत महत्वपूर्ण हैं। इस प्रयास में भा.कृ. अनुं.प.-रा.अ.अनुं.केन्द्र ने अल्ट्रासोनाग्राफी के माध्यम से मद का पता लगाने और ओव्यूलेशन समय का पता लगाने की तकनीकों को मानकीकृत किया है। प्रारंभिक गर्भावस्था में भ्रूण के पुटिका के पूर्व डिंबग्रंथि कूप और आकार से संबंधित डिंबग्रंथि कूपिक गतिकी को चार अलग-अलग स्वदेशी घोड़ियों में मापा गया था और यह देखा गया था कि यह प्रत्येक नस्ल में काफी भिन्न होता है।

एन.सी.वी.टी.सी. की गतिविधियों में पशु रोगाणुओं का अधिग्रहण, प्रमाणीकरण, संरक्षण, प्रलेखन और रिपोर्टिजरी डेटाबेस प्रबंधन प्रणाली शामिल है। वर्ष 2019 के दौरान, रिपोर्टिजरी में कुल 200 रोगाणुओं को जोड़ा गया, जिससे एन.सी.वी.टी.सी. की संचयी शक्ति 3842 तक पहुंच गई। बैक्टीरियल रिपोर्टिजरी (पशु चिकित्सा माइक्रोब घटक) में, वर्ष के दौरान कुल 69 जीवाणुओं का अधिग्रहण किया गया, जिससे रिपोर्टिजरी में पशु चिकित्सा महत्व के 1427 बैक्टीरिया हो गए। इसी प्रकार, वर्ष के दौरान कुल 40 विशाणुओं का अधिग्रहण किया गया, जिससे अब 30 अलग-अलग प्रजातियों के कुल 300 वायरस रिपोर्टिजरी में उपलब्ध हैं। इसके अलावा, 6 बैक्टीरियोफॉज भी गंदे नाले, मिट्टी और किचड़ और पशु फार्म के घोल से अलग किए गए हैं। एन.आई.ए.एन.पी., बेंगलुरु ने रूमेन माइक्रोब रिपोर्टिजरी में 49 रूमेन बैक्टीरिया का अधिग्रहण किया गया, जिससे रूमेन माइक्रोब की कुल संख्या 454 बन गई और एन.डी.आर.आई. में डेयरी माइक्रोब की रिपोर्टिजरी में 36 बैक्टीरिया जोड़े गए, इस तरह डेयरी रोगाणुओं की संख्या 613 पहुंच गई।

वर्ष के दौरान एन.सी.वी.टी.सी. रिपोर्टिजरी में भी न्यू कैसल रोग वायरस (एन = 06), फॉल एडेनोवायरस (एन = 15) और ब्लूसेटॉन्ग वायरस (एन = 19) आदि महत्वपूर्ण वायरस अधिग्रहण किए गए हैं। दो नए एवियन नेफ्रैटिस वायरस के आइसोलेट्स का आंशिक आनुवंशिक लक्षण वर्णन किया गया था और यह पाया गया, कि वायरस जीनोटाइप 2 (एएनवी2) से संबंधित थे और संयुक्त राज्य अमेरिका से एक आइसोलेट (जीए-सीके-एसइपी-एएनवी-364255) से इनकी निकटता है। एक बीओएचवी-5 वायरस को भीलवाड़ा, राजस्थान से मवेशियों के गर्भपात से एकत्र किए गए क्लीनिकल नमूनों से



CK-SEP ANV-364-2005) from USA. A BoHV-5 virus was isolated from clinical samples collected from aborted cattle from Bhilwara, Rajasthan. Phylogenetic analysis of this virus indicated a close association with the Brazilian BHV-5 strains and this is the first report on isolation of BoHV-5 from India. Investigation on the circulation of influenza A viruses (IAVs) in apparently healthy pigs in different parts of the country including north eastern states detected presence of human seasonal (H1N1) pdm09 and H3N2 viruses and upon partial sequence analysis these viruses showed 97 – 99 % identity with human seasonal (H1N1) pdm09 and H3N2 viruses. This indicated the circulation of SwIVs in pig population was likely evolved from the contemporary human seasonal pandemic influenza virus. Further, the circulation of porcine circovirus-2 and 3 (PCV-2 & PCV-3) in apparently healthy pigs and emergence of PCV-3 as an important pig pathogen in the country was also reported during the period.

A triple knockout clone (tKO) HeLa cell line was generated using CRISPR/Cas9-mediated genome editing. Three cellular genes (CBX5, HOXA10 and NR3C2) reported to have antiviral function were knocked out from these cells. Upon virus susceptibility study, the HSV-1-GFP virus replicated at much higher titre in this tKO cells as compared to wild type HeLa cells. Studies on acquisition of antiviral drug resistance against host-targeting agents revealed that drug resistance may be induced against host-targeting antiviral agents as well, but at a slower pace. Whereas, directly acting agents are known to develop a complete resistance with 6-10 passages (P), host targeting antiviral agents (Thapsigargin and CGP57380) used in the study did not induce generation of antiviral drug resistant phenotypes against Newcastle disease virus up to passage 40 (P40) and BPXV upto passage 25 (P25), respectively. However, at further higher passages (~P60 in BPXV and ~ P70 in NDV), a significant resistance was observed.

The bacteriophages offer immense potential in anti-microbial therapy in the changing scenario of emerging antibiotic resistance. In this regard, a bacteriophage (VTCCBPA118) which is having better stability at 37°C was isolated against

अलग किया गया था। इस वायरस के फाइलोजेनेटिक विश्लेषण ने ब्राजील के बीएचवी-5 उपभेदों के साथ निकट संबंध का संकेत दिया और यह भारत से बीओएचवी-5 का पहला आइसोलेट है। इन्फ्लूएंजा ए वायरस (आईएवी) के संचलन पर जांच देश के विभिन्न हिस्सों में स्वस्थ सूअरों में की गई, जिनमें उत्तर पूर्वी राज्य भी शामिल है और इनमें मानव मौसमी (एच1एन1) पीडीएम09 और एच3एन2 वायरस की उपस्थिति का पता चला है और आंशिक अनुक्रम में इस वायरस की मानव मौसमी (एच1एन1) पीडीएम09 और एच3एन2 वायरस के साथ 97-99 प्रतिशत समानता देखी गई। यह दर्शाता है, कि सुअर की आबादी में एसडब्ल्यू/वीएस का संचलन समकालीन मानव मौसमी महामारी इन्फ्लूएंजा वायरस से विकसित होने की संभावना है। इसके अलावा, स्पष्ट रूप से स्वस्थ सूअरों के सिरवायरस-2 और 3 (पीसीवी-2 और पीसीवी-3) का प्रचलन भी इस अवधि के दौरान देखा गया है।

कृशस्पर/कैस9-मध्यस्थता जीनम एडिटिंग का उपयोग कर एक ट्रिपल नॉकआउट क्लोन (टीकेओ) हेला सेल लाइन उत्पन्न किया गया था। तीन कोशिकीय जीन (सीबीएक्स5, एचओएक्सए10 और एनआर3सी2) में एंटीवायरल फंक्शन देखा गया है और इन तीन जीन को इन कोशिकाओं से नॉक आउट किया गया। वायरस की संवेदनशीलता के अध्ययन पर, एचएसवी-1-जीएफपी वायरस ने इस टीकेओ कोशिकाओं में जंगली प्रकार की हेला कोशिकाओं की तुलना में बहुत अधिक टाइटर पर दोहराया गया। होस्ट-टारगेटिंग एजेंटों के खिलाफ एंटीवायरल ड्रग रेजिस्टेंस के अधिग्रहण पर किए गए अध्ययनों से पता चला है, कि ड्रग रेजिस्टेंस को होस्ट-टारगेटिंग एंटीवायरल एजेंटों के साथ-साथ धीमी गति से भी प्रेरित किया जा सकता है। जबकि सीधे काम करने वाले एजेंटों को 6-10 पसोंज के साथ एक पूर्ण प्रतिरोध विकसित करने के लिए जाना जाता है। अध्ययन में उपयोग किए जाने वाले मेजबान लक्ष्य एंटीवायरल एजेंट (टेस्पिगैरिन और सीजीपी 57380 न्यू कैसल रोग वायरस 40 पसोंज और बीपीएक्सवी 25 पसोंज के खिलाफ एंटीवायरल ड्रग फिनोटाइप की पीढ़ी को प्रेरित नहीं करते थे। हालांकि, आगे के उच्च पसोंज (बीपीएक्सवी में - पी60 और एनडीवी में - पी70) पर, एक महत्वपूर्ण गतिरोध देखा गया।

बैक्टीरियोफॉज उभरते एंटीबायोटिक प्रतिरोध के बदलते परिदृश्य में रोगाणुरोधी चिकित्सा में अपार संभावनाएं प्रदान करते हैं। इस संबंध में, एक बैक्टीरियोफॉज (वीटीसीसीबीपीए118) जिसकी 37 डिग्री सेल्सियस पर बेहतर स्थिरता है, को एफओपी185ए-क्लैबसिएला निमोनिया तनाव के खिलाफ एनसीवीटीसी रिपॉजिटरी (पशु बाड़े की मिट्टी से) में पृथक



FOP185A- *K. pneumonia* strain at NCVTC repository (from animal farm soil). The isolated phage was stable in the temperature range of 4-55°C and within a narrow pH range of 5 to 9. In another study, the presence of antibiotic resistance genes (*CTXM*, *SHV*, *NDM*, *TetA*, *TetB*, *TetG*, *TetO*, *TetW*, *qnrA*, *qnrS* & *bla-Tem*) in different bacteriophage DNA ($n = 16$) isolated from animal farm soil was investigated, however none of the phage DNA (BPA-106 to BPA-119) was observed to carry any ARG in their genome. Assessment of antibiofilm activity of a bacteriophage (VTCC-BPA-139) isolated against MDR *Proteus mirabilis* strain was also carried out during the period.

The surveillance of antimicrobial resistance in bacteria from cattle, buffalo, sheep, goat, pig and poultry was carried out by collection of milk samples from cattle/buffalo, cloacal swabs from chicken and rectal swabs from sheep and pig from Hisar and Hansi blocks of Haryana state. From overall 103 samples, 62 *E. coli*, 16 *S. aureus* and 14 CoNS were isolated and tested against oxyminocephalosporins and monobactams. *Escherichia coli* isolates from poultry, pigs and sheep were ESBL and ACBL positive. None of the *S. aureus* showed methicillin resistance. A *Clostridium* strain was isolated from foal mortality cases at Nehla village, Bhuna, Fatehabad. Although 16S rRNA sequence did not confirm the species of bacteria, the whole genome sequencing using Illumina platform identified the isolate as *Clostridium botulinum*. An opportunistic rodent bacterium *Rodentibacter pneumotropica* was isolated from intestines of BALB/c mouse during the period and upon genetic characterization the bacteria was found to have close similarity with *Rodentibacter rattiormrazii*. Polyphasic characterization of *Achromobacter* sp. nov. Isolated from stem-cell derived primary cell culture of a term buffalo was carried out using 16S rRNA gene sequencing, commercially available miniaturized identification systems API 20NE, API20E, and API CH (Biomerieux) and MALDI-TOF MS analysis. Efforts are also going on the development of a repository of mycobacterial species at NCVTC repository.

क्रिया गया था। यह फॉज आइसोलेट 4-55° सी के तापमान रेंज में और 5-9 की संकीर्ण पीएच सीमा के भीतर स्थिर था। एक अन्य अध्ययन में पशु बाड़े की मिट्टी से अलग किए गए विभिन्न बैक्टीरियोफॉज डीएनए (एन = 16) में एंटीबायोटिक प्रतिरोध जीन (सीटीएक्सएम, एसएचवी, एनडीएम, टीईटीए, टीईटीबी, टीईटीजी, टीईटीओ, टीईटीडब्ल्यू, क्यूएनआरए, क्यूएनआरएस एण्ड बीएलए-टीईएम) की उपस्थिति की जांच की गई। हांलाकि, किसी भी फॉज डीएनए (बीपीए-106 से बीपीए-119) को किसी भी एआरजी को उनके जीनोम में ले जाते नहीं देखा गया। एमडीआर प्रोटीस मिराबिलिस स्ट्रेन के विरुद्ध एक बैक्टीरियोफॉज (वीटीसीसीबीपीए139) की एंटीबायोफिल्म गतिविधि का आंकलन भी इस अवधि के दौरान किया गया था।

हरियाणा राज्य के हिसार एवं हांसी ब्लॉक की गाय, भैंस, भेड़, बकरी, सुअर और मुर्गी से बैक्टीरिया में रोगाणुरोधी प्रतिरोध की निगरानी गाय, भैंस के दूध के नमूनों के संग्रह द्वारा, चिकन के क्लोकल स्वैब, भेड़ और सुअर के रेक्टल स्वैब द्वारा की गई। 103 नमूनों से, 62 ई० कोलाई, 16 एस० ऑरियस और 14 सीओएनएस को पृथक किया गया और ऑक्सिमिनो-सेफलोस्पोरिन और मोनोबैक्टम के खिलाफ परीक्षण किया गया। मुर्गी, सूअर और भेड़ से प्राप्त की गई ई० कोलाई आइसोलेट ईएसबीएल और एसीबीएल पॉजिटिव थे। एस० ऑरियस में से किसी ने भी मेथिसिलिन प्रतिरोध नहीं दिखाया। फतेहाबाद के नेहला गांव के मृत अश्व फोल से एक क्लोस्ट्रीडियम स्ट्रेन को आइसोलेट किया गया। यद्यपि 16 एसआरएनए अनुक्रम में बैक्टीरिया की प्रजातियों की पुष्टि नहीं हुई, इलुमिना प्लेटफॉर्म का उपयोग कर पूर्ण जीनोम अनुक्रमण से क्लोस्ट्रीडियम बोटुलिनम आइसोलेट की पहचान की। एक अवसरवादी कृतक जीवाणु रोडेंटिनबैक्टर न्यूमोप्रोपिका को इस अवधि के दौरान बाल्ब/सी चूहों की आंतों से पृथक किया गया था और आनुवांशिक लक्षण वर्णन पर इस बैक्टीरिया की रोडेंटिनबैक्टर रैटिअुरमराजी से समानता पाई गई थी। 16 एसआरएनए जीन अनुक्रमण का प्रयोग कर, एक भैंस के स्टेम सेल व्युत्पन्न प्राथमिक सेल कल्चर से अक्रोमोबैक्टर स्प0 नोब0 को आइसोलेट किया गया। इसके लिए व्यवसायिक रूप से उपलब्ध मिनिएच्युराइज्ड पहचान प्रणाली एपीआई 20 एन ई, एपीआई20ई और एपीआईसीएच (बायामेरिक्स) और माल्डी-टॉफ एमएस विश्लेषण का उपयोग किया गया था। एनसीवीटीसी रिपॉजिटरी में माइक्रोबैक्टीरियल प्रजातियों के भंडार के विकास पर भी प्रयास चल रहे हैं।

Zanskari Stallion at ICAR-NRCE,
EPC, Bikaner





01

Introduction

Horses have been domesticated since pre-historic times and hold a special place in our history and culture. Domestication of wild horses played a key role in the rise of larger human settlements and great civilizations. The horses have been a symbol of bravery and power since ancient time. In India, the Aryan started domestication of horses and since then many wars were won by cavalry of this species. Mules and donkeys have been a better choice for carrying packs in the mountains and hence are more popularly known as tractor and truck of the hilly tract of the mountain. With the advent of modern means of transportation, utility of equines is decreasing resulting in decline in their population. Horses still remain preferred means of transport in hilly and desert terrains for the rural poor, nomadic tribes in the north, north-west and north-eastern parts of India.

To cater to the needs of equine health and augment equine productivity in the country, Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on November 26, 1985 at Hisar (Haryana). The main campus of NRCE has state-of-the art laboratories and facilities for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry and biotechnology. The research activities are supported by centralized services such as animal and agriculture farms, experimental animal facility, microbial containment laboratory, AKMU cell, ATIC, library and Info-equine museum. Subsequently, Equine Production Campus (EPC) was established in 1989 at Bikaner (Rajasthan) to undertake research on equine production, management, genetics and breeding, reproduction, physiology and nutrition. Bikaner campus has well maintained herd of Marwari, Kathiawari, Zanskari and Manipuri horses and Halari donkeys and exotic donkeys.

The Centre has created repository facility in the form of establishment of The National Centre for Veterinary Type Cultures (NCVTC) in the year 2005 for the conservation of animal microbes. The NCVTC serves the state-of-the-art facility for collection and preservation of microbes of animal origin and veterinary importance for the protection of microbial biodiversity and R&D. Presently, the Centre is working through 14 network units spread throughout the country. A gamut of microbial cultures in the form of authenticated bacteria, viruses, bacteriophages and genetic materials of the microbes are being maintained and supplied to the stakeholders.

MANDATE OF ICAR-NRCE

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

OBJECTIVES OF ICAR-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 under privileged under changing environment & socio-economic scenario.

SALIENT ACHIEVEMENTS OF ICAR-NRCE

During past 35 years, ICAR-NRCE has contributed significantly in the area of diagnosis and control of equine infectious diseases by providing state-of-the-art diagnostics and biologicals. The Centre is striving hard for conservation and characterization of Indian breeds of equines in the country and even established nucleus herds of representative breeds of equines in its Bikaner campus. Some of the achievements and accolades of the Centre are listed below:



Development of diagnostics for equine diseases

The Centre has been recognized as National Referral Centre for diagnosis of important equine infectious diseases by 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 (EHV-1) infection.
- A neutralizing monoclonal antibody-based diagnostic kit 'Equiherpes B-ELISA' for EHV-1 antibody detection.
- A type-specific ELISA and real-time PCR for differentiation of EHV-1 and EHV-4 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 serodiagnosis of glanders and equine infectious anemia.
- Lateral flow assay based a rapid diagnostic for *Theileria equi* infection.
- LFA kit for glanders.
- LFA kit for EIA.
- A nested (gB-nPCR) and real-time PCR (gB-qPCR) targeting gB were standardized for detection of EHV1 latency.
- Indirect ELISA using recombinant protein for detection of JEV specific antibodies in horse and pig. The assay has been transferred to NE region labs and is used for validation on pig serum samples.
- Multiplex PCR standardized to differentiate *Streptococcus equi subsp equi* and *zoepidemicus*.
- Lateral flow assay validated for rapid diagnosis of trypanosomosis using different *T. evansi* antigens.
- ELISA has been developed to detect *T. evansi* antibodies in multiple species.
- Peptide ELISA for serodiagnosis of EHV-1.
- Multiplex PCR for simultaneous diagnosis of *Theileria equi* and *Babesia caballi* infection.
- Recombinant protein (BC-48 gene) based ELISA for detection of *Babesia caballi*.

Development of vaccines and immuno-biologicals

- Inactivated EHV-1 vaccine "Equiherpabort" using indigenous virus for prevention of abortions in mares.
- Updated equine influenza vaccine using indigenous isolate (A/equi-2/Ludhiana/87). The vaccine was updated in 2008-09 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 virus, Japanese encephalitis virus and *Trypanosoma evansi*.
- Improved EHV-1 vaccine was developed using inactivated EHV-1 adjuvanted with Montanide Pet Gel. The modified vaccine tested in horses and showed protective immune responses.

Basic and Strategic Research

- CBX5 (HP1) knockout cells developed by CRISPR/Cas9-mediated genome editing.
- Host genes with antiviral functions (Med23/HOXA10/NR3C2) were knocked out from the HeLa cells for their utility in studying the sensitivity to HSV-1 replication.

Surveillance and monitoring of equine diseases in India

- ICAR-NRCE is involved in nation-wide monitoring and sero-surveillance of important equine infectious diseases with a view to manage, control and eradicate diseases. Some of the salient achievements under sero-monitoring include:
 - India has gained OIE disease-free status for African horse sickness (AHS) in 2006 based on sero-monitoring data generated by NRCE.
 - Clinical cases of equine infectious anaemia (EIA) have not been reported since 1997. Only two sero-positive



cases (one mule from Uttarakhand in 2009 and one horse from Haryana in 2011) were detected and culled. Control of EIA in India was possible due to timely diagnosis and implementing package of practices formulated by ICAR-NRCE.

- Outbreaks of glanders in equines have been detected since 2006-07 from different states and control measures are being adopted for preventing their further spread.
- Effective control of equine influenza outbreak of 1987 (involving 83000 equines) was done by implementing biosecurity and development of effective vaccine. Similarly, a major outbreak of equine influenza that spread in 13 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.

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, A/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 EHV-1 isolates was carried out using NGS. Sequence comparison of Indian EHV1 isolates with other published isolates revealed that Indian isolates are closely related to EHV-1 isolates (OH03 and VA02) from Japan (97.4 to 98.8%).
- Phylogenetic analysis based on US segments classified our isolates into clade 5 along the reference isolates V592.

Phenotypic and genotypic characterization of Indian equine breeds

- Seven equine breeds namely, Marwari, Kathiawari, Spiti, Zanskari, Bhutia, Manipuri and Kachchhi Sindhi have been characterized on the basis of their biometric indices and coat colour.
- High genetic diversity observed between Spiti and Thoroughbred, followed by Spiti and Kathiawari while Zanskari and Manipuri are the least differentiated.
- Indian breeds form three distinctive clusters based on Bayesian analysis: (a) Kathiawari; (b) Zanskari, Spiti & Manipuri ponies and (c) Bhutia.
- Phenotypic parameters of Kachchhi-Sindhi horses were analyzed
- Microsatellite marker based genetic diversity analyzed for proposing effectual population breeding and management strategies for future.

Establishment of nucleus herd

- 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:
- Marwari horses from Rajasthan; Kathiawari horses from Gujarat; Zanskari ponies from Zanskar valley (Jammu & Kashmir) and Manipuri ponies from Imphal (Manipur) and herds of indigenous and exotic donkeys are being maintained:
- Halari donkeys for conservation and improvement of donkeys are being maintained.
- Poitou donkey herd for production of superior mules.

Improvement in production potential of equines

- In order to conserve the germplasm of indigenous equine breeds, cryopreservation of semen of Marwari, Kathiawari, Zanskari and Manipur stallions and Halari & Poitou donkeys has been done.
- 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 donkey and horse 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 have 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

Utilization of equine energy in agricultural activities

- Single animal drawn matching plough, seed drill (two furrow) and harness have been designed and developed for donkeys and mules for agricultural operations like ploughing and sowing.
- Mules have been used for chaff cutting operation with average output capacity of 660 kg/hour of chopped bajra straw in rotary mode chaff cutter.
- Draughtability studies conducted on adult donkeys using conventional pneumatic two-wheel cart showed that Halari donkeys may pull draft of 33 Kg for 3 hour, 44, 55, 66 Kg for 2 hour, 77, 88 Kg for 1 hour without much stress to donkeys.
- Technique of vermicomposting of equine dung has been optimized for use in agricultural fields.

Patents granted

- Nano-drug delivery for quinapyramine sulfate (Patent No. 310429, Application no. 2560/DEL/2011, dated 06.09.2011).

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
- Nano-drug delivery for quinapyramine sulphate. 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 TssA protein for detection of antibodies against *Burkholderia mallei* and uses thereof. Application No. 3610/DEL/ 2015.
- 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).
- Polymeric metal nanocomposites and methods of synthesis thereof Application no.: 201911009696, Dated 13.03.2019

Services

ICAR-NRCE provides following services to the 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.
- Surveillance, monitoring and control of equine infectious diseases in India.
- 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.

- Onsite and online consultancy in equine health and production, including toll-free telephonic advisory at Hisar and Bikaner campuses for farmers and stakeholders.
- Trainings and supply of education materials for equine management, production and health.
- Education and awareness of equine farmers by organization of health camps, awareness campaigns and farmers meet in different areas of the country.

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 14 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.

The activities of NCVTC comprise acquisition, authentication, preservation, documentation, and repository database management system of animal microbes. During the year 2019 (From January- December, 2019), a total of 200 microbes were accessioned in the repository leading to a cumulative strength of 3842 microbial resources. In the bacterial repository at NCVTC, 69 bacteria were accessioned during the year, making cumulative culture collection of 1427 bacteria of veterinary importance. Some of the significant bacteria accessioned are *Lactococcus lactis*, *Enterococcus faecalis*, *Staphylococcus xylosus*, *Flavobacterium mariense*, *Acinetobacter johnsonii*, *Bacillus licheniformis*, *Microbacterium maurum*, *Agrococcus lahulensis*, *Rhodococcus equi*, *Vibrio parahemolyticus*, *Aeromonas enteropelogens*, *Pseudomonas anguilliseptica*, *Pseudomonas peli*, *Pseudomonas cuatrocienegasensis*, *Streptococcus equi* species *zooepidemicus*, *Streptococcus agalctiae*, *Corynebacterium pseudotuberculosis*. The accession also included anaerobic cultures viz., *Clostridium sporogenes*, *Clostridium perfringens*, and *Clostridium difficile*. In the virus repository, 40 viruses were accessioned in the repository. The important virus isolates accessioned includes- New castle disease virus, Fowl adenovirus and Bluetongue virus. The repository also strengthened with collection of characterized bacteriophages against mastitis causing pathogens including: *Staphylococci* species, and *Streptococci* species. A total of 6 bacteriophages were isolated from sewage, soil, sludge and farm yard slurry. In rumen microbial repository at NIANP Bengaluru, with the accessioning of 49 rumen bacteria, the total strength of the rumen microbe's repository has reached to 464 microbes. The 36 bacteria were accessioned in the dairy microbe's repository at NDRI, Karnal and its strength reached to 620 dairy microbes.

The distribution of microbes for teaching, research and development of new technologies is another mandated activity of NCVTC. In this regard, different bacterial cultures including bacteria namely *Pasteurella multocida*, *Streptococcus agalactiae*, *E.coli*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella Gallinarum*, *Klebsiella pneumonia*, *Campylobacter* species); two viruses viz., Bluetongue virus and Infectious bursal disease virus and four cell lines viz., BHK-21, MDCK, A-72 & Vero were distributed to different Institutes/Universities in India for research and teaching purposes. Some of the salient achievements of NCVTC repository are listed below.



Status of microbial cultures at NCVTC

Microbial Resources	2019	Total (31 December, 2019)
Veterinary microbes		
Bacteria	69	1427
Virus	40	300
Bacteriophage	6	143
Recombinant clone	0	573
Phage library	0	27
Genomic DNA	0	288
Total	115	2758
Rumen microbes		
Anaerobic bacteria	49	349
Fungi/Yeast	0	107
Meth. Archae	0	8
Total	49	464
Dairy microbes		
Bacteria	36	620
Total	36	620
Grand Total	200	3842

Veterinary Microbes

- First laboratory confirmed camelpox virus zoonosis.
- First report on isolation and genetic characterization of swinepox virus from India.
- Accessioning of vaccine strains of viruses viz., Peste des petits ruminant's virus, Sheeppox virus (Srinagar strain), Goatpox virus (Uttarkashi strain), Orf virus (Mukteswar strain), NDV (R2B strain) and NDV (F strain).
- Complete genome sequencing of Classical swine fever virus (2), chicken astro virus (2) & porcine circo virus (4).
- First report of isolation and genetic characterization of an BoHV-5 virus from India
- First isolation and characterization of *Bordetella bronchiseptica*, *Actinobacillus equilli*, *Staphylococcus hyicus*, *Trueperella pyogene* and *Moraxella (Branhamella) ovis* methicillin-resistant coagulase negative *Staphylococcus sciuri*.
- Whole genome sequencing of *Pasteurella multocida* sub spp. *multocida* B:2 serotype, *Trueperella pyogenes*, *Bordetella bronchiseptica*, *Pasteurella multocida*, *Actinobacillus equuli* *Salmonella Gallinarum* *Clostridium bothulism*
- Accessioning of rare strains of bacteria: *Campylobacter* spp., *Bacillus megaterium*, *Enterococcus casseliflavus*, *E. cecorum*, *Barrientosiimonas humi*, *Corynebacterium amycolatum*, *Enterococcus devriesei*, *E. hirae faecium*, *Nocariopsis alba*, *Ignatzschineria larvae* *Escherichia hermanii* and *Rodentibacter* spp. from BALB/c mice
- *Isolation of bacteriophages against a variety of pathogenic bacteria* including a novel thermotolerant bacteriophage isolated from Ganga river water. Isolation, characterization and assessment of stability of a *Klebsiella pneumoniae* phage



Rumen Microbes

- Isolation and characterization of seven tannin degrading bacteria-*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 - *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*, *Streptococcus thermophilus*, *Leuconostoc sp.*, *Bifidobacterium sp.*, *Bifidobacterium dentium*, *Bifidobacterium longum*, *Micrococcus sp.*, *Kluyveromyces lactis* and *Saccharomyces bisporus*.
- *Lactobacillus* sp. having phytase degrading potential and strong antifungal activity have been isolated from milk-cereal fermented products (Rabadi samples).
- Anamalytic strain of *Pediococcus acidolactici* isolated has potential as starter culture in preparation of milk cereal fermented products.

Summary of Expenditure & Revenue Generation : ICAR-NRCE

Details	2017-18 Rs. in Lacs	2018-19 Rs. in Lacs
Summary of Expenditure		
Establishment charges including LSP/PF, wages, OTA	908.88	1276.17
Travelling allowances & HRD	13.05	24.64
Others charges including equipment's & recurring charges	827.81	927.36
Works	54.52	71.43
Loans and Advances	2.50	0.0
Total Plan Expenditure	1806.76	2299.6
Summary of Revenue Generation		
Sale of farm produce	5.29	15.47
Sale of livestock	0.65	0.59
Sale of publications and advertisements	0.18	0.0
License fee	1.47	1.98
Interest on loans and advances	3.94	4.72
Interest on short term deposits	17.24	26.69
Contractual diagnostic services	53.97	61.54
Receipt from services	0.0	0.0
Other miscellaneous receipts	22.37	91.56
Eco-tourism	3.50	6.21
Total Revenue Generation	108.61	208.76

Staff Position at NRCE and NCVTC

Name of the Post	ICAR-NRCE			NCVTC		
	Sanctioned	Filled	Vacant	Sanctioned	Filled	Vacant
Director	1	1	0	-	-	-
Scientific	26	14	12	10	7	3
Technical	23	21	2	-	-	-
Administrative	14	11	3	-	-	-
Supporting	22	20	2	-	-	-
	86	67	19	10	7	3

Marwari Stallion at ICAR-NRCE,
EPC, Bikaner





02

Research Achievements

EQUINE VIROLOGY

Stepping towards developing a combined vaccine for EHV-1 and equine influenza

Equine herpesvirus type-1 (EHV-1) infects horses of all ages and breeds causing respiratory and neurologic disease as well as late term abortions in pregnant. On the other hand, Equine influenza A viruses (H7N7 and H3N8 subtypes) are a leading cause of respiratory disease in the horse, which has not been controlled successfully by vaccination and remains today a serious threat to horse welfare and an economic problem for the horse industry. Vaccination is commonly used to control equine respiratory pathogens such as equine herpesvirus type-1 (EHV-1) and equine influenza virus (EIV). Hence, to combat both of these important and prevalent diseases, the present study is to incorporate a BAC of EHV-1 (already prepared in our laboratory) with an immunodominant gene of EIV employing red recombination technology for creating a combination vaccine holding protection against EHV-1 and EIV. This particular bivalent vaccine will save the time and labour of the field farmers and caretakers in the horse industry. Moreover, it will also reduce the number of susceptible equids in India.

For this purpose, an immunodominant gene of equine influenza and certain other required sequences have been fused by fusion PCR to create a cassette. This particular cassette is inserted in an already constructed EHV-1 deletion mutant by *en-passant* red recombination protocol. To check the presence of insert several selected PCR were carried out (primers were designed and brought backhand). RFLP analysis of the construct was also performed to check the gene fragment pattern with respect to the already available deletion mutant of EHV-1 virus. Meanwhile, the 1st red product (colony PCR screened clones) was transfected on RK13 cells to check the reproducibility and expression of the construct. DNA isolated from the transfected cells revealed the presence of all the fragments in the EHV-1 virus.

(Nitin Virmani, BC Bera and Taruna Anand)

Characterization of equine herpesvirus isolates in India for documentation of their genetic diversity

During this period, 218 clinical samples from equines were tested for EHV-1 infection by qPCR and 43 were detected positive for EHV-1 infection. EHV-1 was isolated in RK13 cells from seven positive samples and characterized for neuropathogenicity. All isolates were non-neuropathogenic.

Clinical samples tested for EHV-1 infection during 2019

Sample	Number Tested	Number Positive
Tissues	29	13
Blood	96	7
Cervical swab	45	4
Stomach content	23	8
Heart Blood	25	11
Total	218	43

Whole genome sequencing of EHV-1 isolates: Partial genome sequences of two EHV-1 isolates (EHV-1-14 and EHV-1-Meerut) using NGS were generated covering more than 90% of genome. The sequences have been analyzed and



submitted to GenBank. Phylogentic analysis based on US and UL segments of both the isolates has been analysed for their genetic comparison. Five more EHV-1 isolates were bulk cultivated and purified by differential centrifugation. The DNA samples of these isolates were submitted for NGS and the preliminary data obtained is being analyzed.

Mechanism of inhibition of HSV-1 infection in EHV-1 infected cells

In a co-infection study, it was investigated whether Herpes simplex 1 virus infection is interfered in the lymphoblastoid cells on co-infection with equine herpesvirus-1 infection. On co-infection and super-infection, it was investigated whether EHV-1 interference takes place in case of co-infection with HVS-1 and EHV-1. For this, we employed qRT-PCR to quantify HSV-1-specific genome. It was concluded that EHV-1 interferes with HSV-1 infection, both in superinfection and coinfection (qRT-PCR).

(BR Gulati, Naveen Kumar and Riyesh T)

Epidemiology of Japanese encephalitis in pigs of Assam district

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus which causes significant epidemics of encephalitis in Asian countries, including India. Assam is the most vulnerable state for Japanese encephalitis (JE) accounting for nearly 50 % of total Japanese encephalitis (JE) positive cases reported in India. A study was undertaken to understand the epidemiology of JE in animal and mosquito vectors.

During the year, seroprevalence of JEV in different districts of Assam was done. Serum samples from pigs (n=947) from 8 districts of Assam were tested for JEV antibodies by ELISA & HI and 87 samples (9.18 %) of pigs were positive for JEV antibodies

District-wise prevalence of JEV antibodies in Assam (2019)

Month	District-Wise Number of Samples Tested (Positive)								% Positive
	Kamrup	Sonitpur	Sivsagar	Lakhimpur	Nalbari	Jorhat	Barpeta	Total	
Jan-Feb	20 (0)	15 (0)	14 (0)	11 (0)	18 (0)	20 (0)	11 (0)	109 (0)	0
Mar-Apr	22 (0)	11 (0)	17 (0)	15 (0)	25 (0)	18 (0)	15 (0)	123 (0)	0
May-Jun	46 (4)	25 (6)	30 (8)	27 (5)	25 (3)	12 (2)	11 (0)	176 (28)	15.90
Jul-Aug	53 (7)	20 (0)	30 (0)	22 (2)	37 (4)	20 (6)	22 (3)	204 (22)	10.78
Sep-Oct	20 (0)	16 (0)	22 (0)	18 (5)	28 (0)	15 (2)	20 (0)	139 (7)	5.03
Nov-Dec	31 (7)	35 (10)	25 (0)	32 (8)	30 (0)	21 (0)	22 (5)	196 (30)	15.30
Total	192 (18)	122 (16)	135 (8)	125 (20)	163 (7)	106 (10)	101 (8)	947 (87)	9.18
% Positive	9.37	13.11	6.01	16.0	4.29	9.43	8.24	9.18	

Mosquito collection and testing for JEV: Mosquito samples were collected from Jorhat, Lakhimpur and Kamrup and were identified with the help of State Surveillance Unit, Guwahati, Assam. Most prevalent vectors identified were *Culex tritaeniorhynchus*, *Cx. Gelidus* and *Mansonia* spp. and found significantly higher during summer season (April-September) than in winter months (December-February).

Sentinel pig seroconversion: JEV sero-negative pigs in a village in Kamrup Rural (n=12) and in NRC-Pig Farm, Rani (n=12) were placed and observed for seroconversion. JEV sero conversion was recorded in eight sentinel pigs starting from the month of June with a peak in July and August month.

Clinical samples tested for EHV-1 infection during 2019

Month	JEV Seroconversion	
	Village Condition	Institute Farm
April 19	12 (0)	12 (0)
May 19	12 (1)	12 (0)
June 19	12 (4)	12 (0)
July 19	12 (5)	12 (0)
August 19	12 (7)	12 (0)



Development of real-time PCR: A Taqman-based real-time PCR was developed for detection of JEV infection. The sensitivity of the assay was 23 copies of JEV cDNA. The assay was specific for JEV and did not cross-react with WNV.

(BR Gulati and Seema Rani Pegu)

Development of peptide ELISA for serodiagnosis of EHV-1

A peptide ELISA has been developed for sero-prevalence of EHV-1 infection in equine population and to determine the immune status of animals following vaccination. To increase the signal-to-noise ratio and specificity of the assay, the linear peptide conformation and presentation was modified to develop a multimeric antigenic peptide (MAP) and the validation of assay was completed during the year.

The optimum reagent concentration by checker-board method was worked out to be 250 ng/well of peptide for coating, 1:10000 for anti-horse HRPO. The cut off value was determined to be 0.15 by testing 34 known negative and 108 positive samples. The repeatability was tested on 3 consecutive days with mean CV less than 10%. The sensitivity and specificity of the assay was estimated using known positive and negative equine sera from infected and vaccinated animals.

The relative analytical sensitivity and specificity of this assay was 96.77 % and 97.78 %, respectively). The diagnostic sensitivity of the assay was further validated with 1136 field equine serum samples and 116 (10.21 %) were detected positive for EHV-1 antibodies. There was 99.1% agreement between virus neutralization and MAP-ELISA, with Cohen's *K* (0.952). The diagnostic sensitivity and specificity of MAP-ELISA vis-a-vis VNT was 96.73 % and 99.51 %, respectively.

State-wise seroprevalence of EHV-1 by VNT and MAP-ELISA

States	No Tested	Number (%) Positive by	
		VNT	MAP-ELISA
Jammu & Kashmir	527	81 (15.4)	84 (15.9)
Delhi	290	8 (27.6)	8 (27.6)
Uttar Pradesh	269	15 (55.8)	16 (59.5)
Uttarakhand	20	4 (20.0)	4 (20.0)
Haryana	18	3 (16.7)	4 (22.2)
Meghalaya	12	0 (0)	0 (0)
Total	1136	111 (9.77)	116 (10.21)

(BR Gulati and Nitin Virmani)

Development of refined vaccine for EHV-1

NRCE has earlier developed inactivated vaccine against EHV-1 (Equiherpabort) which is being utilized in the field, however, the vaccine lacks the generation of good cell mediated immune response. Thus, for the refinement of vaccine, adjuvant was replaced with Montanide-Pet-Gel adjuvant. The vaccine showed generation of good humoral and cell mediated immunity in murine model with good protective efficacy. For adjudging the efficacy of the refined vaccine experiments were planned in main host. For this, the vaccine was inoculated into the pregnant and non-pregnant mares (non-pregnant $n=3$ new vaccine and $n=4$ old vaccine) (pregnant $n=4$ new vaccine and $n=3$ old vaccine) at 5, 7 and 9 months of pregnancy at EPC, Bikaner for evaluation of the immunogenicity of the vaccine in natural host.

The serum samples were collected periodically from the immunized horses and the immune response were assayed by serum neutralization assay. The result showed increasing SNT titres in the sera samples at various intervals. At day 30 of first vaccine, the titres for Montanide-Pet-Gel adjuvanted vaccine in non-pregnant and pregnant mares were 44 ± 24 and 26.66 ± 9.23 , respectively in comparison to old EHV-1 vaccine where the titres in pregnant and non-pregnant mares were 36 ± 20.13 and 26.66 ± 5.42 , respectively. At 60day post vaccination the titres were more or less comparable while at 90 and 120 days of vaccination, animals immunized with EHV-1 vaccine adjuvanted Montanide-Pet-Gel showed better titres for antibodies in pregnant (74.66 ± 48.88 & 96 ± 55.42) and non- pregnant animals (56 ± 16 & 88 ± 48). The CMI responses are to be estimated by Interferon gamma assay in lymphocytes.

(Nitin Virmani, BR Gulati and BC Bera)



EQUINE BACTERIOLOGY

Sero-surveillance of equine infectious diseases in India

Surveillance and monitoring of indigenous equine population played a pivotal role in monitoring and control of infectious disease in various states of the country. The Centre is also involved in testing of equine samples from race courses, turf club, stud farm, riding schools, Animal Quarantine Service Station (AQCS). During the year 2019, a total of 2169 equine serum samples from 12 states were tested for various diseases like Equine Infectious Anaemia, Equine Influenza, EHV-1, JEV/WNV, Trypanosomiasis, Piroplasmiasis, *Salmonella Abortus equi* and Brucellosis. Total number of positive cases and seropositive percentage are indicated in table. Highest seroprevalence of 41.40% were observed for Piroplasmiasis followed by 6.52 % for EHV-1, 5.44 % for JE/WNV and 1.98 % for *Trypanosoma evansi*. None of the equines were found positive for equine influenza, equine infectious anemia, brucellosis and *Salmonella Abortus equi*.

Sero-prevalence of important equine diseases among indigenous equines (Jan-Dec. 2019).

States	EIA	EI	Piroplasmiasis (<i>Theileria equi</i>)	EHV-1	<i>T. evansi</i>	JE/WNV	Sal. Ab.equi	Brucellosis
Madhya Pradesh	238	238	238 (90)	238(14)	238(2)	238(32)	238	238
New Delhi	51	51	51 (37)	51	51(1)	51(4)	51	51
Punjab	27	27	27 (9)	27(6)	27(1)	27(4)	27	27
Haryana	74	74	74 (50)	74(1)	74(1)	74(13)	74	74
Uttarakhand	67	67	67 (19)	67(10)	67(3)	67(4)	67	67
Uttar Pradesh	726	726	726 (247)	726(5)	726(5)	726(32)	726	726
Rajasthan	44	44	44 (29)	44	44	44(3)	44	44
Chhattisgarh	124	124	124 (37)	124(14)	124(8)	124(16)	124	124
Jammu & Kashmir	550	550	550 (260)	550(75)	550(22)	550(9)	550	550
Himachal Pradesh	194	194	194(88)	194(7)	194	194	194	194
Bihar	57	57	57(32)	57	57	57	57	57
Karnataka	17	17	17	17 (6)	17	17 (1)	17	17
Total	2169	2169	2169	2169	2169	2169	2169	2169
Number tested positive	0	0	898	138	43	118	0	0
Sero-prevalence (%)	0	0	41.40	6.52	1.98	5.44	0	0

Under disease investigation 22116 equine sera was tested for glanders and 210 glanders positive cases were reported from Uttar Pradesh (135), Uttarakhand (7), Haryana (5), Delhi (9), Jammu & Kashmir (5), Himachal Pradesh (3), Rajasthan (8), Gujarat (7), Chhattisgarh (4), Madhya Pradesh (11), Maharashtra (9), Karnataka (4) and Andhra Pradesh (3). In public health point of view, 219 human serum samples from in-contact equine handlers were tested and all of them were negative for glanders. In addition, testing of 414 samples for equine influenza revealed negative for H3N8 antibodies. For EIA, 2619 serum samples obtained from 13 states were found negative by Coggin's test. For AHS, 56 random samples from 4 states were found negative.

Microbiological analysis was done on 473 clinical samples including nasal swab, tissue, abscess, aborted fetus etc. originating from Rajasthan, Haryana, Uttar Pradesh, Himachal Pradesh, Chhattisgarh, Gujarat, and Delhi yielded 111 isolates including *Burkholderia mallei* (7), *Klebsiella pneumoniae* (31), *E.coli* (40), *Rhodococcus equi* (12) *Streptococcus equi* (7) and *Streptococcus zooepidemicus* (14).



No of sample tested under disease investigation (Jan-Dec. 2019)

Disease	No. of samples tested	No. positive
Glanders	22116	210
EI	414	-
EIA	2783	-
AHS	56	-
Piroplasmosis (<i>Theileria equi</i>)	5	-
<i>T. evansi</i>	2	-
EHV-1	22	1
JE/WNV	28	1

Bacteria isolated from 473 bio-samples yields 111 isolates

Organism	No.	Place
<i>Burkholderia mallei</i>	7	Gujarat (2), Delhi (2), Haryana (2) and Maharashtra (1)
<i>Klebsiella pneumoniae</i>	31 (13 Animals)	Rajasthan (19), Haryana (12)
<i>E. coli</i>	40 (14 Animals)	Rajasthan (28), Haryana (12)
<i>Streptococcus zooepidemicus</i>	14	Maharashtra (2), Haryana (12)
<i>Streptococcus equi</i>	7	Chhattisgarh (1), Uttar Pradesh (1), Haryana (5)
<i>Rhodococcus equi</i>	12	Rajasthan (9), Haryana (3)

(H Singha, BN Tripathi, SC Yadav, BR Gulati, Rajender Kumar, Sanjay Kumar, Nitin Virmani, Sanjay Barua, Rajesh K Vaid, Ramesh Dedar, Anju Manuja, Balvinder Manuja and K Shanmugasundaram)

Glanders Surveillance Report: 2019

Glanders surveillance during last three years (2016-18) revealed glanders outbreaks in territories with previously unknown status like Jammu & Kashmir, Gujarat, Rajasthan, Madhya Pradesh, Maharashtra and Tamil Nadu. This alarming situation of glanders in the country attracts political and administrative attention for devising a national control policy and surveillance plan on glanders. In this direction, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India has approved National Action Plan on Glanders for control and eradication (<http://dadf.gov.in>). The programme is funded by RKVY-RAFTAAR scheme.

In 2019, a total of 34322 equine sera were tested from 15 states under glanders surveillance programme. Zone wise states may be categorized as Northern India (Jammu & Kashmir, Himachal Pradesh, Haryana, Uttarakhand, Delhi and Uttar Pradesh), Western India (Rajasthan, Gujarat and Maharashtra), Central India (Chhattisgarh and Madhya Pradesh), Southern India (Tamil Nadu, Karnataka and Andhra Pradesh) and Eastern India (Bihar).

For rapid and efficient execution of surveillance activities glanders ELISA developed by NRCE has been provided to 11 state diagnostic laboratories/RDDLs namely Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, Karnataka, Jammu & Kashmir, Bihar, Chhattisgarh and Madhya Pradesh at different time point during last three years. In 2019, 12206 equine samples were screened by ELISA at eight State Lab/RDDLs (Gujarat, Haryana, Himachal Pradesh, Punjab, Rajasthan, Maharashtra, Jammu & Kashmir).

During the year, a total of 210 glanders positive cases were reported from Uttar Pradesh (135), Uttarakhand (7), Haryana (5), Delhi (9), Jammu & Kashmir (5), Himachal Pradesh (3), Rajasthan (8), Gujarat (7), Maharashtra (9), Chhattisgarh (4), Madhya Pradesh (11), Karnataka (4) and Andhra Pradesh (3). This year surveillance data revealed that disease has spread across 12 states in Northern, Central, Western and Southern India. District wise glanders cases were reported in 68 districts and Uttar Pradesh had maximum number of glanders reported districts (40). In public health aspect, 219 sera from occupationally exposed humans (Veterinary Officers, equine handlers, laboratory workers) were tested and all of them were negative for glanders.

**Glanders positive cases reported in the year 2019**

Sr. No.	State	Positive Sample	Districts
1	Uttar Pradesh	135	Sharavasti, Agra, Varanasi, Allahabad (Prayagraj), Bareilly, Lakhimpur-Kheri, Muzaffarnagar, Firozabad, Gonda, Mirzapur, Hardoi, Mathura, Gorakhpur, Baghpat, Bhadoi, Fatehpur, Ghazipur, Maharajganj, Barabanki, Moradabad, Kasganj, Unnao, Gautum Buddha Nagar, Bahraich, Kaushambi, Siddharth Nagar, Shamli, Jalaun, Sultanpur, Hathras, Sambhal, Bijnor, Ghaziabad, Sahranpur, Jonpur, Ajamgarh, Etawah, Meerut, Basti, Farrukhabad (40 Districts)
2	Haryana	5	Gurugram, Mahendergarh, Rohtak
3	Jammu & Kashmir	5	Reasi, Udhampur
4	Uttarakhand	7	Dehradun, Udham Singh Nagar, Chamoli, Haridwar
5	Delhi	9	North West Delhi, South West Delhi
6	Madhya Pradesh	11	Bhopal, Indore, Khargone, Tikamgarh
7	Chhattisgarh	4	Raipur, Rajnandgaon
8	Maharashtra	9	Pune, Thane, Buldhana, Raigarh
9	Rajasthan	8	Alwar, Bharatpur
10	Gujarat	7	Ahmedabad
11	Karnataka	4	Mysore, Bidar
12	Andhra Pradesh	3	Vijaywada
	Total	210	67

Number of equine samples tested at State Diagnostic Lab/RDDLs

Sr. No.	State Laboratories/RDDLs	No. of equines tested	No. District Surveyed
1	Ahmedabad, Gujarat	1062	20
2	Sonipat, Haryana	665	09
3	Shimla, Himachal Pradesh	2326	12
4	Jaipur, Rajasthan	3171	27
5	WRDDL, Pune	2318	25
6	NRDDL, Jalandhar	562	12
7	DDRP, Jammu	1297	6
8	DDRP, Kashmir	805	09
	Total	12206	120

It was found that many states did not take part in the surveillance programme. No samples were received from Odisha, West Bengal, North-East states and Kerala. Similarly, very less number of samples were received from Karnataka, Andhra Pradesh, Tamil Nadu and Telengana. Therefore, mandatory participation of all State Animal Husbandry Department in the surveillance programme is necessary to achieve the objectives of national action plan.



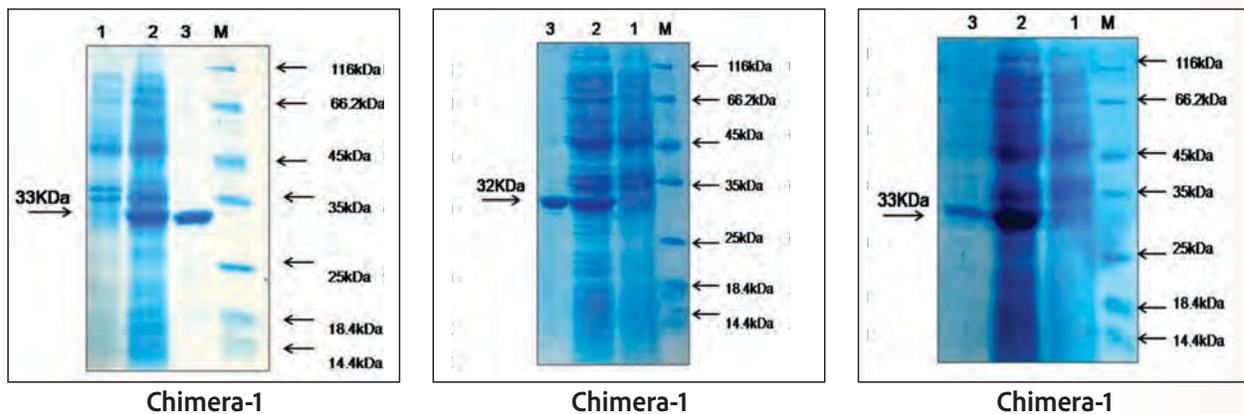
Representative photographs of glanders affected equines detected at different places during 2019.

(HS Singha, K Shanmugasundaram and BN Tripathi)



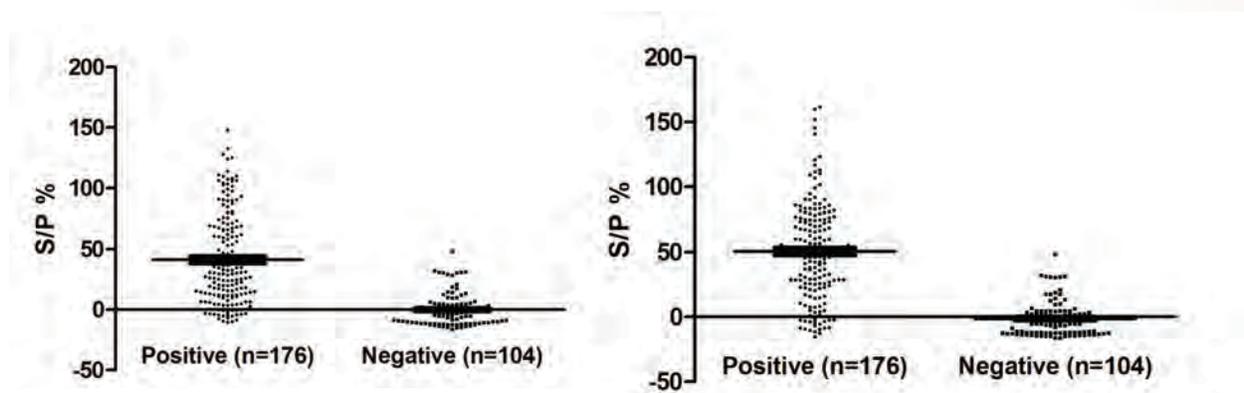
Expression of recombinant fusion chimera of *Burkholderia mallei* Type 6 Secretory proteins

The work was done under the project entitled 'Seroproteome analysis of recombinant secretory proteins of *Burkholderia mallei* towards development of multiple antigen immunoassay for improved diagnosis of Glanders' funded by LSRB-DRDO, Ministry of Defence, Govt of India. The project was proposed with the aim of identification of diagnostic antigen(s) and development of multiple antigens based serodiagnosis of glanders. A total of 24 secretory proteins belonged to T2SS, T3SS, T5SS and T6SS secretory systems of *B. mallei* were selected from DBSecSys2.0 data base. Amino acid sequences of these secretory proteins were assessed for screening immunogenic epitope using suitable bio-informatics software. Finally, selected epitope sequences were assembled and six chimeric fusion gene(s) were commercially synthesized in cloning vector. Subsequently, inserts were sub-cloned into prokaryotic expression vector for production of recombinant proteins. Four fusion proteins were produced in *E. coli* and purified by Nickel affinity chromatography. These purified recombinant proteins showed specific reactions to glanders positive sera by Western blot.



Expression and purification of recombinant *B. mallei* fusion chimera.

Two chimeric proteins were evaluated by indirect ELISA using glanders-positive ($n=176$) and negative sera ($n=104$). Optimal coating concentration of both chimeras was 125 ng/well. Sensitivity and specificity of chimera 1 was 92.14 % and 93.69 %, respectively. For chimera 2, sensitivity and specificity were 81.48 % and 91.22 %, respectively.



Reactivity of fusion chimera with glanders positive and negative sera is depicted in scatter plots.

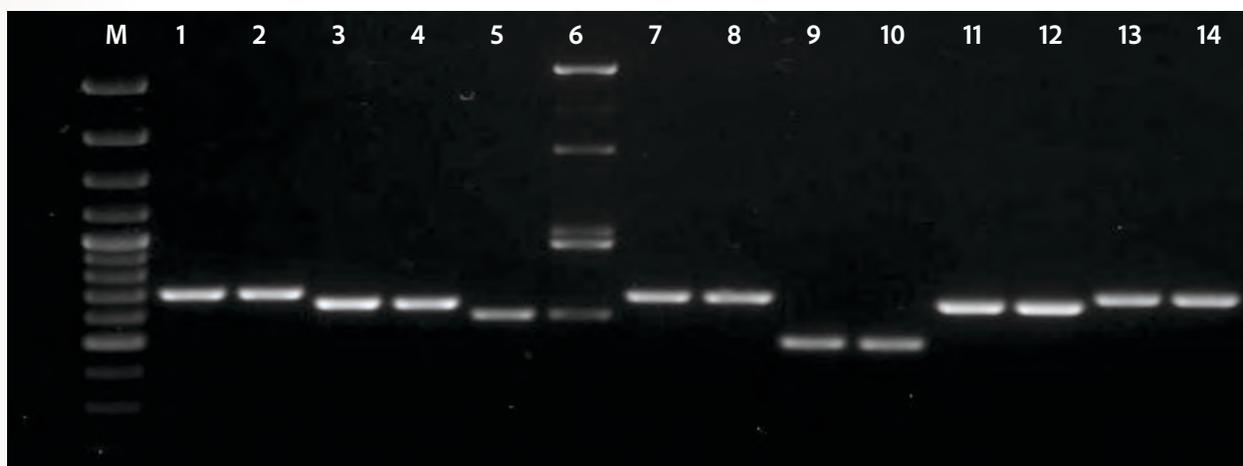
(HS Singha, Sheetal Saini, K Shanmugasundaram and BN Tripathi)

Genomic diversity of *Streptococcus equi* causing strangles in Indian equine population

Strangles is the most contagious and infectious disease of horses, mules and donkeys. The disease is caused by *Streptococcus equi* and is economically important due to occurrence in large proportion of affected herds in India. *S. equi* has a protein known as SeM protein or M-like protein on the surface of bacteria which is important in molecular characterization of strangles outbreaks and guiding selection of strains to manufacture vaccine. Multi-locus sequence typing (MLST) is a molecular technique that allows genetic comparison of bacterial strains and is based on sequencing seven highly conserved housekeeping genes. An MLST scheme has been developed for the



β -hemolytic, Lancefield group C streptococcal bacterium *S. equi* subspecies *zooepidemicus*. In order to study the genomic diversity, forty five isolates of *S. equi* subsp. *equi* and *S. equi* subsp. *Zooepidemicus* collected from field cases were analyzed by sequencing SeM gene and seven conserved genes namely carbamate kinase (*arcC*), ribonucleoside-diphosphate reductase (*nrdE*), prolyl-tRNA synthetase (*proS*), signal peptidase-I (*spi*), thymidylate kinase (*tdk*), triosephosphate isomerase (*tpi*) and acetyl-CoA acetyltransferase (*yqiL*) prescribed for multilocus sequence typing of *S. equi* and *S. zooepidemicus*. Thirteen sequences having novel SeM allele were submitted to BIGSdb and assigned novel allele no 190 from Indian *S. equi* isolates. The sequences of seven conserved genes have been analyzed and indicated the presence of unique *S. equi* isolates circulating in Indian equine population. The information generated through this study can be very useful for devising vaccination strategies for indigenous equine population.



Lanes: 1, 2-*arcC*; 3, 4-*nrdE*; 5,6-*proS*; 7, 8-*spi*; 9,10-*tdk*; 11,12-*tpi*; 13-14-*yqiL*

MLST of Streptococcus equi indicating amplification of seven conserved genes

In addition, in prevalence studies, seventy serum samples were tested for *S. equi* antibodies from three organized farms along with ten field samples. Seventy percent field samples were positive for antibodies while only one sample from organized farms (1/60) was positive. Forty-seven SeM and HSP60 gene sequences have been submitted and accessioned by NCBI GenBank.

(Balvinder Kumar, RK Vaid, Anju Manuja, K Shanmugasundram and H Singha)

Strengthening of surveillance on zoonotic diseases in animals and human population

under the programme on "Strengthening of intersectoral coordination for prevention and control of zoonotic diseases" ICAR-NRCE conducted active surveillance on zoonotic diseases in India. During the year, 137 human cases living in contact with equines were examined for Glanders and no clinical case of Glanders was detected. In addition, 13696 equines were screened for Glanders and 94 positive cases were detected. For Japanese encephalitis, 157 equines were tested for Japanese encephalitis antibodies and 13 were detected positive.

Diagnostic services to humans' biological samples during 2019.

Month	Number of human samples tested (ELISA & CFT)	Numbers Positive
July 2019	29	0
August 2019	18	0
September 2019	11	0
October 2019	30	0
November 2019	23	0
December 2019	26	0
Total	137	0



Diagnostic services to equid sector for Glanders during 2019

Month	Number Tested	Numbers Positive
July 2019	1852	19
August 2019	2592	19
September 2019	2240	19
October 2019	1898	6
November 2019	2952	25
December 2019	2162	6
Total	13696	94

Diagnostic service for Japanese encephalitis samples (equines) during 2019

Month	Number Tested	Numbers Positive
November 2019	104	11
December 2019	53	2
Total	157	13

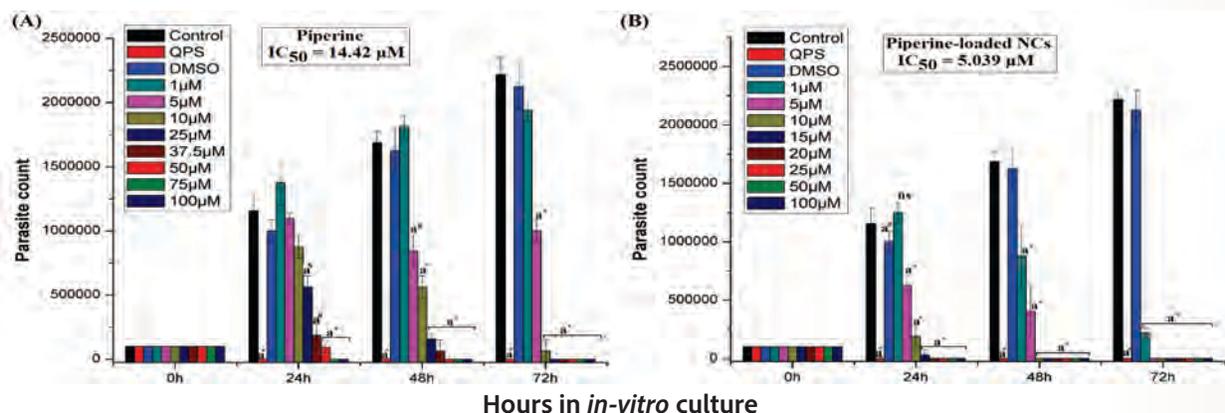
(BN Tripathi, BR Gulati, Naveen Kumar, HS Singha, Shanmugasundaram and Riyesh T)

EQUINE PARASITOLOGY

Evaluation of target specific novel drug molecules against *Trypanosoma evansi* infection using nanotechnology approach

Two bioactive compounds named piperine and emetine were selected based on targeted approach. Both, piperine and emetine are alkaloids and isolated from the fruits of *Piper nigrum* and from root of *Carapichea ipecacuanha*, respectively. Both the compounds have also been reported for many pharmacological activities including antiprotozoal activity. Both the compounds were evaluated *in vitro* against *T. evansi* by growth inhibition assay. IC_{50} (50% inhibition concentration) for piperine and emetine were found to be 14.42 μ M and 183.5 μ M, respectively. Based on inhibitory drug concentration, piperine was selected for nanoformulation.

Piperine-loaded nanocapsules (NCs) were prepared by using emulsion-diffusion method and characterised for particle size, zeta potential, transmission electron microscopy. Further drug release profile was also checked using dialysis method and was found sustained drug release of piperine from its NCs as compared to pure piperine. The NCs were then evaluated for growth inhibition assay and IC_{50} was found 5.039 μ M, which was approximately one-third of the IC_{50} of pure piperine. It was concluded that piperine-loaded NCs have more significant inhibition of parasite growth as compared to pure piperine. Furthermore, the cytotoxicity profile for both, piperine as well as its NCs was dose dependent, but when compared in-between, then piperine showed more cytotoxicity as compared to piperine-loaded NCs.



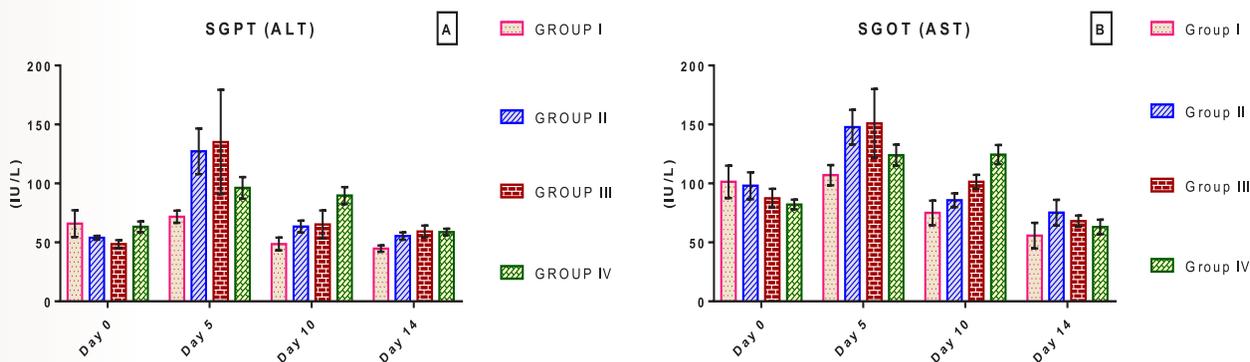
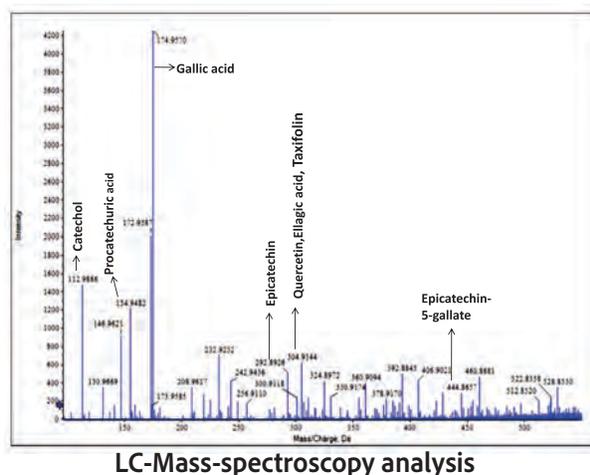
in vitro growth inhibition pattern of piperine (left side) and piperine-loaded NCs (right side) against *Trypanosoma evansi*.

(Ruma Rani and Rajender Kumar)



Identification of principal drug molecules in herbal plant extracts which showed *in-vitro* *Theileria equi* growth inhibitory efficacy

Theileria equi and *Babesia caballi* are haemoprotozoa responsible for causing equine piroplasmiasis. This disease is primarily transmitted by tick's species of *Dermacentor*, *Hyalomma* and *Rhipicephalus*. The currently available drugs are not suitable to completely clear the *T. equi* or *B. caballi* parasite from latently infected animals. Indian medicinal plants have been attributed for different therapeutic purposes including analgesic, anti-inflammatory, immunomodulation, anti-oxidant, hepato-protective and anti-malarial properties. We undertook this study to establish anti-*T. equi* activities of Indian medicinal plant extracts and analyse their phytochemical properties for correlating their anti-piroplasmic properties. Previously, we reported anti-*T. equi* properties of ANMEA fraction of *Acacia nilotica* in MASP in-vitro culture system. Now we characterized this fraction and identified the lead molecules. ANMEA fraction was analysed on LC/MS spectroscopy and different major peaks were identified. The important lead molecules were identified as – catechol, procatechuric acid, gallic acid, epicatechin, quercetin, ellagic acid etc. Further organ toxicity of this fraction was also accessed in in-vivo studies in mice model. The LD₅₀ dose of ANMEA fraction on mice was determined following the OECD guideline, which was 500 mg/ml. Therapeutic dose (TD) for mice trial was derived from LD₅₀, as 1/10th of the calculated LD₅₀ dose in mice. The therapeutic dose for ANMEA in this study was calculated as 50 mg/kg of body weight. Organ toxicity trial in mice model was conducted. Three groups (8 no in each groups) of mice were injected (i/p) ANMEA fraction @ 25 mg/kg b.wt, 50 mg/kg b.wt, 100 mg/kg b.wt, respectively for continuously 3 days. Fourth group of mice was taken as control and administered only with PBS in i/p route for 3 days. Different parameters – body weight, haematology, biochemical (liver fn, kidney fn) analysis was performed at different intervals (0th, 5th, 10th, 14th). The blood glucose level was high in group II & group III animals at end of the study period. The ALT & AST level were significantly higher in group II & group III animals after 5th day of treatment and recovered in subsequent periods. Serum creatinine level was also increased in all the four groups after 5th day of the treatment commencement and recovered in subsequent periods. There was no mortality or signs of toxicity recorded in the study period in any of the three groups. The transient increase in liver enzymes and BUN, creatinine level in serum indicating temporary effect of ANMEA extract on liver and kidney at doses of 50 mg/kg b.wt and 100 mg/kg b.wt. As such promising results were observed with this fraction of the herbal plant.



Graph representing SGPT (A) and SGOT (B) levels (Mean±SEM) observed in experimental mice of different group treated by different doses of ANMEA extract.

(Sanjay Kumar and Rajender Kumar)



Development of diagnostics for *Theileria equi* and *Babesia caballi* as per OIE guidelines

Equine piroplasmiasis is a tick transmitted haemoprotozoan disease caused by *Theileria equi* and/or *Babesia caballi* and poses a serious threat in international movement of the infected horses. These parasites are widely distributed in the world, including Asian continent, Europe, Africa, and South America, and the prevalence corresponds to the presence of the tick-vectors. In Asia *T. equi* infection has been reported to be endemic. These latently infected horses are responsible for spread of this parasite to other non-infected horses. In our previous studies, we developed IFAT diagnostics as per OIE guidelines. NRCE has also developed MASP in-vitro culture system for *Theileria equi*. Recently, we expressed BC48 gene of the *Babesia caballi* in pGEX-4T-1. Expressed protein was purified on Sepharose beads and protein concentration was determined. ELISA diagnostic was optimized with this recombinant protein and samples were tested for diagnosis of *B. caballi*. Multiplex PCR was also optimized for simultaneous diagnosis of *T. equi* and *B. caballi* infection in a single PCR reaction. Species specific primers were designed and PCR conditions were optimized. A PCR product of size 392 bp and 540 bp were amplified specific for *Theileria equi* and *Babesia caballi*. More samples need to be tested for its validation.



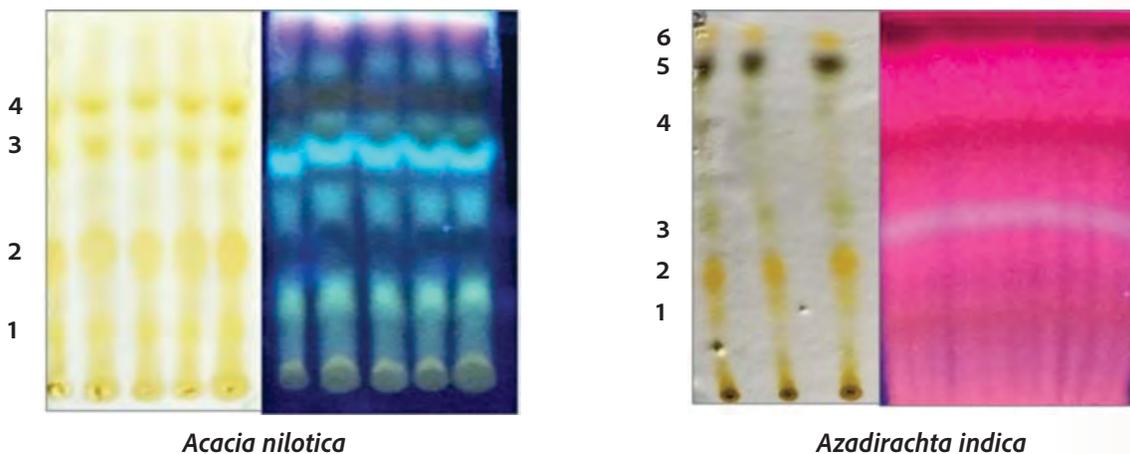
Multiplex PCR for simultaneous diagnosis of *T. equi* and *B. caballi* infection

(Sanjay Kumar and Rajender Kumar)

EQUINE MEDICINE

Anti-*Rhodococcus equi* activity of some Indian herbal medicinal plant extracts and their cytotoxicity and phytochemicals analysis

The growing problem of resistance to antibiotics has limited the use of already available drugs thereby necessitated the search for new antimicrobial compounds. Ethano-pharmacology has provided a new approach to the development of drugs using traditional plants. It has led to the advancement in health care and also helps to find new ways to deal with the problem of emerging resistance because of the presence of active metabolites present in the plant extracts. In India, medicinal plants have been utilized for centuries for the cure of various diseases because of the presence of bioactive compounds such as flavonoids, tannins, phenols, alkaloids etc. that have pharmacological effects and serve as a potential source for the development of new herbal drugs.



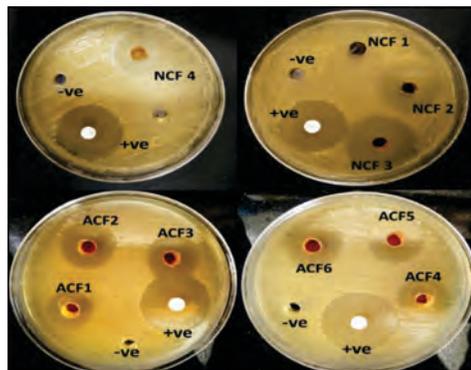
Acacia nilotica

Azadirachta indica

TLC slides with spots (Iodine spotted left side and in UV right side) observed in NRCE-AN-CHL and NRCE-AZI-CHL



Rhodococcus equi has been considered as an important pathogen of the foals of age between 2 to 5 months. The disease is insidious and are at risk. The growing problem of antibiotic resistance has limited the use of antibiotics. This has necessitated a search for new antimicrobial substances from other sources including plants. We investigated anti-*Rhodococcus equi* activity of the plant extracts in this study. Extracts of three plants viz *NRCE-AN-CHL*, *NRCE-AZI-CHL* and *NRCE-TRC-MTL* were having the highest inhibition potential against *Rhodococcus equi*. MIC of the three plants was recorded upto 625µg/ml in resazurin microtiter assay. All the three plants were non-toxic to peripheral blood mononuclear cells (PBMCs) isolated from horse blood and these cells were viable upto 100µg/ml of herbal drug concentration. The presence of phytochemical compounds of these selected plants were analyzed both qualitatively and quantitatively. The total phenolic content of 483.6 µg equivalent to gallic acid/mg of the extract was observed in *NRCE-TRC-MTL* and highest flavonoid content of 124.84 µg equivalent to catechin was found in *NRCE-AN-CHL*. Further the anti-oxidant activity of the three plants was estimated by DPPH and *NRCE-TRC-MTL* was found to have maximum anti-oxidant activity. So, these plants were found to be highly effective against *Rhodococcus equi*.



Plant extract fractions tested for antibacterial activity against *Rhodococcus equi*.

(Sanjay Kumar, RK Dedar, Rajesh Vaid and HS Singha)

Herbal disinfectant against *Rhodococcus equi*

Rhodococcus equi is an important bacterial respiratory pathogen of young foals. Aerosol infection via soil is most important route of infection to the foals and effective management of land by reducing aerosol exposure can reduce incidence of *R. equi*. We found that leaves of *Tamarindus indica* and *Eucalyptus globules* have potential in vitro antibacterial activity against *R. equi*. Both the plants found widely so leaves of these plants were tested for their disinfectant activity against *R. equi* present in farm soil. Study was carried out to find an herbal disinfectant for farm soil against *Rhodococcus equi* and its active principal. Aqueous extracts of leaves of *Tamarindus indica* and *Eucalyptus globules* were fractionated on silica column by using ethanol methanol and water serially. Different fractions were tested against pure colonies of *R. equi* in vitro to find out active principal. To find out disinfectant property of leaves in presence of farm soil, pure colonies of *R. equi* were mixed in soil and efficacy of leaves boiled water were tested against control. Plant leaves boiled water in concentration 10% and above were found effective against *R. equi* present in farm soil. Active principal of *T. indica* was matching with the previous reports while active principal of *E. globulus* was cannot be matched with previous reports, however it was found that active principal of *E. globulus* is a very polar compound. Efficacy of herbal disinfectant was compared with chloroxylenol a para-chloro-meta-xyleneol. Commonly used household disinfectant dettol contains 4.85 % chloroxylenol and shopkin hand sanitizer contain 0.05 % chloroxylenol. In present study (Eucalyptus leaves boild water) ELBW and (*Tamarindus indica* leaves boiled water) TLBW 5 % (W/V) were compared in-vitro with 1 % dettol (0.05 % chloroxylenol) and 5 % dettol. Antimicrobial efficacy of ELBW was found comparable to 1 % dettol and efficacy of TLBW was equal to 5 % Dettol. Efficacy of the combination of ELBW and TLBW was also tested in vitro and efficacy was found lower than their individual efficacy. Same efficacy of stored powder was observed till 6 months.

(RK Dedar, Lalit Kumar, Dinesh Barsiwal, RA Legha and Sanjay Kumar)

Retrospective study of colic incidences at an organized horse farm

Five years incidences of colic from 2014 to 2019, at an organized horse farm situated at sub-tropical desert climate of Rajasthan were analysed. Incidences of colic were closely related with nutrition and management of horses. Horse in this study were being managed with following nutritional and managerial conditions. Dry fodder containing Wheat straw and Groundnut straw in 1:1 ratio, was available round the year. Green fodders availability in different seasons containing supply of lucerne and oats in December, January, February, and March. Dependency on



complete dry fodders was in April, May, and June. Sorghum green fodder was available in July, August, and September. Again, complete dry fodders were being available in October and November. Concentrate mixture was containing Wheat bran, Barley and Gram. Deworming was done once a year and repeated if parasites were found on repeated faecal examination. Concentrate was fed twice a day after soaking. No dentistry work was carried out. Average horses and pony's strength of the farm remained 69 ± 4.74 during the study period. In five years, period total 243 incidences of colic occurred at farm. Chances of colic cases per animals per year was 0.77. Case fatality rate (CFR) for colic was 1.67 % (Excluding colitis). Ponies were having higher chances of colic, 1.14 colic case per pony per year than the horses 0.58 colic cases per horse per year. Incidences of spasmodic colic were highest (88 %), no mortality was occurred due to spasmodic colic. Incidences of impactive colic were 5.76%, CFR for spasmodic colic was (14 %). Incidences of obstructive colic (intussusceptions) occurred in 0.82 % cases with CFR 100 %. Incidences of cases having diarrhoea, colic and increase in neutrophils percentage were categorized into enteritis related colic (2.88 %). Cases having profuse watery diarrhea, severe pain, frequent rolling of body, frequent up and down, high sweating, congested visible mucus membranes, dehydration, high rise in heart rate (75 to above 100 per minute) were kept in category colitis. Colitis was observed in (1.65 %) of colic cases with CFR of 75 %.

(RK Dedar, J Singh, T Rao and RA Legha)

Studies on usage of *Aerva javanica* plant extract in treatment of equine diseases

A. Habronemiasis in donkeys

Habronemiasis is a caused by invasion of *Habronema species* larvae in moist skin tissues and wounds, characterized by proliferative, moist and granulomatous wounds below eyes, over face and extremities. *Habronema species* is a helminth parasite of stomach of horses and donkeys. Adult parasite lays eggs in stomach and the eggs are passed in the faeces. In faeces eggs hatch and first stage larvae of *Habronema* appears. This larva infests the maggots in faeces and further development occurs in maggot. In a one-week period the maggots turn into adult fly (Stable fly and house fly) and the infested *Habronema* larvae reaches in the mouth part of the fly. When these flies feed on the wounds and moist skin surfaces, leaves the *Habronema* larvae on skin. These *Habronema* larvae feed on the tissues and causes non healing wounds and granulomatous tissue reaction. Surgical removal of granulation tissue, topical or systemic use of corticosteroids are used to treat the clinical cases, regular deworming with ivermectin and fly control are used to control the incidences of habronemiasis. Treatment of clinical cases become difficult in many instances due to immune reactions initiated by the dead larvae. Growth of excessive granulation tissue may lead to chronic ulceration and proud flesh.

B. Proud flesh in horses

Wound healing in horses often complicated by the excessive growth of granulation tissue, commonly known as proud flesh and is similar to keloids in human beings. At present there is no satisfactory treatment for proud flesh in horses. We for the first time demonstrated that leaf extract of *Aerva javanica* suppresses excessive growth of granulation tissue in horses. We hypothesized that *Aerva javanica* may have high levels of kaempferols to survive in stressful conditions of desert. Those kaempferols may suppress the growth of granulation tissue by their anti-angiogenesis property and ecdysteroids may control the larvae of habronema, if associated. Extract was prepared using aqueous extraction, solvent based fractionation and silica gel column flash chromatography. Topical application of the leaf extract once daily for 20 to 60 days on the proud flesh growth in horses suppressed growth of granulation tissue along with restoration of normal skin function.

With the help of a series of purification steps and mass spectrometry, kaempferol or its derivative was identified as active compound for exerting anti-granulation tissue activity. Hence it was concluded that *Aervajavanica* leaves extract containing kaempferol or its derivative has a potent activity against excessive growth of granulation tissue in horses.

In our previous study, we have found that leaf extract of *Aerva javanica* which was containing kaempferol and beta ecdysone found effective in the treatment of injury associated proud flesh and *Habronema* associated proud flesh both in horses. Meanwhile, we have treated 20 cases of Habronemiasis in donkeys and used using leaf extract of *Aerva javanica* found clinical recovery in all the cases.



Complete suppression of proud flesh growth in horses.

C. Alopecia in Horses

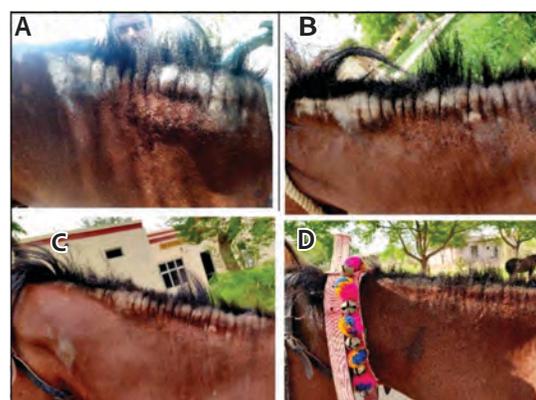
Hairs have important role in protection, thermoregulation and perspiration in mammalian animals. In horse's hair loss is usually found associated with inflammatory and pruritic diseases of the skin. Hair loss due to insect bite hypersensitivity in summer season is common in horses. In present cases report we have carried out histopathology of 2 cases of alopecia in horses. Instead of eosinophilic infiltration, we observed lymphocytic infiltration, fibroblastic changes, and presences of melanophages in dermis, resembling to the alopecia areata. In non-inflammatory and non-pruritic alopecia in horse's alopecia areata is a frequently reported condition. Alopecia areata (AA) is characterized by non-scarring hair loss and preservation of hair follicle in human beings, horses, dogs, cattle and some other mammals. It is considered as T lymphocyte driven autoimmune disorder. Alopecia also has cosmetic importance in horses and other pet animals. There is no reliable therapy for the treatment of AA in any species. Perifollicular fibrosis and lymphocyte proliferation is a common feature of AA. Kaempferol and its derivatives inhibits fibroblast collagen synthesis and reported for their fibrinolytic activity.

Kaempferol is also reported for its inhibitory effect on skin fibrosis. Kaempferol derivatives also blocks lymphocyte proliferation. We hypothesized that extract containing kaempferol or its derivative isolated from leaves of *Aervajavanica*, which one earlier we have used for the treatment exuberant tissue growth in horses can treat the cases of alopecia areata in horses.

On topical application of the extract, we have treated one cases of confirmed AA and three cases of alopecia in horses. While with pure kaempferol isolated from this extract we treated one confirmed cases of AA in a horse.



Perifollicular fibrosis and lymphocyte proliferation in a clinical case of alopecia



(A). Alopecia at mane before application of herbal extract Figure (B) (C) and (D) showing recovery in Alopecia after 7, 15 and 30 days post-treatment, respectively.

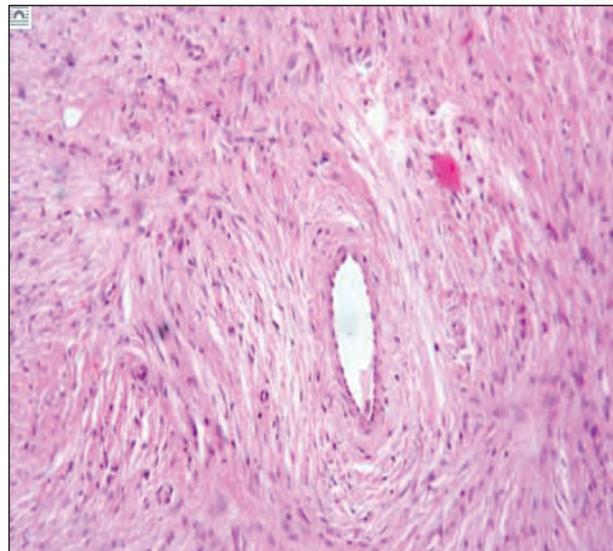
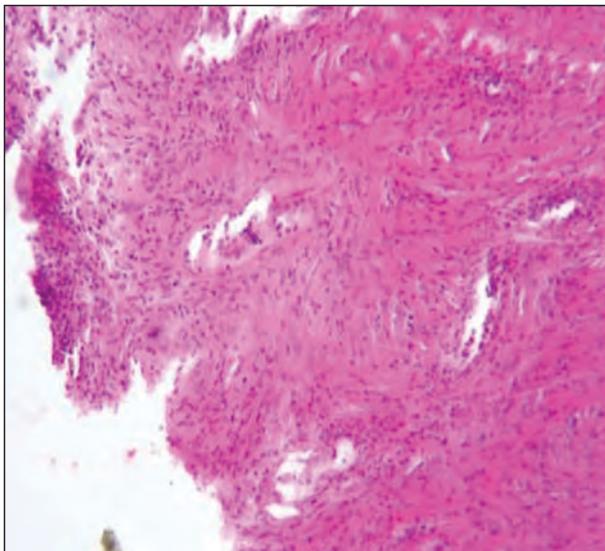


D. Fibroblastic sarcoma in horses

Sarcoma are poorly most common tumors occurring in equids. There is no satisfactory treatment is available to treat fibro sarcoma in horses. Surgical removal is not curative and recurrence is common due to failure of complete removal by surgical excision. In our study against proud flesh and tumors we have found that a kaempferol derivative, kaempferol Odeoxyhexosyl-deoxyhexoside-O-deoxyhexosylhexoside or kaempferol Odeoxyhexosyl-deoxyhexoside-O-coumaroylhexoside was found effective to cure the cases of proud flesh in horses. We used the extract topically in a case of equine sarcoma, where the tumor occurred on maxillary region of face, on histopathological analysis long rete pegs into the dermal fibroblastic tissue containing immature fibroblasts with mitotic figures in a whorled fibrocellular mass were observed that yielded complete clinical recovery.



Sarcoma tumorous growth below jaw, pre- and post-treatment in a mare.



Fibroblastic tissue containing immature fibroblasts with mitotic figures in a whorled fibrocellular mass were in biopsy sample obtained from Sarcoma (H&E stain, 40x)

(RK Dedar, Naveen Kumar, Shirish D Narnaware and BN Tripathi)

EQUINE PRODUCTION

Assessment of genetic variants through SNP mining in indigenous horse breeds

A total of 98 samples of Indian equines (18 Kathiawari horses, 25 Kachchi-Sindhi horses, 30 Marwari horses, 5 Manipuri ponies, 5 Zanskari ponies) and Thoroughbred horses (15) were used for the identification of genome wide SNPs and they were sequenced using Illumina TrueSeq chemistry on Illumina HiSeq 2000 platform. The raw reads obtained were further processed for quality filtration and alignment with the *Equus caballus* genome (GCF_002863925).

Bioinformatics data was analysed by TASSEL. A total of 1789642 SNiPs and 16,98,013 biallelic SNPs found from screening of above 98 samples. Among the biallelic, 1510815 biallelic SNPs at RD10 were taken further into analysis and identified 21197 SNPs for differentiation among different above mentioned equine breeds. These led to a total number of 108367 effects out of which about 0.006% are of high impact effects and the majorities are classified as modifiers.



Genome	Equine
Date	2019-09-13 11:43
SnEff version	SnEff 4.3t (build 2017-11-24 10:18), by Pablo Cingolani
Command the arguments	SnEff - status equine.html equine equine_LD.vcf
Warnings	43,818
Errors	0
Number of lines (input file)	21,197
Number of variants (before filter)	21,197
Number of not variants (i.e. reference equals alternative)	0
Number of variants processed (i.e. after filter and non-variants)	21,197
Number of known variants (i.e. non-empty ID)	0 (0%)
Number of multi-allelic VCF entries (i.e. more than two alleles)	0
Number of effects	108,367
Genome total length	2,506,966,135
Genome effective length	2,411,291,078
Variant rate	1 variant every 113,756 bases

SnEff: Variant analysis for Indian breeds of horse.

Number variants by type

Type	Total	Type	Total
SNP	21,197	INV	0
MNP	0	DUP	0
INS	0	BND	0
DEL	0	Interval	0
Mixed	0	TOTAL	21,197

Number of effect by impact

Type (alphabetical order)	Count	Percent
High	7	0.006%
Low	356	0.329%
Moderate	129	0.119%
Modifier	107,875	99.546%

(Anuradha Bhardwaj, Jay Kumar, SC Mehta and Yash Pal)

Microsatellite marker based genetic diversity analysis of Kachchhi-Sindhi horses

The genetic diversity in Kachchhi-Sindhi horses was evaluated at 30 microsatellite loci using fifty adult, healthy and unrelated animals. Allele frequency data was used to detect genetic diversity and for bottleneck studies. The estimated average number (\pm SE) of 10.1000 ± 3.1878 was obtained with a total of 303 alleles. A high level of genetic diversity within this breed was observed in terms of number of alleles, heterozygosity and polymorphism information content (>0.5). In-breeding coefficient (*Fis*) was -0.17497 ± 0.0102 , suggesting moderately low inbreeding in Kachchhi-Sindhi breed. This genetic information will be valuable for proposing effectual population breeding and management strategies for future.

(Anuradha Bhardwaj, Jay Kumar, SC Mehta Yash Pal and RA Legha)

Development of fatigue-cum-fitness score card for working equines

Three indigenous Mules (average body weight 399.0 kg) were selected for the draughtability trials which carried draft equivalent to 10, 20, and 30 % of body wt and corresponding draft (kg) was 37, 74, 111, and corresponding pay load was 845, 1126, 1406 kg, respectively. The pay loads were tested in mules using conventional pneumatic two-wheel cart on *pucca* road at normal speed of mules. Physiological indices, speed, fatigue symptoms (Tongue Protrusion, frothing, leg un-coordination) were recorded before, during and 20 min after the work. Body weight loss was recorded. In mules carrying 37 kg, 74 kg and 111 kg draft, all the physiological indices increased during carting and significant decrease was observed after 20 min of rest after work. The speed of mules reduced significantly at intervals. It was observed that mules were less tired up to 2 hrs of work as fatigue score was less than 7 out of 28 only. However, mules worked for 4 hour (37 kg and 74 kg), 3rd hr (111 kg) continuously and were tired as fatigue score was less than 14. However, during 4th hour of work the animal was more tired as fatigue score reached 14 in 111 kg draft. During the study, it was observed that the fatigue symptoms (tongue protrusion, opening of mouth,



dribbling of saliva and frothing) were not present in mules as heat loss in mules is by sweating and not by panting. Serum biochemical profile (urea, cholesterol, triglyceride, uric acid, calcium, albumin and total protein) was analysed before and after work in all types of loads in mules. The haematological indices were not affected by the load in mules.

The study shows that mule may pull draft upto 30 % draft for 2 hrs and 30 % of body weight for 3 hrs. Changes in physiological responses, reduction in speed, dragging of feet were observed during pulling of 30 % draft. However, for using the mules in continuous and regular type work the fatigue score should be less than 7 for keeping the animal healthy and productive.

(RA Legha and Yash Pal)

Standardization of energy efficient nutritionally balanced feed for equines (Donkey, Mules, Pony) to enhance energy output.

For the current study 3 of each donkeys and mules were fed with three rations (T_1 -maintenance ration, T_2 - 15% below maintenance and T_3 -15% above maintenance) as per ICAR standards and switch over technique of feeding method was followed. All the physiological and haematological parameters were recorded and estimated for different treatment groups. Haematological parameters were not influenced by the ration fed to the animals. SGOT, SGPT, urea, creatinine, triglycerides, cholesterol, uric acid, calcium, albumin and total protein were not influenced by any feed in donkeys.

(RA Legha and Yash Pal)

Endurance and fertility analysis in indigenous horses using SNP markers

PCR amplification using Amplification Refractory Mutation System (ARMS) was done for – PCR six SNPs associated with endurance or fertility. Three SNPs viz. BIEC2_11782, BIEC2_755603, BIEC2_363958 were associated with endurance, two SNP FKBP6_2 and PLCZ1_2 was associated with fertility and one SNP was associated with growth and development of skeletal muscles. The genotyping by ARMS-PCR for four SNPs has been confirmed by sequencing. Performance recording for endurance racing has been done for Sindhi horses and 22 samples of Sindhi horses have been collected and included in the study. Additionally, 27 samples of Kathiawari horses have also been collected from the breeding tract and included in the study and the studies are in progress.

(SC Mehta and TR Talluri)

Assessment and optimization of equine management in an intensive system

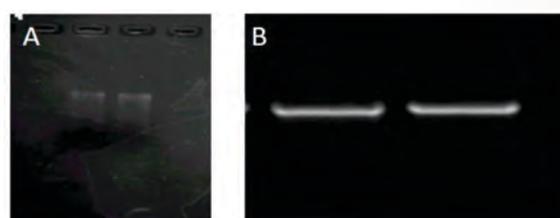
The inventory database (biometry, growth and breeding) of the farm equines has been prepared and updated for the period 1989 to 2019 (inception of the campus to till date). The pedigree, biometry and growth analyses of Marwari horses, Manipuri and Zanskari ponies maintained at the Campus was carried out. There was no inbreeding observed in the Marwari herd and selection criterion was not clear. The body parameters were analysed with respect to the sex and performance over year. The number of animals and generations in ponies' group were not sufficient to make any conclusive remarks on pedigree analysis. The overall growth performance was found to be stable over the years.

A selection differential for body height, colour and independent culling level for body length and heart girth was adopted for selecting the animals and a breeding plan was prepared for Marwari, Manipuri and Zanskari mares. Specific sire was allotted to a dam for breeding keeping in view of above criteria and the pedigree of the animals to be mated. The average height of selected Marwari Stallions was 157.75 ± 0.76 cm and the average height of breed able females was 151.05 ± 0.72 cm. The average body length and heart girth of selected stallions were 150.45 ± 0.94 cm and 171.55 ± 1.59 cm respectively.

(SC Mehta, TR Talluri and Jitendar Singh)

Stallion spermatozoal DNA isolation protocol

The unique DNA packaging of spermatozoa renders them resistant to DNA isolation techniques used for somatic cells, requiring alternative methods that are slow and labor intensive. For the isolation of DNA from stallion spermatozoa, a combination of salting out technique and ethanol extraction methods were adopted which are unique to the spermatozoa DNA isolation. This protocol



(A) Isolation and (B) amplification of DNA from the stallion spermatozoa



resulted in high molecular weight DNA of high quality with an $A_{260/280}$ ratio ranging between 1.8 and 2.0 and an $A_{260/230}$ ratio of 2.0 and greater. The DNA is efficiently digested with restriction enzymes and amplified by PCR.

(TR Talluri and Anuradha Bahradwaj)

Assessment, evaluation and identification of physical, biochemical and genetic factors affecting stallion fertility

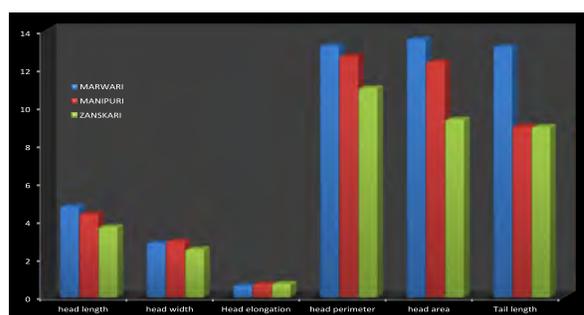
Scrotal biometry and ultrasonography of the scrotal sac of all Marwari stallions of farm were done and no calcification at the testicular sac was identified. Scrotal biometry parameters were significantly correlated with age but not with stallion body weight and height. The values of scrotal biometry parameters were significantly lower in the stallions below 4 years of age. Fresh, pre-freeze and post thaw semen characteristics differed significantly among stallions but did not differ among stallions of different age groups. Various biochemical and hormonal profile for the Marwari stallions were also carried and correlated with that of physical parameters. GOT, GPT, Total Protein and Ca differed significantly in seminal plasma while glucose differed significantly in spermatozoa among stallions. Serum testosterone, GOT, GGT, TP, Ca and P were significantly lower in the stallions below 4 years of age.

Correlation of serum testosterone with seminal parameters

	Testosterone	TTV	SC	Body Weight	Height	Reaction Time	Total Volume	Sperm Concentration	PM
Testosterone	--	0.855**	0.785*	0.419	0.417	-0.618**	0.239	0.467*	-0.150

TTV-total testicular volume; SC- Scrotal circumference; PM- Progressive Motility *Significant $p \leq 0.05$; ** Highly Significant $p \leq 0.01$

Sperm morphometry was also studied across different breeds of stallions. There was a significant difference observed in the parameters like Head length and tail length of Marwari and to that of Manipuri and Zanskari stallions and there were no significant differences observed among the Manipuri and Zanskari stallions.

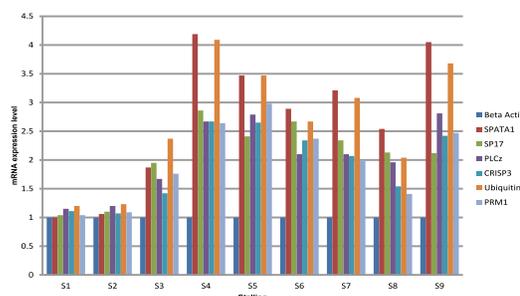


Sperm morphometry in stallions of three breeds

(TR Talluri, Anuradha Bahradwaj, SC Mehta and Yash Pal)

Expression of fertility related genes in Marwari stallions

A study was conducted for screening the expression of fertility related genes viz., PLCz, SPATA, PRM, UBIQUITIN and SP17 during breeding and non-breeding seasons and in different gene groups of stallions. Six fertility related marker genes (CRISP3, UBQ, PLCz1, PRM1, SPATA1 and SP17) showed differential expression between the seasons in Marwari stallions. SPATA1, SP17, CRISP3, PLCz, Ubiquitin B and PRM1 genes were expressed in Marwari stallions and the expression of these genes was lower in the stallions below 4 years of age. CRISP3 and UBQ genes expressed more in the breeding season than the non-breeding season while PLCz1, PRM1, SPATA1 and SP17 were expressed more in the non-breeding season than the breeding season. The expression of these genes was correlated with the DNA integrity, acrosome integrity and mitochondrial membrane potential. The study concluded that there was no significant significance in the expression patterns of these genes in both the seasons.



Over all expression of mRNA for different genes in the semen of different Marwari stallions

(TR Talluri, Anuradha Bahradwaj, SC Mehta and Yash Pal)

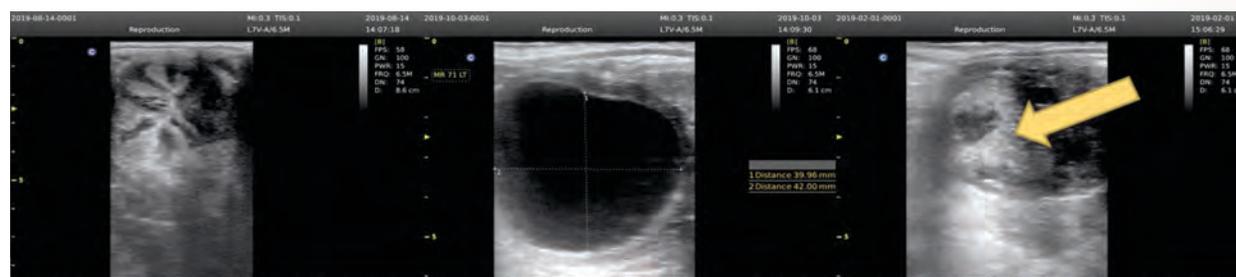


Early detection of ovulation and pregnancy in equines through ultrasonography

Detection of mares in estrus, ovulation time and pregnancy diagnosis are very key managemental and reproductive characteristics in successful breeding programme of equines. In the current project, we monitored the follicular dynamics of four different horse breeds namely Marwari (18), Kathiawari (8), Zanskari (8) and Manipuri (5) breeds throughout their estrus cycles. The study found that there is a significant variation in length of estrus, ovulatory size of the follicle, ovulation time and size of the embryonic vesicle at different pregnancy stages in different breeds.

Comparative follicular dynamics in mares of three different breeds.

Mares	Length of estrus cycle (Days)	Estrus Duration (Days)	Pre-Ovulatory follicle Size (mm)
Marwari (18)	21.01±1.27	6.92±0.28	43.8±1.56
Kathiawari (9)	24.21±0.19	9.19±1.81	40.5±1.35
Zanskari (8)	20.71±2.09	8.72±2.47	40.0±1.34

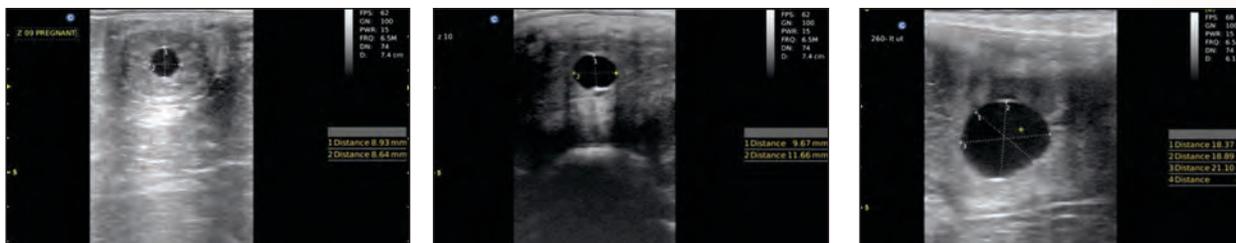


Ultrasound image of uterine edema of a mare in estrus

Ultrasound image of a mature follicle

Ultrasound image of corpus luteum after fresh ovulation

Early pregnancy diagnosis through ultrasonography.



Ultra image of presence of embryonic vesicle of 10 days in a mare

Ultra image of presence of embryonic vesicle of 12 days in a mare

Ultra image of presence of embryonic vesicle of 16 days in a mare

(TR Talluri, Jitender Singh, RA Legha and SC Mehta)

Microsatellite marker based genetic diversity analysis of Kachchhi-Sindhi horses

The native Kachchhi-Sindhi breed of horse has been accessioned (INDIA_HORSE_0417_KACHCHHISINDHI_07007) recently by NBAGR, Karnal (<http://www.nbagr.res.in/reghorse.html> retrieved on 6/9/2019). The genetic diversity in Kachchhi-Sindhi horses was evaluated at 30 microsatellite loci using fifty adult, healthy and unrelated animals. Allele frequency data was used to detect genetic diversity and bottleneck. The estimated average number of alleles (\pm SE) was 10.1000 ± 3.1878 with a total of 303 alleles. A high level of genetic diversity within this breed was observed in terms of number of alleles, observed heterozygosity (0.9058 ± 0.1127), expected Leven's heterozygosity (0.7865 ± 0.0873), expected Nei's heterozygosity (0.7786 ± 0.0864) and polymorphism information content (>0.5). Wright's (1978) fixation index (F_{is}) as a measure of heterozygote deficiency or excess. In-breeding coefficient (F_{is}) was -0.17497 ± 0.0102 , suggesting moderately low in-breeding in Kachchhi-Sindhi breed. Now, only a few thousand pure-bred animals are left. Earlier we reported the phenotypic characterization of Kachchhi-Sindhi horses and this genetic information will be valuable for proposing effectual population breeding and management strategies for future.



Summary of heterozygosity statistics for all loci.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ASB2	100	0.1200	0.8800	0.1525	0.8475	0.8390	0.8390
EB2E8	100	0.0000	1.0000	0.1547	0.8453	0.8368	0.8368
HTG6	100	0.0800	0.9200	0.3267	0.6733	0.6666	0.6666
TKY333	100	0.0000	1.0000	0.2517	0.7483	0.7408	0.7408
UM11	100	0.0800	0.9200	0.0828	0.9172	0.9080	0.9080
ASB17	100	0.0000	1.0000	0.2156	0.7844	0.7766	0.7766
AHT5	100	0.0600	0.9400	0.1281	0.8719	0.8632	0.8632
HMS5	100	0.0400	0.9600	0.3372	0.6628	0.6562	0.6562
HTG3	100	0.0200	0.9800	0.2152	0.7848	0.7770	0.7770
HTG7	100	0.0000	1.0000	0.1929	0.8071	0.7990	0.7990
AHT4	100	0.0400	0.9600	0.1760	0.8240	0.8158	0.8158
TKY321	100	0.2600	0.7400	0.2178	0.7822	0.7744	0.7744
TKY394	100	0.0000	1.0000	0.3582	0.6418	0.6354	0.6354
UM32	100	0.2000	0.8000	0.1598	0.8402	0.8318	0.8318
HMS3	100	0.1400	0.8600	0.1921	0.8079	0.7998	0.7998
TKY337	100	0.0200	0.9800	0.1075	0.8925	0.8836	0.8836
ASB23_	100	0.1600	0.8400	0.1339	0.8661	0.8574	0.8574
LEX33_	100	0.1600	0.8400	0.1327	0.8673	0.8586	0.8586
TKY297	100	0.0600	0.9400	0.1424	0.8576	0.8490	0.8490
TKY312	100	0.1600	0.8400	0.1877	0.8123	0.8042	0.8042
VHL20	100	0.0000	1.0000	0.1731	0.8269	0.8186	0.8186
ASB43	100	0.0000	1.0000	0.3251	0.6749	0.6682	0.6682
LEX78_	100	0.4800	0.5200	0.4438	0.5562	0.5506	0.5506
HMS2_	100	0.0000	1.0000	0.1830	0.8170	0.8088	0.8088
COR69	100	0.0600	0.9400	0.3911	0.6089	0.6028	0.6028
AHT31	98	0.2653	0.7347	0.1847	0.8153	0.8070	0.8070
COR22_	98	0.0204	0.9796	0.2394	0.7606	0.7528	0.7528
HTG10_	100	0.2600	0.7400	0.2123	0.7877	0.7798	0.7798
SGCV28_	100	0.0000	1.0000	0.1630	0.8370	0.8286	0.8286
COR7_	100	0.1400	0.8600	0.2253	0.7747	0.7670	0.7670
Mean	100	0.0942	0.9058	0.2135	0.7865	0.7786	0.7786
St. Dev		0.1127	0.1127	0.0873	0.0873	0.0864	0.0864

* Expected homozygosity and heterozygosity were computed using Levene (1949)
 ** Nei's (1973) expected heterozygosity

(Anuradha Bhardwaj, SC Mehta and Yash Pal)



NCVTC RESEARCH ACHIEVEMENTS

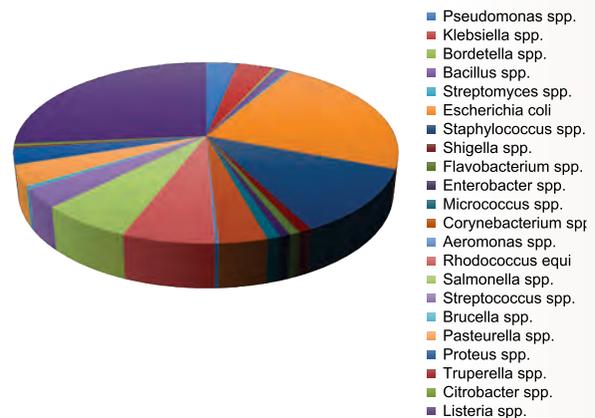
National Centre for Veterinary Type Cultures (NCVTC) is working towards the conservation of the animal microbial diversity in the country. The repository activities comprise of acquisition, authentication, preservation, documentation, and database management of animal microbes. The repository is being populated through a nationwide network of 14 network units located across nine different states viz., Assam, Gujarat, Himachal Pradesh, Haryana, Karnataka, Kerala, Rajasthan, Tamil Nadu and Uttar Pradesh. These network units are contributing towards the conservation of animal microbial diversity in three specialized areas: Veterinary microbes at ICAR-NRCE Hisar; Dairy microbes at ICAR-NDRI, Karnal and Rumen microbes at ICAR-NIANP, Bengaluru.

During the year 2019, a total of 200 microbes were accessioned in the repository leading to a cumulative strength of 3842. In the bacterial repository at NCVTC, a total of 347 bacterial cultures were received from NCVTC network units and allied sources. A total of 69 bacteria were accessioned during the year, making cumulative culture collection of 1427 bacteria of veterinary importance. Some of the significant bacteria accessioned are *Lactococcus lactis*, *Enterococcus faecalis*, *Staphylococcus xylosus*, *Flavobacterium mariense*, *Acinetobacter johnsonii*, *Bacillus licheniformis*, *Microbacterium maurum*, *Agrococcus lahulensis*, *Rhodococcus equi*, *Vibrio parahemolyticus*, *Aeromonas enteropelogens*, *Pseudomonas anguilliseptica*, *Pseudomonas peli*, *Pseudomonas cuatrocienegasensis*, *Streptococcus equi* ssp. *zooepidemicus*, *Streptococcus agalctiae*, *Corynebacterium pseudotuberculosis*. The accession also included anaerobic cultures viz., *Clostridium sporogenes*, *Cl. perfringens*, and *Clostridium difficile*. In the virus repository, a total of 62 virus isolates were processed, of which 40 viruses were accessioned in the repository that increases the strength to 300 virus isolates from 30 different species. The important virus isolates accessioned include, New castle disease virus (n=06), Fowl adenovirus (n=15) and Bluetongue virus (n=19). During the year, emphasis was on the isolation and characterization of bacteriophages against pathogens of mastitis: *Staphylococci* spp., and *Streptococci* spp. A total of 6 bacteriophages were isolated from sewage, soil, sludge and farm yard slurry. In rumen microbial repository at NIANP Bengaluru, with the accessioning of 49 rumen bacteria, the total strength of the rumen microbes repository has reached to 464. With deposition of 36 bacteria, the dairy microbes repository at NDRI, Karnal has also increased its strength to 620 dairy microbes.

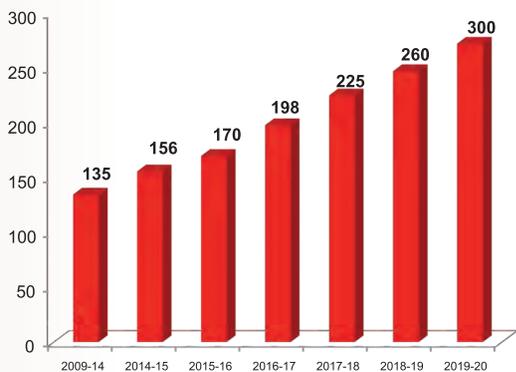
The distribution of microbes for teaching, research and development of new technologies is another mandated activity of NCVTC. In this regard, different bacterial cultures (*Pasteurella multocida*, *Streptococcus agalactiae*, *E. coli*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Gallinarum, *Klebsiella pneumonia*, *Campylobacter* sp.); two viruses (Bluetongue virus and Infectious bursal disease virus) and four cell lines (BHK-21, MDCK, A-72, Vero) were distributed to different Institutes/Universities in India for research and teaching purposes.



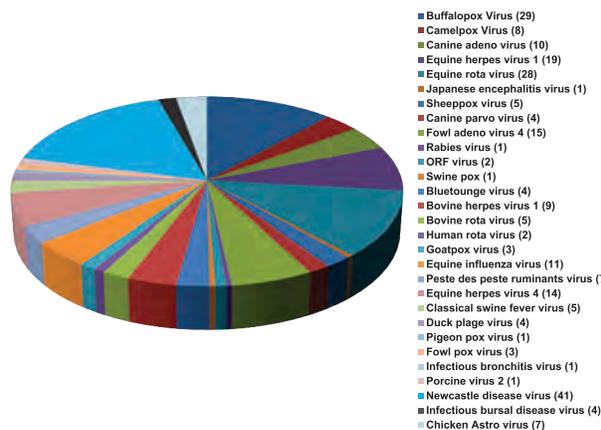
Year-wise progression of Bacteria



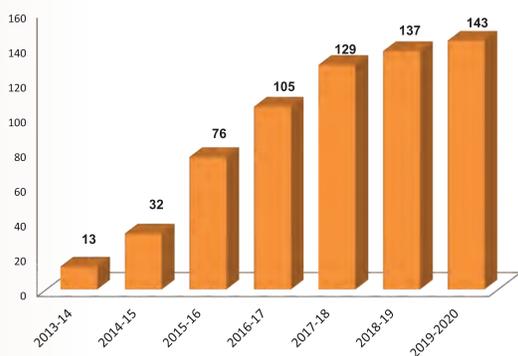
Repository represent a total of 1422 bacterial isolates across 68 different genera



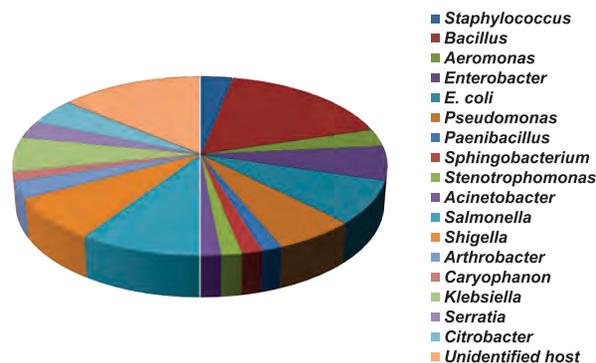
Year-wise progression of virus



30 different species of 300 virus isolates in the repository



Year wise progression of bacteriophage



No. of bacteriophage in repository 143

Microbial Culture Resources at NCVTC till 2019

Microbial Resources	2019	Total (31 Dec. 2019)
Veterinary microbes		
Bacteria	69	1427
Virus	40	300
Bacteriophage	6	143
Recombinant clone	0	573
Phage library	0	27
Genomic DNA	0	288
Total	115	2758
Rumen Microbes		
Anaerobic bacteria	49	349
Fungi/Yeast	0	107
Meth. Archae	0	8
Total	49	464
Dairy Microbes		
Bacteria	36	620
Total	36	620
Grand Total	200	3842

(Sanjay Barua, RK Vaid, Naveen Kumar, BC Bera, Taruna Anand, Riyesh T and Shanmugasundaram K)



Authentication and accessioning of viruses of animal origin

NCVTC virus repository is being strengthened with the addition of viruses from different geographical locations of the country through the deposition/collection of isolates and clinical samples from different animals and poultry. During the year 2019, a total of 110 different biological samples *viz.*, tissue, swabs and blood were collected / received from Udaipur, Delhi, Hansi, Karnal, Hisar and Ranchi. The samples were processed for isolation of different viruses. The details of virus authentications/isolations are mentioned in

Acquisition / Receipt of viral isolates during 2019

Depositor	Names of Virus	Number
NCVTC, Hisar	IBV	03
	NDV	01
	FAdV	15
LUVAS, Hisar	Bluetongue virus	21
	Canine parvo virus	06
TANUVAS, Chennai	BTV	10
	FADV	01
AAU, Khanapara	NDV (received during 2018)	05
Total		62

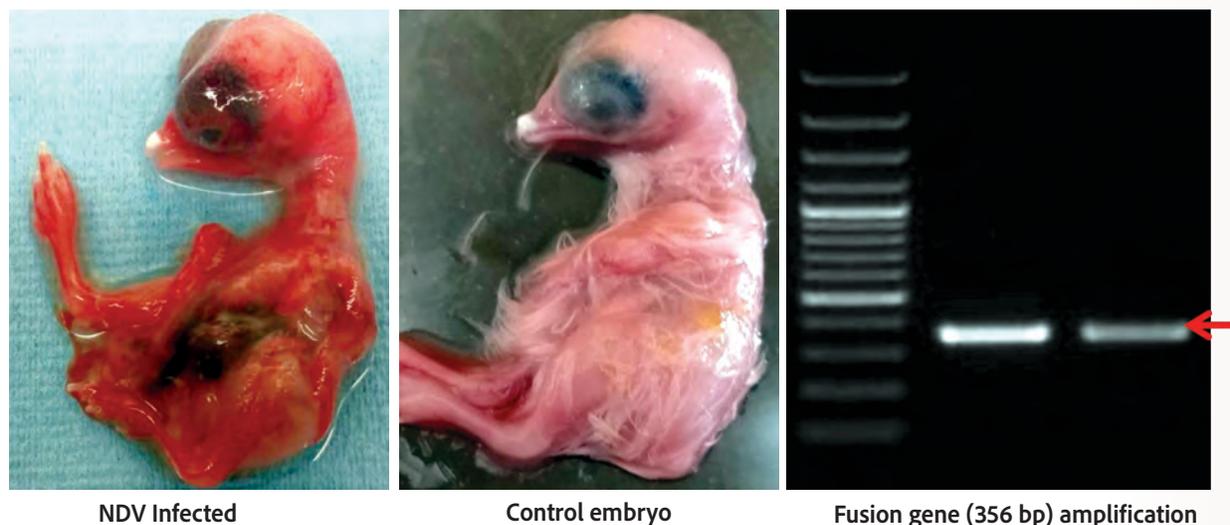
List of Viruses accessioned during the year 2019

Sr. No.	Names of Virus	Number
1	New castle disease virus	06
2	Fowl adenovirus	15
3	Bluetongue virus	19
	Total	40

The various authentication and isolation methods adopted for authentication and accessioning viruses in the repository virus is as follows.

Isolation and accessioning of Newcastle disease viruses

Five NDV isolates received from AAU, Khanapara were authenticated by amplification of fusion (F) gene (356 bp). Similarly, a Newcastle disease virus was also isolated from tissue samples collected from a poultry farm at Udaipur, Rajasthan. Virus isolations were carried out in 10 days old SPF eggs by allantoic route of inoculation.



NDV Infected

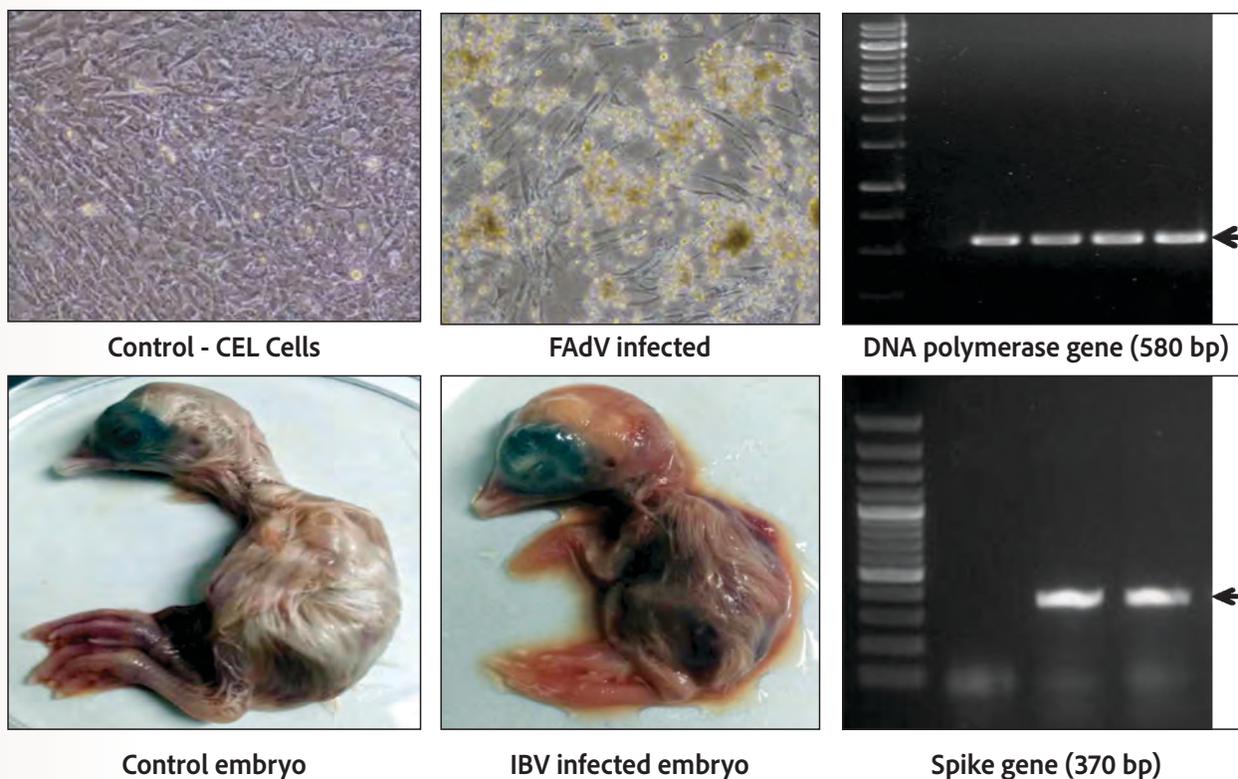
Control embryo

Fusion gene (356 bp) amplification



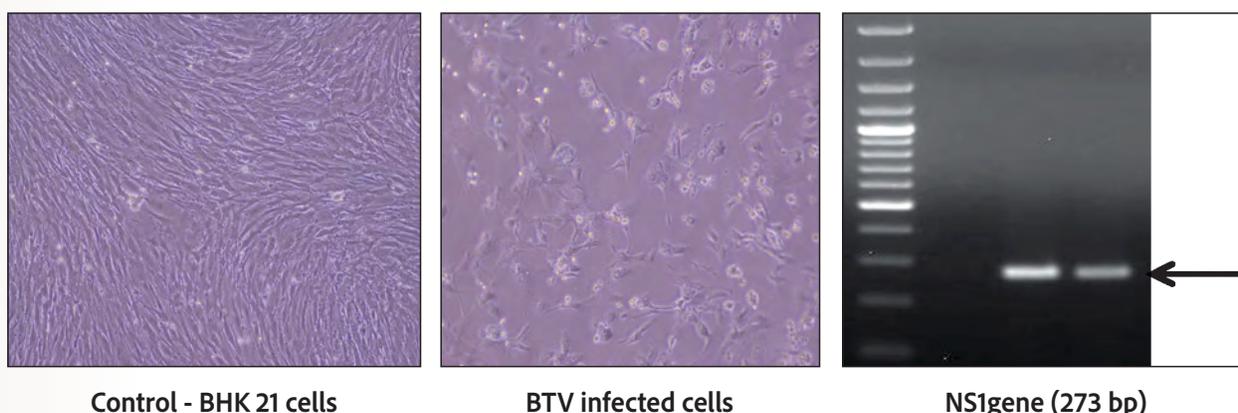
Isolation and accessioning of Fowl adenovirus

Fowl adenovirus (n=15) were isolated from clinical samples collected from different poultry farms and slaughter houses of Haryana state. The viruses were isolated in chicken embryo liver cells and virus identity was confirmed by PCR amplification and sequencing of hexon and DNA polymerase genes.



Authentication and accessioning of Bluetongue virus (BTV)

A total of 23 BTV isolates have been accessioned during the year 2019. LUVAS, Hisar submitted 21 isolates and 18 of them were authenticated by amplification of NS1 gene. Likewise, six isolates of BTV received from TANUVAS, Chennai were also authenticated. The isolates were accessioned in the repository upon confirmation of their viability in BHK21 cells.



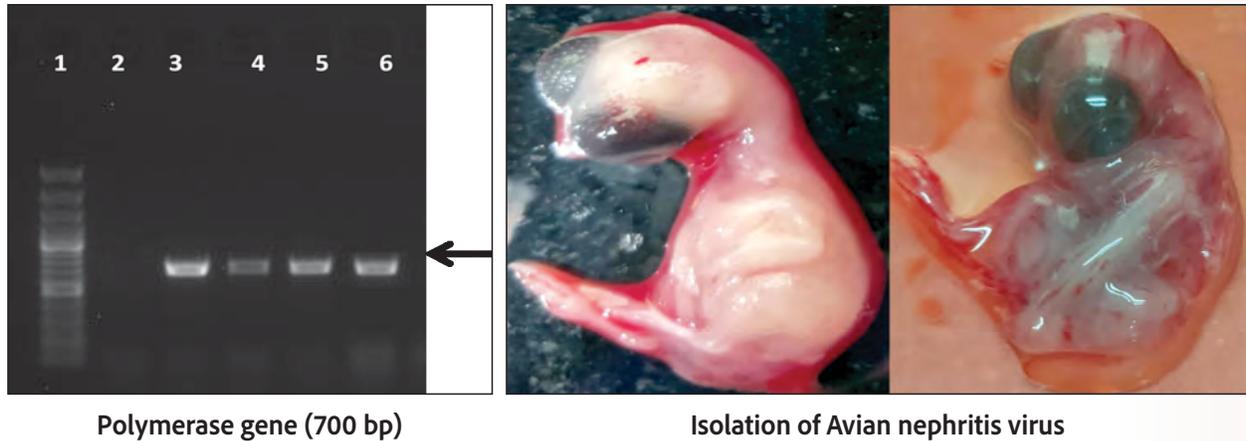
(Sanjay Barua, Riyesh T and Naveen Kumar)

Isolation and genetic characterization of avian nephritis virus from poultry

Avian nephritis is a viral disease of poultry caused by the avian nephritis virus (ANV), characterized by diarrhoea, retarded growth, tubule nephrosis, uricosis (gout) and death in severe cases. ANV belongs to the genus *Avastrovirus* within the family *Astroviridae* and the genome consists of a single stranded positive sense RNA of approximately 7 kb in length. The genome encodes three open reading frames ORF1a, ORF1b, ORF2 and UTRs. At least two serotypes and different genotypes of ANV have been reported. In this study, we attempted to isolate and



characterize the ANV circulating in poultry of different regions/eight districts of Haryana and Rajasthan states. Out of 279 intestinal samples tested 33 were found positive for ANV by PCR targeting RNA polymerase gene (ORF1b). The PCR positive clinical samples were passaged in SPF embryonated eggs by yolk sac route and allantoic route for virus isolation. Virus isolation was successful from four samples inoculated *via* yolk sac route and the infected embryos exhibited lesions *viz.*, dwarfism, gelatinous consistency and diffused haemorrhage. The amplified PCR products were sequenced to further confirm the identity of the virus. Sequence analysis confirmed that the isolated viruses have high nucleotide and amino acid similarity with ANV2 reported (GA-CK-SEP ANV-364-2005) from USA. Circulation of ANV1 has previously been reported from India, however reports on circulation of ANV2 in poultry from India are rare.



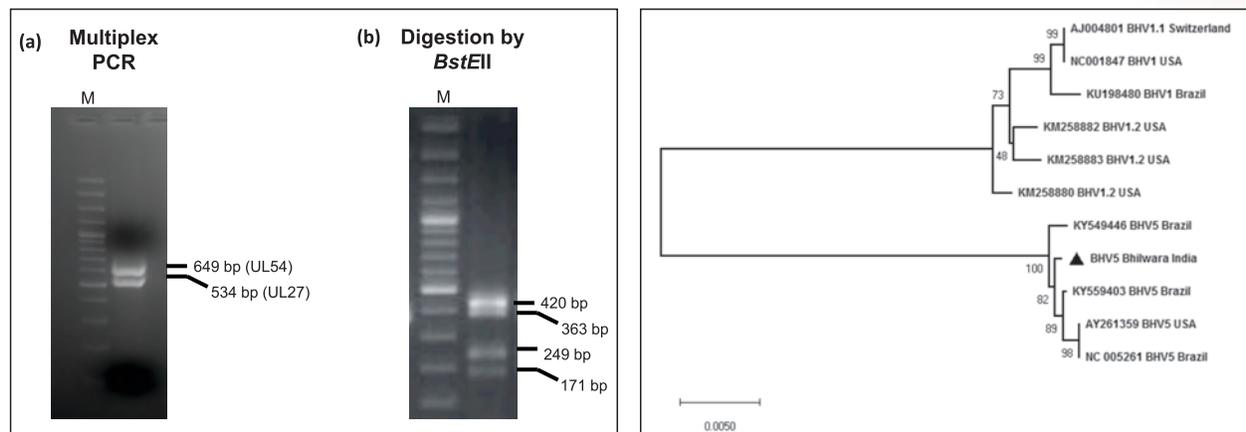
Polymerase gene (700 bp)

Isolation of Avian nephritis virus

(Riyesh T, Naveen Kumar, Naresh Jinal and Sanjay Barua)

Molecular characterization of BoHV-5

Bovine herpesvirus 1 (BoHV1) and 5 (BoHV5) are genetically and antigenically related alphaherpes viruses. However, disease associated with BHV1 and BHV5 varies significantly; whereas BHV1 infection is usually associated with rhinotracheitis and abortion, BHV5 causes encephalitis in cattle. BoHV5 outbreaks are sporadic and mainly restricted to the South American countries and the virus has never been reported from India. Here we for the first time report the isolation and genetic characterization of an BoHV-5 virus (VTCC AVA 218) isolated from clinical samples collected from aborted cattle from Bhilwara, Rajasthan. The isolate was subjected to molecular characterization by amplification of UL27-gB (glycoprotein B- 534 bp) gene and UL54 gene (649 bp) followed by restriction enzyme analysis (REA) which was confirmed the BoHV isolate as BoHV-5, subtype A. Furthermore, phylogenetic analysis of BoHV-5a indicated a close association with the Brazilian BHV-5 strains. This is the first report of BoHV-5 from India.



Biotyping indicate Biotype A of Indian BHV5 isolate

Phylogenetic analysis of BHV5 indicated close association with Brazilian BHV5 strains

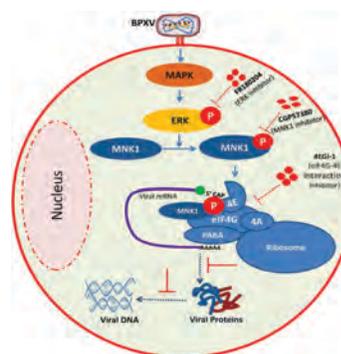
(Naveen Kumar, Riyesh T and Sanjay Barua)



Deciphering role of MNK1 in buffalopox virus replication

A study has been carried out to assess the role of MAPK interacting kinase 1 (MNK 1) in buffalopox virus (BPXV) replication using a small molecule chemical inhibitor CGP57380 that blocks activation of MAPK interacting kinase 1

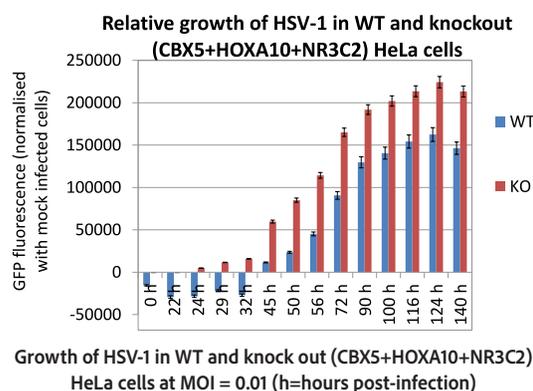
(MNK1) was found to significantly suppress buffalopox virus (BPXV) replication. With the help of time-of-addition and virus step-specific assays, CGP57380 treatment was shown to decrease synthesis of viral genome (DNA). Disruption of ERK/MNK1/eIF4E signaling resulted in reduced synthesis of viral proteins, suggesting that BPXV utilizes cap-dependent mechanism of translation initiation. The study revealed that ERK/MNK1/eIF4E signaling is pre requisite for BPXV replication and BPXV utilizes cap dependent mechanism of translation initiation.



Role of MNK1 in buffalopox virus replication
(Naveen Kumar and Sanjay Barua)

Generation of knockout cells by CRISPR/Cas9-mediated genome editing

We exploited CRISPR/Cas9-mediated genome editing to generate knockout (KO) HeLa cells with disrupted CBX5, HOXA10 and NR3C2 proteins known to play antiviral function in herpes simplex virus 1 (HSV-1) life cycle. The small guide RNAs (sgRNAs) targeting *CBX5*, *HOXA10*, *NR3C2* and *Med23* genes were cloned into pL.CRISPR.EFS.GFP. Upon transfection into HeLa cells, the recombinant constructs expressed respective target CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA RNA) and Cas9, besides expressing green fluorescent protein (GFP). All the GFP expressing cells are likely to express sgRNA (crRNA+tracrRNA)/Cas9 complex that cut the genome at the targeted sites. The GFP expressing cells were sorted by fluorescence activated cell sorter (FACS) and cultured in 96-well tissue culture plate to obtain single clones by limiting dilution assay. After culturing for 1-2 weeks, the wells with a single clone were selected for further propagation. To evaluate gene editing, all the single clones were subjected to amplify target genes by PCR. For further confirmation of the gene editing, the PCR products were subjected to nucleotide sequencing. We simultaneously transfected HeLa cells with plasmid construct expressing sgRNAs against 4 antiviral cellular genes, viz; *CBX5*, *HOXA10*, *NR3C2A* and *Med23*. A single gene was targeted at 3 different sites using 3 different set of sgRNAs. Though a single clone having disruption in all the 4 genes could not be obtained, we were able to isolate a triple knockout clone (tKO) with edition of three cellular genes *CBX5*, *HOXA10* and *NR3C2*. HSV-1-GFP virus replicated at much higher titer in tKO cells than in WT cells. As a proof of concept, for the first time employed CRISPR/Cas9 method to generate engineered cell lines deficient in multiple antiviral cellular genes, thereby supporting high titer growth of the virus. This concept can be employed to scale up virus production for vaccine manufacturing.



Growth of HSV-1 in WT and knock out (CBX5+HOXA10+NR3C2) HeLa cells at MOI = 0.01 (h=hours post-infection)

(Naveen Kumar, Sanjay Barua, Riyes T and Balvinder Kumar)

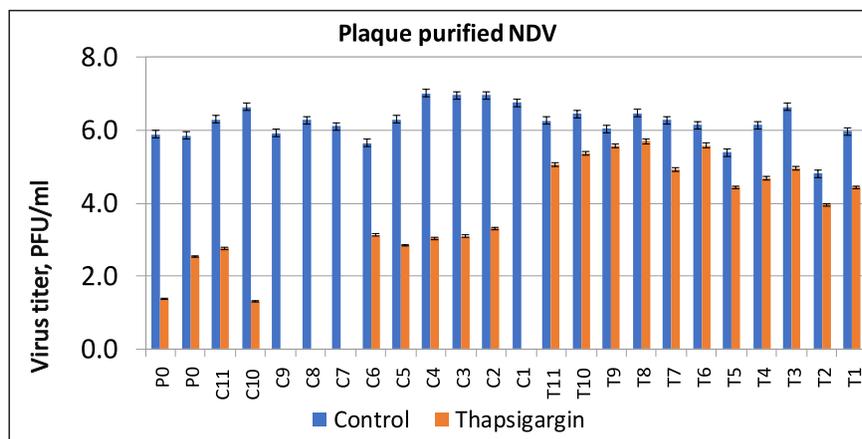
Investigating mechanisms underlying acquisition antiviral drug resistance against host-targeting agents

Classically, antiviral drugs have been developed by directly targeting viral proteins. However, due to high mutation rates, viruses quickly become resistant at the druggable targets. The rise in incidence of drug resistance has prompted a shift towards development of antiviral drugs. Host factors that are dispensable for host but are critical to virus replication may serve as alternate targets for development of novel antiviral therapeutics. We screened a library of small molecule chemical inhibitors and identified antiviral efficacy of sarco/endoplasmic reticulum



calcium-ATPase (SERCA) inhibitor (Thapsigargin) and MNK1 (MAPK-interacting kinase) inhibitor (CGP57380) against paramyxoviruses [(Newcastle disease virus (NDV) and peste des petits ruminant's virus (PPRV)] and buffalopox virus (BPXV), respectively. Whereas directly acting agents are known to develop a complete resistance with 6-10 passages (P), Thapsigargin and CGP57380 (host-targeting antiviral agents) did not induce generation of antiviral drug resistant phenotypes against NDV (up to ~P40) and BPXV (upto ~P25), respectively. However, at further higher passage (~P60 in BPXV and ~ P70 in NDV), a significant resistance was observed. Acquisition of antiviral drug resistance against host-targeting agents (SERCA/MNK1 inhibitor) is intriguing and its mechanisms are not well understood.

One possibility of acquisition of resistance to host-directed antiviral agents may be to generate defective interfering (DI) particles (upon long-term virus culture) which differently suppress virus yield in drug-resistant and drug-susceptible viruses. In order to test this hypothesis, drug-resistant and drug-susceptible NDV at passage level 70 (P70) were plaque purified (n=10 each) and evaluated for their sensitivity to the drug (host-directed antiviral agent-Thapsigargin). Like virus mixtures (viral quasispecies) (P70-Thapsigargin and P70-Control), plaque purified P70-Control viruses showed more sensitivity to the antiviral action off Thapsigargin than plaque purified P70-Thapsigargin viruses. This suggests that acquisition of drug resistance against host-directed antiviral agents is not simply due to the generation of DI particles but rather due the specific events that may involve switch to use alternate host factors or increased affinity of the resistant viruses to its substrate.



Acquisition of drug-resistance by NDV against Thapsigargin is not due suppression of defective interfering particles. NDV was sequentially passaged in Vero cells in the presence of 0.25 μ M Thapsigargin or 0.05% DMSO. At each passage, confluent monolayers of Vero cells were infected with the virus, washed 5 times with PBS before a fresh aliquot of MEM was added and incubated for 48-96 h or until the appearance of cytopathic effect (CPE) in \geq 50% cells. Sixty passages of virus infection were carried out. Ten plaques, each from P60-Control (C1-C10) and P60-Thapsigargin (T1-T10) virus were purified and sensitivity to Thapsigargin was evaluated.

(Naveen Kumar and Sanjay Barua)

Circulation of porcine circovirus 2 and 3 (PCV2 & PCV3) in apparently healthy pigs and investigation of PCV3 associated severe reproductive problem in pigs

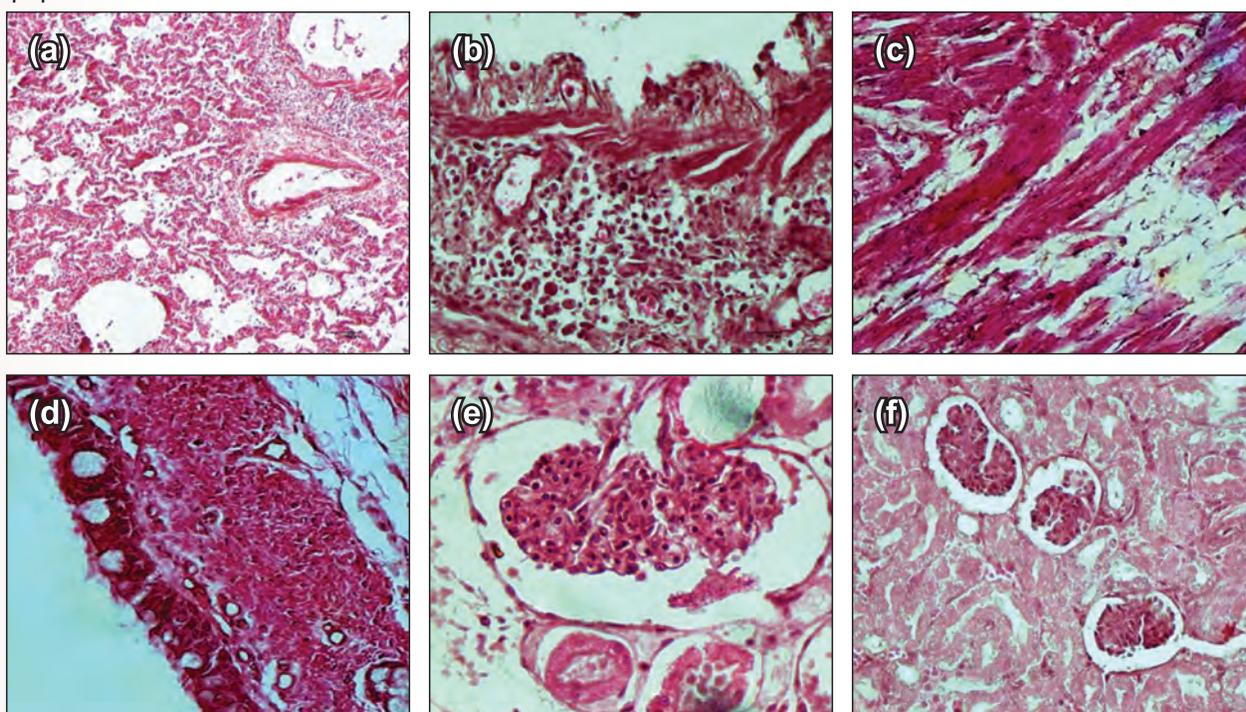
The status of circulation of Porcine circovirus (PCV) and their variants among pig population of the country are being investigated. PCV - a small non-enveloped circular single-stranded DNA virus under the genus Circovirus within the family Circoviridae incurs huge economic losses in swine industry globally. A variant of PCV- PCV type 3 (PCV3) related infection have also been described recently from USA, Europe, China, South Korea and Japan. In present study, lung tissue samples were collected from abattoir in Mumbai and post mortem samples of pigs died due to respiratory problem in a farm in Bhiwani Haryana. PCV3 was detected in lung tissues collected from apparently healthy pigs from abattoir in Mumbai by amplification of PCV3-specific cap gene (263 bp) in 12 samples. The PCV3 load was determined by qPCR and observed 1.2×10^3 to 4.4×10^{10} copies of PCV3/g lung tissues. The post mortem samples were positive for PCV2 as confirmed by amplification of ORF2 region and sequencing.

Further, detailed virological and histopathological investigation was carried on PCV3 infected clinical samples associated with reproductive failure in sow in a pig farm in Chhattisgarh. The presence of PCV3 was further confirmed by sequencing of PCV3 genomes and BLAST homology. Subsequently, the viral loads in the tissues were estimated by qPCR which showed presence of 6.3×10^{11} genome copies/g of lungs, 3.1×10^7 genome copies/g of liver, 4.8×10^{12} genome copies/g of heart, 3.1×10^{11} genome copies/g of kidney and 4.6×10^{12} genome copies/g of naval cord tissues collected from stillborn piglet and 3.05×10^6 genome copies/ml of nasal swabs of sows. The pathological changes in various tissues from stillborn piglet were analyzed and observed specific changes in lungs, heart and kidney. The microscopical observation showed diffused infiltration of alveolar septa with lymphocytes, plasma



cells and macrophages which could be seen spreading and engulfing in peribronchial and perivascular areas in lungs. Oedema could be seen in the alveolar lumen and around the blood vessels. Bronchiolar epithelium was hyperplastic at places and showed areas of degeneration and necrosis. The changes were indicative of interstitial pneumonia. Histological lesions in heart includes: focal necrosis of cardiac muscles in many areas; thickened blood vessels' wall due to fibrinoid degeneration in tunica media, and endothelial cells of blood vessels appeared plump and hypertrophied. In kidneys, hydropic degenerative changes in the tubular epithelium of proximal as well as distal convoluted tubules were observed in the cortex. The glomerular capillary walls appeared moderately thickened with proliferation of mesangial cells in almost all glomeruli indicating membrano-proliferative glomerulonephritis.

The results confirmed the circulation of PCV2 and PCV3 in apparently healthy pigs. Several earlier reports have described the clinical cases of PCV2 infections, however the association of PCV3 with clinical case speculates emergence of PCV3 as an important pig pathogen in the country. This warrants the detail investigation in disease pathogenesis and epidemiology due to the recent prevalence of this virus in major swine population countries.



Histopathological lesions in lung, heart and kidney tissues stained with H&E. (a) Lungs showing diffused infiltration of alveolar septa with lymphocytes, plasma cells and macrophages. (b) Bronchiolar epithelium of lung was hyperplastic at places and showed areas of degeneration and necrosis. (c) Heart showing focal necrosis of cardiac muscles. (d) Endothelial cells of blood vessels of heart appeared plump and hypertrophied. (e) Kidney showing hydropic degenerative changes in the tubular epithelium. (f) Glomerular capillary walls of kidney appeared moderately thickened with proliferation of mesangial cells.

(BC Bera, Taruna Anand and Nitin Virmani)

Emergence of human seasonal H1N1 pdm2009 and H3N2 viruses in apparently healthy swine populations

Respiratory virus infections in pigs are primary problems to swine industry as well as for human health especially due to zoonotic swine influenza virus (SwIV) infection. Information on circulating SwIVs is largely speculative as no detail investigation of causative agents has been thoroughly explored in swine population of the country. We investigated the circulation of IAVs in apparently healthy pigs in different parts of the country including north eastern states having major pig populations. Last year, we have detected presence of human seasonal (H1N1) pdm09 and H3N2 viruses in nasal swabs collected from pigs from Mumbai (abattoir), Guwahati, Mizoram, Manipur and Meghalaya. Around 7% samples were positive for IAVs. The sequences of haemagglutinin (HA) and neuraminidase (NA) genes of representative samples showed 97-99% identity with human seasonal (H1N1) pdm09 and H3N2 viruses. Subsequently, genomic characterization was carried out by cloning and sequencing of

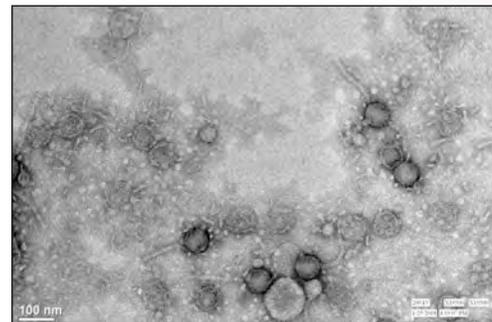


other six genomic segments (PB2, PB1, PA, NP, M and NS) of both (H1N1) pdm09 and H3N2 viruses. Indian H1N1 SwIV showed signature mutations (S74R, S164T and I295V) which also observed in human strains including vaccine strain (A/Michigan/45/2015). Phylogram of HA gene of H1N1 revealed clustering of Indian strains with human seasonal (H1N1) pdm09 viruses circulating during 2017-19 in India, USA, Europe, Kenya and other countries. Further, Indian H1N1SwIVs grouped with recent swine isolates from USA. Indian H3N2 SwIVs grouped with H3N2 SwIV isolate from USA and human isolate from China. Internal genes showed almost similar identity as observed for HA and NA gene analysis. This indicates the circulation of SwIVs in pig population was likely evolved from the contemporary human seasonal pandemic influenza virus. This suggests, thorough surveillance for understanding the ecology of circulating SwIVs for implementation of the control strategies.

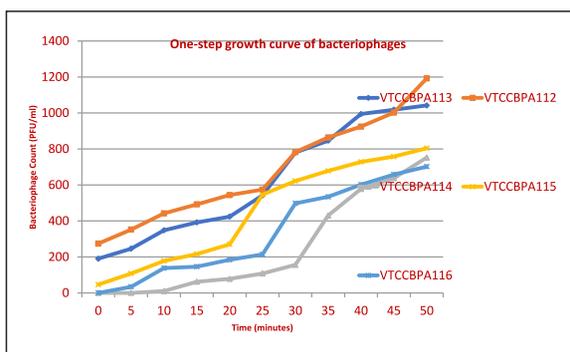
(BC Bera, Taruna Anand and Nitin Virmani)

Isolation, characterization and assessment of stability of a *Klebsiella pneumoniae* phage kept for longer duration

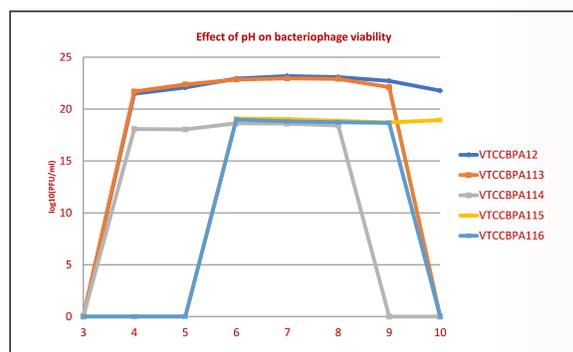
Bacteriophages are the obligate parasites of bacteria. The bacteriophages offer immense potential in various applications in the changing scenario of emerging antimicrobial resistance. Keeping in view the unprudent use of antibiotics in livestock sector and the urgent need to develop alternatives of antibiotics, phage therapy is being explored for treatment of mastitis. In this context, a bacteriophage - VTCCBPA118 was isolated against FOP185A- *K. pneumoniae* strain. The bacteriophage was isolated from animal farm soil and yielded plaques-2mm in size with a halo zone after overnight incubation at 37°C. BPA118 is observed to be a siphoviridae phage with head diameter of ~75nm and tail length~141nm (as depicted by TEM). BPA118 was stable in the temperature range of 4-55°C and within a narrow pH range of 5-9. The phage was bulk cultured using PEG precipitation and successive purification to yield a titre of 2.3×10^{18} PFU/ml. The phage was kept at 37°C for upto 90 days where the titre was estimated by plaque formation assay at an interval of every 10 days. BPA118 was found to be quite stable with a decrease in log titre of 1 (every month). However, this indicates that certain phages may be best store at 37°C temperature. As such there wouldn't be any need of maintaining the cold chain during their transport to field conditions



Transmission electron micrograph of VTCCBPA118 (*Klebsiella pneumoniae* phage)



Growth curve of bacteriophages



pH sensitivity of different bacteriophages

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

Assessment of ARGs in bacteriophage DNA isolated from animal farm soil

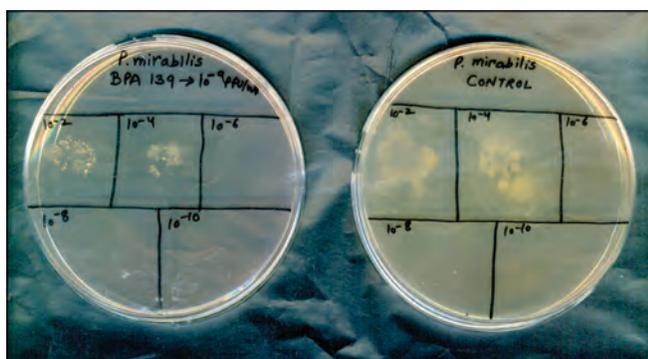
The DNA of bacteriophages was isolated and assessed for the presence of any antibiotic resistance genes (ARGs) including CTXM, SHV, NDM, TetA, TetB, TetG, TetO, TetW, qnrA, qnrS & bla-Tem but none of the phage DNA (16nos. assessed – BPA106 to BPA119) was observed to carry any ARG in their genome. However, when the bacterial genomes were assessed for ARG's, they were found to exhibit CTXM in 3 strains of *S. sciuri* and 1 strain of *S. chromogenes*. TetO was also exhibited in 3 strains of *E. coli* and SHV was found in one strain of *K. pneumoniae*

(Taruna Anand, BC Bera, Nitin Virmani, RK Vaid and Medhavi Vashisth)



Assessment of antibiofilm activity of bacteriophage VTCCBPA139

An MDR *Proteus mirabilis* strain was isolated from poultry farm litter and the corresponding bacteriophage (BPA139) was also isolated. The phage was used to assess the anti-biofilm forming activity against host bacteria. It was observed that the bacteria present in 96hr biofilms were inhibited significantly by 10^4 PFU/ml of bacteriophage suspension when added to preformed biofilms in microtitre wells.



Comparison of growth inhibitory effect of bacteriophage – BPA139

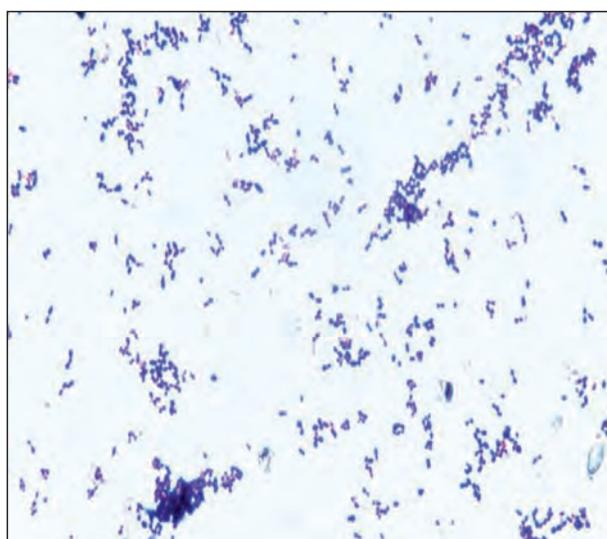
(Taruna Anand, Nitin Virmani, BC Bera and RK Vaid)

Authentication and accessioning of bacteria

During the year of 2019, among the processed cultures, 69 cultures were accessioned in the bacterial repository which has led to total strength of Bacterial Culture Collection to 1427 veterinary bacteria. Cultures were submitted from SKUAST, Jammu; CMVL, Meerut; IVRI, Izatnagar; AAU, Khanapara and CIRG, Makhdoom. Many of the cultures were accessioned from NCVTC itself. In addition, many cultures are ready to be accessioned. 117 pathological/other samples submitted/collected at NCVTC bacteriology laboratory; viz, samples from goat/sheep (6); Cattle (26), buffalo (23); mice (13), poultry (11) and equines (23), dog (12), pigs (9) including samples from contaminated Gurana cell culture used in *Trypanosoma/Theileria* culturing, which led to isolation of 183 cultures, which are preserved in general preservation. Some of the significant bacteria accessioned are *Lactococcus lactis*, *Enterococcus faecalis*, *Staphylococcus xylosus*, *Flavobacterium mariense*, *Acinetobacter johnsonii*, *Bacillus licheniformis*, *Microbacterium aurum*, *Agrococcus lahulensis*, *Rhodococcus equi*, *Vibrio parahemolyticus*, *Aeromonas enterope logens*, *Pseudomonas anguilliseptica*, *Pseudomonas peli*, *Pseudomonas cuatrocienegasensis*, *Streptococcus equi* ssp. *Zooepidemicus* *Streptococcus agalctiae*, *Corynebacterium pseudotuberculosis*. The accession also included anaerobic cultures viz., *Clostridium sporogenes*, *Cl. perfringens*, and *Clostridium difficile*. Some of the cultures accessioned are those which were sent by researchers from GADVASU, Ludhiana; EBS, Hisar; SKUAST, Jammu; and NDRI Karnal. Particularly interesting isolates identified as *Burkholderia contaminans*, *Mannheimia caviae*, *Achromobacter xylosoxidans*, *Clostridium butyricum*, *Gemella morbillorum* (Ss14), *Sphingomonas spaucimobilis* (Fo133), *Ochrobactrum anthropi* (Fo137) and *Gallibacterium anatis*.



Mannheimia caviae



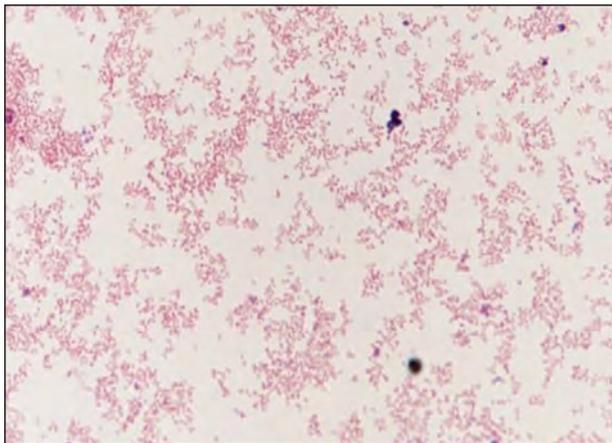
Streptococcus zooepidemicus

(RK Vaid, Taruna Anand, K Shanmugasundaram, T Riyesh and BC Bera)

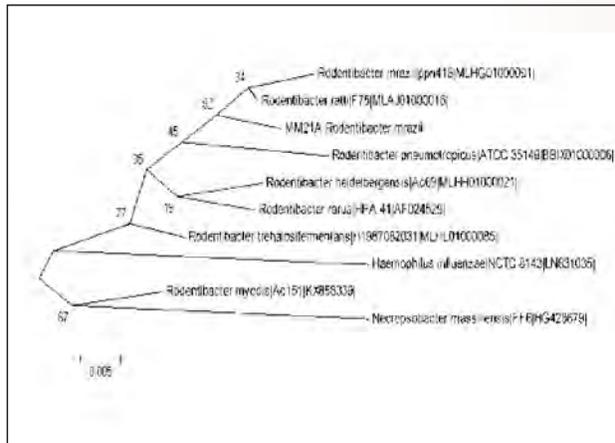


Isolation and identification of *Rodentibacter* spp. from BALB/c mice

Earlier considered a member of *Pasteurella*, *Rodentibacter* spp. has been considered the most important *Pasteurellaceae* members colonizing laboratory rodents. *Rodentibacter* spp. is an opportunistic pathogen that is found in many research and commercial rodent colonies. Testing and reporting of *Rodentibacter* spp. in laboratory rodent colonies is critical due to laboratory rodent colony welfare and experimentation concerns. An isolate of *Rodentibacter pneumotropica* was isolated from intestines of BALB/c mice. The colony grew as small non-hemolytic, circular, <1 mm size on SBA. The cells were Gram-negative cocco-bacillary short rods, however filamentous clustering was also seen on initial isolation. Colonies were oxidase positive. Biochemically, the API system identified the isolate as *Pasteurella pneumotropica*. However, as this taxa has undergone major modifications due to discovery of a number of species, the strain Mm21A was subjected to molecular identification by 16S rRNA sequencing and phylogeny. The NCBI nBLAST of the strain identified it close to *Rodentibacter rattiomrazii*. It is important to screen for *Rodentibacter* spp. in experimental animal like BALB c mice, and a sentinel program therefore should be in place in the animal houses in the country distributing small animals for experimental purpose.



Rodentibacter spp. from BALB/c mice

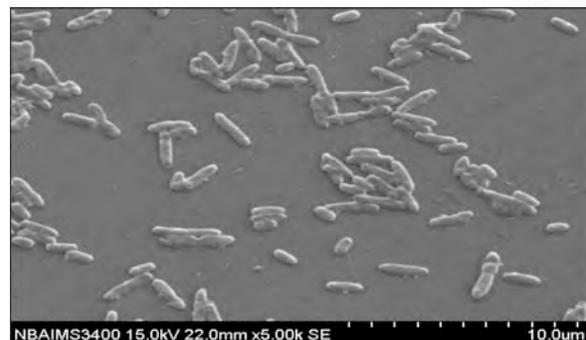


Rodentibacter spp. Phylogeny

(RK Vaid, K Shanmugasundaram, Taruna Anand, Riyesh T and BC Bera)

Polyphasic characterization of *Achromobacter* sp. nov strain FO90

Achromobacter spp. nov. strain Fo40^T is an isolate obtained from stem-cell derived primary cell culture of a term buffalo. Fo40^T was causing persistent contamination in cell culture. The isolate was previously identified as *Achromobacter* spp., and it was found to be multi-drug resistant. However, 16S rRNA phylogeny revealed that it may be novel taxa of Genus *Achromobacter*. The 16S rRNA gene sequences of strain Fo40^T (genogroup 19), was more than 99% similar to those of the neighbouring *Achromobacter* species, a characteristic of the genera. The strain was subjected to polyphasic characterization. The strain Fo40^T was characterized by *nrdA* (ribo-nucleoside-diphosphate reductase 1 subunit α) gene analysis under MLST scheme and it represented a novel sequence type ST-425 with *nrdA*_765 allele number 150. Commercially available miniaturized identification systems-API 20NE, API20E, and API CH (Biomereux) were used for further biochemical tests. In the Gen III plate of Biolog strain Fo40^T has shown most reactions as negative. VITEK analysis of strain Fo40^T gave a report of 'low discrimination'. MALDITOF MS identified strain Fo40^T best match as *Achromobacter ruhlandii* DSM653T, and second match *Achromobacter xylosoxidans* LMG 3429. Scanning electron microscopy of the Fo40^T strain was performed in a Hitachi 3400N VP-SEM model microscope which showed pleiomorphic rods. Strain is preserved in NCVTC.



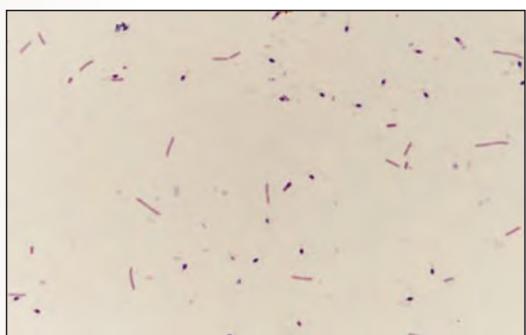
Electron micrgraph of *Achromobacter* sp

(RK Vaid, Taruna Anand and Riyesh T)

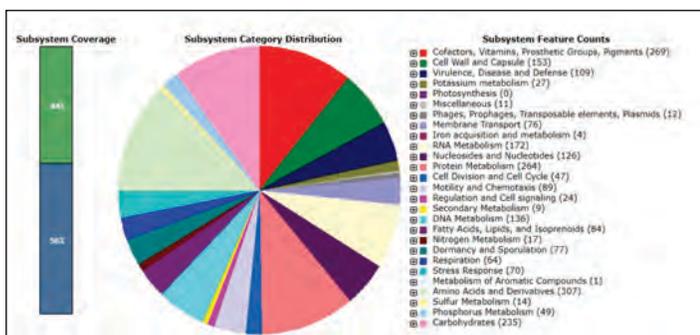


Isolation and NGS of *Clostridium botulinum* strain from mortality cases in horse

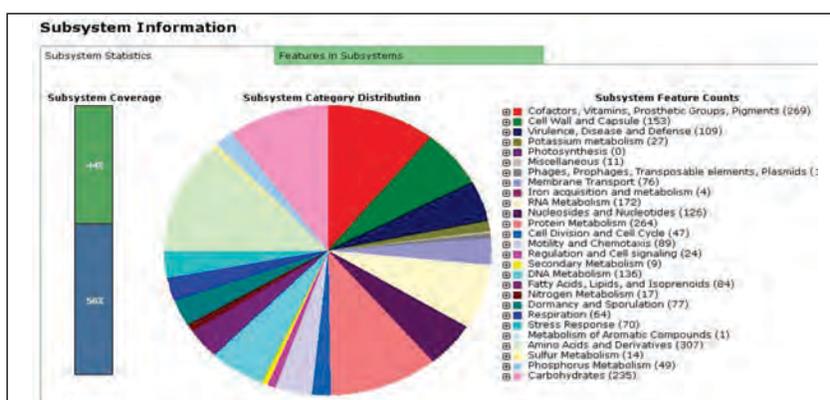
A field outbreak of mortality in brick-kiln horses was reported from Village Nehla, Bhuna, Fatehabad. Out of 8 adult horses, 3 deaths were reported. The acute death of healthy horses with toxemic changes suggested clostridial etiology. The intestinal content of dead horses was subjected to anaerobic bacteriology and 2 strains of clostridia were isolated. Out of which, 1 strain Eq187A was molecularly identified to be either *Clostridium botulinum* or *Clostridium sporogenes*. However, the identification could not be confirmed by 16S rRNA sequencing or by MALDI-tof analysis also. The strain Eq187A was identified as *Clostridium botulinum* on the basis of Whole Genome Sequencing using Illumina platform with 150 bp paired end (PE) library for data generation. Total PE reads data of 4.63 GB was assembled into 33 scaffolds using Velvet v 1.2. Bioinformatic analysis of revealed quality genome of 40,54,056bp, (N50 536032) with a GC content of 27.82%, 3741 CDS and 98 RNAs. The genome was annotated using SEED, and subsystem analysis revealed 109 proteins related to virulence, disease and defence.



Clostridium botulinum



Clostridium botulinum subsystem analysis



Subsystem Classification of *Clostridium botulinum* CDS

(RK Vaid, T Riyesh, Taruna Anand, K Shanmugasundaram and BC Bera)

Indian Network for Fisheries and Animal Antimicrobial Resistance (INFAAR)

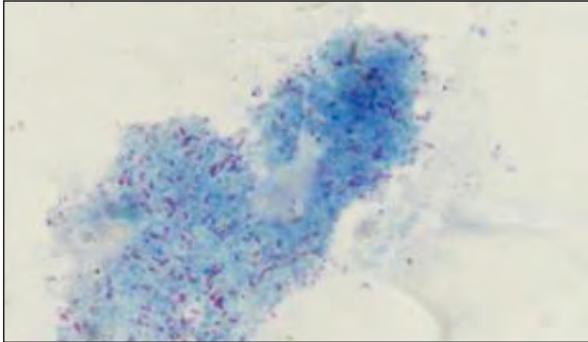
The surveillance of Antimicrobial resistance in bacteria from cattle, buffalo, sheep, goat, pig and poultry was carried out by collection of milk samples from cattle/buffalo, cloacal swabs from chicken and rectal swabs from sheep and pig from Hisar and Hansi blocks. Milk samples were processed for the isolation of *Staphylococcus aureus*, *Staphylococcus* spp. and *Escherichia coli*. From 49 milk samples, 1 *Escherichia coli*, 16 *S. aureus* and 9 Coagulase Negative *Staphylococcus* (CoNS) were isolated. *Staphylococcus aureus* were confirmed by nuclease gene PCR. Among 9 CoNS staphylococci, *Staphylococcus sciuri*, *S. epidermidis* and *S. chromogenes*, *S. hemolyticus* were identified. From 15 cloacal swabs of chickens, 18 *Escherichia coli*, 1 each *Staph. intermedius* and *Staph. xylosum* were isolated. From 10 rectal swabs of sheep, 9 *E. coli* were isolated. From 24 pig samples, 32 *Escherichia coli* isolates and 2 *Staphylococcus* spp. isolates were isolated. Isolates were subjected to AMR testing by Disc diffusion assay against given panel of antimicrobials. From overall 103 samples, 62 *E. coli*, 16, *S. aureus* and 14 CoNS were isolated and tested against oxymino-cephalosporins and monobactams. *Escherichia coli* isolates from poultry, pigs and sheep were ESBL and ACBL positive. None of the *S. aureus* showed methicillin resistance. All isolates are cryopreserved.

(RK Vaid, Taruna Anand, K Shanmugasundaram and HS Singha)

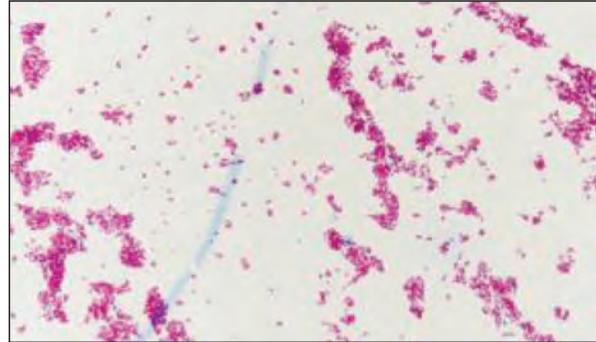


Isolation, characterization and generation of repository of mycobacterial species

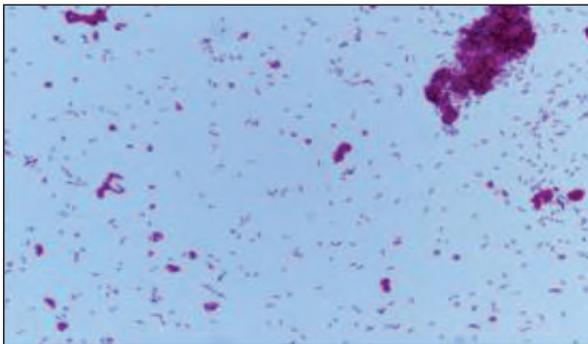
A total of 25 lung samples, 25 intestinal samples, 5 milk samples and a nasal swab were collected for the isolation of mycobacterial species. Decontamination steps for the isolation of *Mycobacterium avium* subspecies *paratuberculosis* from the intestinal samples have been optimized. A concentration of 0.75% HPC for 24 h at room temperature minimizes the bacterial and fungal contaminations. Ten intestinal samples and 10 fecal samples collected from an organized farm were processed for the isolation of *Mycobacterium avium* subspecies *paratuberculosis* and samples are under incubation. MAP has been isolated from sheep intestine after 5 months of incubation and is under subculturing. Acid fast bacilli was isolated from a horse, confirmed by PCR, however, it has been contaminated with fungus during subculture.



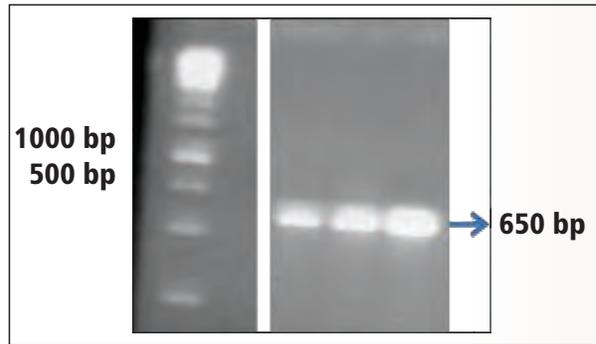
Isolate from sheep Intestine



Sub-cultured MAP from Nodal centre



AFB staining of isolated *Mycobacterium* spp.



PCR amplification of *Mycobacterium* spp.

(Shanmugasundram K., RK Vaid, BC Bera and BN Tripathi)





03

Technology Development, Transfer and Commercialization

Since inception, the Centre has made focused efforts for the development of advanced technologies for improvement in equine health, production and their utilization by the stakeholders and striving for upliftment of equine sector. Many diagnostic kits, vaccines and biologicals developed by the scientists of ICAR-NRCE are being used in the field. Many of the newer technologies are under development, transfer and commercialization.

A. TECHNOLOGY TRANSFER AND COMMERCIALIZATION

a) ELISA for diagnosis of Glanders

ICAR-NRCE has developed a recombinant Hep1 antigen based ELISA as an alternate to Complement-Fixation Test (CFT). The ELISA has been duly validated in India and the OIE Reference Laboratory, Germany and showed excellent sensitivity (97.2%) and specificity (99.6 %). This technology has been transferred to 8 State Disease Diagnostic Laboratories following approval of DADF, Ministry of Agriculture & Farmers' Welfare and commercialized for transformation into ready to use kit. The ELISA has a huge potential of international commercialization as recombinant protein-based ELISA is not available in any other country. This technology will prove to be a milestone in controlling and eradicating Glanders from India.

b) ELISA for diagnosis of Equine Infectious Anaemia

ICAR-NRCE developed recombinant p26 protein-based ELISA as an alternative to Coggin's test for diagnosis of EIA. This technology will provide sustainable and homogeneous source of antigen and harmonized protocol for ensuring regular surveillance of EIA. The kit is highly economical as compared to imported kit.

c) Release of Glanders and Equine Infectious Anaemia Diagnostic ELISA kits

Shri Radha Mohan Singh, Union Minister of Agriculture & Farmers Welfare, released the recombinant ELISA kits (one for Glanders and other for Equine Infectious Anaemia), developed by ICAR-National Research Centre on Equines, Hisar on 9th January, 2019 at Krishi Bhawan, New Delhi. Smt. Krishna Raj, Union Minister of State for Agriculture & Farmers Welfare; Dr. Trilochan Mohapatra, Secretary (DARE) & Director General (ICAR); Dr. J.K. Jena, Deputy Director General (Animal Science); Dr. Ashok Kumar, Assistant Director General (Animal Health); Dr. S. Honnappagol, Animal Husbandry Commissioner and Dr. B.N. Tripathi, Director, ICAR-NRCE were also present in the ceremony.



Release of ELISA kit for EIA



Release of ELISA kit for Glanders

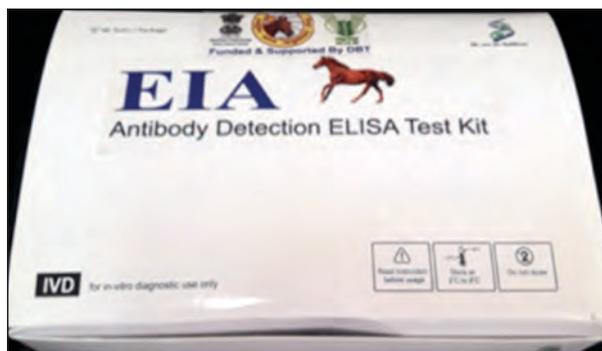


B. TECHNOLOGY DEVELOPMENT & ASSESSMENT

a) Evaluation of Glanders and Equine Infectious Anemia (EIA) ELISA and Rapid Test Kit

The ELISA kits were evaluated under project on 'Scheduling Equines from Fatal Zoonotic Disease- Glanders and Equine Infectious Anemia (EIA) in India using Point of Care Diagnostic (POCD)' funded by Biotechnology Research Assistant Council (BIRAC), Department of Biotechnology, Govt of India. It is an Academic- Industry collaborative project where technology has been transferred to Genomix Molecular Diagnostic Pvt Ltd., Hyderabad for manufacturing ready- to- use diagnostic kits. Diagnostic efficacy, repeatability and reproducibility of these kits were evaluated at ICAR-NRCE and other six laboratories in the country including Central Military Veterinary Laboratory (CMVL), Meerut; Defence Research & Development Establishment (DRDE), Gwalior; State Diagnostic Lab, Ahmedabad; Western Regional Disease Diagnostic Laboratory, Pune; State Disease Diagnostic Lab, Jaipur and State Disease Diagnostic Lab, Shimla. Three batches of Glanders ELISA kit were prepared and internal validation done at different laboratories at ICAR-NRCE. ELISA kit showed 99.3% sensitivity and 99.6% specificity on testing of 152 Glanders positive and 526 Glanders negative samples. Inter-laboratory agreement on panel of blind samples revealed high degree of agreement (97%). Rapid test kit was evaluated using 70 true positive and 30 true negative glanders sera. Specificity and sensitivity of the test were 100% and 80%, respectively. Although, performance of glanders rapid test is in parallel with other rapid test used for onsite diagnosis of various human and animal diseases, it requires improvement to increase test sensitivity to 85-90%.

Three batches of EIA ELISA kit were prepared and internal validation done at different laboratories at ICAR-NRCE. ELISA kit showed 100 % sensitivity and 100% specificity on testing of 15 EIA positive and 450 EIA negative samples. Inter-laboratory agreement on panel of blind samples revealed high degree of agreement (98%).



ELISA kit and Lateral Flow Assay/RDT for Glanders and EIA diagnosis

(HS Singha, Praveen Malik, RK Singh and BN Tripathi)

b) Development of nested and real-time assay for detection of EHV1 latency

In order to diagnose latent EHV1 infection, nested (gB-nPCR) and real-time PCR (gB-qPCR) targeting gB gene were standardized. EHV1 gB-nPCR was developed that amplified 188 bp of glycoprotein B. The assay sensitivity was determined to be 1340 fg or 4.1×10^3 gene copies. For gB-qPCR, standard curve was generated with serial 10-fold dilutions of EHV1/V592 DNA template. The amplification efficiency was calculated to be 87.7%. The sensitivity of the real-time assay was found to be 13.4 fg or 41 copies. Both the assays were found to be specific for EHV1 and did not react with other equine DNA viruses viz. EHV4/Hisar and EAdV/H9. The relative sensitivity and specificity of the gB-nPCR assay for EHV1 latency was estimated by comparing with that of gB-qPCR for detection of EHV1 gB. The sensitivity and specificity of gB-nPCR were 46.66% and 100%, respectively. The findings establish that the real-time PCR is a sensitive and specific assay for ante-mortem detection of EHV1 latency in equine population.

(BR Gulati and Nitin Virmani)

c) Development of recombinant-protein-based iELISA for detection of JEV antibodies

An Indirect ELISA assay was developed using recombinant E protein immunodominant epitope (444 bp) of JEV, for detection of JEV specific antibodies in horse and pig. The recombinant protein produced in bulk, purified and stability was tested and was found to be stable for one year at -20°C . The assay has been transferred to Core Lab-I



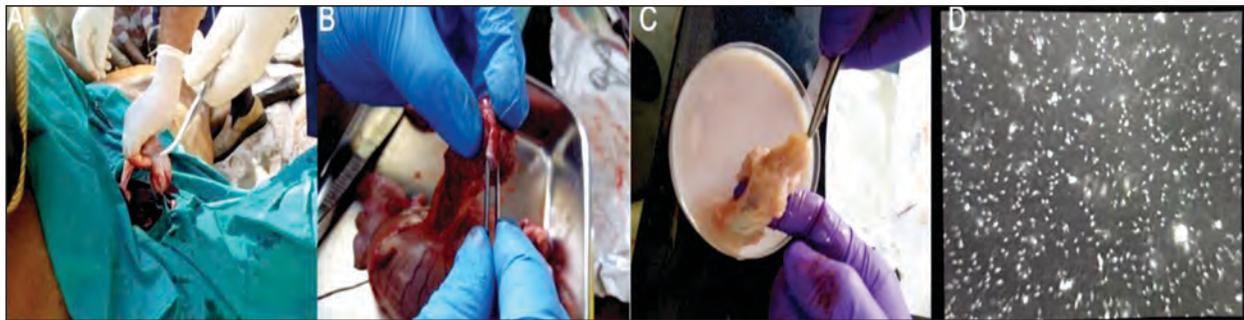
(AAU, Khanapara, Guwahati) and is used for validation on pig serum samples. The results from the Core Laboratory by HI and ELISA indicated that the assay is working satisfactorily.

The ELISA assay was transformed to a diagnostic kit for detection of JEV antibodies in equines. The kit stability has been tested satisfactorily for 6 months. The internal validation of the kit was done from three laboratories and results indicated good agreement. More than 350 samples of horse, donkey and mules and pig have been tested for JEV by ELISA and results have been compared with HI and VNT.

(BR Gulati, Sarika Punia and BN Tripathi)

d) Isolation and cryopreservation of spermatozoa from stallion epididymis

Immediately after gelding, the epididymis from the testis was recovered using microsurgery techniques. From the isolated epididymis, the spermatozoa were successfully recovered either using the retrograde flushing method or floating up technique. The qualitative analysis of the spermatozoa was done using CASA for their morphometry and kinematic properties. Later these spermatozoa were cryopreserved using either Glycerol or DMF as cryoprotectant. The extended epididymal spermatozoa were cryopreserved using INRA and Egg yolk extenders using tradition methods of vapor freezing technique and stored in LN₂. This technique is useful in conserving the elite equine germplasm.



Isolation and cryopreservation of stallion epididymal spermatozoa. a Process of gelding. b. retrograde flushing of epididymis. c. floating up technique for recovery of epididymal spermatozoa. d. CASA analysis of epididymal spermatozoa.

(TR Talluri, Dinesh Jhamb, RA Legha, Jitender Singh and SC Mehta)

e) Prototype of customized Artificial Vagina for semen collection in Stallion

Commercially available artificial vaginas for semen collection of stallions are quite expensive and imported by only authorized firms in India. A prototype of customized artificial vagina for collecting the semen from the stallions was developed using available material in the local market. This AV is being successfully used for routine semen collection at EPC, Bikaner and is proved to be handy and light in weight compared to that of the commercially available AVs.



Prototype of customized AV for stallion semen collection.

(TR Talluri, SK Ravi and RA Legha)

**f) Applications of Donkey milk in terms of Donkey milk based soaps, body butter and lip balms Preparation**

ICAR-NRCE has standardized the formulations and technology for production of Donkey milk based soaps, body butter and lip balms. In view of promotion of donkey milk along with generating awareness among equine farmers and donkey keepers for benefits of donkey milk and getting economic gain from it, ICAR-NRCE made efforts to standardize the formulations and methodology for making Donkey milk soap and body butter for package of practices. The soaps were made by HP and CP methods utilizing the benefits of olive oil, coconut oil, castor oil and essential oils along with other key ingredients. Fresh frozen donkey milk and freeze dried donkey milk was used for making the formulations and soaps have been tested for organoleptic properties, cleansing, and lathering properties. The body butter were made by using freeze dried donkey milk, beeswax, coconut oil, clarified butter oil, Shea butter, vitamin E and aloe vera gel along with essential oils and lip balms were made by using freeze dried donkey milk, coconut oil, clarified butter oil, shea butter, vitamin E and sweet almond oil along with essential oils.



Donkey milk based soaps, body butter and lip balms

(Anuradha Bhardwaj, Yash Pal, Varij Nayan, RA Legha, Hema Tripathi and BN Tripathi)

C. LIST OF TECHNOLOGIES READY FOR TRANSFER / COMMERCIALIZATION

Sr. No.	Name of the Technology
1	Equi Herpes B-ELISA Kit
2	mAb based ELISA kit for diagnosis of rotavirus infection in equines
3	Recombinant antigen based ELISA kit for diagnosis of <i>Theileria equi</i>
4	Updated Equine Influenza Vaccine
5	Equine Herpesvirus-1 vaccine (Equiherpabort)
6	Pregmare kit for pregnancy diagnosis in mares
7	Cryopreservation of equine semen
8	Recombinant protein based ELISA for diagnosis of Glanders
9	Recombinant protein based ELISA for diagnosis of EIA
10	LFA for diagnosis of Equine Piroplasmiasis in equines
11	Recombinant glycoprotein G based type specific ELISA for differentiation of EHV-1 & 4 infections in equines
12	A multiplex PCR targeting glycoprotein G for differentiation of EHV-1 and 4
13	Recombinant protein based ELISA for detection of Trypanosomiasis in animals
14	Improved saddle and harness for working equines
15	Prototype of customized artificial vagina for semen collection in stallion
16	RT-PCR for Equine Influenza diagnosis and typing
17	Japanese Encephalitis Virus Antibody Test Kit- iELISA for equids and pigs
18	Monoclonal antibody-based Sandwich ELISA for detection of Equine Influenza (H3N8) antigen
19	Parentage Analysis in horses
20	LFA for pregnancy diagnosis in mares



D. LIST OF PATENT APPLICATIONS FILED

Sr. No.	Title of Patent	Application No.	Responsible Partners
1	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	DRDE, Gwalior and ICAR-NRCE, Hisar
2	Polynucleotide sequence, processes, composition and methods thereof-	Application No. 1575/CHE/2010, Dated 08.06.2010 and PCT/IB 2011/052475	IISc, Bangalore and ICAR-NRCE, Hisar
3	Nano-drug delivery for quinapyramine sulphate	Application, No. 2560/DEL/2011, Dated 06.09.2011	ICAR-NRCE, Hisar and GJUS &T, Hisar
4	A highly sensitive kit for detection of antibodies against <i>Theileria equi</i> in serum of equids.	Application No. 2763/DEL/2012, Dated 06.09.2012	ICAR-NRCE, Hisar
5	Recombinant TssA protein for detection of antibodies against <i>Burkholderia mallei</i> and uses thereof.	Application No.3610/DEL/2015, Dated 04.11.2015	DRDO and ICAR-NRCE, Hisar
6	Recombinant Hcp1 protein for detection of antibodies against <i>Burkholderia mallei</i> in Equines	Application No.4120/DEL/2015, Dated 15.12.2015	DRDO and ICAR-NRCE, Hisar
7	<i>Aerva javanica</i> extract for the treatment of exuberant granulation tissue and tumors in horses.	Application No.201811048899, Dated 24.12.2018	ICAR- NRCE, Hisar
8	Polymeric metal nanocomposites and methods of synthesis thereof	Application No.201911009696, dated 13.03.2019	ICAR- NRCE, Hisar.

E. LIST OF PATENTS GRANTED

- a) A method for preparation of a diagnostic kit useful in forecasting Equine Herpesvirus-1 disease (Patent has been notified on 25.10.2003 and classified as 55E4-1891278).
- b) A method for preparing complement fixation test based (Cofeb) kit for diagnosis of *Babesia equi* infection of equines (Patent has been granted, 31.07.2009 and Patent No.196690). Later on, patent was abandoned due to poor commercial viability in the present context.
- c) A recombinant haemagglutinin domain-containing protein for the detection and diagnosis of glanders and method of preparation thereof (Patent No. 296824, dated 16.05.2018, Application No.1328/DEL/2010 dated 08.06.2010)
- d) Nano-drug delivery for quinapyramine sulphate (Patent No. 310429, dated 29.03.2019, Application No.2560/DEL/2011 dated 06.09.2011).

F. REVENUE GENERATION UNDER DIAGNOSTIC SERVICES AND CONSULTANCY

During the year 2019, contractual diagnosis services were offered to stakeholders of the country. A total 8653 samples received from race courses, turf club, stud farm, riding schools and other organized sector, were tested on payment basis. Overall 3444 and 4662 serum samples were tested for EIA and glanders, respectively. All the samples tested were negative for EIA and Glanders. Among others, 208 swab samples from Animal Quarantine & Certification Services (AQCS) tested for Contagious Equine Metritis (CEM) were negative. Similarly, 70 samples were tested for Equine Viral Arteritis (EVA), 83 samples each for African Horse Sickness (AHS), and Dourine were found negative. Revenue of about Rs 59.589 lakhs was generated through contractual diagnostic services.

**Samples tested and revenue generation through contractual diagnostic services during 2019**

Disease Diagnostics	No. of samples tested	Amount (in Rs)
EIA	3444	1894200.00
Glanders	4662	3263400.00
EHV-1	3	6000.00
<i>Trypanosoma evansi</i>	2	1100.00
EI	7	3850.00
Dourine	83	91300.00
Equine Piroplasmosis	44	88000.00
AHS	83	91300.00
EVA	70	140000.00
CEM	208	332800.00
Culture Isolation	47	47000.00
Total sample	8653	5958950.00



04

Education and Trainings

ICAR-NRCE encourages its staff for capacity building in advanced scientific field, administrative updating and personality development. This year following scientists, technical and administrative staff were trained as detailed below:

Name of trainees	Designation	Training programme (Title) attended	Organizing Institute	Duration (Days)
Scientists				
Dr Sanjay Kumar	Pr. Scientist	National Workshop on Computation for Biomedicine and Healthcare	IIIT-Delhi	5
Dr Taruna Anand	Sr. Scientist	Genome Manipulations, Editing and Interference by VIGS, CRISPR and RNAi	GJU S&T, Hisar	10
Dr B.C. Bera	Sr. Scientist	Genome Manipulations, Editing and Interference by VIGS, CRISPR and RNAi	GJU S&T, Hisar	10
Dr B.C. Bera	Sr. Scientist	Training of ISO 17025 at BIS Noida	BIS, Noida	4
Technical				
Sh Sanjeev Kumar	STO	Motivation, Positive Thinking and Communication Skill for Technical Officer of ICAR	ICAR-NAARM, Hyderabad	7
Sh Joginder Singh	TO	Motivation, Positive Thinking and Communication Skill for Technical Officer of ICAR	ICAR-NAARM, Hyderabad	7
Sh Raghubir Singh	Sr Technician	Auto mobile maintenance road safety and behavioural skills CIAE, Bhopal	CIAE, Bhopal	7
Sh Gopal Nath	Technician	Auto mobile maintenance road safety and behavioural skills CIAE, Bhopal	CIAE, Bhopal	7
Administrative Staff				
Smt. Shammi Tyagi	AF & AO	Goods and Service Tax	NIFM, Faridabad	3
Sh. Subhash Chander	Assistant	Establishment and Financial Matter for Asst./AAO/JAO/AF&AO/F&AO/ Section Officer of ICAR	ICAR-NAARM, Hyderabad	6
Sh. Subhash Chander	Assistant	Goods and Service Tax	NIFM, Faridabad	3
Sh. Ram Pal	AAO	Goods and Service Tax	NIFM, Faridabad	3
Sh. Sunil	Assistant	Establishment and Financial Matter for Asst./AAO/JAO/AF&AO/F&AO/ Section Officer of ICAR	ICAR-NAARM, Hyderabad	6



Sh. Sunil	Assistant	Goods and Service Tax	NIFM, Faridabad	3
Sh. Om Parkash	UDC	Goods and Service Tax	NIFM, Faridabad	3
Sh. Ashok Arora	PA	Enhancing Efficiency and behavioural skills of stenographers Grade-III, PA,PS,PPS and Sr. PPS of ICAR	ICAR-NAARM, Hyderabad	6
Sh. Dinesh Dutt Sharma	UDC	Goods and Service Tax	NIFM, Faridabad	3

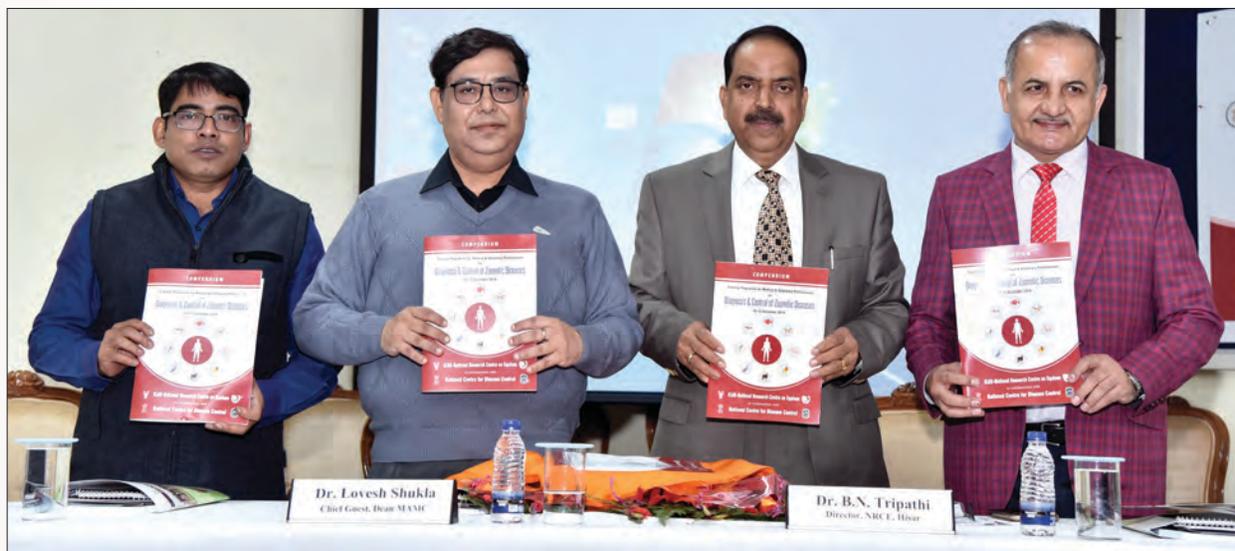
Training of medical and veterinary professionals on zoonosis

Due to rapid rise in population and urbanization, human beings live in close contact with animals and therefore get the exposure to diseases transmitted from animals to humans called 'Zoonotic Diseases'. The important zoonotic diseases of public health importance occurring in India are Anthrax, Scrub typhus, Brucellosis, Kyasanur Forest Disease, Crimean-Congo haemorrhagic fever, Rabies, Nipah virus, Avian Influenza, buffalopox, Japanese encephalitis, Glanders, Leptospirosis, Cysticercosis, Hydatid disease, Trypanosomiasis and toxoplasmosis.

For prevention and control of zoonotic diseases, National Centre for Disease Control (NCDC), New Delhi under the aegis of Department of Health and Family Welfare, Ministry of Health and Family Welfare, Govt. of India has initiated a new scheme "Strengthening of intersectoral coordination for prevention and control of zoonotic diseases" to facilitate sharing of disease information within stakeholders for taking appropriate actions and development of Laboratory capacity for diagnosis of Zoonotic diseases, and creating awareness among health and veterinary professionals about zoonotic diseases of public health importance. ICAR-National Research Centre on Equines (NRCE), Hisar has been recognized as one of the Regional Coordinators and has been given the responsibility to liaison with Medical and Veterinary Professionals of this region for reporting and sharing of zoonotic disease outbreak information between animal and public health sectors, capacity building and creating awareness among health and veterinary professionals about Zoonotic Diseases of Public Health Importance.

Activities conducted in this programme included Orientation Workshop and Training of Medical and Veterinary Professionals on Zoonotic Diseases; Surveillance of Zoonotic Japanese encephalitis and Glanders; and development of information, education and communication (IEC) materials for creating awareness on zoonotic diseases.

Under this programme, ICAR-NRCE organized a workshop-cum-orientation training program on 18 September, 2019 for medical and veterinary professionals in the region to combat zoonotic diseases. Dr Baldev Gulati and Dr Naveen Kumar, Organizing Secretaries of the Workshop informed that 37 doctors (17 medical and 20 Veterinary doctors) from various Districts of Haryana and Rajasthan participated in the workshop. In addition, faculty members from medical and veterinary colleges and scientists from disease diagnostic laboratories also participated in the workshop.



Release of compendium during workshop for Medical and Veterinary Professionals



In order to upgrade diagnostic capacity of laboratory professionals of the region for zoonotic diseases, 3 days "Training Programme for Medical and Veterinary Professionals on Diagnosis and Control of Zoonotic Diseases" was organized from 10-12 December, 2019. The training programme included expert lectures on prevention and control of zoonotic diseases and hands-on-training on diagnosis of zoonotic diseases, viz, Glanders, Japanese Encephalitis and Pox Viruses. In this training programme, 37 professionals, including 20 medical doctors, 14 veterinary doctors and 3 senior laboratory technicians involved in laboratory diagnosis from different states of India took part.



Participants in hands-on-training for Medical and Veterinary Professionals on Zoonotic Diseases

Training of Veterinary Officers on Glanders Diagnosis

As part of capacity building and strengthening of state diagnostic lab on glanders surveillance, ICAR-NRCE conducted a 'Hands on Training on Glanders Diagnosis' from 13-15 November, 2019. In this training program 10 Veterinary Officers from three state disease diagnostic laboratories namely Bihar, Chhattisgarh and Madhya Pradesh have been imparted hands-on training on glanders diagnosis. They were trained in recombinant Hcp1 ELISA, Lateral Flow Assay (rapid test) and Complement Fixation Test. After successful completion of training glanders ELISA was provided to these three states for carrying out glanders surveillance. State DI labs have been instructed to submit all record of sample and ELISA data to NRCE. ELISA positive samples shall be further tested by CFT at NRCE for confirmation of glanders. In addition, participants also visited various laboratories of the NCRE and NCVTC and interacted with scientists. Dr B.N. Tripathi, was the Chairman, Dr Harisankar Singha and Dr Shanmugasundaram were organizing secretaries of the training.



Veterinary officers attended Glanders Training



Post Graduate Student's Research and Guidance

Sr. No.	Student Name	Name of the Guide/Co-guide	Title of the Thesis
Ph.D Students			
1	Ms Medhavi Vashiasth, CCS, HAU, Hisar	Dr. Taruna Anand	Characterization of bacteriophages against ESKAPE pathogen and assessment of their synergy with antibiotics.
2	Ms. Sushma, CCS, HAU, Hisar	Dr. BR Gulati	Immunological based detection methods of stored mites and their damage potential in equine feed.
3	Mr. Deepak Verma, CCS, HAU, Hisar	Dr. BR Gulati	PCR based detection methods of stored mites and their damage potential in cattle feed.
4	Dr. VK Pal, ANDUAT, Ayodhya	Dr. Rajendar Kumar	Molecular characterization and sero-prevalence studies on equine protozoan diseases with special reference to <i>Trypanosoma evansi</i> .
5	Dr. Snehil Gupta, LUVAS, Hisar	Dr. Rajendar Kumar	Screening, identification and evaluation of some novel target specific therapeutic compounds against <i>Trypanosoma evansi</i> .
6	Mr. Yogesh Chander, GJUS & T, Hisar	Dr. Sanjay Barua	Role of p38 MAP kinase in buffalopox virus replication.
7	Dr. Nitin Khandelwal, GLA, Mathura	Dr. Naveen Kumar	Studies on the antiviral activity of Apigenin against buffalopox virus.
8	Dr Ram Kumar, RAJUVAS, Bikaner	Dr. Naveen Kumar	Studies on the role of ROCK signaling pathway in Buffalopox virus replication.
9	Dr. Dinesh Jhamb, CVSC, Navania, Vallabh Nagar, RJUVAS, Bikaner	Dr. TR Talluri	Effect of L-arginine and Trehalose supplementation to the semen extender on quality and fertility of cryopreserved stallion semen.
MVSc/MSc			
1	Ms Dharvi Chhabra, LPU, Phagwara.	Dr. Balvinder Kumar	Molecular diversity of Indian isolates of <i>Streptococcus equi</i> .
2	Ms Parbha Kumari, LPU, Phagwara	Dr. Anuradha Bhardwaj	Determination of Antioxidant Activity of Indigenous and Poitu Donkey Milk and Donkey Milk Powder.
3	Dr. Sanjeev Kumar, RAJUVAS, Bikaner	Dr. SC Mehta	Study of genetic variation in interleukin-6 (IL-6) and interleukin-8 (IL-8) genes among equine species.
4	Ms. Priyanka, GJUS&T, Hisar	Dr. Taruna Anand	Isolation and characterization of host specific bacteriophages against <i>Pseudomonas</i> spp.



MoU for cooperation in Research and Education

The ICAR-NRCE, Hisar inked the Memorandum of Understanding with the Chaudhary Charan Singh Haryana Agricultural University, Hisar; Bihar Animal Sciences University, Patna, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya; Lovely Professional University, Phagwara (Punjab) and Guru Jambheshwar University of Science and Technology, Hisar for the cooperation in the areas of Research and Education. Dr. BN Tripathi, Director, ICAR-NRCE, Hisar and Vice Chancellors of the Universities signed the Memoranda.

The NRCE and Universities have agreed for collaborative programs in the fields of research, education, training and capacity building, extension, consultancy and other areas of national interest. Both the partners have also agreed for mutually recognizing the faculty of both the institutes for the research and teaching purposes, wherein, the students and faculties can carry out the specific, research and outreach activities at the laboratories of these institutions.

Sr. No.	University	MoU Date
1	CCS Haryana Agriculture University, Hisar (Haryana)	05.01.2019
2	Bihar Animal Sciences University, Patna (Bihar)	07.06.2019
3	Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.)	26.08.2019
4	Lovely Professional University, Phagwara (Punjab)	26.11.2019



Orientation of School Students

Capacity Building programme conducted for 200 school students by Samagra Shiksha Abhiyan (SSA), Hisar on 23-24th December and 200 school students by Samagra Shiksha Abhiyan (SSA) Sirsa on 28th December 2019.





Trainings imparted

Training of Veterinary Doctors for Artificial Insemination (AI) in Equines was given under the “Network Approach for Genetic Improvement of Indigenous Horses in the Country”. Overall 15 participants from Punjab, Uttarakhand, MP, Kerala, Rajasthan were imparted training from July 18-20, 2019. Another training was conducted on Aug., 7- 9, 2019.



Release of compendium of AI Training from 18-20 July, 2019



Hands-on training on AI in Equines



Participants attended the training on AI



Release of compendium of training on AI from 7- 9 August, 2019



05

Workshops, Seminar and Institutional Activities

Organized National Workshop on “Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety”

National workshop was organized on “Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety” in collaboration with The Indian Council of Medical Research (ICMR) during 29th to 31st July, 2019. A total of 28 participants from 7 states (Assam, Karnataka, Tamil Nadu, Maharashtra, Haryana, Punjab, & Delhi) participated in the workshop. A total of ten experts of medical and veterinary backgrounds delivered lectures on various aspects of phage research and their applications. Hands on training sessions were conducted to provide practical exposure to all the participants.



Release of workshop manual

Organized Workshop on “Strengthening Intersectoral Coordination for Prevention and Control of Zoonotic Diseases”

One day Workshop-cum-orientation program was organized on “Strengthening Inter-sectoral Coordination for Prevention and Control of Zoonotic Diseases” at ICAR-NRCE, Hisar in collaboration with National Centre for Disease Control (NCDC), New Delhi on 18 September, 2019.



Joint Orientation workshop for Medical and Veterinary Professionals



Symposium organized on advances in Equine Health Management

ICAR-NRCE organised symposium on “Advances in Equine Health and Management” in collaboration with Indian Association of Veterinary Microbiologists, Immunologists and specialists on Infectious Diseases, on February 27, 2019.

ICAR-NRCE celebrated Foundation Day

ICAR-National Research Centre on Equines, Hisar celebrated the glorious 35th Foundation Day on 26 November, 2019. ICAR-NRCE is the sole research centre exclusively caters-to the well-being of the equine sector of the country. The function was organized enthusiastically by the Chairman-Dr. Nitin Virmani, Pr. Scientist and Co-Chairman- Dr. R.K. Vaid, Pr. Scientist of the institute through arranging lectures on burning issues and Kishan-gosthi. On this occasion, the Director - Dr. B. N. Tripathi highlighted the role played by NRCE during past years towards serving the healthcare and management of the equines to various stakeholders. The eminent dignitaries adorned the function included Prof. Rajendra Kumar Anayath, Hon'ble Vice Chancellor of Deen Bandhu Chhotu Ram University of Science & Technology (DCRUST), Murthal, Sonapat as Chief Guest; Dr. Mehar Chand Gehlot, Vice-Chairman, Livestock Development Board, Govt. of Haryana, Panchkula as Guests of Honour and Dr. S.S. Dahiya, Director, ICAR-Central Institute for Research on Buffaloes (CIRB), Hisar. The renowned Prof. Rajender Kumar Anayath delivered a talk on an important issue of “Significance of values in the Modern Media World” highlighting the precious role of the media in technological era. In this occasion, a Farmer-Scientist interface meet was organized at the Centre for discussing the various issues of rearing and healthcare of equines at field condition. The scientists provided solutions to the problems facing by the farmer for quality management of equine health and increasing the equine production.



Celebration of foundation day at ICAR-NRCE

Celebration of foundation day and organization of farmer-scientific meet at Equine Production Campus, Bikaner

The Foundation Day of the Equine Production Campus, ICAR-NRCE, Bikaner was celebrated on September 28, 2019. The program started with the lighting of the lamp by the horse farmers. A workshop was organized on this occasion with horse owners and tourism stakeholders. The issues raised by equine owners were addressed by the scientists of the Campus. A scientific quiz was organized in this workshop. A horse show was organized and prizes were distributed to the winners. On this occasion, a poster on ban on single use plastic was also released by the guests. The Chief Guest of the occasion was Sh.Narayan Chopra, Mayor, Municipal Corporation, Bikaner and Guest of Honour was Dr. (Mrs.) Vimala Dukwal, Dean, PG Studies, SKRAU, Bikaner.



Foundation Day address by the Chief Guest Sh. Narayan Chopra, Mayor, Municipal Corporation, Bikaner



Farmers scientific interactions



Distribution of prizes to the winners of Equine Husbandry Quiz organized during the stake holder meet.

Organized Equine Health Camps

Various equine health camps were organized for farmer welfare and for creating awareness about different technologies for better equine practices among owners. The equine camp was organised at village Degana in district Nagoreaur where . A total of 30 horses were examined in the health camp. An exhibition on Equine Husbandry at farmer's fair was organized on July 5, 2019 at ICAR-NRC on Camel, Bikaner. Another exhibition was organized during Farmer's conference organised by ICAR-CIAH, Bikaner on 30 August, 2019. Shri Kailash Choudhary, honourable MoS Agriculture graced the occasion and interacted with farmers during exhibition.



Equine Health Camp at Degana Nagore



Exhibition on Equine Husbandry at ICAR-CIAH, Bikaner



Interaction with Sh. Kailash Choudhary, honourable MoS Agriculture and Farmer Welfare and Sh. Arjun Ram Meghwal, honourable MoS Water Resources and Parliamentary Affairs during Farmers Conference organised by CIAH, Bikaner



Mera Gaon Mera Gaurav activities by ICAR-NRCE

ICAR-NRCE constituted six teams of scientists for Mera Gaon Mera Gaurav (MGMG) related activities in selected villages. The scientists of the Centre made efforts to coordinate agriculture, animal health related activities and social awareness programme through developing linkages with government officials (Agriculture Development officers, Veterinary officers and Anganwari officials & workers) and local village Panchayats. During the period, scientists made 14 visits and conducted 23 field activities in their adopted villages. The teams organized various scientific activities viz., interface meetings, exhibitions, animal health camps, students programmes, entrepreneurship development at village etc. Pregnancy diagnosis for mares and distribution of medicines were conducted. Animals were treated for infertility, skin infections, wounds, debility, ectoparasites. School students were educated on fundamental rights and duties and hindi essay competition on "Mahatma Gandhi Jayanti was also carried out. Animal Health treatment/advisories were given to needy farmers and interactive meeting with equine farmers were conducted. General awareness were created by apprising the villagers about various Govt programmes viz., clean milk production, value of vaccination, sex ratio, single use plastics, swachha bhara abhiyaan etc.



Discussion with farmers regarding animal related problems and vaccination at Gurana village



NRCE Scientists at Village Dandur, Hisar discussing the general issue and societal problems



Addressing the Udairamsar villagers in Bikaner on the importance of clean milk production

Sr. No.	Name of the activity	No. of activities conducted	No. of farmers participated & benefited
1	Visit to village by teams	14	486 (402 farmers, 68 students and 16 teachers)
2	Interface meeting/ Goshties	13	
3	Training organized	-	
4	Demonstrations conducted	-	
5	Mobile based advisories (No of message)	1	
6	Literature support provided (No)		
7	Awareness created (No)	15	
8	Other, if any		
	Total	43	486 (402 farmers, 68 students and 16 teachers)
	Linkages developed with other agencies	9	

Swachhta Abhiyan activities at ICAR-NRCE

Swachhta Abhiyan activities were carried out in the Centre. Various related activities including Shramdaan for general swachhata, awareness generation in school, general public awareness in the villages and farmers, awareness on ban on single plastic use and general cleaning activities in the ICAR-NRCE, Hisar and EPC, Bikaner were carried out between September 11, 2019 and October 2, 2019. Under this activity, importance of Swachhata in the day-to-day life and its benefits have been briefed by our Director, Dr B N Tripathi. At EPC, Bikaner, Shramdaan for general swachhata programme was conducted at Sujandesar village, by team of scientists from EPC, Bikaner. A lecture on "Single Use of Plastic, effects on environment and solutions" was delivered at Govt. High School, Kajla, Hisar on 21 September, 2019 under "Swachhta Hi Sewa" by Dr Yash Pal, Principal Scientist, NRCE. Awareness on ban of single use plastics were created among the staff members of the both campus and residential colonies located in the NRCE, Hisar.



Account Officer, Smt Shammi Tyagi had briefed about the issues with single use plastic and requested to all the participant to avoid use of single plastic. All the staff members were actively participated in the events.



Organized *Swachhta Hi Sewa* at ICAR-NRCE

A swacchata programme was conducted at Sujandesar village, Bikaner by EPC, MGGM team on 30.09.2019. Places around the village and temple premises were cleaned by the team and awareness was created against ban on single use plastic. A general Swacchata/cleaning drive was conducted at the premises of Main building, Guest house and in front of New Museum (under construction) and other surrounding areas at EPC Bikaner. All the staff of the campus participated and cleaned the premises. A general swacchata programme was organised and cleaned the premises of Main building and other surrounding areas on 02.10.2019. All the staff have participated with zeal and enthusiasm.



Cleanliness drive at the campus



Awareness programme against Plastic use

Celebration of Birth Anniversary of father of our nation "*Mahatma Gandhi*"

The birth anniversary of father of our nation - Mahatma Gandhi was celebrated at the Centre. During this program Director, Dr B N Tripathi briefed about the importance of *Swacchata* and *Mahatma* Gandhi's vision and mission on *Swacchata*. In addition, he briefed about Mahatma Gandhi's role and journey in India's independence and requested to follow his foot prints for the betterment of human life and peace. Scientist and administrative staff were also briefed about the importance of *Swacchata* and dreams of Gandhi on general cleanliness, removal of untouchability, swaraj and humanity among people.



Celebration of birth anniversary of Mahatma Gandhi Jayanti



Equine Health Camp

International Yoga Day celebrated at ICAR-NRCE

International Yoga Day was celebrated at ICAR-NRCE, Hisar at the lawns of the Centre on 21st June, 2019. The Staff members of the Centre actively participated in Yoga Day celebrations. Yoga activities were conducted as per Common Yoga Protocol by Yoga expert, Sh. Sukhbir Singh from Pattanjali Yog Samiti, Hisar. He explained the benefit of each yoga *asnas* for the health. Congratulating staff members on the occasion of 5th International Yoga Day, Dr. B. N. Tripathi, Director, NRCE emphasized the importance of Yoga for improving health and productivity of the employees. He encouraged staff members to regularly organize such activities at the NRCE Campus.



Yoga activities at ICAR-NRCE, Hisar



06

IRC, RAC, IMC and Scientific Review Meetings

Annual Institute Research Committee (IRC) Meeting

The annual meeting of Institute Research Committee (IRC) was held under the chairmanship of Dr BN Tripathi, Director, ICAR-NRCE during 22-23 April, 2019 at NRCE, Hisar campus and on May 21, 2019 at Equine Production Campus, Bikaner for appraisal of the research achievements of the ongoing research projects and also to consider new research project proposals. A total of 36 research projects (24 institutional and 12 externally funded) and two new concept notes were discussed in the meeting.

The Chairman in his opening remarks apprised that research activities in the institute were satisfying; however, there is scope for further improvement. The chairman opined that 30-40% of research projects in an institute should be basic type. The chairman encouraged the scientists to take a challenging research project and to do some innovative research through intra and inter-institutional collaboration. He also urged the scientists to publish their research papers in high impact factor (>5) international journals. He expressed satisfaction over the number of technologies released from the institute. The Chairman suggested that more efforts need to be made for commercialization of technologies already available. The scientists were asked to participate in extension activities and MGMG programs of the Centre.



IRC meeting of ICAR-NRCE in progress

Half Yearly Institute Research Committee (IRC) Meeting

Half yearly IRC meeting of ICAR-NRCE was held during 30-31 October, 2019 under the chairmanship of Dr BN Tripathi, Director, ICAR-NRCE, Hisar. In this meeting, 26 research projects from NRCE/NCVTC including 10 externally funded projects were discussed. Besides these projects, three concept notes were also presented and discussed in the meeting. The Chairman in his opening remarks asked all the scientists to discuss about research activities and also to raise any issues that they are facing in their research. He further asked the scientists to submit all the reports including monthly and weekly progress reports on time. He encouraged that the scientists should always work for the betterment of the institute and also to diversify the areas of their research to open new avenues. The RPPs of the research projects should be submitted within the time schedule fixed in ISO documents.

Research Advisory Committee (RAC) meeting of ICAR-NRCE

The 22nd RAC meeting of ICAR- National Research Centre on Equines was held under the Chairmanship of Dr MP Yadav (Former Vice Chancellor & Director IVRI) during 11-12 February, 2019 to review the research achievements of the ongoing research projects for the year 2017-18 and also to consider new research project proposals. Dr BN Tripathi, Director ICAR-NRCE delivered a brief presentation on the overall achievement of the Centre for the year 2017-18 which was followed by presentations on equine health, equine production and NCVTC.



RAC interacting with ICAR-NRCE scientists



The key recommendations of the committee included:

- I. Establishment of a jenny dairy unit at the Centre.
- II. Evaluation of donkey milk constituents for medicinal, microbiological and nutraceutical properties.
- III. Assessment of the impact of disease diagnostics and technologies developed by NRCE at field level.
- IV. Initiating the processes for achieving disease free status for Equine infectious anemia.
- V. More emphasis on basic research and bacteriophage research .
- VI. Establishment of nucleus units of Bhutia, Spiti, Kachchhi-Sindhi breeds of horses for their conservation.
- VII. Regular meetings with stakeholders for popularization of equine keeping and dissemination of technologies developed by ICAR-NRCE.

In his concluding remarks chairman urged the scientists to work hard for the upliftment equine sector in the country and also to explore the areas of value addition technology so that equine keeping will be more remunerative and attractive to the farmers.

Quinquennial Review Team (QRT) meetings

A Quinquennial Review Team (QRT) was constituted by the Secretary DARE & Director General, ICAR under the chairmanship of Maj Gen (Dr.) Shri Kant former Vice-Chancellor, LUVAS, Hisar to review the work done by the ICAR-NRCE, Hisar for the period from April, 2013 to March, 2018. The QRT constituted by the Council had members from diversified spectrum of Veterinary and Animal Science Education and Research, Economics and Management. The Chairman held preliminary discussion with DDG (AS) before review meetings.



QRT meeting of ICAR-NRCE in progress

The QRT held five meetings to review the progress of research work at ICAR-NRCE Hisar, NCVTC and EPC Bikaner. In addition, a meeting with stakeholders was held at Directorate General RVS, IHQ of MOD (Army) at RK Puram New Delhi in which DG RVS, Addl DG RVS, Director RVS (Tech), Commissioner Animal Husbandry (GoI), ADG (AH) ICAR, representatives of Race Club, Brooke India and chairman, members of the QRT participated to discuss the expectations of the stakeholders.

Institute Management Committee (IMC) Meeting

The 39th meeting of the IMC of ICAR-NRCE was held on 19th March, 2019 under the Chairmanship of Dr BN Tripathi, Director, NRCE, Hisar. Mrs Shammi Tyagi, FAO, CIRB was special invitee to the meeting. Member Secretary presented the agenda items and the following recommendations were given by the committee:

- I. Engagement of AMA (Part-Time Doctor) for medical treatment of employees and their dependents.
- II. Repair/Repainting of first phase of NCVTC Building.
- III. Re-carpeting of Roads at EPC, Bikaner.
- IV. Opening of Equine Clinics at ICAR-NRCE, Hisar & EPC, Bikaner.
- V. Establishment of a Jenny Dairy Unit (Donkey Nucleus herd), at ICAR-NRCE, Hisar.
- VI. Condemnation of old vehicles and writing off losses of damaged library publications.



07

Visit of Dignitaries

Shri Giriraj Singh, Hon'ble Minister of Fisheries, Animal Husbandry and Dairying of India and Dr. Sanjeev Kumar Balyan, Hon'ble Minister of State of Fisheries, Animal Husbandry and Dairying visited the Centre on 16 August, 2019. During their visit, they interacted with scientific, technical and administrative staff of the Centre. The ministers advised the NRCE family to work for the betterment of poor people in the country. Ministers expressed their interest in the research on donkeys and some of the donkey milk-based products developed at ICAR-NRCE (soaps, body butter and lip balms) and the products were released during the visit.



Sh. Giriraj Singh, Hon'ble Minister of Fisheries, Animal Husbandry visiting NRCE Campus

Dr. Parveen Malik, Director, NAIH, Bhagpat (UP) visited the Centre on 28 January, 2019. During his visit he interacted with scientist of the Centre and highlighted the need for commercialization of vaccines & diagnostics developed by the Centre.

Dr. SPS Ahlawat, Former Vice-Chancellor, Vikram University, Ujjan; Director cum Vice-Chancellor IVRI, Izatnagar; Director, NBAGR, Karnal and Director CARI, Port Blair visited the Bikaner campus. He visited the equine museum, farm and different laboratories of ICAR-NRCE, Bikaner Campus.



Dr SPS Ahlawat visiting the Equine Museum of the Bikaner, Campus.

Quinquennial Review Team (QRT) under the Chairmanship of Major General Shri Kant visited the ICAR-NRCE Main campus on 10 May, 2019 and the Bikaner Campus on 19 May, 2019. The members of QRT visited various laboratories, museum, farm area, farm animals, and dispensary and also interacted with scientists and technical officers. A meeting with equine stakeholders was also arranged on this occasion at ICAR-NRCE, Bikaner Campus and the members of QRT interacted with them.



Interaction of QRT Chairman and Members with scientists and technical officers during farm visit at EPC, Bikaner.

Prof. Vishnu Sharma, Vice-Chancellor, RAJUV AS, Bikaner visited the Campus on 18 July, 2019 and inaugurated the training of Veterinary Officers on



"Artificial Insemination in Equines" under the Network Approach for Genetic Improvement of Indigenous Horses.

Prof. MP Yadav, former Vice-Chancellor, SVPDAT, Meerut and Director, IVRI, Izatnagar visited ICAR-NRCE on 12 February, 2019. He motivated the scientists to work in collaborative mode and appreciated the research achievements and technologies generated by the ICAR-NRCE scientists.

Prof. PK Uppal, Former Director, ICAR-NRCE, Hisar visited the Centre on 12 February, 2019. He appreciated the efforts of ICAR-NRCE to address the issues related to equine stakeholders and congratulated scientists for their research attainments.

Prof AK Mishra, Honorable Chairman ASRB, New Delhi visited ICAR-NRCE, Bikaner Campus on 8 August, 2019. Upon his visit he interacted with scientists of the campus and also visited the equine farm.

Prof. RP Singh, Vice-Chancellor, SKRAU, Bikaner visited the Campus on 20 December, 2019. During his visit, he addressed the staff on the occasion of Hindi workshop and also participated in plantation in Herbal Garden.



Felicitation of Prof. Vishnu Sharma, Vice-Chancellor, RAJUV AS, Bikaner was done by Dr SC Mehta (Officer In-charge, Bikaner campus) on the occasion of inaugural function of training Programme.



Prof. AK Mishra, Chairman, ASRB, New Delhi examining the farm animals



Prof. RP Singh, Vice-chancellor, SKRAU, Bikaner addressing the Campus staff on the occasion of Hindi workshop.



08

Infrastructure and Developmental Activities

Accreditation of ISO:17025-2005 to Equine Piroplasmosis lab of ICAR- NRCE

Equine piroplasmosis laboratory at ICAR-NRCE was accredited as per ISO:17025-2005. The scope of the accreditation includes – diagnosis of the bio-samples with ELISA, cELISA and IFAT for *Theileria equi* and *Babesia caballi* infection.

ICAR-NRCE re-certified for ISO 9001: 2015

In an effort towards continuous quality improvement, a surveillance audit was conducted for re-certification of ICAR-NRCE for ISO9001:2015 certification in recognition of its Quality Management System in the area of 'Research and Development for Improving Equine Productivity, Disease Diagnosis and Microbial Conservation'. The institute awarded with certification of ISO 9001: 2015 for quality management system and the certificate is valid till 10 March, 2020.



Equine herd strength at ICAR-NRCE, Hisar compus during 2019

Sr. No.	Kind of Animal	O.B. as on 01.01.2019	Addition		Disposal		C.B. as on 31.12.2019
			Birth	From EPC	Death	To EPC	
(A) Horses							
1	Stallion	01	-	-	-	-	02
2	Mare	10	-	-	-	03	07
3	Colt	03	01	-	-	-	03
4	Filly	01	01	-	-	-	02
	Total	15	02	-	-	03	14
(B) Ponies							
1	Mare	01	-	04	01	-	04
(C) Donkey							
1	Stallion	03	-	-	-	02	01
2	Mare	02	-	03	01	-	04
3	Colt	-	01	-	-	-	01
4	Filly	-	01	-	-	-	01
	Total	05	02	03	01	02	07
	Grand Total	21	04	07	02	05	25



Equine herd strength at EPC, Bikaner during 2019

Category	HORSES				PONIES				DONKEYS				MULES		TOTAL
	Marwari		Kathiawari		Zanskari		Manipuri		Exotic		Indigenous		M	F	
	M	F	M	F	M	F	M	F	M	F	M	F			
Stock as on 01.01.2019	18	32	01	04	07	12	07	08	14	18	07	09	04	01	142
Birth	04	05	-	-	-	-	-	-	-	03	-	-	-	-	12
Death	04	-	-	-	-	-	-	-	-	-	-	01	-	-	05
Transferred from Hisar	-	03	-	-	-	-	-	-	02	-	-	-	-	-	05
Transferred to Hisar	-	-	-	-	-	02	-	02	-	-	-	03	-	-	07
Purchased	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auctioned	02	10	-	-	04	03	03	03	02	06	03	-	02	-	38
Balance as on 31.12.2019	16	30	01	04	03	07	04	03	14	15	04	05	02	01	109
G. Total	46		05		10		07		29		09		03		109

Farm Production

Production of crops: - During the period under report, about 125-acre land was used rotationally for growing of different types of crops. In spite of high-water table and salinity in most of the farm area, vigorous efforts were made to produce maximum feed & fodder. A sum of Rs. 28,45,599.00/- (Rs Twenty-eight lakh forty-five thousand five hundred ninety nine only) was generated through sale of surplus farm produce.

Production of Crop at ICAR-NRCE, Hisar

Sr. No.		Area (Acre)	Production (Qt.)
	A) Green Fodder		
1	Oat	10.0	396.5
2	Sorghum sudan grass + Cowpea	} 12.0	547.5
3	Sorghum sudan grass		151.0
	Total Green Fodder	22.0	1095
	B) Grain		
1	Oat Grain	40	278.58
2	Wheat Grain	50	540.90
3	Paddy Grain	9.5	125.00
	Total Grain	99.5	944.48
	C) Dry Fodder	-do-	673.85

Production of Crop at EPC, Bikaner

Sr. No.	Crops	Area in Acre	Production (Qtl.)		
			Green Fodder	Dry Fodder	Grain
1	Oats	10	935.45	0	0
2	Lucerne	5	338.40	0	0
3	Sevan	8	27.05	0	0
4	Jawar	10	88.10	0	0
5	Barley	25	0	302.00	201.00
6	Gawar	40	0	164.00	78.50
	Total	80	1389.00	466.00	279.50



09

Awards, Recognitions and Personal Milestones

Dr Anju Manuja was conferred the "*Panjabrao Deshmukh Outstanding Women Scientist*" award by Indian Council of Agricultural Research, New Delhi for the year 2018-19 for her outstanding research achievements during ICAR Foundation Day and Annual Award Ceremony on 16 July, 2019 at NASC auditorium, New Delhi by the Hon'ble DG, ICAR.



Dr Anju Manuja bestowed with Best Researcher Award in "International Scientist Awards 2019 in Engineering, Science, and Medicine" held at Chennai in September, 2019.

Dr Anuradha Bharadwaj was bestowed with Make in India Award Bharat Nirmaan foundation at IIC-New Delhi on 14th December, 2019.

Dr Anuradha Bhardwaj received Reviewer excellence award from IJAR-ARCC and ASD-ARCC, Karnal, India.

Dr Balvinder Kumar received International Veterinary Vaccinology Network Laboratory Exchange Award by Roslin Institute, University of Edinburgh, United Kingdom.

Dr Naveen Kumar has been appointed as an external member for the University Research Committee at Shri Krishna Ayush University, Kurukshetra, Haryana.

Dr SC Mehta has been elected as Vice-President (2019-2021) of "Society for Conservation of Domestic Animal Biodiversity" in the General Body meeting of the Society for Conservation of Domestic Animal Biodiversity (SOCDAB) held at National Bureau of Animal Genetic Resources, Karnal, Haryana .

Dr TR Talluri (as a co- author) and his team received Best Oral presentation award for their presentation of a paper entitled "*Effect of cholesterol loaded cyclodextrin on oxidative parameter of Poitou jack (Equus asinus) spermatozoa during cooling and cryopreservation*" presented at XXXV Annual Convention of The Indian Society for Study of Animal Reproduction and International Symposium on "Global Perspectives to Enhance Livestock Fertility through Modern Reproductive Techniques for Doubling Farmer's Income" held Department of Veterinary Gynaecology and Obstetrics Veterinary College and Research Institute Namakkal - 637 002, Tamil Nadu, India on 18-20 Dec, 2019.

Dr TR Talluri has been selected as "Associate Member" of National Academy of Veterinary Sciences (India) for his significant contribution in veterinary sciences on 26 December, 2019 at Kamdhenu University, Gujarat.





Dr TR Talluri received reviewer excellence award from Indian Journal of Animal Research-Agriculture Research Communication Centre on 22 October 2019.



Appreciation Certificate and Award on the occasion of Republic Day 2019 for their outstanding performance/contribution to the ICAR-NRCE during the year 2018 (Jan-Dec).

Sr. No.	From NRCE, Hisar campus	
1	Scientific category	Dr. Harisankar Singha, Scientist
2	Technical category (Lab)	Sh. PP Choudhury, STO
3	Technical category (Farm)	Sh. KS Meena, ACTO
4	Driver category	Sh. Raghbir Singh, Tech. Assistant
5	Administrative	Sh. Dinesh Dutt, UDC
6	Supporting category	Sh. Om Parkash, SSS
7	Bilateral Category	Sh. Sajjan Singh, Office Asstt. (ESM)
8	Best MGMT Team	Dr. Yash Pal
		Dr. Anju Manuja
		Dr. Anuradha Bhardwaj
		Dr. Harisankar Singha
9	Best Contractual person	Sh. Deen Dayal
From EPC Bikaner campus		
10	Technical category	Dr. RA Pachori, Sr. Tech. Officer
11	Supporting category	Sh. Ashok Kumar, SSS

Felicitation of staff members on Independence Day 2019 : Considering their contributions following staff were received special appreciation awards.

Sr. No.	NRCE, Hisar	
1	Scientific category	Dr Balvinder Kumar, Principal Scientist Dr Anju Manuja, Principal Scientist Dr Naveen Kumar, Principal Scientist
2	Technical category (Lab)	Sh DD Pandey, Senior Technical Officer
3	Driver category	Sh Suresh Kumar, Technical Officer
4	Administrative	Smt. Shammi Tyagi, F&AO
5	Bilateral Category	Sh Dharampal Singh, Security Guard Sh Dalip Singh, Security Guard



Promotion of Scientific staff

Sr. No.	Name/Designation of the Scientist	Recommendation of the Committee	Date from which recommended for promotion
1	Dr Naveen Kumar, Sr Scientist (Veterinary Microbiology)	Recommended to the next higher grade of Rs. 37400-67000 + RGP of Rs. 10000/-) as Pr. Scientist	19. 07. 2017
2	Dr. Harishankar Singha, Scientist (Biotechnology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	07. 01. 2017
3	Dr. Anuradha Bhardwaj, Scientist (Biotechnology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	07. 01. 2017
4	Dr. Taruna Anand, Scientist (Biotechnology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	07. 01. 2017
5	Dr. B C Bera, Scientist (Biotechnology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	26. 02. 2017
6	Dr. RK Dedar, Scientist (Vety. Medicine)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	08. 01. 2017
7	Dr. Prokasanand Bala, Scientist (Animal Nutrition)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs.8000/- (Revise Level- 12) as Sr. Scientist	08. 01. 2017
8	Dr. TR Talluri, Scientist (Animal Reproduction & Gynaecology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs. 8000/- (Revise Level- 12) as Sr. Scientist	07. 01. 2018
9	Dr. K Shanmugasundaram, Scientist (Vety.Pathology)	Recommended to the next higher grade Rs. 15600-39100 + RGP Rs.7000/-	07. 01. 2013

**Superannuation of NRCE Staff**

Sr.No.	Name of the staff	Category	Designation	Date of Superannuation
1	Sh DD Pandey	Technical	Sr Technical Officer	30 th September, 2019
2	Sh AG Barapatre	Administration	Administrative Officer	30 th November, 2019
3	Dr SC Yadav	Scientist	Pr Scientist	31 st December, 2019

नगर राजभाषा कार्यान्वयन समिति द्वारा सम्मानित

अशु अनुसंधान परिसर को वर्ष 2018-19 में हिन्दी में उत्कृष्ट कार्य करने के लिए नगर के लगभग 25 लघु कार्यालयों में से प्रथम स्थान पर चुना गया एवं नगर राजभाषा कार्यान्वयन समिति द्वारा नगर राजभाषा शील्ड (लघु कार्यालय) प्रदान की गई।



नगर राजभाषा कार्यान्वयन समिति, बीकानेर के अध्यक्ष मण्डल रेल प्रबंधक, बीकानेर श्री संजय श्रीवास्तव से नगर राजभाषा शील्ड (लघु कार्यालय) प्राप्त करने हुए केन्द्र के बीकानेर परिसर के प्रभारी अधिकारी डॉ० शरतचन्द्र मेहता



10

Research Publications

RESEARCH PUBLICATIONS OF ICAR-NRCE DURING THE YEAR-2019

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2. Bera BC, Choudhary M, Anand T, Virmani N, Sundaram K, Choudhary B and Tripathi B.N. 2019. Detection and genetic characterization of porcine circovirus 3 (PCV3) in pigs in India. *Transboundary and Emerging Diseases*, doi: 10.1111/tbed.13463.
3. Bhardwaj A, Kumar S, Nayan V, Sharma P, Pal Y and Yadav SC. 2019. Expression and characterization of recombinant single chain beta-alpha equine chorionic gonadotropin in prokaryotic host. *Indian Journal of Animal Research*, 53 (5): 587-593.
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11. Grace MR, Dhanze H, Pantwane P, Sivakumar M, Gulati, BR and Kumar A. 2019. Latex agglutination test for rapid on-site serodiagnosis of Japanese encephalitis in pigs using recombinant NS1 antigen. *Journal of Vectorborne Diseases*, 56:105-110.



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2. Chaudhary AK, Purohit GN, Mehta JS, Ravi SK and Talluri TR. Serum testosterone profile in Marwari stallions and its relationship with testicular parameters, semen characteristics, reaction time, stallion age, bodyweight, and height. *Reproduction, Fertility and Development*. 2019. 32(2) 198.
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8. Talluri TR, Naresh Selokar, Papor sharma, Ananth Krishna, Ravi SK and Dharmendra Kumar. Interspecies somatic cell nuclear transfer: Hopes and challenges. Mid-Term National Symposium of Indian Society for the Study of Reproduction & Fertility (ISSRF) on "Impact of Lifestyle and Environmental factors on the Reproductive Health and Fertility" held at College of Veterinary Science, SVVU, Tirupati on 16 Oct 2019. 54-64.

BOOKS, BOOK CHAPTERS, TECHNICAL BULLETINS and POPULAR ARTICLES

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3. Anand Taruna, Virmani N, RK Vaid, BC Bera and BN Tripathi. 2019. Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis An update on bacteriophages: Classification, Characterization & Applications. Technical Bulletin of National Workshop on Clinical and Translational Research on Bacteriophages as and Food Safety" 29th to 31 July 2019. 16-20.
4. Anand Taruna, Virmani N, Vaid RK, Bera BC and Tripathi BN. 2019. An update on bacteriophages: Classification, Characterization & Applications. Technical Bulletin of National Workshop on Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety". 29- 31 July. 16-20.
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7. Bera BC, Taruna Anand, Nitin Virmani and Tripathi BN. 2019. CRISPR weaponized phage system as novel living antimicrobial to combat multi-drug-resistant (MDR) bacteria. Technical Bulletin of National Workshop on Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety". 29- 31 July. 66-70.



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9. Chaudhary AK, Purohit GN, Mehta JS, Ravi SK and Talluri TR. 2019. Serum testosterone profile in Marwari stallions and its relationship with testicular parameters, semen characteristics, reaction time, stallion age, bodyweight, and height. *Reproduction, Fertility and Development*. 32(2)198.
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14. J Singh, TR Talluri and BN Tripathi. 2019. Chronic Pyometra in an Arabian Mare and its treatment. Accepted for publication in Special Issue on 'Racing and Working Animals'. *Intas Polivet*.
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16. Kumar R. and Kumar S. 2019. Emerging and important zoonotic parasitic infections in India. Compendium, training Programme for Medical and Veterinary Professionals on Diagnosis & control of Zoonotic Diseases organized by ICAR-NRCE, Hisar in collaboration with NCDC, New Delhi. 10-12 Dec 2019. 34-44.
17. Kumar R. and Kumar S. 2020. How to save horses from surra (*Trypanosoma evansi* infection). Souvenir published by *All India Marwari Horse Society*. 106-108.
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20. Mehta SC, Talluri TR, Singh J. and Tripathi, B.N. 2019. Network approach for genetic improvement of indigenous horses. In : *Network approach for genetic improvement of indigenous horses, Training of veterinary officers on artificial insemination in equines*. Published by Director, ICAR-NRCE, Hisar, August 7-9. 1-21.
21. Nayan V, Bhardwaj A, Lyngdoh EL, Bhatia T, Pawaria S and Jasmer. 2019. Clinical applications of reproductive hormones in animals. In *Buffalo production and performance recording techniques*. Published by ICAR-CIRB, Hisar. 101-107.
22. Virmani N, Pavulraj, S., B.C.Bera, Taruna Anand, R.K.Singh and B.N.Tripathi. 2019. Equine Influenza, Emerging and Transboundary Animal Viruses. Chapter 9, Springer Nature Singapore Pte Ltd.
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24. Virmani N, Anand T, Bera BC and Tripathi BN. 2019. Tripathi Developing Bacteriophages as therapeutics: Animal Models and Formulations for Delivery. Technical Bulletin of National Workshop on Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety". 29- 31 July. 48-51.
25. RA Legha, TR Talluri, RK Dedar and Yash Pal. 2019. Status and conservation of Equine Genetic resources in India. ICAR Sponsored Winter School on *Current status, emerging issue and future scenario regarding conservation of indigenous breeds of livestock* 05 - 25 Nov. 25 – 29.
26. Shanmugasundaram K, Virmani N, Singha HS, Vaid RK, Indu R and Tripathi BN. 2019. Sample collection, processing and dispatch for diagnosis of important Zoonotic diseases. In Training Programme for medical and veterinary professionals on diagnosis and control of Zoonotic diseases. 10-12 Dec. 10-16
27. Singha H, Shanmugasundaram K, Sheetal Saini and Sita Ram. 2019. Serological diagnosis (ELISA, CFT and Rapid Test) of glanders. Compendium for training programme for Medical & Veterinary Professionals on Diagnosis & Control of Zoonotic Diseases organized by NRCE in collaboration with National Centre for Disease Control, New Delhi from 10-12 Dec. 65-68.
28. Singha H, Shanmugasundaram K, Sheetal Saini and Tripathi BN. 2019. Status of glanders in India, diagnosis and control. Compendium for training programme for Medical & Veterinary professionals on Diagnosis & Control of Zoonotic Diseases organized by NRCE in collaboration with National Centre for Disease Control, New Delhi from 10-12. 3-9.
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30. Singha HS, Shanmugasundaram K, Khurana SK and Tripathi BN. 2019. Surveillance of equines and in-contact humans in India for Glanders -a zoonotic and notifiable equine disease. Department of Biotechnology (DBT), Ministry of Science & Technology, SCOPE Complex, Lodhi Road, New Delhi. 18-19. 68-69.
31. Sumant Vyas, GN Purohit and TR Talluri. 2019. Effect of photoperiodicity on reproductive efficiency of equines, "Network approach for genetic improvement of indigenous horses". Training of Veterinary officers on artificial insemination in equines. 18 – 20 July & 7 – 9 Aug 2019. 36 – 44.
32. Talluri TR, Dedar RK, Legha RA and Yash Pal. 2019. Application of assisted reproductive technologies in conservation and optimizing production in livestock. ICAR Sponsored Winter School on *Current status, emerging issue and future scenario regarding conservation of indigenous breeds of livestock* 05 - 25 Nov. 147 – 152.
33. Talluri TR, Legha RA, Yash Pal, Mehta SC and Tripathi BN. 2019. Collection and cryopreservation of stallion semen. "Network approach for genetic improvement of indigenous horses". Training of Veterinary officers on artificial insemination in equines. 18 – 20 July & 7 – 9 Aug. 23 – 30.
34. Talluri TR, Singh J, Legha RA, Yash Pal, Mehta SC, Dedar RK and Tripathi BN. 2019. Pregnancy diagnosis in equines. "Network approach for genetic improvement of indigenous horses". Training of Veterinary officers on artificial insemination in equines. 18 – 20 July & 7 – 9 Aug. 83 – 92.
35. Talluri TR and S K Ravi. 2019. Artificial Insemination in Equines. *Indian Farmer* 6(1): 51-62.
36. Talluri TR and J Singh. 2019. Diagnosis and treatment of endometrial cysts in mares. Accepted for publication in Special Issue on 'Racing and Working Animals'. *Intas Polivet*.
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38. Talluri TR, Naresh Selokar, Papor sharma, Ananth Krishna, SK Ravi and Dharmendra Kumar. 2019. Interspecies somatic cell nuclear transfer: Hopes and challenges. *ISSRF Newsletter*. 23. 21-23.
39. Tripathi BN and Shanmugasundaram K. 2019. Threat from mycobacterial infection in animals and human



- population in India. In Training Programme for medical and veterinary professionals on diagnosis and control of Zoonotic diseases. 10-12 Dec. 45-49.
40. Vaid RK, Shanmugasundaram K and Taruna Anand. 2019. Antimicrobial resistance: Modern approaches in management and role of bacteriophages. Technical Bulletin of National Workshop on Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety". 29- 31 July. 52-59.
 41. Pal Y, Legha RA and Talluri TR. 2019. Current scenario of donkeys in India and their conservation. ICAR Sponsored Winter School on *Current status, emerging issue and future scenario regarding conservation of indigenous breeds of livestock* 05 - 25 Nov. 53 – 56.
 42. Yash Pal, Anuradha Bhardwaj, RA Legha and BN Tripathi (2019). Donkey conservation through donkey dairy farming. In compendium of ICAR Sponsored Winter School on Current Status, Emerging Issues and Future scenario Regarding Conservation of Indigenous Breeds of Livestock organized by Department of LPM, COVAS, RAJUVAS, Bikaner during 5-25 November pp30-33.
 43. Yash Pal and Sanjay Kumar (2019). Pregnancy diagnosis in mares through hormone assays. In compendium of Network Approach for Genetic Improvement of Indigenous Horses for Training of Veterinary Officers on "Artificial Insemination in Equines" during July 18-20 and August 7-9, pp 124-128.

Ritik- A Marwari Stallion
at ICAR-NRCE, EPC, Bikaner





11

Participation, Presentation in Seminars, Conferences and Symposia

PARTICIPATION AND PRESENTATION OF ICAR-NRCE SCIENTISTS IN SEMINARS, CONFERENCES AND SYMPOSIA

1. Anand Taruna delivered a lead paper on "Careful Cocktail designing after assessment of diversity, host specificity and biological characterization of bacteriophages against mastitis causing Staphylococci in bovines", in International Conference on Bacteriophage Research and Antimicrobial Resistance at Vellore Institute of Technology, Vellore, 12-13 December, 2019.
2. Anand Taruna presented a paper on "Careful designing of bacteriophage cocktail for biocontrol of MDR poultry pathogens and preliminary results on the encapsulation strategies for safe delivery in poultry gut, in VIROCON 2020: International Conference on "Evolution of Viruses and Viral Diseases" at Indian National Science Academy, New Delhi, 18-20 February, 2020.
3. Anand Taruna presented invited paper on "Bacteriophage Banks - A unique therapeutic platform providing personalized medicine against emerging infectious agents" in 60th Annual Conference of Association of Microbiologists of India & International Symposium on Microbial Technologies in sustainable development of energy, environment, agriculture and health, Central University of Haryana, Mahendergarh, 15-18 November, 2019.
4. Bhardwaj A presented a paper on "Antioxidative properties of indigenous and exotic donkey milk in comparison to goat, camel and buffalo milk by TAC assay and its entrepreneurship possibilities for cottage enterprises" for Golden Jubilee International Conference on "New Millennia Agriculture- Novel Trends and Future Scenario", CCS-HAU, Hisar, 6-8 November, 2019.
5. Bhardwaj A presented a paper on "Assessment of genetic variants and SNP mining in indigenous horse breeds through advanced bioinformatics approaches" in 25th International Conference (CONIAPS XXV on "Physical and Biological Sciences at Cross-roads: Interdisciplinary Explorations and Exciting Challenges", GJU, Hisar, 29-31 December, 2019.
6. Bhardwaj A presented a paper on "Microsatellite markers based parentage verification in indigenous equine population" in XVI Annual Convention of Society for Conservation of Domestic Animal Biodiversity (SOCDAB) and National Symposium on "Sustainable Management of Livestock and Poultry Diversity for enhancing the Farmers' Income", NBAGR, Karnal, Haryana, 7-8 February, 2019.
7. Bhardwaj A presented a paper on "Studies on major morphometric parameters of Kachchhi-Sindhi horses" in XVI Annual Convention of Society for Conservation of Domestic Animal Biodiversity (SOCDAB) and National Symposium on "Animal Genetic Resources for Food and Social Security", NBAGR, Karnal, 7-8 February, 2019.
8. Bhardwaj A presented invited lecture on "Comparative genomics and Molecular evolution" in two-week Faculty Development Program on "Use of ICT in Life Sciences", School of Basic & Applied Sciences, Maharaja Agrasen University, Baddi (Solan) in association with National Institute of Technology, Patna, 28 November – 07 December, 2019.
9. Bera BC attended National Workshop on "Clinical and Translational Research on Bacteriophages as



- Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety” at National Centre for Veterinary Type Cultures, ICAR-NRC on Equies, Hisar, Haryana from 29-31 July, 2019.
10. Bera BC delivered guest speaker lecture on the topic entitled “CRISPR/Cas System as novel living antimicrobial to combat multi-drug-resistant (MDR) bacteria” in National Workshop on “Clinical and Translational Research on Bacteriophages as Promising Antimicrobial Alternatives for Therapeutics, Prophylaxis and Food Safety” at National Centre for Veterinary Type Cultures, ICAR-NRC on Equies, Hisar, Haryana.
 11. Bera BC presented a paper on “Emergence of porcine circovirus 2 and influenza A viruses in pigs: a retrospective study, in VIROCON 2020: International Conference on “Evolution of Viruses and Viral Diseases” at Indian National Science Academy, New Delhi, 18-20 February, 2020.
 12. H Singha attended Workshop on ‘National animal disease control programme and important emerging diseases of livestock’ organized by Animal Husbandry Department, Govt of Madhya Pradesh at Bhopal on 14.02.2019.
 13. H Singha delivered an expert lecture on ‘New Trends in the diagnosis & control of Glanders in equines with reference to National Glanders Control Programme’ in Workshop on ‘National animal disease control programme and important emerging diseases of livestock’ organized by Animal Husbandry Department, Govt of Madhya Pradesh, Bhopal, 14 February, 2019.
 14. H Singha participated in Global-Bio India Conference 2019 organized by Biotechnology Industrial Research Assistance Council (BIRAC), Department of Biotechnology, Ministry of Science and Technology, Govt of India at Aerocity, New Delhi, 21-23 November, 2019.
 15. H Singha participated in One Day Symposium on Advances in Equine Health and Management organized by ICAR-NRCE and IAVMI on 27 February, 2019
 16. H Singha presented paper on “Surveillance of equines and in-contact humans in India for Glanders - a zoonotic and notifiable equine disease” in One Health India Conference -2019 organised by Department of Biotechnology (DBT), Ministry of Science & Technology, SCOPE Complex, Lodhi Road, New Delhi, 18-19 February, 2019.
 17. K Shanmugasundaram delivered a lecture on "Sample collection, processing and dispatch for diagnosis of important Zoonotic diseases". In Training Programme for medical and veterinary professionals on diagnosis and control of Zoonotic diseases, ICAR-NRCE, Hisar, 10-12 December, 2019.
 18. Kumar N delivered invited lecture on “CRISPR/Cas9-mediated genome editing: Generation of knockout cells to scale up virus production” in ICAR sponsored summer school organized at Department of Veterinary Microbiology, LUVAS Hisar, 20 November – 10 December, 2019.
 19. Kumar N delivered invited lecture on “Virus quantitation techniques – Methods and applications” in ICAR sponsored summer school organized at Department of Veterinary Microbiology, LUVAS Hisar, 20 November – 10 December, 2019.
 20. Kumar N participated in symposium on “Advances in Equine Health and Management” organized by ICAR-National Research Centre on Equines, in collaboration with Indian Association of Veterinary Microbiologists, Immunologists and specialists on Infectious Diseases, ICAR-NRCE, Hisar, 27 February, 2019.
 21. Virmani N presented a paper on “Glycoprotein E Gene Deletion Mutant of Equine Herpes Virus 1 as Attenuated Live Vaccine Candidate” in VIROCON 2020: International Conference on “Evolution of Viruses and Viral Diseases”, at Indian National Science Academy, New Delhi, 18-20 February, 2020.
 22. RA Legha delivered expert lecture on “Status and conservation of Equine Genetic resources in India.” in ICAR Sponsored Winter School on Current status, emerging issue and future scenario regarding conservation of indigenous breeds of livestock, 5-25 November, 2019.



23. RA Legha presented a paper on "Pulling capacity of Hallari donkeys in pneumatic two-wheel cart" in XVI National Symposium on Animal Genetic Resources for Food and Social Security, ICAR-NBAGR, Karnal, 7-8 February, 2019.
24. Barua S participated in AMI-2019 International conference at Central University of Haryana-Mahendergargh held on 15-18 November, 2019.
25. Mehta SC delivered expert lecture on "Current status, emerging issue and future scenario regarding conservation of equines" in ICAR sponsored winter school on "Current status, emerging issue and future scenario regarding conservation of Indigenous breeds of livestock", CVAS, RAJUVAS, Bikaner, 14 November, 2019.
26. Mehta SC delivered expert lecture on "Prospectus of equine farming and research". In : Orientation programme on teaching methodology", organised by Director of Extension, Rajasthan University of Veterinary and Animal Sciences, Bikaner, 28 March – 17 April, 2019 and 21 June -11 July, 2019.
27. Mehta SC delivered lead lecture on "Utilization of indigenous animal genetic resources for rural socio-economic security" in National Symposium on Animal Genetic Resources for Food and Social Security, organised by the Society for Conservation of Domestic Animal Biodiversity and National Bureau of Animal Genetic Resources, Karnal, Haryana, 7-8 February, 2019.
28. Talluri TR delivered expert lecture on "Application of assisted reproductive technologies in conservation and optimizing production in livestock." in ICAR Sponsored Winter School on Current status, emerging issue and future scenario regarding conservation of indigenous breeds of livestock held at Dept of LPM, College of Veterinary and Animal Science, RAJUVAS, Bikaner, 05- 25 November, 2019.
29. Talluri TR delivered expert lecture on "Collection and cryopreservation of stallion semen" in Training of Veterinary officers on artificial insemination in equines held under "Network approach for genetic improvement of indigenous horses" at Equine Production Campus, ICAR-NRC on Equines, Bikaner, 18-20 July and 7-9 August, 2019.
30. Talluri TR delivered expert lecture on "Pregnancy diagnosis in equines" in Training of Veterinary officers on artificial insemination in equines held under "Network approach for genetic improvement of indigenous horses" at Equine Production Campus, ICAR-NRC on Equines, Bikaner, 18-20 July and 7-9 August, 2019.
31. Talluri TR invited to deliver guest lecture on "Interspecies somatic cell nuclear transfer: Hopes and challenges" at Mid-Term National Symposium of Indian Society for the Study of Reproduction & Fertility (ISSRF) on "Impact of Lifestyle and Environmental factors on the Reproductive Health and Fertility" held at College of Veterinary Science, SVVU, Tirupati, 16 October, 2019.

Zanskari Mares in their natural habitat at Leh & Ladakh





12

On-going Research Projects

Equine Health

Sr. No.	Title	Team	From	To	PIMS Code
1	Surveillance, Monitoring and Control of Emerging and Existing Diseases of Equines	HS Singha*, SC Yadav, BR Gulati, Rajender Kumar, Sanjay Kumar, N. Virmani, Sanjay Barua, Rajesh Vaid, Ramesh Dedar, Anju Manuja, Balvinder Kumar and B N Tripathi	April, 1997	Continuous Service Project	IXX00257
2	Nano-based therapeutic interventions against osteoarthritis	Anju Manuja*, Balvinder Kumar and Riyesh T	April, 2016	March, 2020	IXX12559
3	In vitro growth inhibitory efficacy of different herbal plant extracts against <i>Theileria equi</i> and identification of principal drug molecule (s) thereof	Sanjay Kumar* and Rajender Kumar	September, 2017	August, 2019 Extend upto 6-month March, 2020	IXX14012
4	Development of recombinant EHV-1 viruses employing bacterial artificial chromosome mediated mutagenesis and their pathological evaluation in murine model	Nitin Virmani*, BC Bera, Taruna Anand and BN Tripathi	April, 2017	March, 2020	IXX14011
5	Diagnosis and sequence typing of strains <i>Streptococcus equi</i>	Balvinder Kumar*, RK Vaid, Anju Manuja, K Shanmugasundram and HS Singha	April, 2018	March, 2021	IXX14584
6	Characterization of Equine herpesvirus isolates in India and documentation of their genetic diversity	BR Gulati*, Naveen Kumar, Riyesh T	September, 2018	August, 2021	IXX14746
7	Development of multi-species ELISA for diagnosis of <i>Trypanosoma evansi</i> infection in different livestock species.	Rajender Kumar*, Sanjay Kumar and BN Tripathi	November, 2019	October, 2020	IXX15308

* Principal Investigator



Equine Production

Sr. No.	Title	Team	Date of Start	Date of Completion	PIMS Code
1	Genetic characterization of Marwari horses for selection of true to breed animals	Anuradha Bhardwaj*, Yash Pal, SC Mehta (w.e.f. May 2018), AK Gupta, Mamta Chauhan, and Vijay Kumar (AK Gupta (w.e.f. Oct. 2017), Mamta Chauhan (w.e.f. June 2018) and Vijay Kumar (w.e.f. March 2017))	July, 2015	June, 2019	IXX12220
2	Assessment and optimization of equine management in an intensive system (Service Project)	SC Mehta*, RA Legha, RK Dedar, TR Talluri and J Singh.	June, 2016	Service Project	IXX13192
3	Area specific mineral mixture for equine of Rajasthan	RK Dedar* and R Nehra (RAJUVAS) (PA Bala was PI upto 7th Aug., 2018)	May, 2016	Oct., 2018 Extended upto March, 2020	IXX13195
4	Assessment of risk factors of equine laminitis and colic	RK Dedar* and Sakar Palecha (RAJUVAS)	Sept., 2016	August, 2019	IXX12693
5	Endurance and fertility analysis in indigenous horses using SNP (single nucleotide polymorphisms) markers	SC Mehta*, RK Dedar and TR Talluri	Oct., 2017	Sept., 2020	IXX13995
6	Assessment, evaluation and identification of physical, biochemical and genetic factors affecting stallion fertility	TR Talluri*, SC Mehta, Yash Pal, Anuradha Bhardwaj	April, 2018	March, 2021	IXX14589
7	Studies on antitumor and antiviral potential of some plant extracts	RK Dedar*, Naveen Kumar and BN Tripathi	Nov., 2018	Oct., 2019	IXX14758
8	Studies on bioactive components of donkey milk and its application	Anuradha Bhardwaj	Dec., 2018	March, 2020	IXX14933

* Principal Investigator

NCVTC

Sr. No.	Title	Team	Duration	To	PIMS Code
1	Authentication and accessioning of viruses of animal origin (Service Project)	Sanjay Barua*, Naveen Kumar, BC Bera, Riyesh T and Taruna Anand	May, 2015	Service Project	IXX11882
2	Phenotypic and genotypic authentication and preservation of network bacterial isolates	RK Vaid*, Taruna Anand, BC Bera, Riyesh T and K Shanmugasundaram	June, 2015	March, 2020	IXX11884
3	Development of bacteriophage repository and exploring the therapeutic potential of phages and their encoded endolysin	Taruna Anand*, Nitin Virmani, RK Vaid and BC Bera	April, 2017	March, 2020	IXX13982



Sr. No.	Title	Team	Duration	To	PIMS Code
4	Isolation, characterization and reposition of enteric viruses of poultry	NCVTC: Riyesh T*, Naveen Kumar, Sanjay Barua and LUVAS: Naresh Jindal	June, 2017	May, 2020	IXX13988
5	Isolation, characterization and generation of repository of <i>Mycobacterium</i> species	Shanmugasundaram K*, RK Vaid, BC Bera and BN Tripathi	Oct., 2017	Sept., 2020	IXX13994
6	Development of Knockout cell by CRISPR/Cas9-mediated genome editing	Naveen Kumar*, Sanjay Kumar, Sanjay Barua, Riyesh T, Balvinder Kumar	April, 2018	March, 2021	IXX14586
7	Prevalence studies for porcine respiratory viruses and development of their repository	BC Bera*, Sanjay Barua and T Anand (N Virmani was Co-PI upto 8th Aug., 2018)	Jan., 2016	March, 2020	IXX12436

* Principal Investigator

External Funded Projects

Sr. No.	Title	Team	From	To	PIMS Code
1	All India Coordinated Research Project on Utilization of Animal Energy with enhanced system efficiency (AICRP on UAE)	RA Legha* and Yash Pal	July, 2009	March, 2020	OXX00486
2	DBT-NER Centre for Advanced Animal Diagnostics and Services on Animal Health and Diseases (ADSAHD)	BN Tripathi*, Sanjay Barua, Nitin Virmani, SC Yadav, BR Gulati, Rajender Kumar, RK Vaid, BC Bera, Taruna Anand & Riyesh T	Sept., 2013	Sept., 2019	OXX02933
3	All India Network Programme on Neonatal Mortality in Farm Animals	Sanjay Kumar*, RK Dedar R.K. Vaid and H Singha	Jan., 2015	March, 2021	OXX03934
4	CRP on Vaccines and Diagnostics	BR Gulati*, Component-I (BR Gulati & Nitin Virmani) Component-II (BR Gulati & BC Bera, (Nitin Virmani was Co-PI upto 8th Aug., 2018) Component-III (Sanjay Kumar & Rajender Kumar)	May, 2015	March, 2020	OXX03182
5	Seroproteome analysis of recombinant secretory proteins of <i>Burkholderia mallei</i> towards development of multiple antigen immunoassay for improved diagnosis of glanders	HS Singha* & K Shanmugasundaram	July, 2017	July, 2020	OXX03948
6	Molecular epidemiology of Japanese Encephalitis Virus in Pigs and Mosquitoes in Assam (DBT Twinning Programme)	PI from Parent Institute: Seema Rani Pegu*, Dilip Kumar Sarma, Swaraj Rajkhowa PI from Collaborative Institute: BR Gulati	Jan., 2017	Jan., 2020 Extended July, 2020	OXX03737



Sr. No.	Title	Team	From	To	PIMS Code
7	Scheduling Equines from Fatal Zoonotic disease-Glanders and Equine Infectious Anemia (EIA) in India using Point of Care Diagnostic (POCD)	HS Singha*and BN Tripathi	March, 2018	March, 2020	OXX04100
8	Elucidating therapeutic role of bacteriophages and encoded endolysins against multidrug resistant enteric pathogens of poultry	Taruna Anand*	June, 2018	May, 2021	OXX04448
9	Exploration of genomic signatures for indigenous horses using next-generation sequencing approaches (DST-SERB)	Anuradha Bhardwaj*	Dec., 2018	Nov., 2021	OXX04453
10	Investigating mechanism underlying acquisition of antiviral drug resistance against host targeting agents.	Naveen Kumar* and Sanjay Baura	March, 2019	March, 2022	OXX04469
11	Regional Coordination center under program for Inter-Sectoral Coordination for prevention and control of Zoonotic Diseases	BN Tripathi* Bacterial Diseases: H S Singha, K Shanmugasundram Viral Diseases: BR Gulati, Naveen Kumar, T Riyesh	June, 2019	May, 2020	OXX04686

* Principal Investigator



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Staff at ICAR-NRCE (as on 31.12.2019)

Director : Dr. B.N. Tripathi

Sr. No.	SCIENTIFIC STAFF
ICAR-NRCE Main Campus, Hisar	
1	Dr. SC Yadav, Principal Scientist
2	Dr. Yash Pal, Principal Scientist
3	Dr. BR Gulati, Principal Scientist
4	Dr. Rajender Kumar, Principal Scientist
5	Dr. Nitin Virmani, Principal Scientist
6	Dr. Sanjay Kumar, Principal Scientist
7	Dr. Anju Manuja, Principal Scientist
8	Dr. Balvinder Kumar, Principal Scientist
9	Dr. Anuradha Bhardwaj, Sr. Scientist
10	Dr. Harishankar Singha, Sr. Scientist
ICAR-NRCE, Equine Production Campus, Bikaner	
11	Dr. SC Mehta, Incharge-cum- Principal Scientist
12	Dr. RA Legha, Principal Scientist
13	Dr. TR Talluri, Sr. Scientist
14	Dr. RK Dedar, Sr. Scientist
ICAR-NRCE, NCVTC, Hisar	
15	Dr. Praveen Malik (on deputation)
16	Dr. Sanjay Barua, Principal Scientist
17	Dr. RK Vaid, Principal Scientist
18	Dr. Naveen Kumar, Principal Scientist
19	Dr. Taruna Anand, Senior Scientist
20	Dr. BC Bera, Senior Scientist
21	Dr. K Shanmugasundaram, Scientist
22	Dr. Riyesh T, Scientist
Sr. No.	TECHNICAL STAFF
ICAR-NRCE Main Campus, Hisar	
1	Sh. KS Meena, ACTO
2	Sh. PP Chaudhary, ACTO
3	Sh. DD Pandey (upto Sept. 2019)
4	Sh. Sita Ram, Sr. Tech. Officer
5	Sh. Sanjeev Kumar, Sr. Tech. Officer
6	Sh. Ajmer Singh, Tech. Officer



Sr. No.	TECHNICAL STAFF
6	Sh. Joginder Singh, Tech. Officer.
7	Sh. Sajjan Kumar, Tech. Officer
8	Sh. Suresh Kumar, Sr. Technical Assistant
9	Sh. Mukesh Chand, Tech. Officer
10	Sh. Raj Kumar Dayal, Tech. Officer
11	Sh. Arun Chand, Sr. Technician
12	Sh. Raghbir Singh, Tech. Assistant
ICAR-NRCE, Equine Production Campus, Bikaner	
13	Dr. Jitender Singh, ACTO
14	Sh. KK Singh, ACTO
15	Sh. RA Pachori, ACTO
16	Sh. Narender Chauhan, Sr. Technical Officer
17	Sh. Brij Lal, Technical Officer
18	Sh. Om Parkash, Technical Officer
19	Sh. SN Paswan, Sr. Technical Assistant
20	Sh. Rajender Singh, Technical Assistant
21	Sh. Gopal Nath, Sr. Technician
Sr. No.	ADMINISTRATIVE STAFF
ICAR-NRCE Main Campus, Hisar	
1	Sh AG Barapatre (Retired on 30.11.2019)
2	Sh. Ram Pal, Administrative Officer (From 26-09-2019) *(as AAO 23-9-2010 to 25-09-2019)
3	Smt. Shammi Tyagi, Finance & Account Officer* (*FAO of CIRB, Additional duty at NRCE)
4	Sh. SP Kaushik, Assistant Admn. Officer
5	Sh. Subhash Chander, AAO
6	Sh. Ashok Kumar, Personal Assistant
7	Sh. Sunil, Assistant
8	Sh. Pratap Singh, Assistant
9	Sh. Dinesh Datt Sharma, UDC
10	Sh. Om Parkash, UDC
11	Sh. Deepak Kumar, LDC
ICAR-NRCE, Equine Production Campus, Bikaner	
12	Sh. Mahender Singh, LDC
Sr. No.	SKILLED SUPPORTING STAFF
ICAR-NRCE Main Campus, Hisar	
1	Sh. Ishwar Singh (FMS ID-012618)
2	Sh. Guru Dutta Sharma
3	Sh. Jai Singh
4	Sh. Mahabir Prasad
5	Sh. Ramesh Chander
6	Sh. Mardan
7	Sh. Desh Raj



Sr. No.	SKILLED SUPPORTING STAFF
8	Sh. Ishwar Chander
9	Sh. Om Parkash
10	Sh. Hanuman Singh
11	Sh. Subhash Chander
12	Sh. Ishwar Singh (FMS No. 012622)
13	Sh. Ram Singh
14	Smt. Santra
15	Sh. Sant Ram
16	Smt. Soma Devi
17	Sh. Lilu Ram
ICAR-NRCE, Equine Production Campus, Bikaner	
18	Sh. Raju Ram
19	Sh. MP Meena
20	Sh. Ashok Kumar

CAR-NRCE IN NEWS

पशुओं से मनुष्यों में फैलने वाले रोगों पर आज से होगा मंथन

जागरण संवाददाता, हिसार : कई पशु, जोका पावर, स्टाइन वगैरह पशुओं से इन बीमारियों के नाम हैं जो पशु-मनुष्यों में से इंसानों में फैली हैं। इससे लेकर भारत सरकार के एनआरसी के राष्ट्रीय अल्प अनुसंधान केंद्र (एनआरसी) को हरियाणा, पंजाब, राजस्थान, चंडीगढ़ और दिल्ली का नेशनल सेंटर बनाया गया है।

इन जूनोटिक बीमारियों पर काम करने के लिए एनआरसी में मंगलवार से तीन दिवसीय जैमिन असेंबली होगी। जिसमें देशभर से 30 प्रोफेशनल भाग लेंगे। जिसमें 14 सिक्योरिटी फंड से चिकित्सक तो 16 पशु चिकित्सक शामिल हैं। इस ट्रेनिंग में एनआरसी के वैज्ञानिक

चिकित्सकों के साथ जूनोटिक बीमारियों पर रोकथाम लगाने के लिए योजना तैयार करेंगे। इस प्रोजेक्ट के तहत पशुओं और इंसानों की बीमारियों का उपाय करने वाले चिकित्सक एक साथ मिलकर काम करेंगे।

उपचार के दौरान बहुत ही महत्वपूर्ण उपाय यथा सही है तब ही पशु-मनुष्यों को जूनोटिक बीमारियों पर नियंत्रण किया जा सके। एनआरसी के डिप्युटी डायरेक्टर डॉ. विद्यालाल ने बताया कि इस अभियान में मुख्य अतिथि अखिल भारतीय कृषि के चिकित्सक डॉ. गोपाल सिन्हा रहेंगे।

भारत सरकार के नेशनल सेंटर फॉर डिजिज कंट्रोल ने एनआरसी को बनाया चीनल कोऑर्डिनेटर, 10, 11 और 12 दिवस तक चलेगी बैठक, देशभर के 30 चिकित्सक लगे भाग लेंगे।

खो बड़ रही हैं जूनोटिक बीमारियां

- जापान में पहिले से
- ओसिगोकिरुस
- जनरलवा ब्रुसेल
- पशुओं और इंसानों की संख्या बढ़ने से
- पशुओं और इंसानों के करीब रहने से
- ग्लोबल वार्मिंग में अत्यधिक मात्रा में पशु पक्षियों का उदय होने से।

40 वर्षों से आर्मी के घोड़ों में नहीं कोई गंभीर बीमारी, ग्लैंडर्स फ्री जोन में शामिल होंगे

दशा के दिल्ली, हरियाणा, उत्तर प्रदेश, राजस्थान सहित कई राज्यों में ग्लैंडर्स के मामले में खामोश आ रहे हैं। इन क्षेत्रों को ग्लैंडर्स फ्री जोन में शामिल करने का फैसला किया जा रहा है।

इस राज्य में मिल चुके हैं ग्लैंडर्स के मामले में दिल्ली, हरियाणा, उत्तर प्रदेश, राजस्थान सहित कई राज्यों में ग्लैंडर्स के मामले में खामोश आ रहे हैं। इन क्षेत्रों को ग्लैंडर्स फ्री जोन में शामिल करने का फैसला किया जा रहा है।

राष्ट्रीय अल्प अनुसंधान केंद्र ने मनाया 34वां स्थापना दिवस

जागरण संवाददाता, हिसार : राष्ट्रीय अल्प अनुसंधान केंद्र ने सोमवार को अपना 34वां स्थापना दिवस मनाया। इस मौके पर मुख्य अतिथि डा. सुप्रियो बाबूव रहे। इस अवसर पर विधिपूर्वक अतिथि डा. अनिल कुमार श्रीवास्तव ने 2.64 बिलियन भारतीय रुपए के लिए खाद्य एवं पौषणिक सुरक्षा को प्रस्तुत किया। उन्होंने अल्प विज्ञान के क्षेत्र में संस्थान के योगदान तथा केंद्र को सतत सहाय्य एवं अनुसंधान संबंधित उपलब्धियों के लिए केंद्र की प्रशंसा की। राष्ट्रीय अल्प अनुसंधान केंद्र के निदेशक डा. बीपूत्र त्रिपाठी ने कहा कि केंद्र ने अल्प विज्ञान के क्षेत्र में उत्कृष्ट अनुसंधान एवं वैज्ञानिक सेवाएं प्रदान करने में अहम भूमिका निभाया है। इस अवसर पर केंद्रीय भैंस अनुसंधान केंद्र डा. सुप्रियो बाबूव ने सभी को 34 वें स्थापना दिवस की बधाई दी। कार्यक्रम में अल्प पालकों और वैज्ञानिकों के मध्य परिसंवादन का आयोजन किया गया। इस दौरान सुकुमार द्वारा अनेक कर्तव्य भी दिखाया गए। कार्यक्रम का चीरबोजक डा. संजय बरुआ ने किया व मंच संवादन डा. अंजु मनुजा द्वारा किया गया।

संक्रामक रोगों से लड़ने के लिए पब्लिक हेल्थ जैसे विभागों को भी साथ जोड़ेंगे एनसीडीसी

जागरण संवाददाता, हिसार : देशभर की पब्लिक हेल्थ विभागों को एनसीडीसी के विभागों के साथ जोड़ने का फैसला किया जा रहा है। एनसीडीसी के निदेशक डॉ. अनिल कुमार श्रीवास्तव ने कहा कि पब्लिक हेल्थ विभागों को एनसीडीसी के साथ जोड़ने का फैसला किया जा रहा है। एनसीडीसी के निदेशक डॉ. अनिल कुमार श्रीवास्तव ने कहा कि पब्लिक हेल्थ विभागों को एनसीडीसी के साथ जोड़ने का फैसला किया जा रहा है।

1.34 बिलियन भारतीयों के लिए खाद्य एवं पौषणिक सुरक्षा जरूरी : प्रो. अनिल

राष्ट्रीय अल्प अनुसंधान केंद्र में 34वां स्थापना दिवस के उपलक्ष्य पर दिग्गज व्याख्यान

जागरण संवाददाता, हिसार : राष्ट्रीय अल्प अनुसंधान केंद्र में सोमवार को 34वां स्थापना दिवस के उपलक्ष्य पर दिग्गज व्याख्यान हुआ। इस अवसर पर प्रो. अनिल कुमार श्रीवास्तव ने 1.34 बिलियन भारतीयों के लिए खाद्य एवं पौषणिक सुरक्षा की आवश्यकता पर बोलते हुए कहा कि खाद्य एवं पौषणिक सुरक्षा की आवश्यकता है।

ग्लैंडर्स फ्री कंपार्टमेंट में शामिल होने को कमेटी से लेनी होगी एनओसी, एनआरसीई ने बनाए नए नियम

जागरण संवाददाता, हिसार : घोड़ों में ग्लैंडर्स नामक जानघोरा बीमारी के बहरी मामलों के बाद केंद्रीय पशुपालन मंत्रालय राष्ट्रीय ग्लैंडर्स फ्री जोन में शामिल करने के लिए नए नियम बनाया है। राष्ट्रीय ग्लैंडर्स फ्री जोन में शामिल करने के लिए नए नियम बनाया है।

अल्प अनुसंधान केंद्र ने बनाया इंडो-इक्वाइन मोबाइल एप

जागरण संवाददाता, हिसार : राष्ट्रीय अल्प अनुसंधान केंद्र ने एक मोबाइल एप बनाया है। इस एप का नाम 'इंडो-इक्वाइन मोबाइल एप' है। इस एप का नाम 'इंडो-इक्वाइन मोबाइल एप' है।

ग्लैंडर्स बीमारी को लेकर दिल्ली सरकार गंभीर नहीं एनआरसीई ने सभी मुख्य संस्थाओं को भेजा पत्र

दिल्ली में इस तरह हुआ ग्लैंडर्स बीमारी का प्रसार, एनआरसीई ने दिल्ली सरकार को भेजा पत्र। एनआरसीई ने दिल्ली सरकार को भेजा पत्र।

घोड़ों में होने वाले बैबेसियोसिस रोग का अब सिर्फ 10 मिनट में पता चलेगा, 50 रुपये में मिल सकेगी किट, हर्बल प्लांट्स से दवा तैयार करने में जुटे वैज्ञानिक

केंद्रीय अल्प अनुसंधान केंद्र दिल्ली के वैज्ञानिकों ने एनआरसीई के सहित एक किट तैयार की है। इस किट का नाम 'बैबेसियोसिस किट' है। इस किट का नाम 'बैबेसियोसिस किट' है।

ऑनलाइन पोर्टल से जुड़े देशभर के वैज्ञानिक और पशु चिकित्सक, मैसेज मिलते ही उतर पर कार्य में संलग्न, वैडियो कॉन्फ्रेंस के माध्यम से भी हो सकेगी चर्चा

ऑनलाइन पोर्टल से जुड़े देशभर के वैज्ञानिक और पशु चिकित्सक, मैसेज मिलते ही उतर पर कार्य में संलग्न, वैडियो कॉन्फ्रेंस के माध्यम से भी हो सकेगी चर्चा।

प्रशिक्षण केंद्रीय अल्प अनुसंधान संस्थान में पहुंचे देशभर के 35 चिकित्सक

सतर्क रहना ही जूनोटिक बीमारियों से बचने का एकमात्र उपाय : डॉ. त्रिपाठी

अमर उजाला ब्यूरो

हिसार। केंद्रीय अल्प अनुसंधान संस्थान के निदेशक डॉ. बीपूत्र त्रिपाठी ने कहा कि पशुओं से मनुष्यों में संचारित होने वाले रोगों से सतर्क रहना ही जूनोटिक रोगों से बचाव का एकमात्र उपाय है। इन रोगों की जानकारी पशु चिकित्सकों के साथ अन्य चिकित्सकों को भी होनी जरूरी है, ताकि जूनोटिक रोगों पर जल्द लगाम लगाई जा सके।

डॉ. त्रिपाठी वीरवार को संस्थान में आयोजित देशभर के 35 चिकित्सकों व पशु चिकित्सकों की ट्रेनिंग के समापन समारोह में बोल रहे थे। डॉ. त्रिपाठी ने बताया कि भारत सरकार ने जूनोटिक रोगों की रोकथाम और नियंत्रण के लिए अंतर-क्षेत्रीय समन्वय के लिए एक परियोजना शुरू की है। इस परियोजना के तहत, एनआरसीई हिसार को नेशनल सेंटर फॉर डिजिज कंट्रोल, हेल्थ एंड फैमिली वेलफेयर के लिए जूनोटिक बीमारी की जानकारी सझा करने की जिम्मेदारी दी है। इसी के तहत यह प्रशिक्षण कार्यक्रम आयोजित किया गया।

तीन दिन में हुए सात व्याख्यान और बीमारियों के निदान का दिया प्रशिक्षण एनआरसीई में 10-12 दिवस तक जूनोटिक रोगों के निदान और नियंत्रण पर आयोजित किया गया।

आयोजित तीन दिवसीय प्रशिक्षण कार्यक्रम में हरियाणा, पंजाब, जम्मू-कश्मीर, उत्तर प्रदेश, राजस्थान और केरल के 35 पशु चिकित्सकों और चिकित्सकों ने भाग लिया। प्रशिक्षण कार्यक्रम में सात व्याख्यान हुए और प्रतिभागियों को ग्रंथियों, तपेदिक, जापानी एन्सेफलाइटिस, परजीवी रोगों, रैब्स आदि जैसे जूनोटिक रोगों के निदान पर प्रशिक्षण दिया गया।







