Annual Report 2004-2005

राष्ट्रीय अश्व अनुसंधान केन्द्र

National Research Centre on Equines

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Dream, Dream, Dream
Dreams transform into thoughts
And thoughts result in action.

- A.P.J. Abdul Kalam

Director's foreword

An organization is only as good as the services it offers. Our mandate is to improve health, performance and production potential of equines in India for our customers, the poor farmers who own equines to support their livelihood. To serve these customers is the entire philosophy embedded within the very fabric of the organization- National Research Centre on Equines. It is our endeavour to improve our offerings for the equine owners, to anticipate problems of equines, to alleviate their sufferings - in short to stay true and firm to our strong commitment of welfare of equines and their owners.

At NRCE, our focus is on major problems confronting equine health and production. Our efforts during the recent years have been concentrated on generation of indigenous and cost-effective technologies for diagnosis, prevention and control of major equine diseases. As a result of these initiatives, we have developed diagnostics and vaccines for some of the infectious diseases confronting majority of indigenous equines. With an aim of improvement of equine germplasm, the technique of artificial insemination using cryopreserved semen of Poitu jacks and Marwari stallions has been demonstrated to field veterinarians and farmers. Serological assay has been developed to facilitate early pregnancy diagnosis in mares for the benefit of poor equine owners.

I feel immense pleasure in forwarding the Annual Report of NRCE for the year 2004-05. As is reflected in this report, this year has been very vibrant for the centre. The work towards development of diagnostics for various equine infectious diseases continued yielding good results. Research carried towards developing immunoassay for pregnancy diagnosis in mare was recognized as one of the nine cutting edge researches in agriculture sector in India. A monoclonal antibodies-based diagnostic kit for equine herpes virus infection has been developed and successfully demonstrated to some of the user's laboratories. Characterization of Marwari breeds of equines was accomplished.

The centre continued to go ahead on active equine disease surveillance and monitoring in different parts of the country. The centre also organized seven equine health camps and a number of farmer meets 'kisan goshthis' to enlighten the equine owners on various aspects of disease control and management practices. Feedback from farmers was also obtained for further research and developmental activities in equine health and production. Foundation day of the centre was celebrated with great zeal and enthusiasm on 26th November 2004. On this occasion, a horse show was organized in which indigenous horses from various states participated in different equestrian events.

Work towards infrastructure improvement at the centre got impetus with the initiation of construction work of the laboratory-cum-office building at our Bikaner campus and laying of foundation stone for the second wing of laboratory-cum-administration building at Hisar campus by Hon'ble Director-General ICAR, Dr. Mangala Rai on January 11, 2005. A veterinary type culture centre has been sanctioned to be established at the centre. ICAR established internet connectivity at NRCE using ERNET backbone with broad band VSATs/leased lines under National Agricultural Technology Programme.

On this occasion, I would like to record my sincere thanks to Indian Council of Agricultural Research, New Delhi, particularly Dr. Mangala Rai (DG, ICAR and Secretary-DARE), Dr. V.K. Taneja (DDG, Animal Sciences) and Dr. Lal Krishna (ADG, Animal Health) for their continuous support to this centre to improve equine health and production. The efforts of scientists entrusted the additional responsibility of ARIS and PME cells are worth mentioning. I compliment the efforts of the chairman and members of publication committee for giving new look and for timely printing of this report.

Dr. S.K. Dwivedi

Executive Summary

Indigenous equines including donkeys, mules and ponies constitute 98% of the entire equine population of approximately 1.77 million in India. These animals provide livelihood to the landless, small and marginal farmers of our rural and semi-urban society. NRCE is committed to improve the performance of these equines by ensuring freedom from various ailments and by exploiting their production potentials. Therefore, during the year, our efforts were focused towards development of improved diagnostics and biologicals for major equine ailments, nation-wide monitoring of equine diseases, improving equine production by timely pregnancy diagnosis & adapting artificial insemination using cryopreserved semen and providing advisory & consultancy services to the equine farmers and breeders. In addition, the centre earned revenue of approximately 14.82 lacs by providing health certificates to thoroughbred equine industry and polo associations.

The scientists of the centre are currently working in four research projects in the area of equine health and seven in the area of equine production. Keeping in view the recommendations of the research advisory committee of the centre, three research projects that were concluded during the year include molecular characterization of Marwari breed, development of diagnostics and technologies for EHV-1 infection and development of herbal drugs for trypanosomosis.

Marwari breed of horses constitutes an elite group of indigenous horses, which are known for their sturdiness, swiftness, elegance and beauty. In order to conserve its germplasm,

कार्यकारी सारांश

भारत के लगभग 17.7 लाख अश्व प्रजाति के पशुओं में से लगभग 98 प्रतिशत स्वदेशी जाति के घोड़े, गदर्भ, खच्चर एवं टट्टू हैं। ये पशु भूमिहीन छोटे एवं सीमान्त किसानों एवं दूसरे निर्धन ग्रामीण एवं अर्द्धशहरी अश्वपालकों की जीविका–यापन के साधन हैं। रा० अ० अनु० के० इन अश्वों की कार्य क्षमता, स्वास्थ्य एवं उत्पादन में सुधार हेतु वचनबद्ध है। अतः इस वर्ष के दौरान केन्द्र की मुख्य गतिविधियाँ अश्व रोगों के निदान एवं रोकथाम हेतु जैविकी विकास करना, राष्ट्रव्यापी अश्व रोग सर्वेक्षण तथा हिमीकृत वीर्य द्वारा कृत्रिम गर्भाधान व शीघ्र गर्भाधारण निदान द्वारा अश्व उत्पादन में सुधार एवं अश्व पालकों को सलाह एवं परामर्श सेवा प्रदान करना रही। इसके अतिरिक्त केन्द्र ने अश्व उद्योग एवं पोलो संघों को स्वास्थ्य प्रमाण पत्र प्रदान करके 14.82 लाख रुपये अर्जित किये।

वर्तमान में केन्द्र के वैज्ञानिक इन प्राथमिकताओं को ध्यान में रखते हुए अश्व स्वास्थ्य सुधार के क्षेत्र में चार शोध परियोजनाओं तथा अश्व उत्पादन के क्षेत्र में सात शोध परियोजनाओं में कार्यरत हैं। इस वर्ष के दौरान जिन तीन शोध परियोजनाओं का कार्य सफलता–पूर्वक पूर्ण हुआ उनमें मारवाड़ी जाति के अश्वों में आनुवांशिक लक्षण वर्णन; अश्वों में ई०एच०वी–1 संक्रमण के निदान हेतु नैदानिक विधि एवं प्रोद्यौगिकी का विकास करना; एवं अश्व ट्राइपैनोसोमा के ईलाज हेतु हर्बल औषधि का विकास करना शामिल है।

मारवाड़ी प्रजाति के स्वदेशी अश्व घोड़े सहनशीलता, चुस्ती, सुन्दरता एवं रोग–अवरोधक गुणों के कारण प्रसिद्व हैं। इस प्रजाति के अश्वों के संरक्षण के लिए लक्षण वर्णन का कार्य जैवमितिक, जैव–रसायनिक एवं आनुवांशिक

Marwari breed was characterized in a recently completed project by studying their bio-metrical, bio-chemical and molecular characteristics. A total of 114 true-to-breed Marwari horses comprising 98 mares and 16 stallions from seven different locations were evaluated. Among biochemical indices, significant difference due to sex and age groups were observed. DNA polymorphism studies revealed high level of heterozygosity and low level of heterozygosity deficit in the Marwari horse population which reflects high genetic variability in Marwari equine population that can be exploited by horse breeders for planning breeding strategies for its conservation. This study also indicated that the present Marwari equine population had not experienced recent bottleneck which is very informative and important for equine breeders

Considering the significance of EHV-1 associated abortions in mares, a research project undertaken by the centre for the development and evaluation of improved diagnostics for EHV-1 infections was completed. Salient outcomes of this project include development of a neutralizing monoclonal antibodies-based diagnostic kit named Equiherpes B-ELISA Kit. This kit tests the serum sample using single dilution (1:250) thus making it very economical. Different EHV-1 strains were compared by DNA fingerprinting and it was found that more than one genetically variant strains of EHV-1 are circulating in equine population of Northern India. A patent application entitled *"Neutralizing monoclonal antibody-based blocking ELISA diagnostic kit for detection of equine herpes virus-1 specific antibodies"* is being submitted for getting Indian patent for this diagnostic kit.

To develop a drug for treatment of *Trypanosoma evansi* infection of equines, extracts from a medicinal herb, *Lawsonia inermis* were

स्तर पर पूर्ण किया गया। इस के लिए सात विभिन्न क्षेत्रों के 114 शुद्ध नस्ल के मारवाड़ी अश्वों, जिसमें 98 घोड़ियॉ एवं 16 घोड़े शामिल हैं, पर अध्ययन किये गए। जैव–रसायनिक सूचकों में लिंग एवं आयु के अनुसार विविधता पाई गई है। आनुवांशिक बहुरुपता अध्ययन से मारवाड़ी अश्वों के गुण सूत्रों में उच्च–स्तर की विषम प्रारुपता पाई गई, जिससे यह निष्कर्ष निकला कि मारवाड़ी अश्वों में पायी जाने वाली आनुवांशिक विविधता का उपयोग करके अश्व संरक्षण के लिए प्रजनन योजना बनाई जाए। इस अध्ययन से यह भी ज्ञात हुआ कि मारवाड़ी प्रजाति में पिछले कुछ समय में कोई आनुवांशिक गतिरोध नहीं रहा जो कि पशुपालकों के लिए महत्वपूर्ण जानकारी है।

घोड़ियों में अश्व हरपीज विषाणु (ई० एच० वी०–1) सम्बन्धित गर्भपात के महत्व को ध्यान में रखते हुए इस संक्रमण के शीघ्र निदान हेतु संशोधित नैदानिक विधि के विकास पर शोध परियोजना का कार्य पूर्ण हुआ। इस परियोजना का मुख्य नतीजा मोनोक्लोनल एन्टीबॉडी प्रयुक्त नैदानिक किट (इक्वीहरपीज बी० एलाईजा किट) का विकास करना रहा। इस किट में सीरम नमूनों की एक ही तनुता (1:250) को प्रयोग में लाया जाता है। अतः यह किट बहुत किफायती है। ई०एच०वी०–1 के विभिन्न पृथ्थकीकृत स्वदेशी विषाणुओं की डी०एन०ए० अंगुली चिन्ह (फिंगर प्रिंट) द्वारा अध्ययन करने यह पाया गया कि उत्तर भारत के अश्वों में एक से अधिक प्रकार के ई०एच०वी०–1 के विषाणु विद्यमान हैं। इस किट का भारतीय पेटैन्ट प्राप्त करने हेतु एक आवेदन 'अश्व हरपीज विषाणु–1 की रोग प्रतिकारकों के पहचान हेतु उदासीनीकृत मोनोक्लोनल एन्टीबॉडी प्रयुक्त अवरोधक एलाईजा किट' भेजा जा रहा है।

अश्व ट्राईपेनोसोमा के ईलाज हेतु वनस्पतिक औषधि विकास के लिए लासोनिया इनरमिस जड़ी–बुटी के अंशों के मूल्यांकन पर एक शोध परियोजना के अन्तर्गत कार्य

evaluated in a recently completed research project. Using activity-guided separation approach, antitrypanosomal components from this herb exhibiting antitrypanosomal activity *in vitro* were identified and were tested for their efficacy in mice model. Out of 8 fractions tested, two fractions could increase survival period of mice from 3 to 17 days when administered orally but none could clear blood form of *T.evansi* completely. The results indicate that the constituents of *L. inermis* responsible for trypanocidal activity *in vitro* might have been degraded *in vivo* system(mice model) when administered by oral or intraperitoneal route. For *in vivo* activity, a suitable drug delivery system needs to be evaluated to protect the allelochemicals.

Amongst various etiological agents, rotavirus is reported to be the predominant cause of foal diarrhoea world over. For rapid diagnosis of rotavirus-associated diarrhoea, a sandwich ELISA was standardized for detection of rotavirus from stool samples. This ELISA was found to be 100% sensitive and highly specific as compared to virus isolation and RNA-PAGE. Using this assay, the rotavirus was found to be prevalent in 23 of the 72 (32%) diarrhoeic foals.

Equine piroplasmosis caused by *Babesia equi* is a serious problem of equines in India. In order to develop improved diagnostics for this ailment, a truncated gene segment of one of the merozoite surface proteins, EMA-2 of *B. equi* was expressed in *E. coli* and the expressed soluble GST fusion protein was purified. An ELISA was standardized using this recombinant protein as antigen. The assay quantitatively differentiated the reference positive and negative serum samples. The assay was found specific in detecting *B. equi* antibodies only.

Molecular diagnostics were developed for differentiation of different equine

पूर्ण किया गया। सक्रियता–निर्देशित पृथक्कीकरण विधि से उन ट्राईपेनोसोमा–नाशक अंशों की पहचान हुई जो कृत्रिम वातावरण में प्रभावी थे और इन अंशों की अन्तर्जीवी जांच चूहों में की गई। आठ अंशों में से केवल दो अंशों को मुख द्वारा खिलाने से संक्रमित चूहों का जीवनकाल 3-17 दिन तक बढ़ गया, लेकिन ये अंश ट्राईपेनोसोमा की रुधिर अवस्था का पूर्ण सफाया करने में असमर्थ थे। इस शोध से यह निष्कर्ष निकला कि इन प्रभावी अंशों के अन्तर्जीवी सक्रियता के लिए उपयुक्त औषधि–वितरण प्रणाली के मूल्यांकन की आवश्यकता है।

अतिसार प्रभावित अश्व शावकों में अधिकतर रोटा वायरस विषाणु का संक्रमण पाया जाता है। इस के शीघ्र निदान हेतु एक सैंडविच एलाईजा विधि का मानकीकरण किया गया। इस विधि की संवेदनशीलता 100 प्रतिशत तथा विषाणु पृथक्कीकरण के मुकाबले विशिष्टता लगभग 96 प्रतिशत पायी गयी। इस एलाईजा द्वारा जांच करने पर 72 अतिसारित अश्व शावकों में से 23 (32 प्रतिशत) रोटा वायरस संक्रमित पाये गये।

अश्वों में बबेसिया का संक्रमण एक गम्भीर समस्या है। इसे व्याधि के नैदानिक विधि के विकास के लिए इस परजीवी की बाह्य प्रोटीन (ई०एम०ए०–2) के गुण सूत्र अंशों को ई०कोलाई में प्रतिलिप्त करके अभिव्यक्त किया गया। इस प्रतिलिप्त अभिव्यक्त प्रोटीन का शुद्धिकरण करके एक एलाईजा विधि के मानकीकरण हेतु उपयोग किया गया। यह एलाईजा संक्रमित एवं असंक्रमित रक्तोद नमूनों की तुलनात्मक पहचान करने में समर्थ है। यह विधि केवल बबेसिया इक्वाई की प्रतिकारकों की विशिष्ट पहचान करती है।

केन्द्र द्वारा अश्व के विभिन्न रोगजनों के विभेदन के लिए आणविक नैदानिक विधियों का विकास किया जा रहा है। इसके अन्तर्गत ई०एच०वी०–1 एवं 4 के विभेदन के लिए प्रोटीन–जी गुण सूत्र–आधारित एक बहुलकीकरण

pathogens/strains. For differential diagnosis of EHV-1 and EHV-4 viruses, a PCR was standardized using primers designed from the region of glycoprotein G. In addition, a multiplex PCR was standardized using primers designed in such a way that these amplified 186 bp of nucleotide 865 to 1050 of gG gene of EHV-1 while 279 bp of 979 to 1257 of gG gene of EHV-4. Similarly, a PCR was developed for differentiation of two subspecies of *Streptococcus equi* i.e. *S. equi* subspecies *equi* and *S. equi* subspecies *zooepidemicus* employing different primers. In addition, a PCR was standardized for detection of *T.evansi* infection in equines.

Monoclonal antibodies serve as important tool in diagnosis. During the year, monoclonal antibodies were developed for improvement of various diagnostics developed by the centre. To further improve the sensitivity and specificity of previously developed serum-based sandwich ELISA for pregnancy diagnosis in mares, monoclonal antibodies against eCG were developed for use in the ELISA. Of the 16 secretory hybrids developed, one of them was cloned. Similarly, six clones secreting monoclonal antibodies against equine rotavirus were developed so as to employ them for use in sandwich ELISA developed for diagnosis of equine rotavirus infection. Further characterization of these monoclonal antibodies is in progress.

Seromonitoring of important equine diseases is being undertaken with special emphasis on indigenous equines to study the magnitude of existing and emerging equine diseases in different states of the country. During the year, active sero-surveillance was conducted in 12 states/ UTs of India, namely Maharashtra, Rajasthan, Chandigarh, Delhi, Gujarat, Haryana, Himachal Pradesh, Punjab, Tamil Nadu, Chattisgarh, Uttar Pradesh and West

श्रृंखला अभिकिया (पी०सी०आर०) तथा एक बहुविध पी०सी०आर० का मानकीकरण किया गया। बहुविध पी०सी०आर० से ई०एच०वी०–1 के 186 बेस जोड़े विस्तृत हुए जबकि ई०एच०वी०–4 के 276 बेस जोड़े विस्तृत हुए। इसी तरह स्ट्रेप्टोकोक्स इक्वाई के दो उप–वर्णो इक्वाई व झूएपिडैमिक्स के विभेदन के लिए पी०सी०आर० विधि का विकास किया गया। इसके अतिरिक्त अश्वों में ट्राईपेनोसोमा के निदान के लिए भी एक पी०सी०आर० का मानकीकरण किया गया।

एकल प्रतिलिपित प्रतिकारक (मोनोक्लोनल एन्टीबॉडी) नैदानिक विधियों के विकास के लिए लाभदायक साधन सिद्ध हुए हैं। इनके महत्व को ध्यान में रखते हुए इस वर्ष के दौरान, केन्द्र में दो विभिन्न मोनोक्लोनल एन्टीबॉडियों का विकास किया गया। घोड़ियों में शीघ्र गर्भाधारण निदान के लिए विकसित की गयी एलाईजा विधि की संवेदनशीलता एवं विशिष्टता में सुधार के लिए ई०सी०जी० हारमोन–विरोधी मोनोक्लोनल एन्टीबॉडी बनाई गई। इसी प्रकार अश्व रोटा वायरस विषाणु के निदान के लिए विकसित सैन्डविच एलाईजा में सुधार हेतु अश्व रोटा वायरस–विरोधी मोनोक्लोनल एन्टीबॉडी की 6 प्रतिलिपियॉ बनाई गई। इन मोनोक्लोनल प्रतिकारकों के अन्य अभिलक्षणों का अध्ययन कार्य प्रगति पर है।

देश के विभिन्न राज्यों के स्वदेशी अश्वों में विद्यमान एवं सम्भावित अश्व रोगों की रक्तोद जांच द्वारा सर्वेक्षण का कार्य 'राष्ट्रीय अश्व रोग सर्वेक्षण कार्यक्रम' के अन्तर्गत प्रगति पर है। इस वर्ष के दौरान देश के 12 विभिन्न राज्यों महाराष्ट्र, राजस्थान, चण्ड़ीगढ़, दिल्ली, गुजरात, हरियाणा, हिमाचल प्रदेश, पंजाब, तमिलनाडु, छत्तीसगढ़, उत्तर प्रदेश एवं पश्चिम बंगाल में सक्रिय अश्व रक्तोद सर्वेक्षण किया गया। सीरम की जांच करने पर 1069 में से 22 (2.05 प्रतिशत) नमूने ई०एच०वी०–1 से संक्रमित पाये गये, जबकि 1055 में से 230 (21.8 प्रतिशत) नमूने बबेसिया से संक्रमित

Bengal. EHV-1 antibodies were detected in 22 of the 1069 (2.05 %) samples, while *Babesia equi* sero-prevalence was detected in 230 of the 1055 (21.80 %) serum samples tested. None of the samples tested for equine infectious anemia, African horse sickness, glanders, brucellosis and *Salmonella* Abortus equi was detected positive.

Rhodococcus equi is one of the major causes of foal pneumonia. A study was initiated to understand the extent of problem in indigenous foals. Ten isolates of *R. equi* were obtained including 8 from respiratory swabs, one from tissues of foal died of respiratory problem and one from soil.

An equine herpes virus-1 (EHV-1) killed vaccine incorporating indigenous strain (Hisar-90-7) of EHV-1 developed at this centre was evaluated in indigenous non-descript ponies for immune response and protection against challenge. Following vaccination, virus neutralizing antibody appeared after first week of the vaccination and peak antibody titers were observed 3-5 weeks post-vaccination. On challenge, abortion and other clinical manifestations could not be seen in non-vaccinated ponies. Therefore, the experiment needs repetition by inducing and ascertaining virulence of the EHV-1 virus to be used for challenge purpose.

In order to conserve the germplasm of Marwari breed, the technique for cryopreservation of semen of Marwari stallions was standardized. During the year, work was done to determine the optimum glycerol concentration and thawing method. Our findings established that freezing media containing 3% glycerol and thawing at 45^{o}C for 15 seconds gave better post-thaw motility in frozen stallion spermatozoa.

During the year, the scientists of the centre published 33 original research articles in international and national journals and 28

थे। इस दौरान जांचे गए किसी भी रक्तोद नमूनों में अश्व एनीमीया, अफ्रीकन अश्व व्याधि, गलैन्डरस, ब्रुसेला एवं सालमोनैला का संक्रमण नहीं पाया गया।

अश्व शावकों में रहोडोकोक्स इक्वाई निमोनिया का एक प्रमुख कारण है। स्वदेशी अश्वों में इस समस्या के प्रसार को जानने के लिए एक अध्ययन शुरु किया गया। इस अवधि के दौरान 9 श्वास रोगी अश्वों सहित 10 आर०इक्वाई जीवाणुओं का पृथक्कीकरण किया गया।

इस केन्द्र में विकसित ई०एच०वी०–1 (हिसार–90–7) के निष्क्रिय विषाणु–प्रयुक्त टीकों की प्रतिरक्षण एवं बचाव क्षमता का गर्भवती टट्टुओं में परीक्षण किया गया। टीकाकरण के एक सप्ताह के उपरान्त घोड़ियों में विषाणु निष्प्रभावीकृत प्रतिकारक (न्यूट्रैलाइजिंग एन्टीबॉडी) पाई गयी तथा 3-5 सप्ताह पर इनकी मात्रा चरम सीमा पर थी। ई०एच०वी०–1 के विषाणुओं से बिना टीकाकृत अश्वों को संक्रमित करने पर भी गर्भपात तथा बीमारी के लक्षण नहीं दिखाई दिए। अतः इस अध्ययन को और अधिक विषाक्त विषाणुओं को प्रयोग में लाते हुए दुबारा करने की आवश्यकता है।

मारवाड़ी अश्वों के जीव द्रव्य के संरक्षण की दिशा में केन्द्र द्वारा इन अश्वों के वीर्य के हिमीकरण संरक्षण विधि का मानकीकरण किया गया है। इस वर्ष के दौरान वीर्य तनुकारक में ग्लीसरोल की उचित मात्रा एवं तरलीकरण विधियों पर शोध कार्य किया गया। इन नतीजों से यह निष्कर्ष निकला कि ग्लीसरोल की मात्र 3 प्रतिशत प्रयुक्त करने पर तथा तरलीकरण हेतु 15 सैकेण्ड के लिए 45 सेंटीग्रेड का तापमान देने पर तरलीकरण–उपरान्त शुक्राणु गतिशीलता बेहतर रहती है।

इस वर्ष केन्द्र के वैज्ञानिकों ने 33 मूल शोध पत्र एवं 28 शोध सार राष्ट्रीय एवं अन्तर्राष्ट्रीय शोध पत्रिकाओं में प्रकाशित किए। केन्द्र के वैज्ञानिकों ने 16 शोध पत्र विभिन्न सम्मेलनों एवं गोष्ठियों में भी प्रस्तुत किये। इस

research abstracts in different conferences and symposia. Scientists also participated and presented papers in 16 different conferences/ symposia. Two of the scientists from the centre completed their Ph.D. during the year. In addition, a number of post-graduate students from state university acquired trainings from the centre. A Ph.D. thesis completed at the centre was awarded Dr. Patri Rama Rao award by the Indian Association of Veterinary Pathologists. Scientists and other staff members participated in 9 different training programmes for their skill upgradation during the year.

Work towards infrastructure improvement at the centre got impetus with the initiation of construction work for the laboratory-cum-office building at our Bikaner campus and laying of foundation stone for the second wing of laboratory-cum-administration building at Hisar campus by Hon'ble Director-General ICAR Dr. Mangala Rai on January 11, 2005. Indian Council of Agricultural Research entrusted the centre with an additional responsibility of establishing Veterinary Type Culture Facility at the centre with an outlay of Rs 780 lacs. ICAR established internet connectivity at NRCE using ERNET backbone with broad band VSATs/leased lines under National Agricultural Technology Programme.

Field trials and in-house validation of various technologies developed at the centre were done during the year. Serum-based sandwich ELISA for pregnancy diagnosis was demonstrated to the veterinarians in U.P. This technology was recognized as one of the cutting edge technologies in the area of agriculture during 75th Annual General Meeting of ICAR Society. Validation of EHV-1 diagnostic kit named Equiherpes B-ELISA was got done from three external and three in-house research laboratories.

During this period, the centre took equine

वर्ष केन्द्र के दो वैज्ञानिकों ने पीएच०डी० की डिग्री प्राप्त की। इसके अतिरिक्त राज्य के विश्वविद्यालयों से कई स्नातकोत्तर छात्रों ने केन्द्र की प्रयोगशालाओं में प्रशिक्षण प्राप्त किया। केन्द्र के एक वैज्ञानिक को पीएच०डी० में उत्कृष्ट कार्य के लिए भारतीय पशु रोग वैज्ञानिक संघ द्वारा डा० पत्री राम राव पुरस्कार से सम्मानित किया गया। केन्द्र के वैज्ञानिकों एवं अन्य कर्मचारियों ने अपनी कार्य कुशलता में सुधार हेतु इस वर्ष विभिन्न प्रशिक्षण कार्यक्रमों में भाग लिया।

ढ़ांचागत सुविधाओं में सुधार के अन्तर्गत इस अवधि में हमारे बीकानेर परिसर में प्रयोगशाला–एवं –प्रशासनिक भवन के निर्माण कार्य में काफी प्रगति हुई तथा हिसार परिसर में प्रयोगशाला–एवं–प्रशासनिक भवन–2 का शिलान्यास आदरणीय डा० मंगला राय जी, महा निदेशक, भा०कृ०अनु०प० ने अपने कर–कमलों द्वारा 11 जनवरी 2005 को किया। भा०कृ०अनु०प० ने इस केन्द्र को दसवीं पंचवर्षीय योजना में 7.80 करोड़ रुपये के प्रावधान के साथ पशु चिकित्सा जीवाणु–संवर्धन केन्द्र स्थापित करने की अतिरिक्त जिम्मेवारी सौंपी। राष्ट्रीय कृषि प्रौद्योगिकी कार्यक्रम के अन्तर्गत भा०कृ०अनु०प० के इन्टरनेट नेटवर्क की ब्रोड–बैन्ड सुविधा के जरिये इस केन्द्र को जोड़ा गया।

वर्ष के दौरान केन्द्र द्वारा विकसित प्रौद्योगिकियों का क्षेत्रीय परीक्षण एवं आन्तरिक प्रयोगशालाओं द्वारा सत्यापन किया गया। रक्तोद–आधारित अश्व गर्भाधारण नैदानिक सैंडविच एलाईजा का उत्तर प्रदेश के पशु चिकित्सकों को प्रदर्शन किया गया । भा०कृ०अनु०प० सोसाईटी की 75वीं वार्षिक आम बैठक में इस प्रौद्योगिकी को कृषि क्षेत्र की नौ अति अग्रणी अनुसंधानों में शामिल किया गया। ई०एच०वी०–1 के नैदानिक किट इक्वीहर्पीस बी०एलाईजा का सफलतापूर्वक सत्यापन तीन आन्तरिक एवं तीन बाह्य प्रयोगशालाओं द्वारा किया गया।

welfare activities in different parts of the country by organizing seven equine health camps and a number of farmer meets 'kisan goshthis' to enlighten the equine owners on various aspects of disease control and management. In addition to the treatment of major equine ailments in these camps, deworming and tetanus vaccination was done in equines. Blood and serum samples were collected from all the animals for serological testing of various viral, bacterial and parasitic infections. Feedback from farmers was obtained for further research and development in equine health and production. Foundation Day of the centre was celebrated with great zeal and enthusiasm on 26th November 2004. On this occasion, a horse show was organized in which indigenous horses from various states participated in different equestrian events.

The centre also offers consultancy and diagnostic services for important infectious diseases of equines. Under this programme, 5158 equine serum samples were tested for equine infectious anemia in addition to testing for other diseases. During the year, the centre generated a revenue of Rs. 23.44 lacs from its internal sources, mainly through the diagnostic services rendered and sale of superior quality equines to the farmers.

इस अवधि के दौरान अश्व कल्याणकारी गतिविधियों का देश के विभिन्न भागों में प्रसार हेतु केन्द्र ने सात अश्व स्वास्थ्य शिविर एवं कई किसान गोष्ठियों का आयोजन किया। इनके जरिये किसानों एवं अश्व पालकों को अश्व स्वास्थ्य, अश्व रोग रोकथाम एवं प्रबंधन के बारे में जागृत किया गया। स्वास्थ्य शिविरों में अश्व रोगों के ईलाज के अतिरिक्त कृमि नाशक दवा पिलाई गई तथा टैटेनस के टीके लगाये गए। रक्त एवं रक्तोद नमूनों को विभिन्न विषाणु, जीवाणु एवं परजीवी संक्रमण की जांच के लिए एकत्र किया गया। 26 नवम्बर 2004 को केन्द्र का स्थापना दिवस बड़े उत्साह के साथ मनाया गया। इस शुभ अवसर पर एक अश्व प्रदर्शनी का आयोजन किया गया तथा विभिन्न राज्यों के स्वदेशी अश्वों ने अश्व क्रीड़ाओं में भाग लिया।

केन्द्र अश्वों के मुख्य सक्रामंक रोगों के लिए परामर्श एवं नैदानिक सेवाएं प्रदान करने के लिए प्रमाणित है। इन सेवाओं के अन्तर्गत अन्य बीमारियों के निदान के अतिरिक्त 5158 रक्तोद नमूनों को अश्व एनीमिया के लिए जाँचा गया। इस वर्ष केन्द्र ने अपने आन्तरिक स्त्रोतों से 23.44 लाख रुपये अर्जित किए जिसमें अश्व रोग निदान सेवाऐं तथा किसानों को उत्तम नस्ल के अश्वों की बिक्री मुख्य थी।

Introduction

India has 1.77 million equines comprising 0.70 million horses and ponies, 0.29 million mules and 0.78 million donkeys. Majority of the equines (98%) in India comprises indigenous horses, ponies, donkeys and mules. These animals provide livelihood to the landless, small and marginal farmers and other section of our rural and semi-urban society through draught and transport especially in hilly, arid and semi-arid regions. Remaining 2% equines are kept in organized sectors and provide services to the army, police, border security force, racing industry and sports. In order to improve the health, performance and production potential of equines in India, the Indian Council of Agricultural Research established National Research Centre on Equines (NRCE) on 26th November 1985 at Hisar (Haryana).

The main campus of NRCE is located at Hisar (Haryana) and has state-of-the art laboratories for undertaking research in areas of equine virology, bacteriology, parasitology, immunology, pathology, medicine, biochemistry, biotechnology. In addition, NRCE has a sub-campus at Bikaner (Rajasthan) where research laboratories for genetics and breeding, reproduction, physiology and nutrition are established to undertake research on equine production. Research activities are carried out by a team of 18 dedicated scientists under the dynamic leadership of Dr. S.K. Dwivedi, Director NRCE. The research activities are supported by centralized services like animal and agriculture farms, experimental animal facility, library and internet facility. The centre has well-maintained herd of Marwari and Kathiawari horses and exotic donkeys at Bikaner. Efforts are being made to create facilities for various equestrian events for the benefit of equine lovers and those interested in equine sports. In addition, the centre has requisite bio-containment facilities and is in the process of development of BSL-III laboratory.

Mandate of NRCE

- To undertake research on health and production management in equines;
- To develop diagnostics/biologicals for major equine diseases;
- To act as national referral facilities for diagnosis, surveillance and monitoring of equine diseases;
- To provide diagnostic, advisory and consultancy services.

Major achievements of the centre

The centre has made the following salient achievements in a short span since its inception:

- Vaccines for the control of equine diseases: The centre has developed equine influenza vaccine using indigenous isolate (A/Equi-2/Ludhiana/87). Improved bacterin and outer membrane protein-based vaccines have been developed for *Salmonella* Abortus equi. Equine Herpes Virus-1 vaccine is under experimental trial in equines.
- Disease Diagnosis: The centre has been recognized as National Referral centre for diagnosis of important equine infectious diseases by Department of Animal Husbandry and Dairying, Ministry of Agriculture (Government of India). The centre has developed diagnostic kits for equine herpes virus-1 (HERP kit) and *Babesia equi* (COFEB kit) infections. In addition, the centre has developed various tests for diagnosis of equine diseases including equine influenza, EHV-1 & EHV-4 infection, equine rotavirus diarrhoea, equine infectious anaemia, equine piroplasmosis, equine viral arteritis, leptospirosis, mycoplasmosis, streptococci infection, *etc*.
- Equine disease surveillance: NRCE is

involved in nation-wide disease monitoring and surveillance of important equine diseases particularly those that are included in list "A" and "B" of *Office International des Epizooties* (OIE). The database generated on prevalence of equine diseases from different geographical locations is helping in their effective management. For instance, the centre contributed significantly in the control of equine influenza outbreak of 1987 involving 83000 equines. Effective influenza vaccine was developed subsequent to this outbreak. The equine babesiosis and equine herpes virus infection is currently endemic in our country and reported by sero-surveillance in most of the states of the country. Therefore, development of control strategies against these diseases is the main priority of the centre. Control of EIA in India was done by timely diagnosis and adopting package of practices formulated by NRCE. The disease is not reported from India since 1997 in our active surveillance programme.

- Immunobiologicals: Monoclonal antibodies have been developed for diagnosis and characterization of equine herpes, equine influenza and equine rota viruses. Monoclonals have also been developed against equine chorionic gonadotropin hormone.
- Molecular characterization of pathogens: DNA finger printing of EHV-1 virus and sequencing of antigenically important genes of equine influenza virus was done to identify different strains prevalent in equines of India.
- Artificial insemination: The technique of artificial insemination using frozen semen for production of superior quality Marwari horses, mules and exotic donkeys has been perfected. The pure germplasm of endangered indigenous breeds of horses is being conserved using this technology.
- Indigenous breed characterization: Phenotypic and molecular characterization of indigenous breeds of horses has indicated the existence of genetic variability within Marwari breeds and molecular markers for identification of this breed have been established.
- Baseline data has been generated on some of the important haematological, physiological and biochemical indices of Kathiawari horses as well as local donkeys.
- Early pregnancy diagnosis: Pregnancy diagnosis between days 14 and 18 post-insemination has been achieved using ultrasonography in donkey and horse mares. An ELISA for pregnancy diagnosis in mares using serum samples has been developed.
- Donkey fibre has been used to produce carpets by mixing with sheep fibres in the ratio of 40:60.

Patents

- Patent has been granted by the Patent Office, Government of India on application (2199/DEL/96) entitled *"A method for preparation of a diagnostic kit useful for forecasting Equine Herpes Virus-1 disease".*
- A patent has been filed for "Complement fixation test (CFT) based diagnostic COFEB-Kit developed for the detection of *Babesia equi* antibodies".

Services

NRCE provides following services to the farmers and equine breeders:

- Disease diagnosis : The centre provides disease diagnostic services for various infectious and non-infectious equine diseases to equine owners, breeders, state animal husbandry departments, police and army horses.
- Artificial insemination to augment the production of superior quality Marwari horses, mules and donkeys.
- Quality jacks and jennies are supplied to various states, breeding societies and

farmers, for production of superior quality mules and donkeys.

- Regulation of movement of equines: NRCE is providing health certification for movement of equines within and outside the country. This facility has helped in promotion of export of horses.
- Extension activities: Assessment and transfer of technology using the latest know-how of information technology is also given due importance to extend the technologies to the end-users. The scientific and technical staff provides clinical and diagnostic (including pregnancy diagnosis) services and consultancy to the farmers on demand related to equine health and production. Farmers are imparted trainings and supplied educational materials for equine management, production and health.

Thrust areas

- Surveillance and monitoring of important equine diseases including emerging and existing diseases with special emphasis on foal mortality and production losses.
- Development of effective and preferably field-based diagnostics and potent immunoprophylactics against major equine diseases threatening equine population in India.
- Development of effective plant-based products for management of some economically important equine diseases and to enhance performance in equids.
- To provide diagnostic and consultancy services for beneficiaries particularly equine farmers and breeders.
- Application of artificial insemination techniques in horse production using frozen semen of true to breed indigenous stallions for the conservation of threatening species in India.
- Breed characterization and *in situ* conservation of various indigenous breeds of horses.
- Exploiting importance of equine draught power for economically weaker section of the society.
- Achieving the status of 'OIE International referral laboratory' for diagnosis of equine rhinopneumonitis and piroplasmosis.

Staff Position

Name of the post	Number of posts		
	Sanctioned	Filled	Vacant
Director	1	1	-
Scientific	20	18	2
Technical	23	21	2
Administrative	11	11	-
Supporting	23	22	1
Total	78	73	5

Summary of Expenditure

		(Rupees in Lacs)
Expenditure	2003-04	2004-05
NON-PLAN		
1. Establishment charges including		
LSP/PF, Wages, OTA	110.13	131.63
2. Traveling allowances	2.11	2.40
3. Other charges including equipments	94.11	85.21
4. Works	23.17	6.27
Non-Plan Total	*229.52*	*225.51*
PLAN		
1. Establishment charges including	0.59	0.63
LSP/PF, Wages, OTA		
2. Traveling allowances	1.91	0.67
3. Other charges including equipments	36.77	47.81
4. Works	119.99	150.84
Plan Total	*159.26*	*199.95*
Total Expenditure	388.78	425.46

Summary of Revenue Generation

		(Rupees)
Revenue Source	2003-04	2004-05
1. Sale of Farm Produce & auction of dry trees	5304	4400
2. Sale of Livestock	318582	200202
3. Sale of Publication and advertisements	1050	2600
4. License Fee	64535	60745
5. Interest on loans and advances	23760	77142
6. Interest on short term deposits	95900	94289
7. Leave salary & pension contribution	-	136155
8. Income from internal resource generation	1190600	1490150
9. Auction of old materials	92617	-
10. Receipt from services	11652	6600
11. Other misc. receipts	845676	272202
Total Revenue	2649676	2344485

Research Achievements

Molecular characterization of horses of Marwari breed

Marwari breed of horses constitutes an elite group of indigenous horses, which are known for their sturdiness, swiftness, elegance and beauty. The quality horses (stallion and mares) of this breed are decreasing day by day due to lack of proper breeding policy and indiscriminate breeding practices adopted by equine owners. Indiscriminate breeding has resulted in the loss of morphological, genetic and economical characteristics of Marwari horses. Qualitative and quantitative characterization of blood protein and DNA polymorphism may be useful in identification and characterization of Marwari horses. Keeping this in view, Marwari germplasm was characterized by studying their bio-metrical, bio-chemical and molecular characteristics.

For phenotypic characterization of the Marwari horses located in different parts of Rajasthan, the bio-metrical parameters from different body regions were measured and analyzed. A total of 114 Marwari horses comprising 98 mares and 16 stallions from seven locations were utilized for the present study. To estimate each trait, data were analyzed separately for both sexes and location wise. The least squares means for average height at withers in Marwari stallions was 154.38±0.1.062 cm where as the height of mares was 150.39±0.392 cm.

Among blood bio-chemical indices, the significant differences due to sex and age groups were observed. The GOT was higher in adult animals where as ALP was higher in young ones. The tri-glycerides and GPT were significantly higher in females than in males.

Genetic characterization was attempted using known polymorphic microsatellites for studying the genetic variability among the population of this breed. Out of 26 known polymorphic microsatellite markers in exotic breeds of horses, three markers (AHT16, AHT44 and UM021) were observed to be monomorphic in Marwari horses. These three markers seem to be located in the highly conserved region of genome and probably can differentiate Marwari breed from the other horse breeds .

A total of 130 alleles were observed at all the loci ranging from 3 (HTG2, HTG4, HTG6) to 12 (UM007) with a mean of 5.9±2.24 alleles per locus. The mean effective number of alleles in Marwari horse population was 3.3±1.27, ranging from 1.2 (HTG2) to 7.5 (UM007) (Fig. 1). The mean observed and expected Levene's and Nei's heterozygosities were 0.5306 (±0.22), 0.6612 (±0.15, Levene's) and 0.6535(±0.14, Nei's), respectively. This basic information indicated the existence of high genetic variability among Marwari equine population. The PIC values that provide informativeness of a genetic marker suggest that 81.8 % markers were highly informative (PIC>0.5) in terms of their suitability as marker of choice for genetic diversity studies and remaining loci were reasonably informative. On testing the neutrality of each

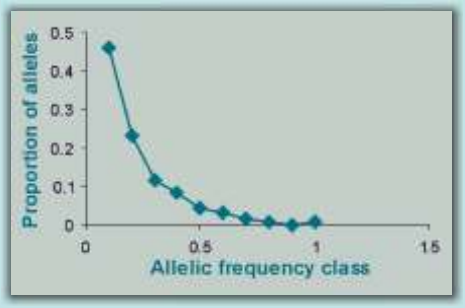


Fig. 1. Proportion of alleles and their distribution in Marwari horses

marker by Ewens-Watterson test indicated that all the microsatellite loci except HTG6 and UM004 were neutral and unlinked to any trait. Mean value of within population inbreeding estimates (0.2433±0.05) indicated the low level of inbreeding in the population. High level of heterozygosity, PIC and low level of heterozygosity deficit in the Marwari horse population reflect high genetic variability that can be exploited by horse breeders for planning breeding strategies and prioritizing the breed for its conservation.

DNA polymorphism studies revealed high level of heterozygosity, PIC and low level of heterozygosity deficit in the Marwari horse population which reflect high genetic variability in Marwari equine population that can be exploited by horse breeders for planning breeding strategies and prioritizing the breed for its conservation. This study also indicated that the present Marwari equine population had not experienced recent bottleneck which is very informative and important for equine breeders.

(S.N. Tandon, A.K. Gupta, R.A. Legha, R.C. Sharma and Mamta Chauhan)

Development of diagnostics and technologies for EHV-1 infection

Considering the significance of EHV-1 associated abortions in mares, a five-year research project funded by National Agricultural Technology Project grants of ICAR was undertaken by this centre for the development of improved diagnostics for EHV-1 infections, evaluation of efficacy of the diagnostic reagents so developed and their validation for field use.

Under this project, work on refinement of previously patented HERP Kit was done so as to develop a specific and quantitative assay to minimize cross-reactions with other equine herpes viruses. Under this project, various tests standardized to detect EHV-1 antibodies and antigen included indirect enzyme-linked immunosorbent assay, sandwich ELISA, immunoperoxidase techniques and blocking dot-ELISA. PCR was also standardized for detection of EHV-1 DNA.

Salient outcomes of this project included development of a neutralizing monoclonal antibodies-based blocking ELISA for specific serodiagnosis of EHV-1 infection. This assay employed neutralizating monoclonal antibodies (Mabs) from available clones 1H6 and 9C6. This B-ELISA is simple to perform and less time consuming than virus neutralization test for diagnosis of EHV-1 infection. The B-ELISA showed 100% specificity and sensitivity with 9C6 Mab. Kappa, a measure of agreement beyond chance, was 0.9438. The correlation coefficients (*r)* between B-ELISA percentages inhibition and VN antibody titre (VNT) was 0.850 with 9C6 Mab, which was highly significant ($P<0.01$).

Based on this assay, a diagnostic kit named Equiherpes B-ELISA Kit was developed for field use (Fig. 2). The reagents of the kit have been stabilized in liquid buffer to increase its shelf-life at 4°C and parts of kit reagents can be used on different days as per the availability of the serum samples. This kit tests the serum sample using single dilution (1:250) thus making it very economical. For precision/validation of Equiherpes B-ELISA Kit, a total of 1224 serum

samples from 17 states were tested and results compared with the conventional virus neutralization test. The agreement between the results of two tests on 1224 serum samples was 85.86%. The validation of results of the kit was got done from six different laboratories and

Fig. 2. Equiherpes B-ELISA kit showing different components

there was more than 95% agreement in results of different laboratories.

In addition, different EHV-1 strains were compared by DNA fingerprinting and it was found that more than one genetically variant strains of EHV-1 are circulating in equine population of Northern India. Field veterinarians of different diagnostic laboratories were imparted training in diagnosis of EHV-1 infection in equines and a short course on "*Diagnosis of equine abortion (EHV-1 infection) using monoclonal antibodies based enzyme-linked immunosorbent assays and by polymerase chain reaction*" was also organized from January 28-February 11, 2002 in which 17 scientists from different institutes of India were imparted training in molecular and ELISA-based diagnostics for equine abortions.

A patent application entitled *"Neutralizing monoclonal antibody-based blocking ELISA diagnostic kit for detection of equine herpes virus-1 specific antibodies"* is being submitted for getting Indian patent for this diagnostic kit.

(B.K. Singh, Baldev R. Gulati and Nitin Virmani)

Effect of *Lawsonia inermis* constituents on blood form of *Trypanosoma evansi* in mice

Trypanosoma evansi is a protozoan parasite causing severe mortality and morbidity among equines in India. No new drug from allopathic system of medicine is available since last 60 years. To develop a drug for treatment of *T.evansi* infection of equines, extracts from a medicinal herb, *Lawsonia inermis* were evaluated in the project undertaken by this centre. To purify the active ingredients, activity-guided separation of antitrypanosomal components from this herb was done using different chromatography techniques (HPLC and TLC) (Fig. 3). Using these methods, major components exhibiting *in vitro* antitrypanosomal activity have been identified.

Based on the promising *in vitro* anti-trypanosomal activity, *L.inermis* constituents were tested for their trypanocidal activity in mice. The constituents of *L.inermis* leaf were fractioned and purified using column chromatography and high performance liquid chromatography. Swiss albino mice were used for *in vivo* testing and mice were infected by intra-peritoneal inoculation of *T.evansi* (10000 per mice). The parasitaemia was monitored and test drugs were administered in different groups

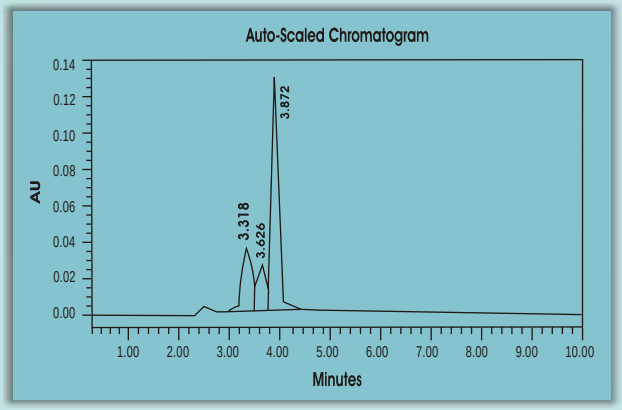


Fig. 3. Fractions of *L.inermis* exhibiting antitrypanosomal activity, as obtained by HPLC

at the onset of parasitaemia. All the 8 purified fractions of *L.inermis* leaf extract were tested in three different dosage (50, 100, 200 µg/kg) daily by oral and intra-peritoneal routes.

Out of these 8 fractions, 2 fractions in oral route could increase survival period of mice from 3 to 17 days but none could clear blood form of *T.evansi* completely. However, none of these test fractions showed any toxic effects during the 30 days observation period. These extracts failed to exert trypanocidal activity when given by intra-peritoneal route.

The results indicate that the constituents of *L.inermis* responsible for trypanocidal activity *in vitro* might be degraded *in vivo* system when administered by oral and intra-peritoneal route. For *in vivo* activity, a suitable drug delivery system needs to be evaluated to protect the allelochemicals.

(S. Dey and S.K. Dwivedi)

A sensitive and specific sandwich ELISA for detection of equine rotavirus from diarrhoeic foals

Diarrhoea is one of the important causes of mortality and morbidity in infants of several animal species including humans and foals. Most of the foals have at least one episode of diarrhoea before they are 6 months old, which may cause loss of condition or even be fatal. Amongst various etiological agents, rotavirus is reported to be the predominant cause of foal diarrhoea world over. Rapid diagnosis of rotavirus-associated diarrhoea is important in managing the outbreak and for further spread of disease in the herd. During the year, a sandwich ELISA was standardized for detection of rotavirus from 20% stool suspension by capturing the antigen in hyperimmune rabbit anti-rotavirus coated ELISA strips and further detection by employing monoclonal antibody raised against group-specific antigen. All the known positive rotavirus isolates of different origin (equine=4, bovine=3 and porcine=2) were detected strong positive (Mean OD_{450} above 0.5) by this ELISA, indicating that this ELISA is able to detect all group A rotaviruses belonging to different species of animals. This ELISA was found specific for rotavirus since other equine viruses (equine herpes virus-1, equine influenza and equine arteritis viruses) were found negative when tested in this ELISA.

Stool samples (n=72) collected from diarrhoeic foals below 2 months of age from an organized farm were tested by the monoclonal-antibody based sandwich ELISA and results were compared with virus isolation and RNA-PAGE. The sandwich ELISA was found to be 100% sensitive whereas RNA-PAGE was only 52.38% sensitive in comparison to virus isolation ($30.34 \leq \pi \leq 73.61$, at 95% confidence interval). The sandwich ELISA was also quite specific test giving a specificity of 0.9608 ($0.8541 \leq \pi \leq 0.9932$) (Table 1). The agreement between the results of ELISA and virus isolation for

Table 1. Analysis of sensitivity and specificity of sandwich ELISA and RNA-PAGE as compared to virus isolation in detection of equine rotavirus

Screening test	Results	Virus Isolation +	Virus Isolation -	Sensitivity*	Specificity*
RNA-PAGE	Positive	11	0	0.5238	1
	Negative	10	51	(0.303-0.7361)	(0.9127-1)
Sandwich	Positive	21	2	1	0.9608
ELISA	Negative	0	49	(0.8076-1)	(0.8541-0.9932)

*Figures in parenthesis are values calculated at 95% confidence interval.

detection of rotavirus in diarrhoeic foal stool samples (n=72) was 97.22%, including 21 positive and 49 negative samples by both the tests.

In conclusion, the monoclonal antibody-based sandwich ELISA developed in the present study was more efficient for detection of rotavirus from stool samples than RNA-PAGE and virus isolation since it is rapid to perform, simple, highly sensitive and specific for detecting rotavirus from equine stool samples.

(Baldev R. Gulati and B.K. Singh)

Development of sensitive and specific diagnostic tests for detection of equine piroplasmosis

Piroplasmosis caused by *Babesia equi* is a serious problem of equines in India. In *B. equi,* two kinds of merozoite surface proteins, EMA-1 (equi merozoite antigen-1, 34 kDa) and EMA-2 (30 kDa) have been identified as most immunodominant antigens. EMA-1 and -2 genes have 52% amino acid identity with each other and have glycosyl-phosphatidylinositol (GPI) anchor-specific motifs in the sequence, suggesting that these proteins might express on the outer surface of merozoite with GPI anchor, similar to merozoite surface antigens in other *Babesia* (MSA-1). Additionally, it was shown that the EMA-1 and EMA-2 are mutually expressed on the surface of extraerythrocytic merozoite and also that the intraerythrocytic merozoite shed only EMA-2 antigen in the infected erythrocytic cytoplasm or inside membrane surface. In the present study, we amplified a truncated EMA-2 gene in a PCR reaction by using specific primers (Forward primer: 5'ACGAATTCGATGAGGCACCAAAG3'; Reverse Primer 5'ACGAATTCGGCGGTGAAGGT GTGCTT3'). A PCR product of about 432 bp was amplified and inserted into the EcoR1 cloning site of the pGEX-4T-1 expression vector (Fig. 4a). The resultant plasmids, pGEX/EMA-2t was used to produce the gene products fused with glutathione S-transferase (GST) in *E.coli,* DH5 strain, according to standard techniques. Gene was expressed under IPTG induction and finally the supernatant containing soluble GST fusion protein was purified with glutathione-sepharose 4B beads. The product was checked on SDS-PAGE

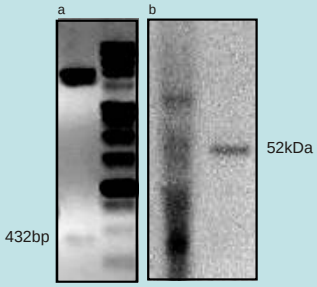


Fig. 4. Cloning & expression of EMA-2 gene

(Fig. 4b). ELISA was standardized using this recombinant protein as antigen. The assay quantitatively differentiated the reference positive and negative serum samples. The assay was found specific in detecting *B.equi* antibodies only and no cross-reaction was observed with *B.caballi, Trypanosoma evansi* antibodies.

(S. Kumar, R. Kumar, S. Dey
A.K. Gupta and S.K. Dwivedi)

Standardization of PCR for differentiation of EHV-1 and EHV-4

While EHV-1 is known to cause respiratory infections, abortions, neurological disorders and perinatal foal mortality, EHV-4 mostly causes respiratory infections and sometimes also leads to abortions. The differential diagnosis of EHV-1 and EHV-4 viruses is often complicated due to antigenic cross-reactions between the two viruses. A PCR-based diagnostic for differentiation of EHV-1 and 4 was standardized using primers designed from the region of glycoprotein G which showed maximum divergence between the two viruses. In this PCR, a final product of 192 bp and 289 bp was obtained for EHV-1 and EHV-4 viruses, respectively. Sensitivity of this assay was found to be ~1ng of DNA for both the viruses.

Further a multiplex PCR was standardized using primers designed in such a way that these amplified 186 bp of nucleotide 865 to 1050 of gG gene of EHV-1 while 279 bp of 979 to 1257 region of gG gene of EHV-4. Based on the difference in size of this PCR product, the two viruses could be differentially diagnosed (Fig. 5). On analysis of 42 samples from equines of Tamil Nadu and Haryana by multiplex PCR, one sample was detected positive for EHV-1 while none was found positive for EHV-4.

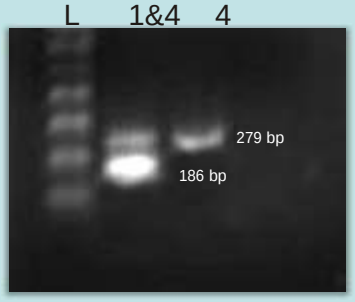


Fig. 5. Multiplex PCR for EHV-1 and EHV-4

(Nitin Virmani, A.S. Panisup,
B.K. Singh and B.R. Gulati)

PCR standardized for *Streptococcus equi* subspecies differentiation

Two subspecies of *Streptococcus equi* are commonly reported from equines. These are *S.equi* subspecies *equi*, the causative agent of strangles, and *S.equi* subspecies *zooepidemicus*, associated with abortions, endometritis, cervicitis, pneumonia, abscesses, joint infections and other lower respiratory disease. In an attempt to rapidly differentiate the two subspecies, we standardized a PCR employing two pairs of primers already cited in the literature. These primer pairs A and B amplified SeM region and 16S-23S RNA gene intergenic spaces, respectively. DNA of the two reference UK strains of both the subspecies were employed for standardization of PCR. With primer pair A, PCR product of ~700 bp specifically from *S. equi* and ~1.2 kbp product in case of *S. zooepidemicus* was amplified. With primer pair B, a PCR product

of ~900 bp were amplified using *S. equi* UK strain and of ~800 bp for *S. zooepidemicus* UK strain. Using These PCRs, various known *Streptococcus* isolates of Indian origin are being tested to confirm the specificity of the assay.

(Praveen Malik and Mamta Chauhan)

PCR amplification of Ro Tat 1.2 VSG gene in *Trypanosoma evansi*

Diagnosis of surra in equines usually depends on demonstration of the parasites in the blood of infected equines. When parasitemia is low, a majority of cases remain undiagnosed due to low sensitivity of routinely used parasitological tests. The variant surface glycoprotein (VSG) Ro Tat 1.2 which is predominant antigen type thought to be expressed specifically in a majority of *T. evansi* isolates of diverse geographical regions. A PCR was standardized using primers from the Ro Tat 1.2 VSG gene sequences. On amplification, this PCR yielded 761 bp product (Fig. 6). Further cloning and sequencing of this PCR amplified fragment (761 bp) is in progress.

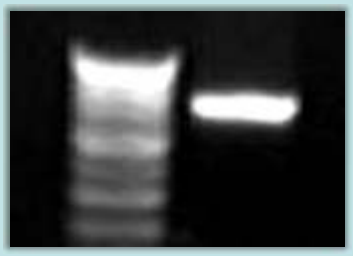


Fig. 6. PCR for *T. evansi*

(Rajender Kumar, Sanjay Kumar, A.K.Gupta, S.Dey and S.K.Dwivedi)

Monoclonal antibodies against equine chorionic gonadotropin

We previously developed a serum-based sandwich ELISA for pregnancy diagnosis in mares that can detect pregnancy in mares as early as 35 days of gestation. This test is animal friendly as it does not involve the transport of pregnant animal to a diagnostic centre for pregnancy diagnosis and is based on the detection of a hormone (equine chorionic gonadotropin) specifically present in the serum of pregnant mares. To make this test easy, sensitive and specific, an effort was made to develop monoclonal antibodies against eCG to use in ELISA for pregnancy diagnosis. For this, BALB/c mice were immunized with purified eCG and fusion was done as per standard protocol. Out of 960 fusion wells, 16 hybrids were selected on the basis of their secretory nature. All hybrids were observed to be low secretory. One of them was cloned by limiting dilution (Fig. 7). Further characterization including isotyping of monoclonal antibodies (mAb) produced by the clones and production of highly secretory clones is under progress. Based on the findings of these characterizations, mAb will be applied in ELISA for pregnancy diagnosis.

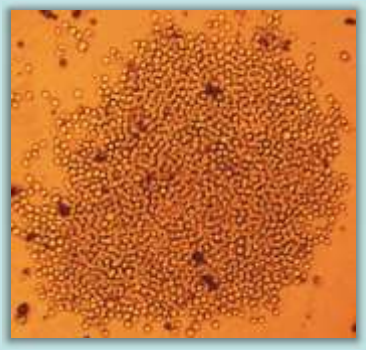


Fig.7. Monoclonal antibody clone against eCG

(A.K. Gupta, Yash Pal, Sanjay Kumar and S.K. Dwivedi)

Seromonitoring of important equine diseases

Sero-surveillance was conducted for various infectious diseases of known importance in equines. Serum samples were collected from 12 States/ UTs of India, *viz.* Maharashtra, Rajasthan, Chandigarh, Delhi, Gujarat, Haryana, Himachal Pradesh, Punjab, Tamil Nadu, Chattisgarh, Uttar Pradesh and West Bengal.

During the year, 22 of the 1069 (2.05 %) samples were found positive for EHV-1 by virus neutralization test. Antibodies to *B.equi* (piroplasmosis) were detected in 230 of the 1055 (21.80 %) samples tested (Fig. 8).

For EIA, 5158 sera samples from thoroughbred as well as indigenous equines were examined by Coggins test and none of the samples was found positive for the disease. In our continuous surveillance and monitoring programme, not a single EIA-positive case has been recorded since 1998-99. Haemagglutination inhibition test for Equine Influenza against A/equi-2 and virus neutralization test for equine viral arteritis revealed negative results on all the serum samples obtained from various states. None of the serum samples from indigenous equines tested for African horse sickness, glanders, brucellosis and salmonellosis (*Salmonella* Abortus equi) was positive for these infections.

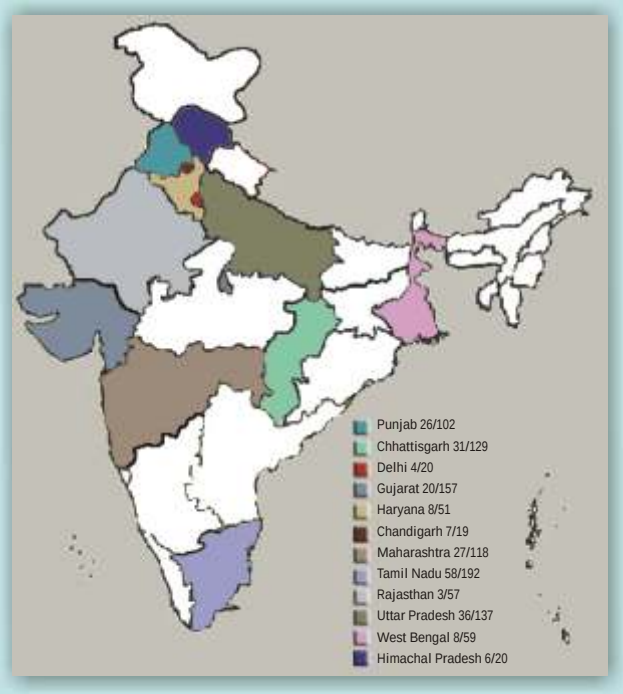


Fig. 8. Sero-surveillance of *Babesia equi* in different states of India

(S.K. Dwivedi, S.K. Khurana and other scientists)

Isolation of *Rhodococcus equi* from foals with respiratory infection

Rhodococcus equi is one of the major causes of foal pneumonia and is associated with pyogranulomatous lesions in lungs in addition to extra-pulmonary involvement. In order to understand the extent of problem in indigenous foals, nasal swabs from 27 foals with respiratory problem and tissues from a foal which died due to respiratory problem were collected. Swabs from in-contact foals and soil samples were also collected for organism isolation. Ten isolates of *R. equi* were obtained including 8 from respiratory swabs, one from tissues of foal died of respiratory problem and one from soil. Antibiotic sensitivity testing revealed that these isolates were sensitive to chloramphenicol, erythromycin, ciprofloxacin, neomycin and rifampicin.

Histopathological investigation of sections of lungs of an affected foal showed diffused areas of necrosis, proliferation of type II pneumocytes, lymphocytes, macrophages and plasma cells along with marked fibrous tissue proliferation in the parenchyma and around the blood vessels and bronchioles. Lumen of some of the bronchioles was completely necrosed and desquamated and a few bronchioles were full of exudates comprising necrotic debris, lymphocytes, neutrophils and macrophages.

(S.K. Khurana, Praveen Malik and Nitin Virmani)

Response of non-descript native pregnant ponies to immunization with killed Equine Herpes Virus-1 immunogen and virus challenge

During the previous year, equine herpes virus-1 (EHV-1) killed vaccine developed at this centre incorporating indigenous strain (Hisar-90-7) was evaluated in experimental BALB/c mice. The findings in mice indicated that EHV-1 immunogen (25 µg per mice) provides good immune response and protection against EHV-1 challenge in BALB/c mice. During the current year, oil adjuvanted, mannide monooleate emulsified EHV-1 killed vaccine was tested in pregnant non-descript ponies. Pregnant ponies (n=9) at approximately 7 months of gestation were immunized with the 50µg EHV-1 immunogen through intra muscular route. Pregnant ponies (n=5) of approximately matched gestation were kept as non-vaccinated control. Following vaccination, no adverse reaction was noticed in the animals. Booster vaccination was done 7 week-post primary vaccination.

Immune response of these ponies after vaccination of EHV-1 killed vaccine is given in Table 2. Virus neutralizing antibody appeared after first week of the vaccination and peak antibody titers were observed 3-5 weeks post-vaccination. Booster effect of the vaccination was noticed one week-post booster immunization.

Vaccinated and non-vaccinated animals were divided in to 2 groups each and one group of each was challenged after 16 days of booster immunization with 2 ml of EHV-1 virus (Raj-98 EHV-1 strain containing $10^{6.5}$ $TCID_{50}$) through intranasal route. There was slight increase in body temperature on day 2-6 in unvaccinated group of ponies compared to vaccinated ponies. Nasal discharge was seen from 6-10 days in unvaccinated compared to 6-8 days in vaccinated group. Vaccinated group after virus challenged showed sharp rise in antibody titre (VNT>1.4) as compared to unvaccinated and challenged control ponies.

Abortion could not be established in pregnant, unvaccinated control ponies on challenge with EHV-1 virus. Therefore, the experiment needs repetition by inducing and ascertaining virulence of the EHV-1 virus to be used for challenge purpose.

(B.K. Singh, Nitin Virmani and Baldev R. Gulati)

Table 2. Serological response to ponies vaccinated with EHV-1 killed vaccine

Group	VN antibody titre Log10 value weeks after								
No. of ponies	Primary immunization							Booster immunzation	
	0	1	2	3	4	5	6	7	8
Vaccinated (n=9)	<0.3	1.05	1.05	1.2	1.2	1.25	1.05	1.1	1.1
Non-vaccinated (n=5)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

Phosphorus supplementation restores folliculogenesis in mares

A study was done to observe the effect of phosphorus supplementation on folliculogenesis in six phosphorus-deficient mares. The blood phosphorus level in these six mares with subnormal reproductive performance ranged between 1.65 and 2.4 mg/dl. A thorough clinical examination of the reproductive system using ultrasound scanner daily for 3 consecutive cycles showed no evidence of developing follicles in both the ovaries of these animals. However, no cyst or persisting corpus luteum was observed in these mares. These animals were administered phosphorus (sodium salt of 4-dimethylamino-2 methyl phenyl-phosphinic acid) intramuscularly twice weekly till the blood phosphorus level was maintained to normal level of 4.5±0.5 mg/dl, followed by oral supplementation with mineral mixture. During 120 days of observation period, developing follicles were observed in 3 out of 6 mares (Fig.9). Further, on conception, embryonic development was recorded in 2 mares. These findings suggest that phosphorus deficiency might be one of the reasons for sub-normal reproductive performance in these mares.

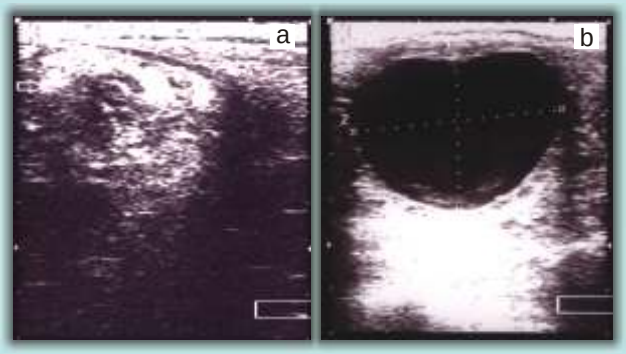


Fig. 9. Ovary of a phosphorus-deficient mare showing no evidence of folliculogenesis (a) and following phosphorus therapy, mature Graaffian follicle observed in the same ovary (b).

(S. Dey, S.K. Dwivedi and Jitender Singh)

Genotyping of major histocompatibility complex class II genes in Marwari horses

Major histocompatibility complex (MHC) genes that code primarily for cell surface glycoproteins, play a key role in the regulation of immune response in the animals. The MHC provides a major genetic component of resistance/ susceptibility to infectious or autoimmune diseases and regulates the basic immune response in higher animals. In horses, MHC is localized to chromosome 20q14 – q22. There are three functional and expressed MHC class II loci (DP, DQ, DR) and each locus contains class II A and B genes.

Fig. 10. MHC-DRB3 gene amplification in Marwari horses

In this study, the DNA from the blood samples of 24 Marwari horses was isolated and the MHC class II DR B gene was amplified using specific set of primers (LA31, LA32). Gene fragment of desired size (309 bp) was successfully amplified (Fig. 10). Further studies are being done with more number of samples to correlate the expression of MHC gene with immune response.

(R. C. Sharma and S. C. Mehta)

Cryopreserved semen of Marwari horses for field trials

Equine semen is far less tolerant to the process of cryopreservation as compared to other species. Presently, no single technique is available for successful freezing of semen of different equine breeds in India. Keeping this in view, various primary and secondary extenders were evaluated to get better post-thaw motility of spermatozoa during the previous year.

Concentration of glycerol and equilibration time affects the post-thaw motility of frozen semen. A study was conducted to optimize glycerol concentration and equilibration time for freezing of Marwari stallion semen. Semen was collected from three Marwari stallions using artificial vagina, centrifuged after mixing with primary extender and re-suspended in freezing media containing 3% and 5% glycerol separately. After filling the semen in straws, half of the straws were given equilibration time of 1hr and the other half for 3hr. Post-thaw motility with 1 hr equilibration time was 41.25±3.15 and 36.25±4.73% for freezing media containing 3% and 5% glycerol, respectively. The post-thaw motility was 40.00±2.04 and 33.75±2.39% for freezing media containing 3% and 5% glycerol with 3 hr equilibration time, respectively. Hence, freezing media containing 3% glycerol was observed to be superior than containing 5% glycerol on the basis of post-thaw motility, where as equilibration time had no effect on stallion semen freezability.

Thawing protocol for frozen stallion spermatozoa plays a major role in the post-thaw motility of spermatozoa. Investigations were carried out to study the effect of various thawing temperatures and time on post-thaw motility of spermatozoa. Post-thaw motility was observed on thawing at 37^{0}C for 30 seconds, 37^{0}C for one minute and at 45^{0}C for 15 seconds. It was observed that thawing at 45^{0}C for 15 seconds was superior for obtaining better post-thaw motility in frozen stallion spermatozoa.

(Yash Pal, R. A. Legha, S. N. Tandon, A. Arangasamy and S. K. Khurana)

Technologies Assessed

Field demonstration of pregnancy detection assay

This centre has developed a serum-based sandwich ELISA for pregnancy diagnosis in mares that can detect pregnancy in mares as early as 35 days of gestation. This test is very much in demand by the field veterinarians. On the request from field, a practical demonstration of this assay was organized for field veterinary officers on August 26, 2004 at Lucknow (UP). In this demonstration, 17 senior level officers of the state animal husbandry department and veterinarians from different parts of the Uttar Pradesh participated. The practical demonstration was successful and the results obtained both by NRCE staff and the participating veterinarians correlated well. The demonstration was highly appreciated by the Director, Animal Husbandry, Govt of UP.

Field trials of improved kit for EHV-1 diagnosis

Field trials of neutralizing monoclonal antibodies (Mabs)-based blocking ELISA (Equiherpes B-ELISA Kit) for serodiagnosis of EHV-1 infection were undertaken in different laboratories. The Equiherpes B-ELISA kit along with 88 horse serum samples were given to three different in-house laboratories and three external laboratories (2 state agricultural universities and one state animal husbandry department) for its validation/precision. The results from these laboratories were compared with the results of our laboratory and there was nearly 95% percent agreement between our laboratory results and the results of other in-house/external laboratories (Table).

B-ELISA kit developed at NRCE for EHV-I diagnosis

Table: Results of field validation of Equiherpes B-ELISA Kit by different Laboratories

Laboratories	Results of testing samples (n=88) by different laboratories			
	Positive	Negative	Variation	Agreement (%)
In-house Lab 1	43	42	3	96.59
In-house Lab 2	39	33	16	81.18
In-house Lab 3	42	42	4	95.45
External Lab 1	42	42	4	95.45
External Lab 2	42	42	4	95.45
External Lab 3	42	43	3	97.72

Education & Training

Two scientists completed their PhD research at NRCE during 2003-2004. In addition, a number of students from state universities acquired trainings from this centre.

PhD theses completed at NRCE

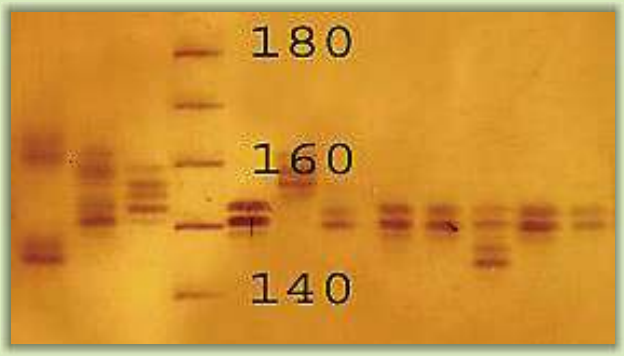
Mamta Chauhan: *"Molecular characterization and establishment of genetic relationship between two different breeds of indigenous equines using microsatellite markers".*

Summary of work: On the basis of geographical localization of horses, six different breeds of horses namely Kathiawari, Marwari, Manipuri, Zanskari, Bhutia and Spiti have been identified. These breeds of horses are distinct not only because of their adaptability to different agro climatic conditions prevailing in the country but they have unique performance traits. Kathiawari horses are known for sturdiness, stiffness, elegance and beauty, whereas Spiti horses have the capability to survive at very low temperature conditions. However, owing to indiscriminate breeding and lack of sound breeding policies, the breed characteristics of these breeds are being diluted. Therefore, there is a need for characterization of indigenous horse populations so that policies can be formulated for their conservation and breeding. Characterization at morphological and genetic level is the first step towards formulating breeding policies and prioritizing the breeds. The research was carried out to characterize two breeds of Indian horses i.e. Kathiawari and Spiti, at molecular level using microsatellite based markers.

Microsatellite markers, which are highly polymorphic, locus specific and amenable for PCR based analysis, have been abundantly used for genome characterization and for population genetic studies in various livestock species. Such efforts have not yet been made in equines of Indian origin.

In this study, 25 microsatellite markers found polymorphic in exotic equines were evaluated across two horse population i.e. Kathiawari and Spiti. Of these 21 robust polymorphic loci were selected for genotyping in both populations. The genetic diversity between the two populations was assessed using measures like mean number of alleles, heterozygosity and F-Statistics. The average number of alleles across all the 21 loci in both Kathiawari and Spiti populations was found to be 5.5 indicating that this set of 21 equine microsatellite markers could be used to study genic variation among other breeds of horses as well. The mean observed heterozygosity for the entire dataset was 0.5360. The Polymorphism Information Content (PIC) values for all the 21 markers revealed that 95% of them were highly informative. Some variation between both populations was observed on the basis of private alleles and some common alleles with varying allele frequencies.

Further studies by gene flow and genetic distance measures indicated low differentiation between Kathiawari and Spiti animals. The standard genetic distance between the two populations was found to be 0.4426 and the

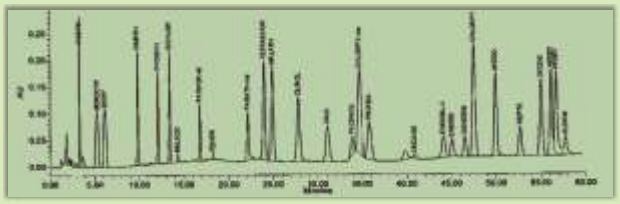


PAGE gel showing PCR product at locus TKY19 in Spiti and Kathiawari horses

genetic identity was found to be 0.6423. The number of generations of divergence between these two horse populations was estimated to be 2213 generations, indicating that Kathiawari and Spiti horses originated from the same population about 2213 generations ago. The estimates of gene flow indicated little flow between these two populations. Due to geographical barriers, gene flow between the two populations is quite unlikely. Thus, the present study on Kathiawari and Spiti horse populations represents a much-needed preliminary effort, which should be extended to other equine populations as well.

Pramod Singh: "Organophosphorus pesticide residues in animal feeds and their excretion in milk".

Summary of Work: Under the Ph.D. project entitled "Organophosphorus pesticide residues in animal feeds and their excretion in milk" a



HPLC showing organophosphorus residues in animal feed

binary gradient elution programme on HPLC was developed for the determination of 18 commonly used organophosphorus pesticides under Indian conditions and 10 persistent organochlorine pesticides. Various samples of feeds and milk were screened for pesticide residues. The degradation of most common pesticide residue, monocrotophos was studied *in vitro*. About 85 percent of monocrotophos was degraded within 48 h of incubation. Under *in vitro* conditions activated charcoal was found a better antidote than zeolite. On supplementing activated charcoal to a diet contaminated with monocrotophos in lactating goats, improvement in nutrient digestibility and blood biochemical profiles like acetylcholinesterase, serum creatinine and serum transaminases (GOT and GPT) was observed. There was 27% reduction in the excretion of monocrotophos in milk.

Trainings for post-graduate students:

- Training on *Studies on profiling of bacterial and viral proteins and nucleic acids* was conducted from February 21-May 26, 2004 for students of Guru Jambeshwar University.
- Training on *DNA polymorphism in Marwari horses and revival of EHV clones* was conducted from January 5-April 29, 2004 for students of Guru Jambeshwar University
- A four-month training on *'Development of RT-PCR based diagnostics for equine rotavirus in diarroheic foals'* was conducted for post-graduate students of Guru Jhambeshwar University from January -April 2005.

Awards & Recognitions

Veterinary Type Culture Centre to be established at NRCE

Recognizing the excellence of the centre in the area of equine health, Indian Council of Agricultural Research entrusted NRCE with the additional responsibility of establishing veterinary type culture collection centre at Hisar campus with an outlay of Rs. 780 lacs during 10th Five Year Plan. The mandate of the Veterinary Type Culture is to establish national repository of micro-organisms of animal origin; identification, characterization & documentation of microorganisms and their conservation and utilization; and surveillance of indigenous and exotic microorganisms.

Technology developed at NRCE applauded by ICAR

NRCE has developed a serum-based sandwich ELISA for pregnancy diagnosis in mares. This technology has been published on 19th October 2004 on the occasion of 75th Annual General Meeting of the ICAR Society, chaired by of Hon'ble Minister of Agriculture (Government of India) Sh. Sharad Pawar Ji. This assay is especially beneficial for equine owners who live in rural areas, as they can get the pregnancy diagnosed in mares by just sending the serum sample to the laboratory and thus saving the cost on transport of the animals to a veterinary hospital for pregnancy diagnosis.

Ph.D. work of Dr. Nitin Virmani awarded

Dr. Nitin Virmani, Scientist (Veterinary Pathology)

was awarded Dr. Patri Rama Rao award for the best Ph.D. thesis in the discipline of Veterinary Pathology in year 2004 by Indian Association of Veterinary Pathologists at XXI Annual Conference of Indian Association of Veterinary Pathologists held at West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal from 23-25 November, 2004. The research work was undertaken at NRCE.

NRCE Kabbadi team wins second prize

NRCE team was adjudged second in the zonal sports meet held at National Dairy Research

NRCE Kabaddi team along with the Director NDRI after winning second prize

Institute, Karnal from 7-10 December 2004. Twenty teams from north India participated in this meet and NRCE team played final against NDRI. Dr. S.K. Dwivedi, Director NRCE felicitated the winning participants and encouraged the employees of the centre to actively participate in various sports activities to keep body and mind healthy.

List of Publications

Research articles

1. Arangasamy A., Singh L.P., Ahmed N., Ansari M.R. and Ram G.C. 2005. Isolation and Characterization of Heparin and Gelatin Binding Buffalo seminal plasma proteins and their effect on in vitro fertilizing ability (BCMPT and HOST) with cauda epididymal spermatozoa. Animal Reproduction Sciences: In press.
2. Arangasamy A., Singh R. and Singh L.P.2005. Bilateral aplastic testes in adult buffalo bull (*Bubalus bubalis*) – an incidental finding. The Indian Journal of Animal Reproduction: In press.
3. Arora A. L., Sharma R. C. and Narula H. K. 2004. Evaluation of Awassi x Malpura half bred sheep in semi- arid region of Rajasthan. Indian Journal of Animal Sciences 74: 1219-1222.
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5. Bork S., Yokoyama N., Ikehara Y., Kumar S., Sugimoto C. and Igarashi I. 2004. Growth inhibitory effect of heparin on *Babesia equi* parasites. Antimicrobial Agents and Chemotherapy. 48:236-241.
6. Chhabra A. and Singh P. 2005. Antinutritional factors and contaminants in animal feeds and their detoxification: A Review. Indian Journal of Animal Sciences 75: 101-112.
7. Dey S. and Dwivedi S.K. 2004. Lead in blood of urban Indian Horses Veterinary and Human Toxicology 46:194-196.
8. Gulati B.R., Malik P. and Kumar R. 2005. Isolation and electropherotyping of equine rotaviruses from diarrhoeic foals in India. Indian Journal of Animal Sciences: Accepted.
9. Gulati B.R., Pandey R. and Singh B.K. 2005. Development of monoclonal antibodies against group A animal rotaviruses. Indian Journal of Biotechnology: Accepted.
10. Gupta A.K., Kaur D., Rattan B. and Yadav M.P. 2005. Molecular variability in different Indian isolates of equine herpesvirus-1. Veterinary Research Communications: Accepted.
11. Gupta A.K., Pal Y., Tandon S.N. and Dwivedi S. K. 2004. Haematological and biochemical profiles in healthy Indian Spiti horses. Indian Veterinary Journal: Accepted.
12. Gupta A.K., Sharma S. K. and Dwivedi S. K. 2004. Biochemical profiles in exotic horse and donkey stallions – A comparative study. Indian Veterinary Journal: Accepted.
13. Joshi A., Bag S., Naqvi S. M. K., Sharma R. C. and Mittal J. P. 2005. Effect of post-thawing incubation on sperm motility and acrosomal integrity of cryopreserved Garole ram semen. Small Ruminant Research 56:231-238.
14. Khurana S.K., Malik P., Srivastava S.K.and Panisup A.S. 2004. Isolation of

Acholeplasmas from indigenous equines. Indian Journal of Veterinary Research 13: 29-32.

15. Khurana S.K., Malik P., Nandal A. and Srivastava S.K. 2003. Seroprevalence of Leptospirosis in equines in India. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 24: 93-95.
16. Kim J-Y, Yokoyama N., Kumar S., Inoue N., Fujisaki K. and Sugimoto C. 2004. Molecular characterization of *Theileria orientalis* piroplasm protein encoded by an open reading frame (To ORF2) in a genomic fragment. The Journal of Veterinary Medical Science 66:
17. Kim J-Y., Yokoyama N., Kumar S., Inoue N., Inaba M., Fujisaki K. and Sugimoto C. 2004. Identification of a piroplasm protein of *Theileria orientalis* that binds to bovine erythrocyte band 3. Molecular and Biochemical Parasitology: In-press.
18. Kim J-Y., Yokoyama N., Kumar S., Inoue N., Yamaguchi T., Sentoku S., Fujisaki K. and Sugimoto C. 2004. Molecular epidemiology survey of benign Theileria parasites of cattle in Japan: detection of a new type of major piroplasm surface protein gene. Journal of Veterinary Medical Science 66:
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23. Mamta, Gupta A.K. and Dhillon S. 2004. Genetic Characterization of Indian Spiti Horses. Journal of Genetics 83: 291-295.
24. Pal Y. and Gupta A.K. 2004. Comparative physiological and biochemical studies in equids under short term feed deprivation stress. Indian Journal of Animal Sciences 74: 662-666.
25. Pal Y. and Gupta A.K. 2004. Effect of Transient Feed Withdrawal Stress on Physiological Indices and Acid Base Balance in Equid. Annals of Arid Zone 43:1-6.
26. Sharma R. C., Arora A. L., Mishra A. K., Kumar S. and Singh V. K. 2004. Breeding prolific Garole with Malpura sheep for increased reproductive efficiency in semi-arid tropics of India. Asian-Australasian Journal of Animal Sciences 17: 737-742.
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Development of neutralizing monoclonal antibody-based blocking ELISA for detection of equine herpesvirus-1 antibodies. Veterinary Research Communications 28:437-446.

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33. Virmani N., Verma P.C., Panisup A.S., Singh B.K. and Batra M. 2005. Studies on neurotropic properties of indigenous strains on EHV-1 in murine model. Indian J. Anim. Sci 75: In Press.

Abstract in Conferences, Symposium, etc.

1. Arangasamy A. and Singh L.P. 2004. Assessment of sperm motility during *in vitro* capacitation with added heparin and gelatin binding buffalo seminal plasma proteins. In: Proceedings of National Symposium on Newer Concepts and Challenges in Veterinary Science and Animal, COVAS, Bikaner, Rajasthan, December 31, 2004- Jan 1, 2005, pp.116.

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3. Arangasamy, A., Singh L.P. and Chauhan M.S. 2004. Effect of heparin and gelatin binding buffalo seminal plasma proteins on sperm-egg binding assay. In: XX Annual convention and National symposium on advanced reproductive technologies for management of fertility in livestock, College of Veterinary & Animal Husbandry, Anjora, Durg, Chhattisgarh, December 14-16, pp.12.

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6. Dey S. and Dwivedi S.K. 2005. Effect of Phosphorus deficiency on folliculogenesis in Marwari Mares. In: Proc Symp. Scientific advancement for improving animal health and Production, Durg, Chhatisgarh, February 2-4.

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8. Dwivedi S.K. 2004. Application of Ethno-veterinary Medicine in Equines. In: Conference on Application of Indigenous Knowledge in Livestock organized by an NGO- Antri, Pune, Maharashtra, September 14-17.

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12. Khurana S.K. and Malik P. 2004. Status of *Mycoplasma equigenitalium* among indigenous equines in India. In 'XXII Annual Conference of IAVMI and National Symposium on Quality assessment of immunodiagnostics and immuno-prophylactics for livestock diseases in post WTO scenario including impact on mountainous regions', College of Vetereinary and Animal Sciences, HPKVV, Palampur, Oct 18-19.

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14. Kumar R., Dwivedi S.K., Dey S. and Malik P. 2005. Seroprevalence of *B. equi* antibodies in Marwari horses in India. In: XXIII Annual Convention and National Symposium on scientific Advancement for Improving Animal Health and Production, College of Veterinary Science and Animal Husbandry, Ajora, Durg, February 02-04.

15. Malik P., Khurana S.K. and Srivastava S.K. 2004. Humoral immune response in ponies immunized with Outer Membrane Protein of *Salmonella* Abortus equi. In 'XXII Annual Conference of IAVMI and National Symposium on Quality assessment of immunodiagnostics and immuno-prophylactics for livestock diseases in post WTO scenario including impact on

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20. Pal Y. and Singh J. 2004. Use of ultrasonography in equines to assess reproductive organs. In: Short Course on Use of ultrasonography in veterinary practice, CCS HAU, Hisar, December 14-23, p61-65.

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22. Rani S., Singh Y., Kumar A., Gulati B.R. and Kumar A. 2004. Occurrence of enterohaemorrhagic *E.coli* in buffalo-meat. In: 3rd Annual Conference of Indian Association of Veterinary Public Health Specialists, Punjab Agricultural University, Ludhiana, October 26-27.

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24. Singh P. and Chhabra A. 2004. Effect of activated charcoal as an antidote against monocrotophos in lactating goats. Proceedings of National Symposium on "New dimensions of animal feeding to sustain development and competitive-ness", National Institute of Animal Nutrition and Physiology, Bangalore, Dec 24-26, pp 45-46.

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27. Virmani M., Gupta A.K. and Garg S.K. 2005. Detection of equine chorionic gonadotropin(eCG) in pregnant mare serum using haemagglutinin inhibition assay. In: XIV annual conference of society of animal physiologists of India and National symposium on Recent Advances in Cryopreservation of Livestock Germplasm, Durg, India, January 28-29, p.173.

28. Virmani N., Panisup A. S., Malik P. and Chandel K.S. 2004. Uterine exfoliative cytological investigations of the cases of infertility in mares. In: National symposium on "Advances in pathological techniques in diagnosis of animal, bird and fish diseases" and XXIst Annual Conference of Indian Association of Veterinary Pathologists, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, November 23-25.

Participation in Conference/Symposia

1. Dr. S.K. Dwivedi (Director) delivered a paper on "Application of Ethno-veterinary Medicine in Equines" in a conference on Application of Indigenous Knowledge in Livestock organized by an NGO- Anthra held at Pune, Maharashtra, from September 14-17, 2004.
2. Dr. S. K. Khurana (Sr. Scientist) presented a paper in National Symposium on "Quality assessment of immunodiagnostics and immunoprophylactics for livestock diseases in post-W.T.O. scenario including impact on mountainous regions" and XXII Annual conference of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases held at CSK Himachal Pradesh Krishi Vishvadyalaya, Palampur (H.P.) from October 18-19, 2004.
3. Dr. Praveen Malik (Scientist) presented a paper in National Symposium on "Quality assessment of immunodiagnostics and immunoprophylactics for livestock diseases in post-W.T.O. scenario including impact on mountainous regions" and XXII Annual conference of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases held at CSK Himachal Pradesh Krishi Vishvadyalaya, Palampur (H.P.) from October 18-19, 2004.
4. Dr. Nitin Virmani (Scientist) presented a paper in XXI Annual Conference of Indian Association of Veterinary Pathologists held at West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal from November 23-25, 2004.
5. Dr. S.K. Dwivedi (Director) delivered a lead paper on 'Past, present & future of indigenous medicinal plants for management of equine production and health' in International Conference on Agricultural Heritage of Asia held at NAARM, Hyderabad from December 6-8, 2004.
6. Dr. Baldev R. Gulati (Senior Scientist) presented a paper in the 31st Annual Conference of Indian Immunology Society to be organized at Anna University, Chennai from December 15-18, 2004.
7. Dr. S.K. Dwivedi (Director) chaired a session in National Symposium on "Newer Concepts and Challenges in Veterinary Science & Animal Husbandry" held at College of Veterinary & Animal Science, Bikaner from December 31,2004 –January 1, 2005.
8. Dr. R.C. Sharma (Sr. Scientist) participated in National Symposium on "Newer Concepts and Challenges in Veterinary Science & Animal Husbandry" held at College of Veterinary & Animal Science, Bikaner from December 31,2004 –January 1, 2005.
9. Dr. R.S. Bansal (T-9) participated in National Symposium on "Newer Concepts and Challenges in Veterinary Science & Animal Husbandry" held at College of Veterinary & Animal Science, Bikaner from December 31,2004 –January 1, 2005.
10. Dr. S. Dey (Senior Scientist) presented a paper in National Symposium on Scientific Advancement for Improving Animal Health and Production, College of Veterinary Sciences, Anjora Durg, Chhatisgarh from February 2-4, 2005.
11. Dr. S.K. Dwivedi (Director) chaired a session in International Congress of Canine Practice organized by Indian Society for Canine Practice, New Delhi from February 9-11, 2005.

12. Dr. S. Dey (Senior Scientist) presented a paper in International Congress of Canine Practice organized by Indian Society for Canine Practice, New Delhi from February 9-11, 2005.
13. Dr. S.K. Dwivedi (Director) delivered a Lead Lecture on "Equine wealth of India: Present status of selection and Evaluation Programmes for Equines" in VIII National Conference on Animal Genetics & Breeding, Theme: National Livestock Breeding Policy held at CIRG, Makhdoom, Uttar Pradesh, from March 8-10, 2005.
14. Dr. Praveen Malik (Scientist) participated in the first Workshops on "Implementation of Personnel Management Information System Network in ICAR (PERMISnet)" held at Indian Agricultural Statistics Research Institute (IASRI), New Delhi on March 9, 2005.
15. Dr. R.C. Sharma (Sr. Scientist) participated in VIII National Conference on Animal Genetics & Breeding, Theme: National Livestock Breeding Policy held at CIRG, Makhdoom, Uttar Pradesh, from March 8-10, 2005.
16. Dr. R.A. Legha (Scientist) participated in VIII National Conference on Animal Genetics & Breeding, Theme: National Livestock Breeding Policy held at CIRG, Makhdoom, Uttar Pradesh, from March 8-10, 2005.

Participation in Trainings

1. Dr. Praveen Malik (Scientist) participated in training on Personnel Management Information System of ICAR (PERMISnet) organized by Indian Agricultural Statistics Research Institute (IASRI), New Delhi from July 19-20, 2004.
2. Dr. Praveen Malik (Scientist) participated in the training on 'Networking' organized by ERNET-India under NATP, New Delhi from July 26-30, 2004.
3. Dr. R. C. Sharma (Senior Scientist) participated in the training programme on "Gene characterization and biodiversity analysis" organized at NBAGR, Karnal from October 04-13, 2004.
4. Dr. S. K. Khurana (Senior Scientist) participated in the Winter School on "Production of Recombinant Proteins in Heterologous Host System and Purification" organized at IVRI, Hebbal, Bangalore from November 4-24, 2004.
5. Dr. Rajender Kumar (Scientist) participated in the Management Development Programme on "Performance Assessment of Agricultural Research Organisations" organized at NAARM, Hyderabad from Feb.15-19, 2005.
6. Dr. Rajender Kumar (Scientist) participated in sensitization workshop cum training regarding "Role and concept of PME in the context of Agricultural Research System in India" at NCAP, New Delhi from March 18-19, 2005.
7. Sh. Subhash Chander (Sr. Clerk) participated in technical workshop on "Management Tools" organized by Institute of Socio Economic Research & Action (ISERA), New Delhi form August 5-7, 2004.
8. Sh. Pratap Singh, Jr. Clerk participated in technical workshop on "Management Tools" organized by Institute of Socio Economic Research & Action (ISERA), New Delhi form August 5-7, 2004.
9. Sh. Mahender Singh (Jr. Clerk) participated in technical workshop on "Management Tools" organized by Institute of Socio Economic Research & Action (ISERA), New Delhi form August 5-7, 2004.

Consultancy, patents & commercialization of technology

Consultancy

This centre offers consultancy and diagnostic services for important infectious diseases of equines. Under this programme, 5158 equine serum samples received from thoroughbred and indigenous equines were examined for Equine Infectious Anaemia (EIA) by Coggins test. None of the samples tested was found positive. Since 1999, 27829 serum samples have been tested for EIA during and not a single positive case has been recorded.

Contagious equine metritis (CEM) testing by agent isolation and identification was done for 75 samples including 57 vaginal swabs and 18 prepucial swabs and all samples were found negative for CEM.

Bacteriological examination of 167 samples, including nasal swabs, vaginal swabs, ocular swab, faecal samples, wound/lesions, exudates, pus samples and aborted foetus yielded 69 isolates (Table) including *Streptococcus equi* subsp. *equi* (3), *Streptococcus equi* subsp. *zooepidemicus* (6), á-hemolytic Streptococci (4), Staphylococci (12), Micrococci(14), *E. coli* (7), *Serratia* spp (9), one each of *Rhodococcus equi*, *Providentia rustigianii*, other *Providentia* spp, *Actinobacillus equuli* and *Actinomycetes* spp.

Post-mortem examination

On the etiopathological front, 9 necropsies on equines were conducted and the conditions recorded included cases of pneumonia due to *Rhodococcus equi* infection in a foal (1), malpositionig of intestine/ strangulation (1), hemorrhagic gastroenteritis (2), acute tubular necrosis and cirrhosis (2), colitis and passive venous congestion (1), alkaloidosis (1), cardiomyopathy (1). Examination of morbid material revealed viral interstitial pneumonia

Table : Important bacterial isolates recovered and their origin

Isolate name	Number	Equine sample collected from
Streptococcus equi subsp. *equi*	3	Rajasthan (1), Haryana (2)
Streptococcus equi subsp. *zooepidemicus*	6	Punjab (1), Rajasthan (2), Haryana (3)
á-hemolytic streptococci	4	Haryana (4)
Staphylococci	12	Rajasthan (9), Haryana (3)
E. coli	7	Rajasthan (3), Haryana (4)
Rhodococcus equi	1	Rajasthan (1)
Serratia species	9	Rajasthan (9)
Providentia rustigianii	1	Haryana (1)
Providentia species	1	Haryana (1)
Actinomycetes species	1	Haryana (1)
Actinobacillus equuli	1	Gujarat (1)
Micrococci	14	Rajasthan (14)
Yeast	2	Rajasthan (2)
Unidentified bacteria	7	
Total	69	

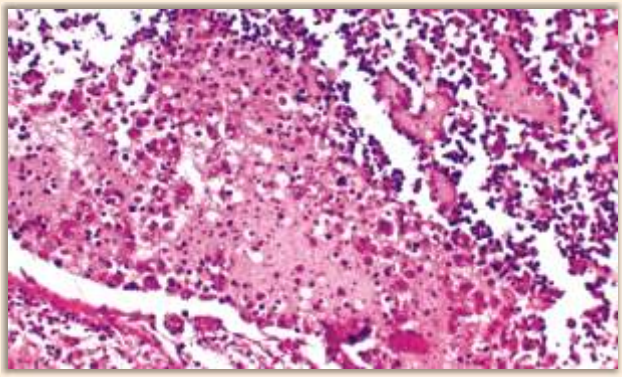


Fig. Section of lung showing focal area surrounded by connective tissue containing necrotic debris along with lymphocytes, macrophages, plasma cells and few neutrohils. (H.E.-132)

(1), Alkaloidosis (1), foetal death due to aspiration of amniotic fluid (1), anoxia due to twisting of umbilical cord (1), non specific (non infectious) causes of abortions (2).

Miscellaneous

Parasitological examination of 34 faecal samples was done, out of which 11 samples were found positive for *Anoplocephala* and 3 samples were found positive for *Strongyles*. Mean fluoride concentration of sera showed significant difference in the range of 0.116 to 0.352 mcg/ ml. in 9 states of the country. Mean fluoride concentration of sera was highest inTamil Nadu followed by Rajasthan and Maharashtra.

Commercialization of technology

The Centre is providing diagnostic services to the equine industry on payment basis. The centre generated revenue to the tune of Rs. 14.82 lacs during the year by testing samples for various diseases including equine infectious anaemia (9.82 lacs), equine viral arteritis (0.89 lacs), contagious equine metritis (0.75 lacs), equine piroplasmosis (0.58 lacs). In addition, vaccine for equine influenza was supplied to equine owners generating revenue of Rs.0.30 lacs and the improved germplasm of equines was provided to the farmers in different parts of the country.

RAC, Management Committee & SRC Meetings

Research Advisory Committee Meeting

The 6th RAC meeting was held under the chairmanship of Dr. V. Gnanaprakasam on April 20, 2004 to discuss various technical, administrative and policy matters of NRCE. The

RAC meeting being held under the chairmanship of Dr. V. Gnanaprakasam

RAC reiterated that the development of containment facilities and getting recognition as international reference laboratory should be the priority of the centre. The RAC strongly recommended that vacant posts of the scientists should be filled up on priority basis and extra technical, administrative and supporting staff should be provided to the centre. Achievements of the scientists of the centre in 11 different on-going research projects were reviewed and future research guidelines were discussed. The RAC strongly recommended that scientists of the centre should be encouraged to go for advanced trainings at national and international institutes for upgradation of their skills.

Staff Research Council Meeting

The Annual SRC meeting was held under the chairmanship of Dr. S.K. Dwivedi, Director on May 1 and 6, 2004 to discuss the progress made in various on-going research projects. Five new research project proposals submitted by various scientists were approved by the SRC.

Annual SRC meeting in progress under the chairmanship of Dr. S.K. Dwivedi, Director

New research projects initiated at NRCE

1. Development of diagnostics for *Rhodococcus equi* infection in foals.
2. Studies on improvement of diagnostics for differentiation between EHV-1 and EHV-4 infections employing molecular techniques.
3. Development of sensitive and specific diagnostic tests for detection of equine piroplasmosis.
4. RFLP- Based genotyping of major histocompatibility complex class II genes in Marwari horses.
5. Isolation of stallion seminal plasma proteins and their effect on *in vitro* fertilizing ability of spermatozoa.

Half-Yearly Staff Research Council Meeting

The half yearly SRC meeting was held under the chairmanship of Dr. S.K. Dwivedi, Director on November 1, 2004 to discuss the progress of various ongoing research projects in the area of equine health and production. The house reviewed the work done and made their specific recommendations for different ongoing research projects.

SRC Meeting in progress to review the research activities of the centre

The following new research project proposals were also approved in half yearly SRC meeting:

1. Superior mule production in the field through frozen semen of exotic Jacks;
2. Molecular markers based parentage testing in horses of Indian origin;
3. Performance evaluation of Marwari horses under different type of shelters.

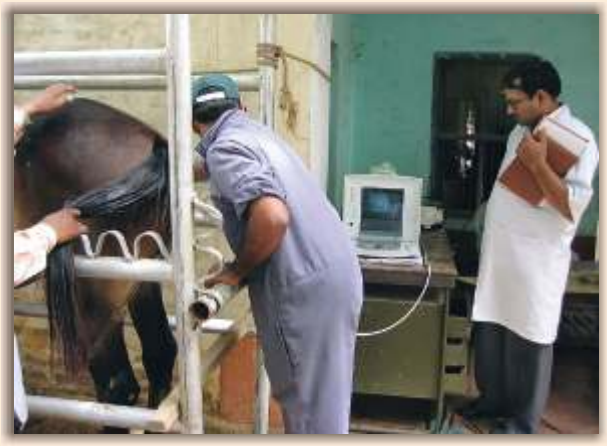
Members of Research Advisory Committee	
Dr. V.Gnanaprakasam, Ex-Vice Chancellor, TNUVAS, Chennai,	Chairman
Dr. S. K. Dwivedi, Director, NRCE, Hisar	Member
Dr. R.P. Mishra, Ex-FAO expert, Bareilly	Member
Dr. N.N.Pathak, Principal Scientist, Dept. of Animal Nutrition, IVRI, Izatnagar,U.P.	Member
Dr. M.C. Goel, Ex-ADR, CCS HAU, Hisar	Member
Dr. O.P. Dhanda, Prof. Animal Produciton Physiology, CCS HAU, Hisar	Member
Dr.Lal Krishna ADG (AH), ICAR, New Delhi	Member
Sh. Arvind Yadav, 208, Sector 3, Rewari (Haryana)	Member
Sh. Ram Kripal Bhadoria, C-20 Dilkhusha, Lucknow (UP)	Member
Dr. Rajender Kumar, Scientist, NRCE, Hisar	Member Secretary

Members of Institute Management Committee	
Dr. S.K. Dwivedi, Director NRCE, Hisar.	Chairman
Dr.Lal Krishna, ADG(AH), ICAR, New Delhi.	Member
Sh. B.K. Bansal, Finance & Accounts Officer, NBPGR, New Delhi	Member
Dr. S.N. Tandon, Principal Scientist, NRCE, Bikaner	Member
Dr. A.K. Gupta, Principal Scientist, NRCE, Hisar	Member
Dr. A.S. Panisup, Principal Scientist, NRCE, Hisar	Member
Dr. B.K. Singh, Principal Scientist, NRCE, Hisar	Member
Sh. R.A. Prashar, AFAO, NRCE, Hisar	Opted member
Sh.Arvind Yadav, 208, Sector 3, Rewari (Haryana)	Member
Sh.Ram Kripal Bhadoria, C-20 Dilkhusha, Lucknow (UP)	Member
Sh. Dilip Kar, AAO, NRCE, Hisar	Member Secretary

Workshops, Seminars, Summer Institutes, Farmers' Day, etc.

Equine Health Camp at Village Barukhera, Sirsa (Haryana)

NRCE organized a clinical health camp at Barukhera village of Sirsa district of Haryana on May 12, 2004. More than 112 farmers along with their equines participated in the camp. Services



NRCE scientists doing ultrasonography in a mare at camp in Barukhera (Sirsa)

of pregnancy diagnosis, ultrasonography, deworming, immunization against tetanus were provided to all needy equines. Treatment for various ailments was also given to sick equines. Various samples from sick animals were collected for laboratory diagnosis of diseases in this region. A kisan goshthi was also organized on this occasion to acquaint the equine owners with the problems of equines of this region and their management.

Equine Health Camp at Julana (Jind, Haryana)

Two clinical health camps were organized at Julana village in Jind District of Haryana on September 18, 2004 and again on March 1, 2005 where sick equines were treated for different ailments, *viz.*, urinary tract infection, parasitism, reproductive disorder, debility and skin disorders. Reproductive tract of female animals were examined using ultrasound and mares suffering from endometritis and pyometra were treated. All the equines were given deworming drugs and vaccinated against tetanus on both occasions. Kisan goshthi was also organized in these camps in which equine owners were educated about the latest

Sick animals being given treatment in an equine health camp at Julana (Jind)

production and health care practices. Literature on management of equines was distributed among farmers. Blood and serum samples were collected from all the animals to assess the health status of equines of this region.

NRCE organizes a horse show on its Foundation Day

National Research Centre on Equines, Hisar celebrated its Foundation Day on 26th November

Dr. R.P.S. Tyagi releasing the souvenir on the occasion of NRCE Foundation Day

2004 with great fanfare. On this occasion, a

horse show was organized in which indigenous horses from various parts of Haryana and Rajasthan participated in different equestrian events like tent pegging, horse dance, *etc*. The Chief Guest, Dr. R.P.S. Tyagi, former Vice-Chancellor, HPKVV, Palampur released a souvenir on this occasion and emphasized on the importance of equines in Army and for livelihood of landless and marginal farmers. Addressing to the equine owners, Dr. S.K. Dwivedi, Director NRCE highlighted the salient achievements of the centre in recent years for improvement in health and production of equines.

A Kisan Goshthi organized at the centre

A Kisan Goshthi was organized on the occasion of the Foundation Day of NRCE at Hisar Campus on 26 November, 2004. In this goshthi, equine

Farmers exchanging views with the scientists of the centre in a kisan goshthi at NRCE, Hisar

farmers from various states, *viz*, Haryana, Rajasthan, Punjab participated and enriched their knowledge on latest equine husbandry practices. The farmers exchanged their views and experiences with the scientists of the centre and were provided solution for various problems faced by them pertaining to equine production and health.

Equine Health Camp-cum-Farmers Meet at Heerwa, Jhunjhunu (Rajasthan)

An equine health camp was organized by EPC Bikaner in collaboration with the Department of Animal Husbandry, Jhunjhunu (Rajasthan) at village Heerwa of district Jhunjhunu on January 20, 2005. In this camp, about 60 equines were treated for various ailments. Pregnancy diagnosis and artificial insemination was also done in mares. A horse show was organized on

Experts replying the queries of farmers at Heerwa (Jhunjhunu)

the occasion and owners of winning horses were encouraged by certificates and prizes. A kisan goshthi was organized to discuss general management and health of equines with farmers. Various experts including scientists of the EPC Bikaner and Deputy Director, State Animal Husbandry, Jhunjhunu replied to the queries of the farmers.

Equine Health Camp at Nagaur (Rajasthan)

EPC Bikaner organised an equine health camp at Nagaur (Rajasthan) on February 15, 2005 where about 40 equines were treated for various ailments. In addition, artificial insemination and pregnancy diagnosis services were provided for mares in the camp.

Dr. R.S. Bansal examining sick animals in a health camp at Nagaur

Equine Exhibition at Katriasar, Bikaner (Rajasthan)

An equine exhibition was organized at village Katriasar on January 24-25, 2005 in which exotic donkeys and indigenous horses of Marwari and Kathiawari breed were displayed. In this exhibition, more than 2000 farmers visited various stalls.

Equine Health Camp at Sardarseher (Rajasthan)

An equine health camp-cum-farmer meet was organized at Sardarseher (Rajasthan) on March

Dr. S.N. Tandon addressing farmers about the management practices for equines at Sardarseher

9, 2005. In this health camp, about 35 animals were given treatment and services of artificial insemination and pregnancy diagnosis. A kisan goshthi was also organized to highlight the importance of rearing equines for farmers and farmers were educated about the developments in management practices for equines.

Republic Day celebrated

The celebrations of 55th Republic Day at the centre began on 26th January 2005 with the

Dr. S. K. Dwivedi, Director addressing the staff on the occasion of Republic Day

hoisting of the National Flag by Dr. S.K. Dwivedi, Director, NRCE. Addressing the employees of the centre on this occasion, Dr. Dwivedi elaborated the salient achievements of the centre towards improvement in equine health and production. He complimented the efforts of scientists and employees of the centre towards betterment of farmers and equine breeders. He emphasized on the need to follow the footsteps of those who sacrificed their lives for the nation and advised to dedicate whole-heartedly for the development of this country. On this occasion, employees and their family members presented a cultural programme.

स्वतंत्रता दिवस समारोह

58वें स्वतंत्रता दिवस समारोह का डा० शैलेन्द्र द्विवेदी, निदेशक ने ध्वजारोहण करके शुभारम्भ किया । इस अवसर पर उन्होने कर्मचारियों को स्वतंत्रता दिवस की शुभकामनाएं दी और शहीदों को शत्–शत् नमन करते हुए

डॉ॰ द्विवेदी स्वतंत्रता दिवस के अवसर पर प्रतिभागियों को पुरस्कार वितरित करते हुए

कहा कि आज का स्वच्छन्द वातावरण अनेक वीरों के बलिदान से प्राप्त हुआ है। इस शुभावसर पर केन्द्र के कर्मचारियों ने सपरिवार रंगा–रंग कार्यक्रम में भाग लिया जिसमें देश भक्ति गीत गायन प्रतियोगिता प्रमुख आकर्षण रही ।

सद्भावना समारोह का आयोजन

केन्द्र के तत्वाधान में 20 अगस्त से 5 सितम्बर 2004 तक सद्भावना समारोह का आयोजन किया गया, जिसका शुभारम्भ 20 अगस्त को केन्द्र के सभी कर्मचारियों एवं अधिकारियों ने एकता एवं भाईचारे की प्रतिज्ञा लेकर किया। इस पखवाड़े में डॉ॰ पी॰ एस॰ वर्मा, सेवानिवृत प्रमुख चिकित्सा अधिकारी, सुश्री आशा पाहूजा, समाज सेवी, श्री नरेन्द्र कश्यप, प्रशासनिक अधिकारी एवं राजयोग शिक्षिका बहन वंदना इत्यादि विभिन्न प्रतिष्ठित वक्ताओं ने सद्भावना बढ़ाने के लिए अपने व्याख्यानों

सद्भावना पखवाड़े के दौरान श्री नरेन्द्र कश्यप जी अपने व्याख्यान देते हुए

द्वारा केन्द्र के कर्मचारियों को प्रेरित किया।

हिन्दी सप्ताह का आयोजन

राष्ट्रीय अश्व अनुसंधान केन्द्र, हिसार के तत्वावधान में 23 से 29 सितम्बर, 2004 को हिन्दी सप्ताह का आयोजन किया गया। इस सप्ताह के दौरान 25 सितम्बर को एक काव्य गोष्ठी भी आयोजित की गई, जिसमें श्री उद्य भानु हंस जी, राज्य कवि हरियाणा ने स्वःरचित रूबाईयां पढ़ कर दर्शकों का मन मोह लिया। हिन्दी सप्ताह में केन्द्रीय सरकार के कर्मचारियों के लिए कविता पाठ तथा भाषण प्रतियोगिता का आयोजन भी किया गया। समापन

हिन्दी सप्ताह के दौरान प्रतिभागी कविता पढ़ते हुए (इनसेट में राज्यकवि श्री उद्यभानु हंस जी)

समारोह में डा० शैलेन्द्र द्विवेदी, निदेशक ने विजयी प्रतिभागियों को पुरस्कार प्रदान किए एवम् केन्द्र के सभी वैज्ञानिकों तथा कर्मचारियों को अधिकाधिक हिन्दी में कार्य करने के लिये प्रोत्साहित किया । उन्होने कहा कि हिन्दी ही एक मात्र भाषा है जो सारे राष्ट्र को एक सूत्र में पिरो कर रखती है।

Personnel Milestones

Promotions

- Dr. Sanjay Kumar as Scientist (Senior Scale) w.e.f. October 31, 2001.
- Dr. Praveen Malik as Scientist (Senior Scale) w.e.f. July 5, 2002.
- Dr. Nitin Virmani as Scientist (Senior Scale) w.e.f. October 14, 2002.
- Dr. Mamta as Scientist (Senior Scale) w.e.f. December 5, 2003.
- Dr. Pramod Singh as Scientist (Senior Scale) w.e.f. March 24, 2004.

New appointments

- Smt. Ram Kali Joined as SSG-I (sweeper) on March 15, 2005.
- Smt. Santra joined as SSG-I (sweeper) on March 15, 2005.

Transfer

- Sh. Dilip Kar, AAO relived from this centre on March 21, 2005 on his transfer to National Research Centre on Groundnut, Junagarh (Gujarat).

Farewell

- Smt. Indu Jyoti (T-3, Hindi Translator) relieved from this centre on 22nd December 2004 on her selection as lecturer in Deptt. of Education Government of Haryana.

Retirements

- Sh. Khiraj Singh, T-2, Driver retired on March 31, 2005
- Sh. Sajjan Singh, SSGr-II retired on March 31, 2005.

Obituary

- Sh. Dalsher Singh, SSG-I expired on June 20, 2004. The centre condoled the family members of the bereaved.

Staff at NRCE

Director

Dr. S.K. Dwivedi, M.V.Sc., Ph.D.

Administrative Staff

1.	Sh. R.A. Parashar	AFAO
2.	Sh. Dilip Kar	AAO (till 21.03.05)
3.	Sh. Hawa Singh	Assistant
4.	Sh. Ram Pal	Assistant
5.	Sh. S.P. Kaushik	Assistant
6.	Sh. Ashok Arora	Jr. Stenographer
7.	Sh. Subhash Chander	Sr. Clerk
8.	Sh. Pratap Singh	Jr. Clerk
9.	Sh. D.D. Sharma	Jr. Clerk
10.	Sh. Om Prakash	Jr. Clerk
11.	Sh. Mahender Singh	Jr. Clerk

Scientific Staff

1.	Dr. S.N. Tandon, M.V.Sc., Ph.D.	Principal Scientist
2.	Dr. A.K. Gupta M.Sc., Ph.D.	Principal Scientist
3.	Dr. A.S. Panisup, M.V.Sc., Ph.D.	Principal Scientist
4.	Dr. B.K. Singh, M.V. Sc., Ph.D.	Principal Scientist
5.	Dr. S. Dey, M.V. Sc., Ph.D.	Senior Scientist
6.	Dr. S.K. Khurana M.V.Sc., Ph.D.	Senior Scientist
7.	Dr. Yash Pal, M.Sc., Ph.D.	Senior Scientist
8.	Dr. R.C. Sharma, M.V.Sc., Ph.D.	Senior Scientist
9.	Dr. B.R. Gulati, M.V.Sc., Ph.D.	Senior Scientist
10.	Dr. Rajender Kumar M.V.Sc., Ph.D.	Scientist (SS)
11.	Dr. R.A. Legha M.Sc., Ph.D.	Scientist (SS)
12.	Dr. Praveen Malik M.V.Sc., Ph.D.	Scientist (SS)
13.	Dr. Nitin Virmani M.V.Sc., Ph.D.	Scientist (SS)
14.	Dr. Deepinder Kaur M.Sc., Ph.D.	Scientist
15.	Dr. Sanjay Kumar M.V.Sc., Ph.D.	Scientist (SS)
16.	Dr. (Ms.) Mamta M.Sc., Ph.D.	Scientist (SS)
17.	Dr. Pramod Singh M.Sc., Ph.D.	Scientist (SS)
18.	Dr. A. Arangasamy M.V.Sc., Ph.D.	Scientist

Technical Staff

1.	Dr. R.S. Bansal, T-9	Farm Manager
2.	Sh. R.K. Chaturvedi, T-5	Technical Officer
3.	Sh. K.S. Meena, T-4	Farm Manager
4.	Sh. K.K. Singh, T-4	Lab. Technician
5.	Dr. Jitender Singh, T-4	Veterinary Officer
6.	Sh. P.P. Chaudhary, T-4	Lab. Technician
7.	Sh. Ajmer Singh, T-3	Stock Assistant
8.	Sh. Brij Lal, T-3	Stock Assistant
9.	Sh. D.D. Pandey, T-3	Lab. Assistant
10.	Sh. Sita Ram, T-3	Lab. Assistant
11.	Sh. S.K. Chhabra, T-3	Lab. Assistant
12.	Sh. N.K. Chauhan, T-3	Farm Technician
13.	Sh. Mukesh Chand, T-2	Lab. Assistant
14.	Sh. Sajjan Kumar, T-2	Staff Car Driver
15.	Sh. Arun Chand, T-2	Tractor Driver
16.	Sh. Suresh Kumar, T-2	Vehicle Driver
17.	Sh. Joginder Singh, T-2	Laboratory Assistant
18.	Sh. Shankar Lal, T-2	Jeep-cum-tractor Driver
19.	Sh. Rajendra Singh, T-1	Lab. Technician
20.	Sh. S.N. Paswan, T-1	Livestock Assistant
21.	Sh. Om Prakash, T-1	Tractor Driver

Supporting Staff

1.	Sh. Ishwar Singh	SSGr. III
2.	Sh. Guru Dutt	SSGr. III
3.	Sh. Jai Singh	SSGr. III
4.	Sh. Mahabir Prasad	SSGr. III
5.	Sh. Ramesh Chander	SSGr. II
6.	Sh. Mardan	SSGr. II
7.	Sh. Balwan Singh	SSGr. II
8.	Sh. Desh Raj	SSGr. II
9.	Sh. Raghubir Singh	SSGr. II
10.	Sh. Ishwar Chander	SSGr. II
11.	Sh. Om Prakash	SSGr. II
12.	Sh. Deepak Kumar	SSGr. II
13.	Sh. Gopal Nath	SSGr. II
14.	Sh. Satbir Singh	SSGr. I
15.	Sh. Hanuman Singh	SSGr. I
16.	Sh. Subhash Chander	SSGr. I
17.	sh. Ishwar Singh	SSGr. I
18.	Sh. Ram Singh	SSGr. I
19.	Sh. Raju Ram	SSGr. I
20.	Sh. Mahabir Prasad	SSGr. I
21.	Smt. Ram Kali	SSGr. I
22.	Smt. Santra	SSGr. I

Distinguished Visitors

Dr. A.S. Faroda visited the centre

Dr. A.S. Faroda, Chairman, Agricultural Scientists Recruitment Board, ICAR visited NRCE

Dr. A.S. Faroda, Chairman, ASRB being apprised of the research activities at NRCE

on April 23, 2004. During his visit, Dr. S.K. Dwivedi, Director apprised him of various research and development activities at the centre for improvement in the health and production potentials of equine. Dr. Faroda was impressed by the technical and expertise available at the centre for the benefit of equine farmers.

Minister of Animal Husbandry, Punjab visits NRCE

Sh. Jagmohan Singh Kang, Hon'ble Minister of Animal Husbandry, Dairy Development, Fisheries and Tourism, Government of Punjab visited this centre on 26th August 2004. Welcoming the guest, Dr. S.K. Dwivedi, Director apprised him of salient achievements of the centre towards improvement in the health and production of equines. Sh. Kang was impressed by the dedicated work done by this centre on conservation of indigenous equines and sought the technical help for initiating artificial insemination and other measures for improving the germplasm of equines in the state of Punjab.

Sh. Jagmohan Kang, Animal Husbandry Minister, Punjab interacting with the Scientists at NRCE

Japanese scientists visited the centre

A delegation of scientists from Obhihiro University of Agriculture and Veterinary Medicine, Obihiro (Japan) visited this centre on 19th November 2004. Dr. Noboru Inoue and Dr. Naoaki Yokoyama of National Research Centre for Protozoan Diseases were highly impressed by the research activities of the centre and proposed a collaborative project on "Epidemiological survey of *Trypanosoma evansi* infection in Asia".

Japanese delegate being apprised of the research activities at the centre

Dr. Mangala Rai, DG ICAR visited the centre

Hon'ble Dr. Mangala Rai, Secretary DARE & Director-General ICAR visited this centre on January 11, 2005. During his visit, Dr. Rai laid the

Hon'ble DG ICAR, Dr. Mangala Rai interacting with scientists at NRCE

foundation stone for extension of laboratory building and planted a tree in the campus. On this occasion, Dr. S.K. Dwivedi, Director NRCE explained to him various ongoing research activities and salient achievements of the centre. Dr. Rai had a personal interaction with all the scientists and provided valuable inputs in different research programmes on diagnostics, vaccines and drug development. Addressing to the scientists of the centre, the Director-General emphasized that scientists should exert more to get excellent results in the research objectives for the benefit of farmers and equine owners. He assured that there will be no dearth of funds for development of infrastructure as per international norms.

Expert team of Ministry of Environment & Forest (GOI) visited the centre

A team of CPCSEA expert members constituted by the Animal Welfare Division, Ministry of Environment & Forest (GOI) visited the centre on February 5, 2005 to inspect the animal house facility of NRCE, Hisar. Expert team comprising Dr. D. Mohanty, Expert Consultant, CPCSEA, New Delhi, Dr. P.K. Yadav, I/C, Primate Research Facility, AIIMS, New Delhi, and Dr P.K. Kapoor, In-Charge, Small Animal House, CCS HAU, thoroughly examined the infrastructural facilities and discussed about experimental procedures and other parameters related to use of small and large animals with the scientists of this centre. On inspection, the team approved the facilities developed at this centre for experimentation on large and small animals.

Infrastructure & Support Section

Construction work for new laboratory-cum-office complex at EPC Bikaner started

The work on the construction of laboratory-cum-office building at Equine Production Campus, Bikaner began with the *Bhoomi-Poojan* on 27th August 2004. The building, with an estimated cost of Rupees 1.07 crores will include state-of-the-art research laboratories, library, conference hall and an administrative section. The funds for the same were approved in the 10th Five Year Plan of the centre by the Indian Council of Agricultural Research. The structure of building has now been completed by the CPWD .

Director and staff of NRCE participating in bhoomi-poojan at EPC, Bikaner

Foundation stone for extension of laboratory building laid

The foundation stone for extension of laboratory building of our campus at Hisar was laid by Hon'ble Dr. Mangala Rai, Secretary-DARE and Director-General ICAR on January 12, 2005. During his address on this occasion, Dr. Rai asked the scientists to work with dedication and attain excellence in their research areas and assured that there will not be dearth of funds for facilities, equipment and other infrastructure for this centre.

Dr. Mangala Rai laying the foundation stone of New Laboratory Wing at Hisar Campus

Internet connectivity upgradation and website updation

National Research Centre on Equines, Hisar is one of the seventy ICAR campuses in which ICAR has established internet connectivity using ERNET backbone with broadband VSATs/leased

lines under National Agricultural Technology Programme. The broadband link was established by installing a C-band VSAT at NRCE and the connectivity under this programme is being provided to the staff on round the clock basis.

National Research Centre on Equines has developed a comprehensive website depicting various programmes, activities, research achievements of the centre. The site is being updated regularly in co-ordination with the National Informatics Centre, Hisar. Apart from several other modifications, a page in Hindi has

also been incorporated in the website.

The centre is also implementing a Personnel Management Information System Network (PERMISNET) software developed by ICAR for monitoring and managing the human resources at all levels. All basic attributes, including the qualifications, experience, specialization, awards, promotion, academic information, family information, *etc*. of all scientific, technical, administrative and supporting staff are included in the software, which is being updated regularly.

Livestock production

The centre has maintained a representative herd of equines comprising indigenous horses of Marwari (n=37) and Kathiawari (n=5) breed, exotic donkeys (n=41) and other equines including ponies and mules (Table 1). During the year, there were 17 foalings (five Marwari, one Kathiawari and 11 exotic donkeys).

Table 1. Equine herd strength at NRCE (Bikaner and Hisar)

Category	Horses		Exotic Donkeys	Other	Total
	Kathiawari	Marwari			
Adult male	0	5	6	4	15
Adult female	2	10	13	4	29
2-3 yrs	1	4	9	-	14
1-2 yrs	1	6	3	3	13
6M-1yr	-	11	2	-	13
0-6M	1	1	8	-	10
Total	5	37	41	11	94

*At Hisar campus

Breeding performance of farm herd at Bikaner centre during the year is given in Table 2. The number of breedable mares and donkeys available were 8 and 14, respectively.

Table 2. Reproductive performance of equine herd at Bikaner

Parameters	Marwari	Kathwari	Jennies
Number of females inseminated	8	2	7
Number of A.I. done	31	6	24
Number of foatings	5	1	11

Agriculture production

During the year 2004-05, the fodder production at our Hisar and Bikaner farms is shown in Table 3. The centre produced 1901.4 quintal of fodder during the year, 1194.4 quintal at Hisar farm and 707 quintals at Bikaner farm.

Table 3. Fodder production at NRCE (Hisar and Bikaner)

Type of fodder	Production in Quintals		Total
	Hisar	Bikaner	
Lucern	479.4	353	832.4
Oat	102	63	165
Millet	80	291	371
Sorghum	309	-	309
Cowpea	60	-	60
Berseem	164	-	164
Total	1194.4	707	1901.4

List of Approved & Ongoing Research Projects

Title of the Scheme	Team	Date of Start	Date of Completion
EQUINE PRODUCTION			
Molecular characterization for studying genetic diversity among Marwari Breed of horses.	S.N. Tandon, A.K. Gupta, R.A. Legha, R.C. Sharma and Mamta Chauhan	Oct. 2001	Sept. 2004
Cryopreservation of stallion semen and perfection of AI in Marwar horses.	Yash Pal, R.A. Legha, S.N. Tandon, A. Arangasamy and S.K. Khurana	May, 2002	June, 2005
Development of equine chorionic gonadotropin (ecg) based ELISA test for pregnancy diagnosis in equines.	A.K.Gupta Yash Pal, Sanjay Kumar and S.K. Dwivedi.	May, 2002	June, 2006
Molecular marker based pilot study for detection of Angiotensin-1-converting enzyme gene (ACE) in indigenous equines.	Mamta Chauhan & A.K. Gupta	July, 2003	August, 2004
RFLP - Based genotyping of major histocompatibility complex class II genes in Marwari horses.	R.C. Sharma & S.C. Mehta (from NRC on camels, Bikaner)	Oct, 2004	May, 2007
Isolation of stallion seminal plasma proteins and their effect on *in vitro* fertilizing ability of spermatozoa.	A. Arangasamy & S.K. Bhure (from NRC on Camel, Bikaner)	Oct, 2004	May, 2006
Molecular markers based parentage testing in horses of Indian origin.	Mamta Chauhan and A.K. Gupta	Dec., 2004	Nov., 2006
Superior mule production in the field through frozen semen of exotic Jacks.	R.A. Legha, R.C. Sharma and A. Arangasamy	Dec., 2004	Nov., 2007
Performance evaluation of Marwari horses under different types of Shelters.	R.A. Legha Pramod Singh and R.C. Sharma	Jan., 2005	Dec., 2008
EQUINE HEALTH			
Development of improved vaccine against equine diseases.			
Development of vaccine(s) against equine herpes virus-I infection.	B.K. Singh, B.R. Gulati and N. Virmani	Nov., 1998	Mar., 2005
Development of improved diagnostics against important viral and bacterial diseases of equines.			
Studies on the improvement of the Diagnostics for differentation between EHV-1 and 4 infections employing molecular techniques.	Nitin Virmani, A.S. Panisup, B.K. Singh & B.R. Gulati	May, 2004	March, 2007
Development of sensitive & specific methods for diagnosis of equine rotavirus from diarrhoeic foals.	Baldev R. Gulati and B.K. Singh	June, 2003	May, 2006
Development of diagnostic(s) for pathogenic *Streptococcus equi* in equines.	Praveen Malik, B.R. Gulati, Nitin Virmani, S.K. Khurana and Mamta Chauhan	June, 2003	March, 2006
Development of diagnostics for *Rhodococococcus equi* infection of equines.	S.K. Khurana, Praveen Malik & Nitin Virmani	May, 2004	March, 2007
Epidemiological studies on emerging and existing diseases of equines.	S.K.Dwivedi, S. K. Khurana, A. S. Panisup, B.K.Singh, A.K.Gupta, S.Dey, B.R.Gulati, Rajender Kumar, P.Malik, Yashpal, Nitin Virmani, Sanjay Kumar& A. Arangasamy	Continuous Service Project	
Chemotherapeutic and diagnostic studies on trypanosomiasis and Babesiosis in equines.			
Isolation and characterization of secondary plant metabolites for the development of an antitrypanosomal drug.	S. Dey, S.K. Dwivedi, A.S. Panisup, Rajender Kumar & Sanjay Kumar	Jan., 2000	Nov., 2004
Development of diagnostic tests for equine trypanosomosis (Surra)	Rajender Kumar, S. Dey, A.K. Gupta & S.K. Dwivedi	June, 2003	March, 2006
Development of sensitive and specific diagnostic tests for detection of equine piroplasmosis.	Sanjay Kumar, Rajender Kumar, S. Dey, A.K. Gupta & S.K. Dwivedi.	May, 2004	March, 2006

Contacts

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