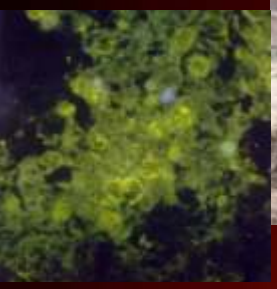
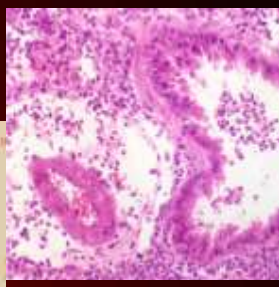
# वार्षिक प्रतिवेदन
# ANNUAL REPORT 2002-2003





## राष्ट्रीय अश्व अनुसंधान केन्द्र
## National Research Centre on Equines

NATIONAL RESEARCH CENTRE ON EQUINES
NRCE-1989

भाकृअनुप
ICAR

EHV-1 Prevalence during 2002-03


"Our mandates include
nation-wide
surveillance and
monitoring of
equine diseases"

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Dr. S.K. Dwivedi, Director

"The centre's invaluable research and scientific discoveries continue to improve equine health and diminish diseases in India."

# Director's foreword

During my two years' tenure as Director, the Centre has attained national and international recognition for quality research on important infectious diseases of equines. During this period, the centre has achieved the status of *National Referral Research Laboratory* on equine diseases through the hard work of the dedicated scientists who are striving to develop technologies for diagnosis treatment and prevention of equine diseases that are threatening the equine population in India. Some of the technologies developed are reflected in this annual report for pursuance of the readers.

During the first few months of my joining, I decided to reorient the on-going research projects with the help of professional peers in the area of equine health and production. The idea behind reviewing the projects was aimed at developing cutting edge technologies that are commercially viable, economically affordable and farmers friendly. These efforts have now started yielding results and at least three technologies are currently under field-testing and would be released shortly for the benefit of the equine industry globally.

Our priorities for the next few years would be to attain international recognition by establishing this centre as international equine reference centre for this region of the world. An *Equine Disease Monitoring System* at national level is under active consideration in collaboration with the Department of Animal Husbandry and Dairying, Govt. of India. This will help in monitoring the national status of important equine diseases and achieving the disease-free status to our country for the equine diseases that are not currently prevalent.

Our major emphasis would also be on formulation and implementation of international collaborative research projects in the area of vaccinology, diagnostics, therapeutics and equine reproduction.

Production of superior quality mules using artificial insemination techniques has been our major emphasis during the year under report in order to augment the socio-economic status of the poorest of the poor equine farmers whose livelihood is dependent on mules, donkeys and ponies. Our endeavour is to extend artificial insemination technique for mule production to various states of the country that are desirous to procure this technology.

During the year 2003-04, the focus will also be on generation of infrastructure facilities in terms of Office-cum Research building at Bikaner campus and development of microbial biocontatment (BSL-3) facility at Hisar campus.

The year is likely to be more challenging to the centre and I look forward to communicate with you on progress in our endeavour in next year's annual report.

I would like to take this opportunity to record my sincere thanks to the Chairman and the Members of the Publication Committee for bringing out this excellent annual report of the Centre with a new look and substantial improvement in the quality of its publication. The whole-hearted support extended to me by Indian Council of Agricultural Research, New Delhi, particularly, Dr. V.K. Taneja (Deputy Director General Animal Sciences) and the encouragement by Dr. Mangla Rai (Director General, ICAR) has generated zeal and enthusiasm in all of us at NRCE to work hard for the global competition in generation of commercially viable technologies and demand-driven research for the benefit of the farmers.

**28 August 2003**

**Dr. S.K. Dwivedi**

# Executive Summary

The Year 2002-03 has been very productive for National Research Centre on Equines. Our researches in the area of vaccinology, diagnostics, drug-development, equine breed characterization and artificial insemination that were initiated a few year ago, made significant progress during the year. Improved diagnostics for equine herpes virus, salmonellosis and pregnancy diagnosis were developed and evaluated. Active components of a novel herbal drug for anti-trypanosomosis were identified. Our efforts in nation-wide disease monitoring system got a shot in the arms with the recognition of this centre as national referral centre by the Department of Animal Husbandry & Dairying, Ministry of Agriculture (GOI). A brief account of achievements of NRCE in 2002-03 is outlined below:

Towards developing improved and indigenous vaccines for infectious equine diseases, we continued evaluation of the immunogenicity of inactivated indigenous EHV-1 strain (Hisar-90-7) emulsified with Tween-80 (OET-80) and mannide monooleate (OEMM). On primary immunization of horses, both the immunogens generated good responses as measured by complement fixing (CF) and virus neutralizing (VN) and responses were comparable with the commercial vaccines. Booster immunization effect of these immunogens was evaluated during the year and findings indicate that OEMM immunogen elicited significantly better VN and CF immune responses than OET-80 and commercial vaccines on booster immunization in horses.

The outer membrane proteins (OMP) of *Salmonella* Abortus equi alone and in combination with EHV-1 vaccines was further evaluated during the year for immune response in pregnant and non-pregnant equines. OMP generated good antibody titres in pregnant mares that remained quite high up to 12 weeks post-immunization. OMP provided good protection as measured by challenge of mice passively immunized with sera of immunized horses. In ponies administered OMP along with commercial EHV-1 vaccine, good immune response against both the immunogens was generated indicating that OMP and EHV-1 vaccines could be used together in equines. The findings establish that OMP is a potent immunogen, which provokes adequate immune responses in ponies against *Salmonella* Abortus equi infection.

Development and improvement of diagnostics has been one of the priority areas of the centre. We developed a-single dilution, sensitive and specific monoclonal antibody-based blocking enzyme-linked immunosorbent assay (B-ELISA) for detection of EHV-1 antibodies in equine sera that was 100 % sensitive and correlated well with virus neutralization test ($r = 0.85$ at $P<0.01$ level). During the current year, B-ELISA was further validated by testing large number of equine field sera (n =523). The B- ELISA detected a total of 271 (51.81%) samples, whereas 259 (49.52%) sera were positive by VNT. There was a very good agreement between results obtained by VNT and B-ELISA (86.61%). The findings establish that the B-ELISA could be used as an alternate to cumbersome VNT for EHV-1 diagnosis.

In an attempt to develop a quick and improved diagnostic for *Salmonella* Abortus equi, outer membrane proteins (OMP) based latex agglutination (LA) test was developed and compared with the tube agglutination test. There was 100% agreement between both the tests.

For pregnancy diagnosis in equines, a sandwich ELISA, that is based on the detection of equine chorionic gonadotropin (eCG) in serum, was developed. The ELISA results were at par with rectal examination and ultrasonogrphy. Inter- and intra-assay variability was also worked out and coefficient of variation was observed to vary from 7.65 to 12.74 % and 1.60 to 7.11% respectively.

With a goal to develop suitable drugs for treatment of *Trypanosoma evansi* infection of equines, extracts from a medicinal herb, *Lawsonia inermis,* were found promising. Activity-guided separation of antitrypanosomal components from this herb was done during the year using different chromatography techniques ( TLC and HPLC) to purify the active ingredients. Using these methods, major components exhibiting antitrypanosomal activity have been identified.

The follicular development and reproductive status of

problematic repeat breeding mares was monitored regularly by ultrasonography. The mares were inseminated at appropriate time and observed till embryonic development. The findings confirmed that the proper use of ultrasonography could improve reproductive performance in mares.

The Centre has initiated disease monitoring in equine population scattered all over the country against a number of diseases, particularly those that are included in list "A" and "B" of OIE. During the year 1333 serum samples from 16 different states of the country were collected and tested for major equine diseases. No incidence of Influenza, Glanders, *Salomonella* Abortus equi, Equine Infectious Anaemia was recorded in equines of the 16 states of India surveyed. EHV-1 was detected in 44 out of 609 samples tested, *Mycoplasma equigenitalium* in 19 out of 436 samples tested. A high prevalence of *Babesia equi* infection (20.9%, 129 out of 598) was reported in different states of India.

The levels of three toxic metals i.e. Fluoride, Lead and Cadmium in the serum samples of indigenous equines from various states were evaluated. Fluoride concentration in 22.96 % (264 out of 1148) of samples was more than WHO recommended physiological limit (0.20 ppm) in serum. Similarly, the sub-clinical lead toxicity was observed in 66.8% animals and 19.5% animals had toxic blood lead level (>0.50 ppm). Majority (78%) of equines had normal cadmium concentration in their blood.

In an effort to better utilize the potentials of indigenous breeds of animals, Marwari breed characterization was initiated using biometrical, bio-chemical and molecular approaches. The results indicate the existence of genetic variability within Marwari breed and molecular markers for Marwari breed identification are being further established.

In order to preserve the germplasm of Marwari horse breed and make available good quality cryopreserved Marwari semen for artificial insemination (AI), work was initiated to standardize frozen semen technique for *ex situ* conservation of Marwari breed of horses. Various physical and biochemical parameters of the semen of Marwari horses were defined during the year.

The technique of cryopreservation of jack semen was standardized in previous year. The cryopreserved semen was evaluated during 2002-03 to estimate the conception rate by AI in the field as well as farm animals. A total of 63 equids (4 jennies and 59 mares) were covered and pregnancy diagnosis after one month of covering yielding a conception rate of 50% and 47.4 % (2 out of 4 jennies; 28 out of 59 mares were pregnant).

As part of our diagnostic, consultancy and advisory services, 393 clinical cases from different parts of Haryana and Rajasthan were provided clinical diagnostic and therapeutic facilities, 123 mares were examined for pregnancy diagnosis and other pathological conditions in reproductive organs by ultrasonography, 954 animals given prophylactic deworming. During the period, 251 clinical samples were processed for bacteriological isolations and 33 isolates belonging to genera *Rhodococcus, Streptococcus, Staphylococcus*, *E. coli*, *Acholeplasma laidlawii* and *A.oculi* were isolated. Necropsies were conducted on 12 equines.

A number of clinical health camps, exhibitions and kissan goshthis were organized in different parts of Haryana and Rajasthan for dissemination of scientific information generated under lab-to-land programme; to get feedback from farmers to assess the priorities for need-based research and to provide health coverage to equines of the area.

Continuing improvements in the NRCE infrastructure, an Experimental Animal Facility as per the guidelines and requirements of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was created. A comprehensive website http://nrce.nic.in of the centre was developed and launched during the year and twenty-four hour internet-connectivity was made available through VSAT.

During the year, the Centre generated a revenue of Rs. 23.42 lacs from its internal sources, mainly through the diagnostic services rendered and sale of livestock to the farmers.

# Introduction

Since its inception on 26th November 1985, National Research Centre on Equines (NRCE) has been continuously marching ahead toward improvement in health and production of equines in India. During last 17 years, the centre has made tremendous achievements in control of important equine diseases including equine influenza, equine infectious anemia. A sub-campus at Bikaner established in 1989 is contributing significantly for the upliftment of the landless and marginal farmers by helping in conservation and improvement of the germplasms of indigenous equine breeds.

The revised mandate of NRCE as approved by the Indian Council of Agricultural Research in 2001 is as:

1. To undertake research on health and production management in equines;
2. To develop diagnostic/biological for major equine diseases;
3. To act as national referral facility for diagnosis, surveillance and monitoring of equine diseases;
4. To provide diagnostic, advisory and consultancy services.

Salient achievements of the Centre include:

- Vaccines: Equine Influenza vaccine using indigenous isolate (A/Equi-2/ Ludhiana/87) and released for commercial use; bacterin and outer membrane protein-based vaccines for *Salmonella* abortus equi.
- Immunobiologicals: Monoclonal antibodies against Equine Influenza and Equine Herpes Virus-1 infection.
- Diagnostic kits: For equine herpes virus (HERP KIT) and *Babesia equi* (COFEB KIT) diagnosis in equines.
- Diagnostics developed at the centre: Equine Influenza (single radial haemolysis, single radial immunodiffusion test, immunostick, strip and plate test ELISA's, virus neutralization, PCR); Equine Herpes Virus-1 (indirect ELISA, blocking-ELISA, polymerase chain reaction, immunoperoxidase and immunofluorescence, virus neutralization); Equine Infectious Anaemia (competitive ELISA and Biotin Avidin ELISA); Equine Piroplasmosis (complement fixation test); Equine Viral Arteritis (virus neutralization); Leptospira and *Mycoplasma equigenitalium* antibodies (Indirect ELISA).
- Molecular characterization of pathogens: DNA finger printing of EHV- 1 virus; sequencing of antigenically important genes of Equine Influenza virus
- Perfection of cryopreservation and artificial insemination for production of superior quality mules and donkeys for their use by landless, small and marginal farmers.
- Disease monitoring: Data on the prevalence of various diseases of equines namely Equine Infectious Anaemia, Equine Influenza, Equine Viral Arteritis, Equine Coital Exanthema, rotaviral diarrhoea in different regions of India generated.
- Repository: The centre has a collection of important indigenous equine isolates viz., EHV-1, equine influenza, *Streptococcus, Salmonella, etc.*
- Drugs: Mare lactoferrin has been purified and characterized for its antimicrobial activity.
- Donkey fibre has been used admixed with sheep fibres in the ratio of 40:60 to produce carpets and named Asheep.
- Indigenous breed characterization: Phenotypic and molecular characterization of indigenous breeds of horses is in progress.
- Baseline data has been generated on some of the important haematological, physiological and biochemical indices of Kathiawari horses as well as local donkey.
- Folliculogenesis studied using ultrasonography in donkey and horse mares and hormonal profiles estimated at different stages of oestrous.
- Draughtability studies: Physical, physiological, biochemical & acid base status of the donkeys during work and rest compared.

## Staff Position

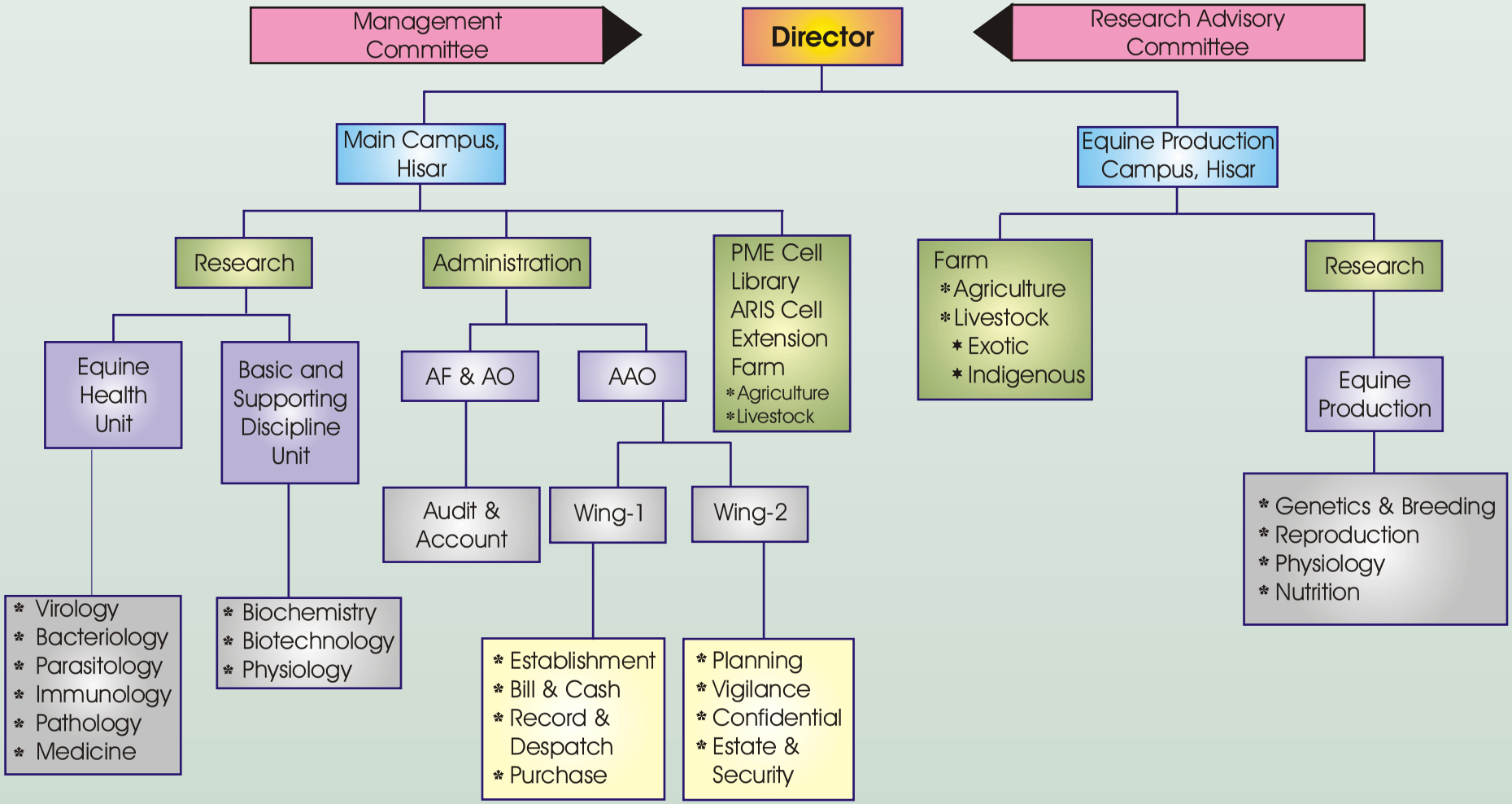
| Name of the post | Number of posts | | |
|---|---|---|---|
| | Sanctioned | Filled | Vacant |
| Director | 1 | 1 | - |
| Principle Scientist | 2 | 1 | 1 |
| Senior Scientist | 6 | 6 | - |
| Scientist | 12 | 10 | 2 |
| Technical | 23 | 23 | - |
| Administrative | 11 | 11 | - |
| Supporting | 25 | 22 | 3 |
| **Total** | **80** | **74** | **6** |

## Summary of Expenditure and Revenue Generation

| | | (Rupees in Lacs) |
|---|---|---|
| **Summary of Expenditure** | **2001-02** | 2002-03 |
| NON-PLAN | | |
| 1. a. Establishment charges including LSP/PF | 93.31 | 91.53 |
| b. Wages | - | - |
| c. O.T.A. | 0.04 | 1.03 |
| 2. a. Travelling allowances | 2.00 | 2.00 |
| b. HRD | - | - |
| 3. Other charges including equipments | 66.71 | 63.14 |
| 4. Information & Technology | - | - |
| 5. Works | 10.00 | 28.66 |
| *Non-Plan Total* | *172.06* | *185.36* |
| PLAN | | |
| 1. a. Establishment charges including LSP/PF | - | - |
| b. Wages | 0.50 | 0.48 |
| c. O.T.A. | 0.16 | 0.26 |
| 2. a. Travelling allowances | 1.99 | 2.00 |
| b. HRD | 0.99 | 0.18 |
| 3. Other charges including equipments | 106.02 | 96.87 |
| 4. Information & Technology | 6.00 | 2.36 |
| 5. Works | 40.31 | 7.81 |
| 6. One time catch up grant | 3.84 | |
| *Plan Total* | *159.81* | *109.96* |
| **Total Expenditure** | **331.87** | **295.32** |

| | | (Rupees) |
|---|---|---|
| **Summary of Revenue Generation** | **2001-02** | **2002-03** |
| 1. Sale of Farm Produce & auction | 56281 | 78542 |
| 2. Sale of Livestock | 260251 | 591200 |
| 3. Sale of Publication and advertisements | 300 | 1550 |
| 4. License Fee | 56906 | 54363 |
| 5. Interest on loans and advances | 218389 | 222 |
| 6. Interest on short term deposits | 96374 | 68234 |
| 7. Income from internal resource generation (EIA service) | 1696714 | 1422500 |
| 8. Other misc. receipts | 198700 | 125145 |
| **Total Revenue** | **2584455** | **2341756** |

# ORGANIZATIONAL STRUCTURE OF NRCE



# Research Achievements

## Effect of booster immunization with inactivated EHV-1 (Hisar-90-7) immunogen in horses

**In an effort to develop killed vaccine against EHV-1, we studied immunogenicity of inactivated indigenous EHV-1 strain (Hisar-90-7) emulsified with Tweeen-80 (OET-80) and mannide monooleate (OEMM). On primary immunization of horses, complement fixing (CF) and virus neutralizing (VN) antibody responses were similar with the two immunogens and were comparable with the commercial vaccines. Booster effect of these immunogens was evaluated during the year and findings indicate that OEMM immunogen elicited significantly better VNT and CFT responses than OET-80 and commercial vaccines on booster immunization in horses.**

Indigenous killed vaccine against EHV-1 abortions is needed for immunoprophylaxis in our country where EHV-1 associated abortions is a big problem and imported commercial vaccines are being used with no data on their efficacy in Indian scenario. We previously studied immunogenicity of inactivated indigenous EHV-1 strain (Hisar-90-7) emulsified with Tweeen-80 (OET-80) and mannide monooleate (OEMM) and compared its efficacy with the commercial vaccine in horses after primary immunization. On primary immunization of horses, no significant difference in complement fixing (CF) and virus neutralizing (VN) antibody responses was observed with the three immunogens. It was felt that the effect of booster immunization of Indigenous strain should be comparatively evaluated with available commercial vaccines in horses.

With this objective in mind, during the current year, two boosters of OEMM and OET-80 based immunogens were given to the horses. A commercial vaccine was also included in the study and immunized as per manufacturers' instructions. Four groups of horses, having 4 horses in each were made. Groups 1-3 were immunized with the three immunogens, while group-4 was kept as control. First

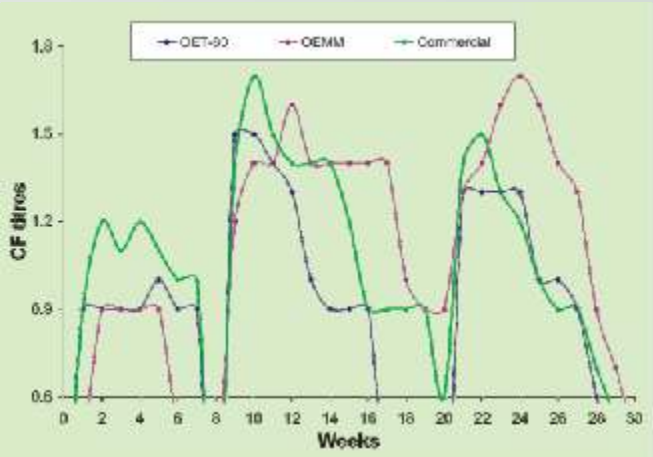



Fig.1. Complement fixing antibody response following booster immunization of horses with different EHV-1 immunoqens

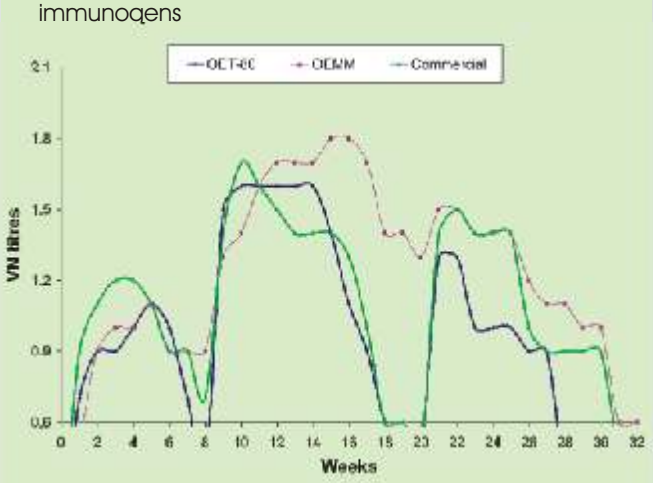



Fig. 2. Virus neutralizing antibody response following booster immunization of horses with different EHV-1 immunogens

boosters in the Kathiawari horses were given sub cutaneously (S/C) after eight weeks of primary immunization and second booster intramuscularly at 12 week after first booster. The animals were observed twice daily for rectal temperature and for any abnormality including nasal discharge. Serum samples from these horses were collected at weekly interval. The sera were tested for EHV-1 antibodies by virus neutralization test, complement fixation test and

blocking ELISA.

CF antibody responses in horses after different immunization schedule are shown in Figure 1. OET-80 immunogen in horses elicited poor response (over all 2-fold rise) after first booster. The CF antibody titre persisted for 8 weeks after first booster of OET-80, while with OEMM, first booster effect lasted for 12 weeks, whereas with commercial vaccine, it lasted for 11 weeks. On second booster immunization OET-80 and commercial vaccine response by CFT lasted for 7 weeks whereas OEMM response was observed till 8 weeks after booster immunization. Control group-4 of horses was seronegative to EHV -1 by CF test.

VN antibody responses in horses after different immunization schedule are shown in Figure 2. OET-80 immunogen in horses elicited satisfactory VNT response ($\geq$ 2 fold rise) after first booster. The VN antibody titre persisted for 9 weeks after first booster of OET-80, while with OEMM first booster effect lasted for 12 weeks, whereas with commercial vaccine, it lasted for 9 weeks. On second booster immunization OET-80 immunization response by VNT lasted for 7 weeks whereas OEMM response was observed till 10 weeks after booster immunization. Commercial vaccine VNT response lasted for 10 weeks after second booster. Control group-4 of horses was seronegative to EHV - 1 by VNT.

No significant difference in the overall B-ELISA response was observed with all the three immunogens after first booster. The B-ELISA using neutralizing Mab indicated that after first booster all the immunogens elicited moderate EHV-1 antibodies. The commercial vaccine appeared to elicit better antibody responses ($\geq$ 65%) from 1$^{st}$ to 3$^{rd}$ weeks post immunization while OEMM immunization elicited strong antibody response on 6$^{th}$ and 7$^{th}$ weeks after first booster (Fig.3). The present findings on comparative booster effect indicate that OEMM immunogen elicited significantly better VNT and CFT responses than OET-80 and commercial vaccines on booster immunization in horses.

In order to further study the protective immune response of OEMM immunogen in experimental animals, challenge studies in mice will be done in the current year followed by evaluation in horses.

***B.K. Singh***

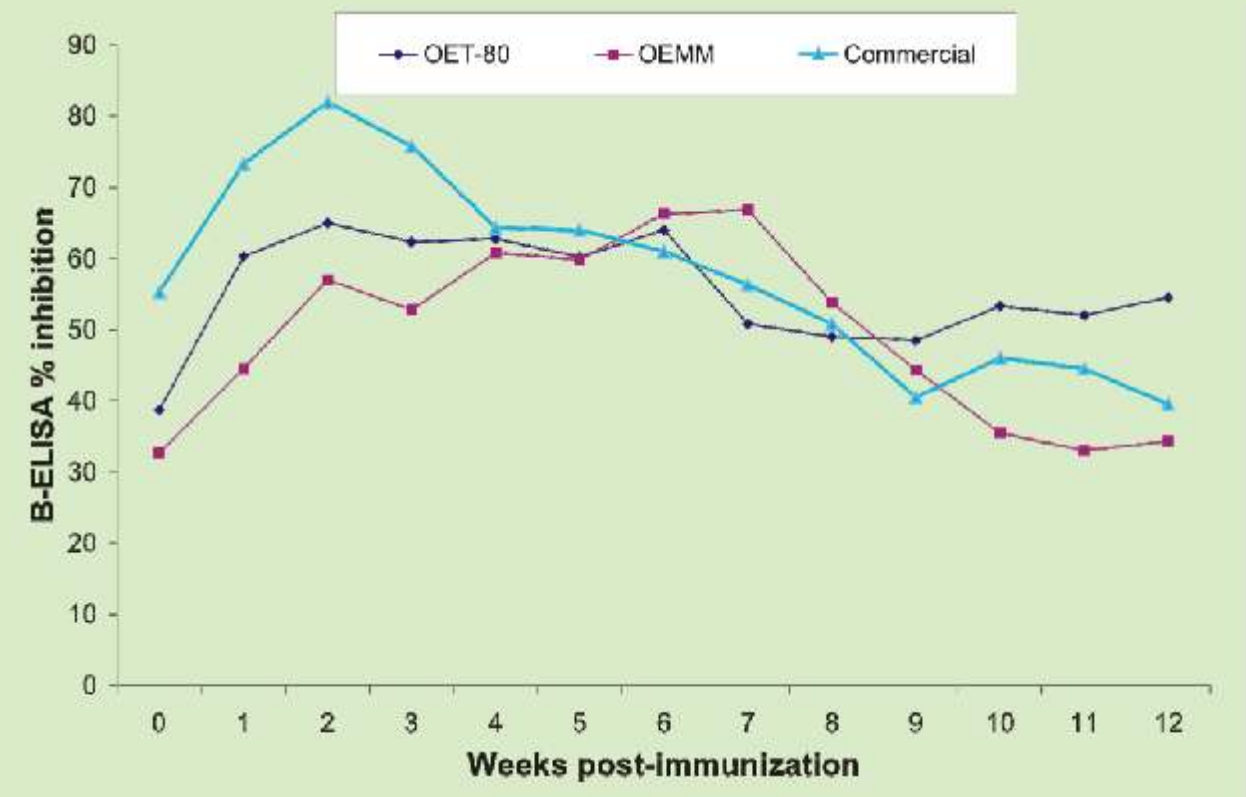



**Fig. 3 B-ELISA titres following immunization with different EHV-1 immunogens**

# Development of candidate for vaccine against *Salmonella* Abortus equi

**The outer membrane proteins (OMP) of *Salmonella* Abortus equi alone and in combination with EHV-1 vaccines was further evaluated during the year for immune response in pregnant and non-pregnant equines. Antibody titres in pregnant mares remained quite high up to 12 weeks post-OMP immunization and equine sera provided protection to mice against challenge on passive immunization. In ponies administered OMP along with commercial EHV-1 vaccine, good immune response against both the immunogens was generated indicating that OMP and EHV-1 vaccines could be used together in equines. The findings establish that OMP is a potent immunogen, which provokes adequate immune responses in ponies against *Salmonella* Abortus equi infection.**

*Salmonella* Abortus equi is considered as one of the important etiological agents responsible for abortion in equines during the last quarter of pregnancy. Seroepidemiological studies at NRCE have revealed that the disease continues to be prevalent in animals at various army and civil studs in several states of the country Inadequate protection of short duration afforded by the currently available commercial vaccines (killed bacterins) along with the problem of adverse reactions caused by its use in equines necessitated the search for a better and potent vaccine candidate. We attempted to use outer membrane proteins from a strain of *S.* Abortus equi as a vaccine in equines and examined its immunogenicity.

The outer membrane proteins (OMP) or the porin proteins of *Salmonella* Abortus equi were extracted and were previously tested for immunogenicity. Since the initial results in mice/ponies were very encouraging, the OMP preparation alone or in combination with EHV-1 vaccines was further tested during the current year for immune response in pregnant and non-pregnant equines. For this, six pregnant animals were administered three doses at 10 day interval with OMP (500 $\mu$g in 1 ml, oil adjuvanted) and another six non-pregnant ponies were administered a combination of OMP (500 $\mu$g in PBS) along with commercial EHV-1 vaccine (Pneumabort K+1b) (2ml) to see the interference, if any, in the simultaneous vaccination of the two. Following immunization, the humoral immune response was monitored for 13 weeks by 'H' agglutination and OMP-ELISA for anti-*Salmonella* antibodies and serum neutralization for EHV-1 antibodies. Cellular immune response was measured by intradermal testing in equines and paw oedema test in mice.

Antibody titres in pregnant mares immunized with OMP alone remained quite high up to a period of 12 weeks

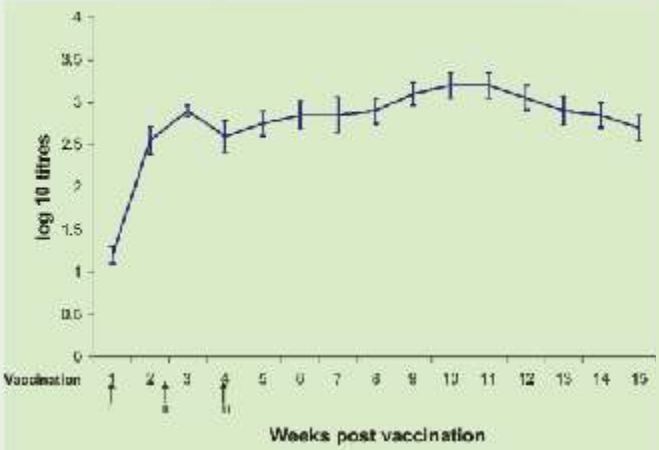



Fig. 1a 'H' titres of ponies (pregnant) administered with OMP in Oil alone

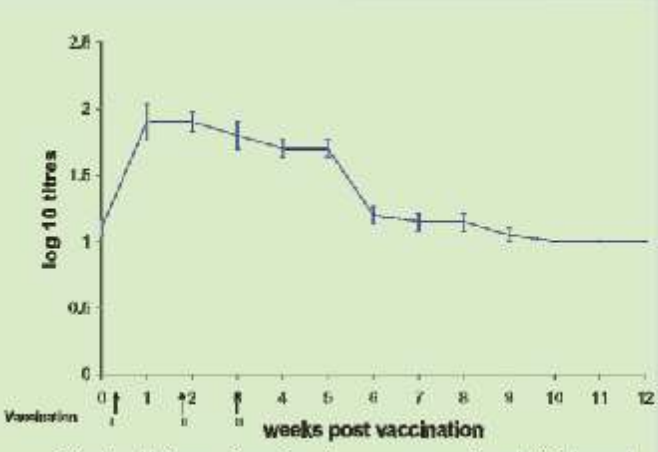



Fig 1b, H titres of ponies (non-pregnant) administered with OMP+Pneumoabort k+1b

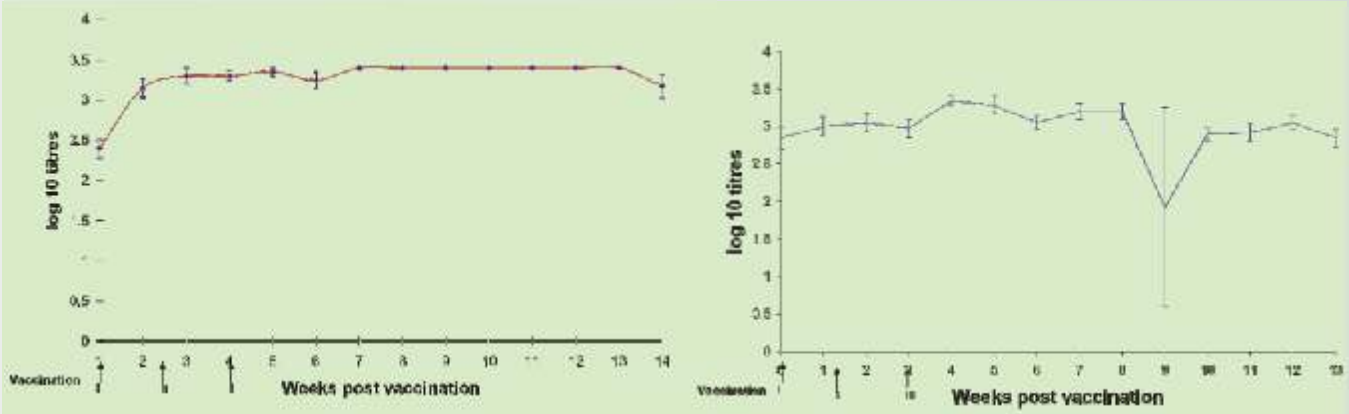



Fig 2a. Anti-OMP ELISA titres of ponies (pregnant) administered with OMP in Oil alone

Fig. 2b. Anti-OMP ELISA titres of ponies (non-pregnant) administered with OMP+Pneumabort k+1b

post first dose of immunization (Fig. 1a). Similarly, in ponies administered OMP in combination with commercial EHV-1 vaccine (Pneumabort k+1b), the anti-OMP antibodies showed reasonably high titres upto 12 weeks post vaccination (Fig. 1b). When the results were compared with the earlier data, OMP appeared to give better and longer humoral immune response as compared to the commercial bacterin vaccine.

Similarly, reasonably high H- titres were observed by agglutination in both the groups. However, when OMP was administered in combination with commercial EHV-1 vaccine (Pneumabort k+1b), the agglutination titres against *Salmonella* Abortus equi H antigen started declining by 9 weeks (Fig 2). The H agglutination response in pregnant mares was almost similar to the earlier observations in non-pregnant ponies indicating that the response of the vaccine candidate was equally effective in both pregnant and non-pregnant animals. No significant decline in the anti-OMP ELISA or H agglutination titres was noticed when OMP was used in combination with Pneumabort k+1b vaccine.

In ponies administered OMP along with commercial EHV-1 vaccine (Pneumabort k+1b), serum neutralization (SN) titres against EHV-1 remained high up to 7-8 weeks post vaccination and after that started declining. Similar SN response has been observed previously when animals were immunized with EHV-1 vaccine alone. This indicated that OMP does not interfere with the immune response to Pneumabort k+1b upon combination. These findings established that OMP and EHV-1 vaccines could be used together in equines.

Six ponies previously administered with OMP alone were examined for delayed hypersensitivity by injecting purified OMP in sterile PBS (0.1ml) on the neck of each animal by intradermal route. At 12h post injection, all animals showed significant swelling (++), which tended to increase/ remained constant till 48 h, thereafter it started declining and ceased completely by 96 h. Redness was maximum between 24-48 h post injection. Skin thickness was increased by 2-4 folds in first 24 h, which reduced slowly and came back to normal in about 7 days post injection. The mean ($\pm$ SE) skin thickness at 0, 24, 48 and 72 h are 2.02$\pm$0.13, 4.92$\pm$0.21, 4.35$\pm$0.25 and 4.13$\pm$0.22 mm, respectively. This suggests that OMP also provokes delayed hypersensitivity response in ponies. However, DTH response to OMP when tested in mice employing paw oedema test did not reveal a discernible reaction as observed in ponies, although some reaction was noticed in mice immunized with bacterin vaccine. Further confirmation using more *in vitro* tests for CMI would be required.

The protective efficacy of sera from OMP immunized horses was studied using passive mouse protection assay and the results were compared with the sera from ponies previously vaccinated with commercial bacterin. Both the sera provided 100% protection (0.2 ml neat serum, i/p) and 50% protection at 1:10 dilutions following challenge with 5 $LD_{50}$ *S.* Abortus equi. The OMP was found to be equally protective in mice as the commercially available bacterin vaccine, based on the assay.

The findings indicate that OMP is a potent immunogen, which provokes adequate immune responses in ponies against *Salmonella* Abortusequi infection. It also renders passive protection in mice comparable to the commercially available vaccine. It is, therefore, a suitable candidate for an improved vaccine against *Salmonella* Abortusequi infection.

# Validation of Blocking ELISA for diagnosis of EHV-1 antibodies

**We developed a-single dilution, sensitive and specific monoclonal antibody-based blocking enzyme-linked immunosorbent assay (B-ELISA) for detection of EHV-1 antibodies in equine sera. The B-ELISA was 100 % specific and correlated well with virus neutralization test ($r$ = 0.85 at P<0.01 level). During the current year, B-ELISA was further validated by testing large number of equine field sera (n =523). The B- ELISA detected a total of 271 (51.81%) samples, whereas 259 (49.52%) sera were positive by VNT. There was very good agreement between results obtained by VNT and B-ELISA (86.61%). The findings establish that the B-ELISA could be used as an alternate to cumbersome VNT for EHV-1 diagnosis.**

Equine herpes virus-1 (EHV-1) is a member of the alpha herpes virus group, which causes abortion, foal mortality, neurological and respiratory diseases worldwide. Although virus neutralization test (VNT) is widely used as serological assay for detecting EHV-1 antibodies, however, it is a cumbersome and time consuming test. Using neutralizing monoclonal antibodies raised in our laboratory against EHV-1, we developed a single dilution, sensitive and specific monoclonal antibody-based blocking enzyme-linked immunosorbent assay (B-ELISA) as an alternative to the VNT for detection of EHV-1 antibodies. The sensitivity of the B-ELISA was 92.5% and 100% with 1H6 and 9C6 Mabs, respectively. A very high correlation coefficient ($r$ = 0.85) was observed between B-ELISA and VNT that was significant at P<0.01 level (Figure 1). There was very good agreement between the results obtained by both VNT and B-ELISA ( = 0.9438). The Mab (9C6) based B-ELISA was found suitable alternative to VNT for screening large number of field sera and enabled confirmatory EHV-1 serodiagnosis.

During the current year, further validation of B-ELISA was done by testing large number of equine field sera (Table 1) by B-ELISA and compared the results with VNT. ELISA and VNT results of field sera (n = 523) are presented in Table

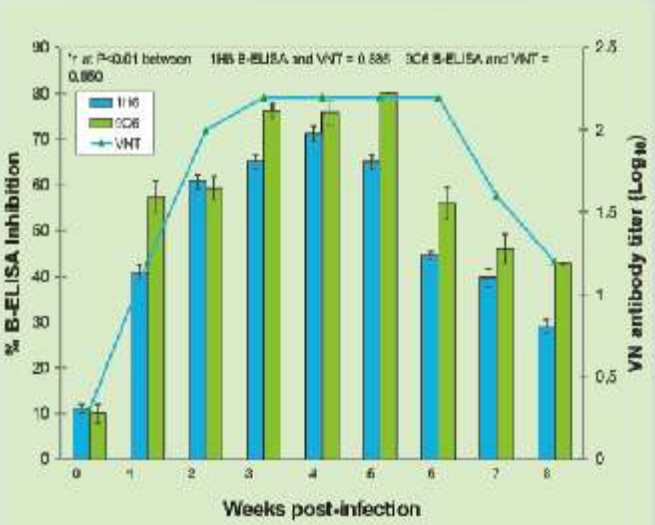



Figure 1: EHV-1 antibody levels as detected by B-ELISA and VNT in experimentally infected ponies (n=3) tested at weekly intervals post-infection using 1H6 and 9C6 monoclonal antibodies.

2. The B- ELISA detected a total of 271 (51.81%) samples, whereas 259 (49.52%) sera were positive by VNT. The agreement between results obtained by VNT and 9C6 based B-ELISA for detection of EHV-1 antibody in field sera (n= 523) was 86.61% including 230 positive and 223 negative sera (Table 3).

| Sr. No. | State | Samples tested |
|---|---|---|
| 1 | Haryana | 35 |
| 2 | Uttar Pradesh | 14 |
| 3 | Uttaranchal | 107 |
| 4 | Himachal Pradesh | 9 |
| 5 | Rajasthan | 327 |
| 6 | Maharastra | 31 |
| | **Total** | **523** |

**Table 1: State-wise details of the field horse serum samples tested for the validation of B-ELISA**

The present findings indicate that the B-ELISA studied at field level using single dilution of the serum is giving good agreement of the result compared to VNT and may serve as an alternative to cumbersome VNT for detection of EHV-1 infection.

In order to make this test available for testing samples at farmers' door and at different testing laboratories

| | Percent positivity of horse serum samples | |
|---|---|---|
| **Status of horses** | **Blocking ELISA** | **VNT** |
| Group 1 (Vaccinated mare, n = 233) | 200 (85.83) | 203 (87.12) |
| Group 2 (Unvaccinated mare, n = 266) | 47 (17.66) | 32 (12.03) |
| Group 3(EHV-1 infected ponies, n = 24) | 24 (100) | 24 (100) |
| Total horses tested =523 | 271 (51.81) | 259 (49.52) |

Note: Figure in parenthesis indicates percentage positive

Table 2: Percentage EHV-1 antibodies positivity of sera collected under experimental condition from different status of horses by blocking ELISA using 9C6Mabs and VN test.

| | Results in B-ELISA and VNT | |
|---|---|---|
| **Number of Sera** | **B-ELISA** | **VNT** |
| 230 | + | + |
| 223 | - | - |
| 41 | + | - |
| 29 | - | + |

Total sera tested =523

% Agreement = {(230+223)/523} x 100=86.61%

Table 3: Agreement between VNT and 9C6 Mab based B-ELISA for EHV-1 antibodies in field horse sera (n= 523).

in the country, a kit entitled **"Neutralizing monoclonal antibody blocking ELISA diagnostic kit for detection of equine herpes virus-1 specific antibodies"** has been developed and its shelf life up to 6 months has been tested. This research work has been carried out under NATP MM project on "*Veterinary diagnostics for prevalent and emerging diseases*".

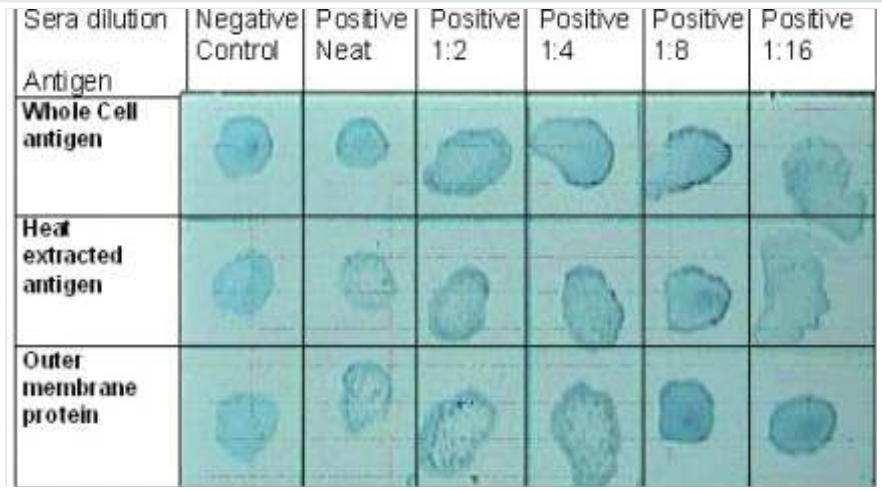
***B.K. Singh***

# Development of a quick diagnostic for sero-monitoring of animals against *Salmonella* Abortus equi

**In an attempt to develop a quick and improved diagnostic for *Salmonella* Abortus equi, outer membrane proteins (OMP) based latex agglutination (LA) test was developed and compared with the tube agglutination test. There was 100% agreement between both the tests.**

*Salmonella* Abortus equi infections continue to be prevalent in animals at various equine studs in several states of the country *viz*. Rajasthan, Andhra Pradesh, Maharastra and Haryana. 'H' and 'O' Agglutination tests are performed as per the standard protocols for the diagnosis of vaccinated/infected animals using the standard H and O antigens of *Salmonella* Abortus equi. The standard tube agglutination tests are relatively time-consuming and a rapid test for screening of large number of samples is required. There is a need for a rapid and more economical diagnostic test for detection of antibodies against *Salmonella* Abortus equi.

In continuation to the earlier attempts to develop a quick diagnostic test for sero-monitoring of vaccinated animals against *Salmonella* Abortus equi, various antigens of *Salmonella* Abortus equi including OMP, whole cell lysate, heat extract, Polymyxin B extract and partially purified cytotoxic factor were used. These different antigens were coated on latex beads (0.45µ) and the efficacy of each reagent so prepared was tested against serially diluted positive sera (with known H agglutination titre). A drop each of the reagent and sera were mixed on a clean glass slide, results read within 30-45 seconds and compared. Based on the results, OMP was selected and used for the development of the latex agglutination (LA) test. The LA was standardized in terms of antigen dose, level of antibody detection, replicability, sensitivity and specificity. In a preliminary study, 60 randomly selected field samples were tested by LA and compared the results with the tube agglutination test. There was 100% agreement between both the tests. Further testing on more field samples to check sensitivity and specificity of test is under progress.




**Latex Agglutination Test for diagnosis of antibodies against *Salmonella Abortus equi* in equine serum**

# Equine Chorionic Gonadotrpin (eCG)-based ELISA for Pregnancy Diagnosis in Equines

**For pregnancy diagnosis in equines, a serum-based sandwich ELISA that is based on the detection of equine chorionic gonadotropin (eCG) was developed. The ELISA results were at par with rectal examination and ultrasonogrphic results. Inter- and intra-assay variability have also been worked out and coefficient of variation was observed to vary from 7.65 to 12.74 % and 1.60 to 7.11% respectively.**

In equines, gestation interval is very lengthy and pregnancy diagnosis is an important issue. If the exact information about the pregnancy status is not known and the empty mares are not covered again in that breeding season then there is always a great economic loss to the poor equine owner. Pregnancy can be detected in mares either by ultrasonography; rectal palpation or imported serum based diagnostic kits. But most of these facilities are out of the reach of the poor equine owners as they are unable to meet the expenses involved in these diagnostic facilities and more than that, there is always a risk involved in transporting the mares to the diagnostic centres for pregnancy diagnosis. Besides this, under field conditions the expertise for pregnancy diagnosis by rectal examination is also limited. In view of these field-oriented difficulties, efforts were made to develop a serum-based sandwich ELISA that is based on the detection of equine chorionic gonadotropin (eCG). It is well known the eCG is released in mare's serum only after their conception. Therefore, this eCG based test could be exploited to detect and confirm the pregnancy in mares.

For standardization of sandwich ELISA, hyperimmune sera were raised against purified eCG in chicken and rabbit and antibodies were purified from both these sera using ammonium sulphate precipitation method. The purified antibodies were titrated in antibody capture ELISA. Using 10.0 mIU of commercial purified eCG, the titres were 1:3000 and 1:8000 for rabbit and chicken sera respectively. Sandwich ELISA was standardized by checkerboard titration and 1:6000 dilution of bird antibodies were selected for coating of ELISA plates to capture eCG present in serum samples (1:250 dilution) that was further detected by rabbit anti-eCG sera at 1:2000 dilution. Goat anti-rabbit IgG-horse radish peroxidase was used as conjugate in 1:20,000 dilutions with tetramethyl bezidine/hydrogen peroxide (TMB/$H_2O_2$) as substrate in 1:20 dilution as per the instructions of manufacturer. Serum samples of non-pregnant mares/stallion were used as negative controls. The optical density was checked in ELISA reader at 540 nm.

Using this sandwich ELISA, 110 serum samples collected from pregnant mares between 35 and 130 days of gestation were tested. ELISA results were at par with rectal examination and ultrasonogrphic results. Inter- and intra-assay variability have also been worked out and coefficient of variation was observed to vary from 7.65 to 12.74 % and 1.60 to 7.11% respectively.

ELISA will be further assessed for its capability to detect pregnancy at the earliest date, say between 30 and 45 days of conception.

***A K Gupta, Y P Sharma and S K Dwivedi***

# Activity-guided separation of antitrypanosomal components in *Lawsonia inermis* leaf extract

**To develop a drug for treatment of *Trypanosoma evansi* infection of equines, extracts from a medicinal herb, *Lawsonia inermis* were found promising. Activity-guided separation of antitrypanosomal components from this herb was done using different chromatography techniques (HPLC and TLC) to purify the active ingredients. Using these methods, major components exhibiting antitrypanosomal activity have been identified.**

*Trypanosoma evansi* is a protozoan parasite causing severe mortality and morbidity among equines in India. Due to emergence of resistance both in the parasite as well as vector, the control of this disease became a matter of great concern in recent days. No new drug from allopathic system of medicine is available since last 60 years. To explore the possibility of developing a new drug from medicinal herbs, *Lawsonia inermis* leaf extract is being evaluated for its antitrypanosomal activity. We previously found that 50% methanol extract possesses antitrypanosomal activity between dosage 1500-2000 µg/ml *in vitro* system. The preliminary attempts to separate the active components revealed solvent system containing chloroform and methanol could separate the active gredient bearing anti-trypanosomal activity from the crude extract.

Detailed studies were conducted for activity-guided separation of antitrypanosomal components from *L. inermis* leaf extract. Thin layer chromatography using a combination of solvents of different polarity was performed to develop a suitable solvent system for fractionation of the components of methanol extract of L *inermis* leaf. It was found that solvent system containing methanol, chloroform and ethyl acetate could separate some components efficiently but for remaining components, a solvent system containing methanol, chloroform, ethyl acetate and acetic acid was found more suitable. The crude extract was separated into 5 distinct fractions (based on similarity of TLC profile) using Column Chromatography and all were tested for their *in vitro* antitrypanosomal activity. Out of 5 column fractions tested, antitrypanosomal activity was recorded in 2 fractions between doses 125 and 250 µg/ml. These fractions were further analyzed using HPLC for their purity and found that these two fractions contained 3 and 7 chemically distinct compounds, respectively (Figs. 1). Both these extracts were subjected to Preparative Thin Layer Chromatography (PTLC) and major components were identified. The anti-trypaonosomal activity of these purified components is being tested.

*In vivo* testing of the fractions exhibiting maximal antitrypanosomal activity *in vitro* is in progress.

***S.Dey, S.K.Dwivedi and A.S.Panisup***

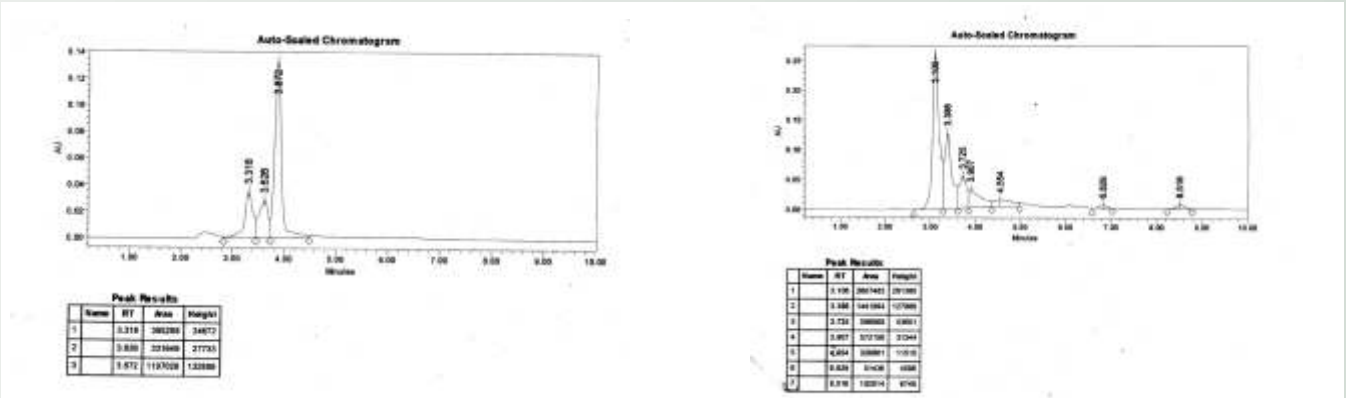


**Fig. 1 Fractions of *L. intermis* as obtained by HPLC**

# Use of Ultrasonography in Mares

**The follicular development and reproductive status of problematic repeat breeding mares was monitored regularly by ultrasonography. The mares were inseminated at appropriate time and observed till embryonic development. The findings confirmed that the proper use of ultrasonography could improve reproductive performance in mares.**

Among all measures, ultrasonography is one of the most useful tools for diagnosing reproductive health in man and animals. A study was therefore undertaken to use ultrasonic imaging to diagnose status of the reproductive organs for the improvement of reproductive performance in mares.

Five mares, which did not conceive inspite of insemination in last three cycles, were used as animals for this study. Two of these animals had history of early foetal death (one) and abortion (one). All these animals were negative to Equine Herpes Virus-1 and *Salmonela abortus equi* infections and were of good health. "Teasing Practice" was followed regularly for recording reproductive behaviour of these animals. All reproductive organs were scanned at regular intervals ultrasonographically to detect follicular and embryonic developments.

The oestrous cycle and oestrous period in these mares were recorded in the range of 21-25 and 5-7 days, respectively. At the beginning of oestrous (day 0) the mean follicular size was measured as 17.73+0.34 mm, which increased to 50.9 mm at the time of ovulation (Fig 1). The Mares were inseminated following the ultrasonographic findings. The embryonic development were detected (6.76 + 0.47 mm) as early as day 9 post insemination (DPI) and was confirmed on day14 with a mean size of 9.7 + 0.40mm (Fig 2a). Increase in embryonic size was recorded on 28 and 34 DPI (Fig 2). The findings of the present study confirmed that the proper use of ultrasonography could improve reproductive performance in mares. Detailed studies are in progress.

It is interesting to note that regression in size of embryonic vesicle was recorded in two mares between 30 and 34 DPI which was cured by progesterone and folic acid therapy as is evident in ultrasonic image on day 37 DPI.

***S.K.Dwivedi, S.Dey, and R.K.Chaturvedi)***

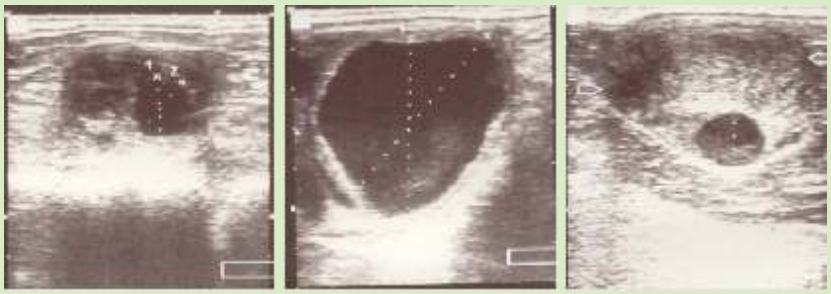


Figure 1. Ultrasonic image of ovarian follicIle at a.onset of estrou; b. near ovulation ; and c. post-ovulation

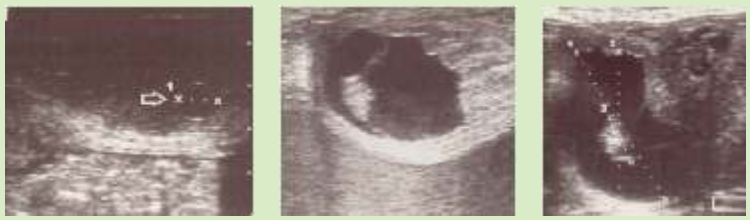


Figure 2. Ultrasonic image of embryonic development at a.day 14; b.day 28; and c.day 34 post insemination

# Seromonitoring of various equine diseases

**National Research Centre on Equines has initiated disease monitoring in equine population scattered all over the country against a host of diseases, particularly those that are included in list "A" and "B" of OIE. During the year 1333 serum samples from 16 different states of the country were collected and tested for major equine diseases. No incidence of Influenza, Glanders, *Salomonella* Abortus equi, Equine Infectious Anaemia was recorded in equines of the 16 states of India surveyed. EHV-1 was detected in 44 out of 609 samples tested, *Mycoplasma equigenitalium* in 19 out of 436 samples. A high prevalence of *Babesia equi* infection (20.9%, 129 out of 598) was reported in different states of India.**

Of about 2.0 million equine population in India, approximately 98% comprises of indigenous equids including donkeys, mules and ponies. Efficient performance of equines for optimal activities like competitive games, sports, draft and transport depends upon a sound health and freedom from various ailments including infectious diseases. The losses due to various diseases among equines are not only direct due to mortality but many indirect factors also come into picture. These include the cost of treatment of ailing animals, expenditure involved in application of control and preventive strategies including isolation and quarantine of affected animals as well as the losses due to

**Table : EIA status in India**

| Sr. No. | State/U.T. | 1987-99 | 1999-2000 | 2000-01 | 2001-02 | 2002-03 |
|---|---|---|---|---|---|---|
| 1. | Delhi | 12054 (19) | 685 | 327 | 197 | 656 |
| 2. | Haryana | 2845 (65) | 102 | 108 | 84 | 214 |
| 3. | Punjab | 1746 (17) | 170 | 130 | 43 | 103 |
| 4. | U.P. | 1791 (5) | 18 | 17 | 129 | 171 |
| 5. | A.P. | 5546 | 718 | 887 | 214 | 771 |
| 6. | Tamilnadu | 8001 (4) | 702 | 659 | 651 | 767 |
| 7. | Karnataka | 19566 (26) | 1440 | 2795 | 1255 | 1306 |
| 8. | W.Bengal | 5627 (80) | 177 | 420 | 192 | 347 |
| 9. | Maharashtra | 11585 (11) | 83 | 132 | 170 | 227 |
| 10. | Chandigarh | 118 | - | 5 | 6 | 34 |
| 11. | Gujrat | 489 | 137 | 19 | 49 | 36 |
| 12. | Rajasthan | 1726(1) | 27 | 12 | 169 | 182 |
| 13. | M.P. | 776 | - | 63 | 30 | 48 |
| 14. | Assam | 166 | - | 7 | - | 5 |
| 15. | Uttaranchal | 68 | - | 21 | 47 | 170 |
| 16. | Himachal Pradesh | 38 | - | - | - | 28 |
| 17. | Bihar | 172 | - | 14 | - | 26 |
| 18. | J & K | 162 | - | - | - | - |
| 19. | Kerala | 15 | - | - | - | - |
| 20. | Others | 1553 | 91 | 7 | 12 | 76 |
| | **Total** | **60823 (228) (0.374%)** | **4350 Nil** | **5623 Nil** | **3248 Nil** | **5167 Nil** |

Fig. 1 - Prevalence of EHV-1 in different states during 2002-2003

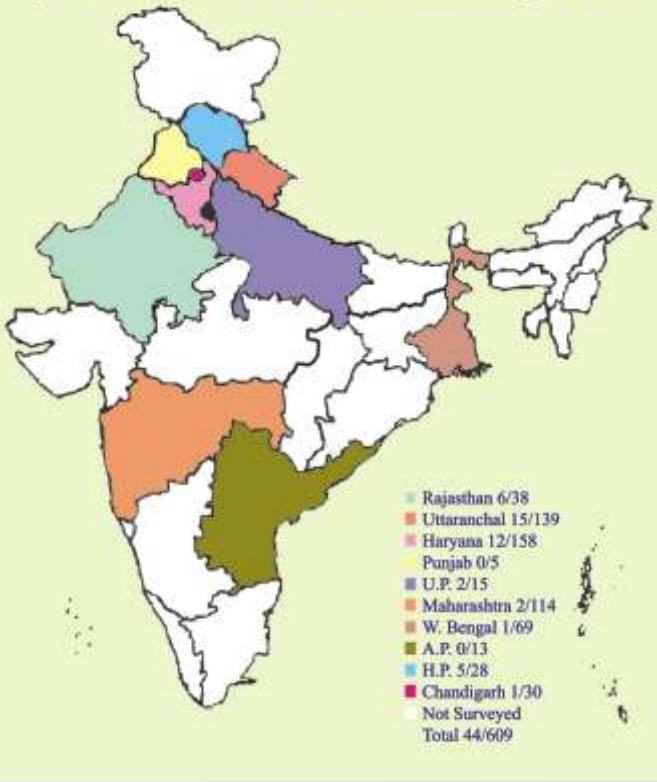
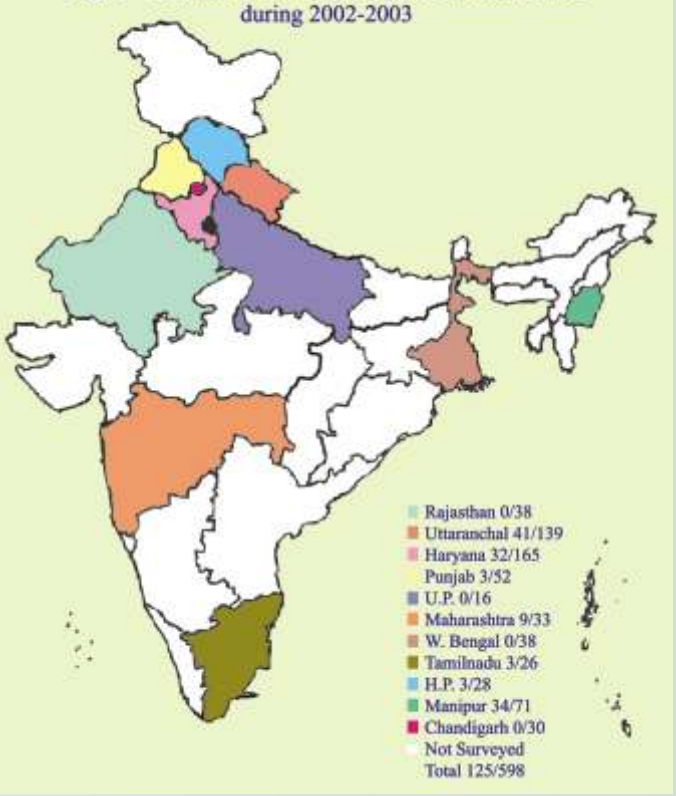



Fig. 3 - Prevalence of *Babesia equi* in different states during 2002-2003




restrictions on the national and international movement of animals (export potential). Thus, regular surveillance of equine diseases at national level, both in organized and unorganized sectors, is not only necessary to ascertain their status but also throws a vision in the area of preparedness against emerging diseases. National Research Centre on Equines has initiated surveillance and monitoring in all the equine population scattered all over the country against a host of diseases particularly those that are included in list "A" and "B" of OIE, *viz.* African Horse Sickness, Equine Infectious Anemia, Equine Viral Arteritis, Equine Babesiosis (*B. equi* and *B. caballi*), Equine Rhinopneumonitis (EHV-1 infection), Dourine, Glanders, Equine Influenza. The prevalence of other equine diseases like Trypanosomasis, *Salmonella* Abortus equi, EHV-4, Brucellosis and Leptospirosis is also being studied.

In this endeavor, during the current year serum samples from various states *viz.* Andhra Pradesh, Assam, Chandigarh, Gujarat, Haryana, Himachal Pradesh Karnataka, Maharashtra, Manipur, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal, Punjab and Uttaranchal were collected and tested for these diseases.

Testing of 1333 samples for Equine Influenza, Glanders, *Salomonella* Abortus equi by Haemagglutination Inhibition test, Complement Fixation Test and H antigen Agglutination Test and Plate Agglutination Test, respectively gave negative status for the diseases. None of the 270 serum samples tested for Leptospirosis employing Indirect ELISA yielded positive result.

Equine Infectious Anaemia is a vector borne retroviral infection and animals once acquire infection remain carriers all through their life. Since the first positive case of EIA detected in 1987, a total of 228 cases have been reported on screening of a population of 60823 equids till March 1999. Since then, EIA has not been reported from the country (Table 1). However, NRCE is regularly monitoring the presence of EIA in thoroughbred and indigenous equines. During 2002-03 a total of 5167 serum samples were screened for EIA by Coggins test and none of the samples was found positive for the infection.

Equine Herpes Virus-1 is one of the serious diseases of the equids, which causes respiratory disease, abortions, neurological disorders and perinatal foal mortality. The disease is widely prevalent in India and during the period of 1989 to 1997, 13.5% (out of 2573) equines were positive for

EHV-1 antibodies. During 2002-03, out of a total of 609 sera samples tested, 44 were positive by ELISA and VNT. Fig.1 depicts state-wise distribution of EHV-1 during 2002-03

Equine Viral Arteritis is another disease associated with abortions, however, there are only a few isolated reports from India and no major outbreak is reported. A limited number of samples (68) from Maharastra, Tamil Nadu, Uttar Pradesh and Bihar were found negative for EVA.

A total of 436 samples of indigenous equines from various states when examined for *Mycoplasma equigenitalium* by indirect ELISA revealed positivity in 19 samples, all of which belonged to Haryana.

On testing 598 sera for *Babesia equi* infection by COFEB kit, 125 were found positive having a rate of infection of 20.9%. Fig. Illustrates the disease pattern in the country. All the sixteen sera tested for B. caballi were found negative. Similarly 16 sera samples tested for dourine were found negative.

***S.K. Dwivedi and all scientists of NRCE Hisar***

## Toxic Metal Residues in the Sera of Indigenous Equines

**The levels of three toxic metals i.e. Fluoride, Lead and Cadmium in the serum samples of indigenous equines from various states were evaluated. Fluoride concentration in 22.96 % (264 out of 1148) of samples was more than WHO recommended physiological limit (0.20 ppm) in serum. Similarly, the subclinical lead toxicity was observed in 66.8% animals and 19.5% animals had toxic blood lead level (>0.50 ppm). Majority (78%) of equines had normal cadmium concentration in their blood.**

Various anthropogenic activities of man affect both biotic and abiotic environment resulting into increase availability of toxic metals (contaminants/pollutants) in water, air, and green and subsequently toxic metal residues may increase in animals sharing such environment and can cause health hazards. A study was therefore undertaken to estimate the level of these toxic metals (Fluoride, Lead and Cadmium) in equine blood.

***Fluoride:*** A total of 1148 equine sera samples collected from different parts of the country were analyzed for fluoride content. It was found that 22.96 % (264 out of 1148) of these sera samples contained more than 0.20 ppm of Fluoride (Highest physiological limit, WHO) in serum.

| Lead Range (unit) | Nos. of Animals | Percentage |
|---|---|---|
| <0.199 | 0 | 0.00 |
| 0.200-0.330 | 23 | 13.61 |
| 0.340-0.499 | 116 | 66.86 |
| 0.500-0.550 | 33 | 19.52 |

***Lead:*** Analysis of blood samples from indigenous equine revealed that 66.863% of these equines are sub clinically exposed to lead and blood lead levels of 19.526 % animals are sufficient enough to produce toxic effects on different vital organs like liver and kidneys (highest physiological limit is 0.200 ppm). The blood lead concentration of 169 Indian horses is presented in Table:

***Cadmium:*** Blood Cadmium concentration in 78.10 % of these equine was within normal range as recommended for this species. Only 21.89% samples had mild increase in blood cadmium concentration.

***S. Dey and S.K. Dwivedi***

# Molecular characterization for studying genetic diversity among Marwari breed of Equids.

**In an effort to better utilize the potentials of indigenous breeds of animals, Marwari breed characterization was initiated using bio-metrical, bio-chemical and molecular approaches. The results indicate the existence of genetic variability within Marwari breed and molecular markers for Marwari breed identification are being further established.**

Marwari breed of horses is one of the potent breed of indigenous horses that needs detailed characterization for its better conservation and for exploitation of its export potential. For the characterization of Marwari breeds, three different approaches i.e. bio-metrical, bio-chemical and molecular are being used.

**Biometrical characterization:** As it is well known that the genotype of an individual with its environment interacts and expresses the phenotype of individual. The biometry is most common method of evaluating the animal for future performance. Therefore the study on biometry is helpful in breed characterization and future performance. The data on biometry of Marwari horses is presented in Table-1. The lower value of height at withers, body length, leg length, face length and ear length reflects that inter-breed crossing is very commonly taking place in theses horses. Stallion's and mare's with higher biometrical value may be selected preserved and conserved.

| S.No | Parameters | Horse Breed (Marwari) (n=29) |
|---|---|---|
| 1. | Height at withers | 149.41±0.80 |
| 2. | Body length | 148.76±0.91 |
| 3. | Heart grith | 172.93±2.08 |
| 4. | Leg length (fore) | 102.86±0.58 |
| 5. | Height at knee | 047.25±0.54 |
| 6. | Face length | 060.14±0.69 |
| 7. | Face width | 017.58±0.41 |
| 8. | Ear length | 015.68±0.29 |
| 9. | Ear width | 009.29±0.18 |
| 10. | Tail length | 046.30±0.25 |
| 11. | Common body colors | Chestnut, Bay, Black, Gray |

Table-1 Biometrical parameters of Marwari horses

**Biochemical characterizaion:** Studies based on bio-chemical polymorphism were initiated to generate data that help in the identification of individual animal or breed identification and other problems connected with animal breeding. Marwari horse population from 5 different equines farms (EPC, Bikaner-07, Sarvodaya Basti, Bikaner-10, Peer Kamadia, Hanumangarh-12, Narlai, Pali-06 and Jodhpu-08) were evaluated for bio-chemical polymorphism of proteins/enzyme systems viz hemoglobin, albumin, transferrin, amylase, carbonic anhydrase and serum esterases using the technique of S. G. E. and PAGE and the results of the phenotypes have been presented in Table-2. The results obtained in the present study are the indicators of genetic variability within Marwari breed and these might

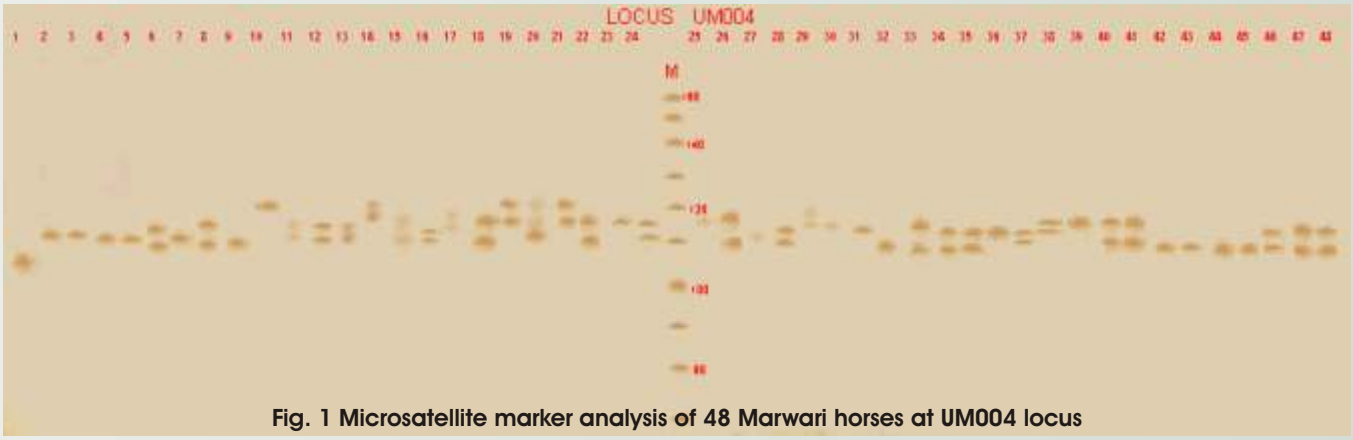



**Fig. 1 Microsatellite marker analysis of 48 Marwari horses at UM004 locus**

help in establishing markers for Marwari breed. Further studies for deciding markers based on biometry and bio-chemical variability of about 50-60 animals will be undertaken in the next year.

| S.No | Protein/enzyme | No. of alleles | No. of phenotypes |
|---|---|---|---|
| 1. | Hemoglobin | 2 | 2 |
| 2. | Albumin | 2 | 2 |
| 3. | Transferrin | 4 | 5 |
| 4. | Amylase | 2 | 3 |
| 5. | Carbonic anhydrase | 2 | 2 |

Table2: Phenotypes of different protein/enzyme systems

**Molecular characterization:** Existing Indigenous Marwari Horses possess certain favorable genetic traits and are known for sturdiness, endurance, swiftness and relatively disease resistance which could have been possible through accumulation of a special combination of certain genes or gene groups required for these traits. However owing to lack of sound breeding policies, the quality of these horses is undergoing rapid deterioration, the breed needs to be conserved. Molecular characterization is an initial step in any conservation program and work was initiated to characterize the genome of Marwari horses and to study the genetic diversity/ variability among them so that policies can be formulated for their conservation and improvement.

Fifty-five unrelated Marwari horses, true to their breed based on the phenotypic characteristics, were selected from different equine farms in Rajasthan. Blood samples were collected and processed for DNA isolation by standard protocol using phenol chloroform extraction followed by precipitation with sodium acetate and ethanol. Quality and quantity of DNA was checked spectrophotometerically as well as by gel electrophoresis.

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 been used in exotic equine genome. We initially selected ten pairs of the primers related to different loci for synthesis from literature based on the maximum number of alleles. Purity of each primer was checked for single major band on denaturing polyacrylamide gel electrophoresis (PAGE). Five microsatellite loci (UM002, UM004, UM021, HTG 6 and VHL 20) were further evaluated across 48 Marwari horses. All the markers amplified the products by PCR, resolved on 6% urea PAGE and silver stained. The gel was dried and documented. The number of detected alleles ranged from 3 (HTG 6) to 6 (VHL 20) with a mean of 4.5 per microsatellite marker. Genotypes at UM-021 locus were found monomorphic, showing only one allele in all the animals. This microsatellite locus seems to be present in highly conservative region of the genome. Heterozygocity values were observed to be 0.56, 0.69, 0.66, 0.74 at HTG6, VHL20, UM002 and UM004 (Figure 1) loci respectively.

The project continues with the objective to screen Marwari horse population at ten more microsatellite loci in order characterize the genome at molecular level.

***S N Tandon, A K Gupta and R A Legha***

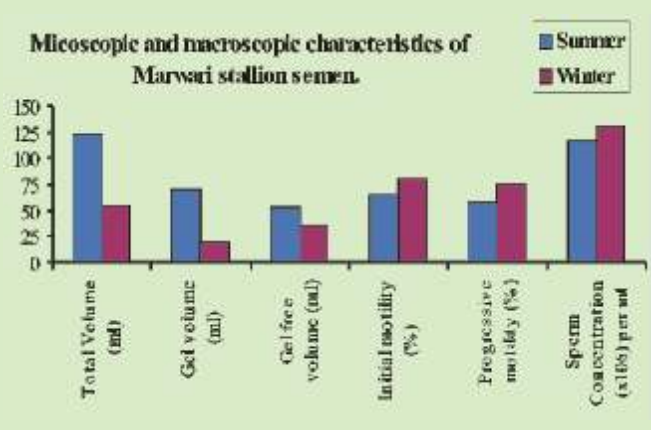
# Cryopreservation of equine semen and Artificial Insemination In Equines

**In order to preserve the germplasm of Marwari horse breed and make available good quality cryopreserved Marwari semen for artificial insemination, work was initiated to standardize frozen semen technique for *ex situ* conservation of Marwari breed of horses. Various physical and biochemical parameters of the semen of Marwari horses were defined during the year.**

**Cryopreservation of Marwari Stallion Semen:** Stallion semen cryopreservation and artificial insemination (AI) is an integral part of equine production in many European countries. Natural service is however is generally adopted as far as equine production is concerned in India except in some organized farms where fresh semen is used for AI. In order to preserve the germplasm of Marwari horse breed and to meet the increasing demand of cryopreserved Marwari semen by farmers, work was initiated to standardize frozen semen technique for *ex situ* conservation of Marwari breed of horses.

Semen was regularly collected using artificial vagina from three healthy adult Marwari stallions maintained at Equine Production Campus, Bikaner In order to draft a suitable extender for semen freezing, physico-biochemical characteristics of filtered gel free semen were studied. Consistency and colour of semen were observed thin to thick and milky white to creamy. The pH varied from 7.0 to 7.5 during summer and winter seasons. Total volume of semen and gel free semen averaged 123.4 and 52.9 ml in summer and 54.0 and 35.0 in winter month's collections of semen, respectively. Average initial and progressive motility was recorded 66.1 & 58.0% and 79.0 & 75.0% respectively during summer and winter months. Average sperm concentration was observed 115.7 $x10^6$/ ml and 131.3 $x10^6$/ ml during summer and winter months, respectively (Figure).

Among biochemical parameters, mean activity of GOT, GPT and ALP in seminal plasma irrespective of season was 145.9, 13.46 and 4027 Unit/l. Since GOT and LDH activity is found in the spermatozoan head, especially in the acrosomal region, or in the mid-piece, elevated free levels of these enzymes are indicative of damage to the




spermatozoan and affect sperm motility. Average total protein (g/dl), cholesterol (mg/dl), uric acid (mg/dl), urea nitrogen (mg/dl) and chloride (mEq/l) contents were 0.64 ±0.25, 4.82 ±0.87, 0.47±0.07, 12.79 ±0.72 and 99.87 ±4.35 in seminal plasma. Proteins provide a protective coating for spermatozoa and hence increase their survival time within the female reproductive tract, and this coating might be a prerequisite to capacitation.

To standardize the procedure of cryopreservation, seminal plasma was removed by centrifuging the semen with Citrate-EDTA that acts as primary extender. The purpose of removing seminal plasma was to avoid the adverse reaction between seminal plasma and egg yolk, a constituent of the main extender. Sperms were finally suspended in lactose-glucose-EDTA-egg yolk extender. The resuspended sperms were filled in polypropylene straws of 0.5 ml capacity, sealed and frozen using programmable bio-freezer. Post-thaw motility of frozen semen ranged between 20 and 40% during the period under report using these extenders. The semen was also tested for the presence of pathogens namely; *Salmonella* abortusequi, *Pseudomonas* spp., *Brucella* spp., *Streptococcus* spp., *Taylorella equigenetalis*, Mycoplasma and found negative.

The study continues with the objective to recommend a suitable extender to achieve better freezibility and post thaw motility of sperms.

***Yash Pal, R A. Legha, S. K. Khurana and S. N. Tandon***

# AI using cryopreserved Jack semen

**The technique of cryopreservation of jack semen was standardized in previous year. The cryopreserved semen was evaluated during 2002-03 for its conception rate by AI in the field as well as farm animals. A total of 63 equids (4 Jennies and 59 mares) were covered and pregnancy diagnosis after one month of covering revealed a conception rate of 50% and 47.4 % (2 out of 4 jennies; 28 out of 59 mares were pregnant).**

Artificial insemination (A.I.) is the most important single technique for genetic improvement of livestocks. This is possible because selected superior male produces enough spermatozoa to inseminate a large number of female per year. Availability of superior jacks, heavy in body weight and large in size is very limited with breeders for the production of superior mules. Exotic jacks are available with military farms and NRCE, for their own requirement of the farm. Artificial insemination with frozen semen is the only way by which animals available with farmers can be inseminated with the semen of superior exotic jacks. The technique of cryopreservation of jack semen was standardized in previous year. The cryopreserved semen was used to inseminate the female animals at livestock farm of Equine Production Campus, Bikaner.

A.I. study was conducted in the field animals as well as in the farm animals to study the conception rate. Under field condition, area covered: Balehra, Pathargarh, Gadhi Basic , Rana Majara, Indri, Luhari, Dhanora, Radore and Deras near Yammuna river and Thermal Power plant in Distt Panipat, Karnal and Yammuna Nagar of State Haryana. Animals were covered on 4$^{th}$ and 6$^{th}$ day of estrous cycle during June to September 2002. A total of 63 equids (4 Jennies and 59 mares) were covered. Pregnancy diagnosis after one month of covering revealed 50% and 47.4 % conception rate (2 out of 4 jennies; 28 out of 59 mares were pregnant). At EPC, Bikaner 23 cycles of 12 Jennies were covered by AI and the conception rate was 52.17%. The results obtained are satisfactory and comparable with other reports.

Next year sufficient semen doses will be preserved and artificial insemination in 100-150 animals in field and farm animals to study the conception rate using frozen semen.

***R. A. Legha and S.N.Tandon***

# Technology Assessed and Transferred so far


## Equine Influenza Vaccine (a low cost, safe and potent vaccine against Equine Influenza virus subtype A/Equi-2)

It is a low cost, safe and potent vaccine effective against equine influenza subtype A/Equi-2 virus. It has been developed from the indigenously isolated virus during an outbreak where more than 83,000 equines were affected throughout the country. The vaccine has been released by the ICAR on 3.11.97. It is a quantified vaccine having haemagglutinin content as 20$\mu$g HA protein per dose per ml. Two doses of vaccine per year after initial vaccination are sufficient to provide the protective cover to the animal. The vaccine is about four times cheaper as compared to other commercially available bivalent vaccines like Prevace and Fluvac on commercial basis. No risk is involved in adopting it, although a minor swelling/lesion at the point of vaccination only in few cases is seen.

Since this vaccine is suitable for all types of breeds of horses, ponies, donkeys and mules above the age of 3 months, it can be adopted as safe preventive measure.

Economically, it is a cheaper and immunologically a safe potent and important preparation, which can increase the total work efficiency of the equines by preventing the occurrence of the disease, thus helping the owners indirectly. There is no social hindrance in its adoption and sustenance as it contains inactivated/killed whole virus mixed with adjuvant only.

The vaccine is recommended for use in all categories of equines including race horses, sports horses, ponies, mules and donkeys as prophylactic measure where equine influenza is present. In addition, all equines requiring movement for racing, sports polo, fairs etc, needs to be vaccinated twice a year but the last vaccination to be done within three months of the movement. The vaccine needs to be produced through recognized drug/vaccine producing company following standard operative procedures.

## *Salmonella* Abortus equi vaccine

An improved concentrated vaccine against *Salmonella abortus equi* with reduced dose has been developed and technology has been transferred to Haryana Veterinary Vaccine Institute, Hisar. Towards further improvement in this vaccine, outer membrane proteins of *Salmonella abortus equi* were tested and found as effective vaccine candidates.

## Equine Herp-Kit (ELISA based diagnostic kit for equine herpes virus-1

It is a field oriented ELISA based diagnostic kit, developed for early detection of equine herpes virus-1 infection and to know the vaccination effect in animals. It helps in controlling the infection in the herd. The kit can be used for detection of antibodies of equine herpes virus-1 (EHV-1). The kit is safe as killed antigens are used, easy to use as no technical know-how or laboratory support is required to use the kit and result is available within 4 hours. The use of the kit may help in reducing the disease due to EHV-1, leading to enhanced productivity. As the kit can be used in all the equine

establishments, it can help in preventing disease due to EHV-1. As the kit can help equine owners in curbing infections due to EHV-1, it will boost the equine production which in turn will make horse breeding and rearing more economical.

The kit is recommended for monitoring the sero-prevalence of EHV-1, vaccine response and to create EHV-1 free herd.

## COFEB-kit (*Babesia equi* diagnostic kit)

The kit based on complement fixation test is intended for diagnosis of equine babesiosis, a tick-borne infection. The disease is widely prevalent in the country and has been kept under list B by the Office International des Epizooties (O.I.E.). Babesiosis causes high economic losses on account of mortality, abortion and loss of condition. The developed kit is cost effective and less time consuming. The kit is highly recommended for testing animals prior to international movement based on WTO agreement and also keeps a vigil on the status of disease within the country. The kit was released on 8th August, 2001 by the Hon'ble Director General, ICAR, New Delhi.

The kit will help in better equine health management and can be adopted in equine establishments of our country e.g. Stud farms, turf clubs, government livestock farms, military, paramilitary and police departments etc. Killed parasite has been used as antigen in the kit so there will be no social hindrance in its adoption and sustenance in use of the kit. Economically the kit is very cheap. This kit will help in better equine health management. So the chance of sustainability of the kit is high. No special technical know how is required to use the kit.

The kit recommended for monitoring of the *B. equi* infection status in equines. It will also help in effective implementation of quarantine practices.
It will save the foreign exchange and help in detection of apparently healthy but disease carrying animals.

## Control of Equine Infectious Anemia Package of Practices

The equine infectious anaemia akin to human AIDS is a retrovirus infection, which broke out during 1987 for the first time in the country. The Centre standardized test for its diagnosis and developed package of practices for field use in collaboration with Department of Animal Husbandry and Dairying, Ministry of Agriculture (Government of India) and helped the nation by controlling the infection. For last 3 years no case of EIA has been reported in the country.

## Enhancement of genetic potentiality of indigenous donkeys

The good quality donkey germ plasm produced by the Centre was supplied at the village level equine breeding societies for increasing the production potentials of the indigenous donkeys and to raise the socio-economic status of the poorest of the poor. The enhancement of their income from 3000-4000 to Rs. 9000-10,000/year per animal has been reported as a feedback.

## Cryopreservation and Artificial insemination in equines:

Technologies for cryopreservation of equine semen has been perfected and using the cryopreserved semen, the methods of artificial insemination has been practiced and perfected for breed improvement of equines, for technique is mainly used as a method for breed improvement as well as for controlling venereal disease in equids.

This technique is safe and more number of foals can be produced in a year by judicious use of the valuable semen of even a single good quality stallion. These techniques are being used for upgradation of donkeys/horses as well as for superior quality mule production along with quick and reliable information of early pregnancy confirmation.

## Assheep-carpet wool Fabric

A carpet wool Fabric named Assheep has been fabricated in collaboration with CSWRI, Avikanagar, Rajasthan, by mixing sheep wool and brown donkey hair. However, it needs commercial exploitation for boosting cottage industry in the country.

# Education and Training

## Education

Under memorandum of understanding signed with CCS Haryana Agricultural University, Hisar, one student completed his Ph.D. research under NRCE scientists and two students are presently working at NRCE for their Ph.D. degree:

### Ph.D. thesis completed

***Nitin Virmani:*** *"Pathobiology of indigenous Equine Herpes Virus-1 infection in pregnant BALB/c mice".*

**Summary of work:** The comparative pathology and tissue tropism of indigenous strains (H-90 and R-98) of EHV-1 was studied in pregnant BALB/c mice. For this, twenty, 8 - 10 weeks old pregnant BALB/c mice at 13-14 days of gestation were divided into three groups namely A, B and C and inoculated intranasally with H-90 (107.4 TCID50/ mice), R-98 strain (107.0 TCID50/ mice) and RK-13 cell lysate, respectively. Mice of groups A and B showed severe symptoms and lesion of respiratory system characterized by dyspnoea, ruffled fur and crouching in corners. However, the mice of group B in addition showed the lesions of vaginal discharge and abortion. Microscopically, lesions in lungs were characterized by presence of intranuclear inclusion bodies, infiltration of neutrophils and lymphocytes, ballooning, rounding and hyperpalsia of epithelial cells of bronchii in early stages followed by necrosis of parenchymal tissue. Lesions in the placenta varied in two strains markedly. H-90 strain could produce only congestion of sinusoids at 3 dpi in mice and patchy necrosis of chorionic plate in one mouse on 5 dpi. R-98 strain produced congestion of sinusoids, chorionic plate necrosis and necrosis in trophoblastic tissue.

For assessing the neurogenic affinity of the two strains (H-90 and R-98) of EHV-1, suckling mice at 2-3 days of age in 3 colonies namely A, B and C were inoculated intracerebrally with H-90, R-98 strain and RK-13 cell lysate, respectively. Mice of colony A and B were inoculated with respective virus at the dose rate of $10^{3.0}$ $TCID_{50}$/mice. Mice inoculated intranasally with H-90 strain failed to produce any lesions whereas R-98 strain produced severe clinical signs and lesions *viz,*. diffuse proliferation of glial cells, degeneration of neurons along with areas of liquefactive necrosis, hyperplasia of endothelium and presence of viral antigen as indicated by IIFT.

The pathodynamics and immune responses of strain R-98 in pregnant BALB/c mice was also evaluated. For this, forty, 8-10 weeks old pregnant female BALB/c mice at 13th14th day of gestation were inoculated intranasally with the R-98 strain at the dose rate of $10^{7.0}$ $TCID_{50}$/ mice where as ten mice were kept as control and inoculated with RK-13 cell lysate by the same route. Mice inoculated with R-98 strain showed severe loss in body weight gain, clinical signs of dyspnoea, ruffled fur, crouching in corners, vaginal discharge and abortions. Virus could be reisolated or its DNA demonstrated through PCR in nasal turbinates (12 hrs to 6 dpi), trachea (I dpi to 6dpi), lungs (1dpi to 2 weeks pi), blood (2 to 6 dpi), uterus (2 to 5 dpi), placenta (2 to 6 dpi), foetus (2 to 6 dpi), brain (3 to 4 dpi), spleen (2 to 4 dpi) and liver (3 to 4 dpi). Histopathological lesions, as observed, in lungs included congestion of blood vessels, presence of eosinophilic intranuclear inclusion bodies, polymorphonuclear cells infiltration, ballooning and rounding of bronchial epithelium and hyperplasia, diffuse mononuclear cells (proliferation of type II pneumocytes) proliferation, necrosis of endothelial cells lining the blood vessels and perivascular and peribronchial lymphocytic infiltration. Upon immunohistochemistry, antigen positive cells could be seen in lung parenchyma, endothelium of the blood vessels and bronchial epithelial lining. Glial cells in brain also exhibited antigen positivity as indicated by apple green nuclear fluorescence in cerebral hemispheres.

Spleen and cervical lymph nodes had no histopathological lesions, however, presence of a few antigen positive cells (large mononuclear cells) by

immunoperoxidase staining could be seen. Endothelium of placental blood vessels as well as trophoblastic tissue lining sinusoidal walls showed fluorescence indicating the presence of viral antigen as early as 3dpi. Trophoblastic tissue also showed similar presence of antigen. Severe involvement of blood vessels led to congestion of sinusoids followed by ischaemia and necrosis of trophoblastic tissue. The activation of immune responses (both humoral and cellular) was demonstrated 6 dpi onwards. Both cellular and humoral immune responses were sufficiently high as compared to the control animals till the end of the experiment i.e. 4 weeks post infection.

### *Ongoing Ph.D. Research titles at NRCE:*

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

***Praveen Malik:*** *"Characterization of Streptococci of Equine origin with Special Reference to M Protein"*

## Trainings

Two trainings on *"Biotechnological tools and their application in equine research"* were conducted for students of different Universities (GJU, Hisar and KU, Kurukshetra) under the ongoing research programmes of the Centre.

1. In a two-month's training (3rd June to 3rd August, 2002), M.Sc Student (1) from the department of Biotechnology, Kurukshetra University, Kurukshetra participated and trained in the biotechnological methods in equine research.

2. In another three month's training (6th January to 10th April, 2003), two M.Sc Students from the department of Biotechnology, Guru Jambheshwar University, Hisar, were trained in different molecular biology tools in equine research.

# Awards and Recognitions

1. Dr. S.K. Khurana was awarded *Certificate of Excellence* for attaining first position in 'Patra-lekhan pratiyogita' organized by 'Nagar Rajbhasha Karyanavayan Samity' under Ministry of Home, Government of India on 5th April 2002.
2. Dr. Sanjay Kumar, Scientist (Veterinary Medicine) awarded JSPS post-doctoral fellowship by Japanese Government w.e.f. 23rd November 2001 for two years.
3. Dr. S.K. Dwivedi was the guest of honour in the symposium on *The Role of Pack Animals in the Economic Development of Uttaranchal Hills* held at G.B. Pant University of Agriculture & Technology, Pantnagar (Uttaranchal) on 21st September 2002. He presented a keynote address on the topic 'Current trends in viral diseases of pack animals'.
4. Dr. S.K. Dwivedi was the Chief Guest at 4th National Indigenous Show organized by Indigenous Horse Society of India and Kathiawari Horse Society at Porbander (Gujarat) on 7th January 2003.

# Linkages and collaborations in India and Abroad including externally funded projects

The Centre has developed effective linkages with various organizations engaged in research and development of equines. These linkages are aimed at overall improvement in the health and production of equines, to and for meeting the stringent criteria for international movement of horses.

The centre has established active liaisons with state animal husbandry departments of different states throughout the country. This collaboration is actively pursued for monitoring and surveillance of major equine diseases. Using this linkage with 16 states of India, monitoring and surveillance of major equine diseases was done during the year and status of various diseases reported. The expertise services of the Centre are sought by various states from time to time for issues related to equines.

Realizing the importance of disease monitoring for entire country, the Department of Animal Husbandary & Dairying, Ministry of Agriculture, Government of India recognized this centre as ***National Referral Centre*** for equine diseases and has entrusted NRCE the responsibility of monitoring and sero surveillance of important equine infectious diseases, *viz.,* African Horse sickness, Equine Infectious anemia, Equine Viral Arteritis, Equine Babesiosis (*B. equi* and *B. caballi*), Equine Rhinopneumonitis (EHV-1 infection), Dourine, Glanders, Equine Influenza. The prevalence of other equine diseases like trypanosomosis, *Salmonella* Abortus equi, EHV-4, brucellosis and leptospirosis.

The Centre has efficient and effective linkages with various organizations involved in breeding and production of equines, like RVC of Indian Army, Riding and Race Clubs, Turf Clubs, Equine Breeders and Breeders' Associations. Based on the feedback from these organizations, the research priorities of the centre are fine tuned and need based new projects are initiated. These linkages are also utilized for the validation of research findings and for conducting collaborative researches.

The Centre has a memorandum of understanding (MOU) for mutual research cooperation with two universities of Haryana, viz., CCS Haryana Agricultural University and Guru Jambheshwar University, Hisar. Under this MOU, three students worked on equine problems for their PhD programme during the year and one thesis was submitted. Trainings were also conducted on biotechnological techniques used in equines for mutual benefits.

The Centre has following projects funded by National Agricultural Technology Programme (NATP) of Indian Council of Agricultural Research, New Delhi:

1. *Development of diagnostic reagents and technologies for EHV infection* under NATP mission mode project on Veterinary Diagnostics for Prevalent and Emerging Disease.
2. *Sero-surveillance and monitoring of animal diseases* under MM project on Animal disease monitoring and surveillance

# Publications

## Research Papers:

i) Dwivedi S.K. and S.Dey 2002. Fluoride concentrations in Thoroughbred Horses in India .*Veterinary and Human Toxicology* **44:**292-293.

ii) Dwivedi S.K. and S.Dey 2002. Medicinal herbs: A potential source of toxic metal exposure to man and animals in India. *Archives of Environmental Health* ***57*:** 229-231.

iii) Gupta, A. K., Kumar, S. and Pal, Y. 2002. Biochemical, haematological and thyroid profiles in Kathiawari horses. Asian Australasian J. Animal Sciences **15:** 1215-1221.

iv) Malik, P, Srivastava, S.K, Khurana, S.K and Yadav, M.P 2002. Isolation of bacterial and fungal pathogens from healthy equines. Indian Vet. J. **79:** 546-550.

v) Malik, Praveen; Srivastava, S.K.; Khurana, S.K. and yadav, M.P. 2002. Isolation of bacterial and fungal pathogens from healthy and diseased equines. Indian Vet. J., **79**: 546-550.

vi) Nandal, A.; Malik, Praveen; Khurana, S.K. and Srivastava, S.K. 2002. Sero-prevalence of leptospirosis in equines in India. Indian J. Comp. Microbiol. Immunol. Infect. Dis. (Accepted).

vii) Pal, Yash., Kumar, S. and Gupta , A. K. 2002. Blood gases, acid-base and physiological indices in donkeys as pack animal. Animal draught News (UK), **37**: 27-33.

viii) Pal Y. and A. K. Gupta. 2002. Effect of Transient Feed Withdrawal Stress on Physiological Indices and Acid Base Balance in Equid. Annals of Arid Zone (Accepted).

ix) Singh, B.K. 2002. Immunological response of ponies to an inactivated Indian strain of equine herpes virus-1. *Indian Journal of Animal Sciences.**72:** 831-835.*

x) Singh, B.K., Gulati, B.R., Tewari, S.C. and Yadav, M.P. 2002. Antigenic differentiation of equine herpes virus- 1 (EHV-1) isolates of Indian origin using Monoclonal antibodies. *Indian Journal of Biotechnology.1, 170-174.*

xi) Singh,B.K., Gulati, B.R. and Poonia, B. 2002. Differentiation of Indian isolates of equine herpes virus (EHV-1) by restriction endonuclease digestion. *Indian Journal of Biotechnology*.**1**: 397-400.

xii) Srivastava, S.K.; Rattan, B.; Malik, Praveen and Yadav, M.P. 2002. Immune response in ponies following vaccinations with *Salmonella* Abortus equi and Equine Herpes Virus-1 vaccines. Indian J. Comp. Microbiol. Immunol. Infect. Dis. **23:** 33-35.

xiii) Tomer, P., Chaturvedi, G. C., Minakshi, Malik, Praveen and Monga, D. P. 2002. Comparative analysis of outer membrane protein profiles of isolates of the *Pasteurella multocida* (B:2) associated with haemorrhagic septicaemia. Veterinary Research Communications. **26:** 513-522.

xiv) Virmani, N**.,** Sengupta, P.P. and Panisup, A.S. 2003. Pathomorphological studies on *Trypanosoma evansi* infection in rats isolated from heterologous host. *Indian J. Anim. Res.* (In press).

## Consultancy, patents, technology:

Invention entitled " A method for preparation of a diagnostic kit useful for forecasting equine herpes virus 1 disease" Patented through Patent Office, New Delhi, in June 2003.

## Popular articles/ Leaflets/ bulletins/ training/ manuals:

1. Khurana, S.K. 2002. Epizootic lymphangitis: A chronic mycotic disease. In: NRCE News. Vol 9. No. 2 ( July to December).
2. Khurana,S.K and Srivastava, S.K 2002. Asvon ke shvasan tantra se sambandhit sankramak rog tatha unki roktham.( NRCE Publication ).
3. Legha,R.A. and Tandon, S.N. 2002. Navjat Asav Shishu (Shavak) Ki Dekhbhal, Upyogi Jankari. Dainik

Krishi Prabhat,Bikaner September,25:p-1.

4. Pal Y, Gupta A.K. and Kumar S. 2002. Ashav Madaon mein Kritrim Grabhadhan. Krishi Chayanika 23:4 (October December) pages 15-16 &18.

5. Singh, B.K. 2002. Abortion in mares: A brief description (Ghorion mein garbhapat: Ek sansipt varnan (In Hindi lipi). *Kheti.* (Accepted) published from ICAR in Hindi magazine.

6. Singh, B.K. and Dwivedi, S.K. 2002. Abortion in equines. Livestock international.**6:** 14-18.

7. Singh, B.K.2002. Abortion in Equines (A technical bulletin in Hindi, NRCE, Hisar)

1 यशपाल एवं ममता (2002) खच्चर पालन एवं उत्पादन ऐसे करें।

2 यशपाल एंव शैलेन्द्र कुमार द्विवेदी (2002) अश्व पशुओं में गर्भाधान।

3 श्रीकुमारन पी, यशपाल एवं संजय कुमार (2002) घोड़ों के स्वास्थ्य पर फाइलेरिया कीटाणुओ का प्रभाव।

4 श्रीकुमारन पी, यशपाल एवं संजय कुमार (2002) घोड़ों के स्वास्थ्य पर बोट्स कीटाणुओ का प्रभाव।

5 श्रीकुमारन पी, यशपाल एवं संजय कुमार (2002) घोड़ों में परजीवी कृमियों द्वारा उत्पन्न बीमारियों की रोकथाम।

6 श्रीकुमारन पी, यशपाल एवं संजय कुमार (2002) घोड़ों के पेट के कीड़े।

7 संजय कुमार एवं शैलेंद्र कुमार, अश्वों में पेट दर्द का रोग।

8 संदीप खुराना, धनुस्तम्भ – एक घातक रोग।

9 संदीप खुराना, गेस्ट्रोफिलस रोग।

10 अतर सिंह पाणिसुप एवं नितिन विरमानी, अश्वों में संचारी रोगों की रोकथाम के उपाय।

11 सहदेब डे, अपने अश्वों को परजीवी रहित रखिये।

12 यशपाल एवं ममता, खच्चर पालन एवं उत्पादन कैसे करें।

13 यशपाल एवं ममता, नवजात अश्व बच्चों की देखभाल कैसे करें।

14 यशपाल, अशोक गुप्ता एवं संजय कुमार, अश्व मादाओं में कृत्रिम गर्भाधान।

# AICRP/Coordination Unit/ National Centre

Presently, the Centre is not having any AICRP, National Centre or Coordination Unit. However, a Regional Station, namely Equine Production Campus (EPC) for undertaking researches in equine production is existing at Bikaner in Rajasthan. The research and related activities of EPC, Bikaner has been included alongwith the main campus, Hisar under various chapters of this report.

**"An equine exhibition organised at Bikaner campus on 17-02-2003**

**"Farmer getting treatment for sick equines at a Health Camp organised by NRCE"**

# List of approved ongoing research projects

| Scheme Code | Title of the Scheme | Team | Date of Start | Date of Completion |
|---|---|---|---|---|
| 2000443002 | Development of improved vaccine against equine diseases. | B.K.Singh, S.K. Khurana, P.Malik. and N.Virmani | 1.11.1998 | Continuous |
| 2000443002.1 | Development of Vaccine against Salmonella Abortus Equi infection | Praveen Malik and S.K.Khurana | 1-11-1998 | 31.03.2003 |
| 2000443002.2 | Development of vaccine(s) against equine herpes virus-I infection. | B.K.Singh and N.Virmani | 1-11-1998 | 31.03.2003 |
| 2000448001 | 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, P.Malik, Yashpal Nitin Virmani and Rajender Kumar. | 1.4.1995 | Continuous |
| 2000446002 | Chemotherapeutic and diagnostic studies on trypanosomiasis and Babesiosis in equines. | S.Dey, S.K. Dwivedi, O.P.Sharma (Proposed) and A.S.Panisup. | 11.1.2000 | Continuous |
| 2000446002.1 | Isolation and characterization of secondary plant metabolites for the development of an antitrypanosomal drug. | S.Dey, S.K.Dwivedi, A.S.Panisup and O.P.Sharma (Proposed). | 11-1-2000 | 30-11-2003 |
| 2000414001 | Molecular characterization for studying genetic diversity among Marwari breed of horses. | S.N.Tondon, A.K.Gupta and R.A.Legha. | Oct., 2001 | Sept. 2004 |
| 2000414002 | Standardisation of procedure and techniques for cryopreservation of Jack's semen. | R.A.Legha and S.N.Tondon | April, 1997 | Sept.,2001 (extended for 2 years) |
| 2000414003 | Cryopreservation of stallion semen and perfection of AI in Marwari horses. | Yash Pal, R.A.Legha and S.N.Tondon. | May, 2002 | June, 2005 |
| 2000414004 | Development of equine chorionic gonadotropin (ecg) based ELISA based test for pregnancy diagnosis in equines. | A.K.Gupta, Yashpal and S.K.Dwivedi. | May, 2002 | June, 2004 |

# Consultancy, Patents, Commercialization of Technology

## Consultancy

One of the mandates of the Centre is to act as national referral facility for disease diagnosis and to provide diagnostic, advisory and consultancy services. In this direction, various activities were undertaken during the year 2002-2003:

**1. Diagnosis and treatment of equine diseases:** During the year 393 clinical cases from different parts of Haryana and Rajasthan were provided clinical diagnostic and

Fig. 1 An equine showing symptoms of mineral deficiency

therapeutic facilities. At field level colic, lameness, repeat breeding, retention of urine, mineral deficiency (Fig 1), debility and digestive disorders were the predominant health problems observed during the year. Incidence of clinical cases of colitis (idiopathic), tetanus and strangles were successfully diagnosed and treated.

**2. Pregnancy diagnosis and artificial insemination:** Pregnancy diagnostic service using ultrasonography was provided free of cost to the farmers. One hundred and twenty three mares were examined for pregnancy diagnosis and other pathological conditions in reproductive organs and provided appropriate therapy and many of them conceived after receiving therapy (Fig 2). Besides pregnancy diagnosis, best germplasm of stallion and jack were made available for farmers. Adoptions of ultrasonography (detection of ovulation time, embryonic development) coupled with artificial insemination have significantly improved reproductive performance in mares.

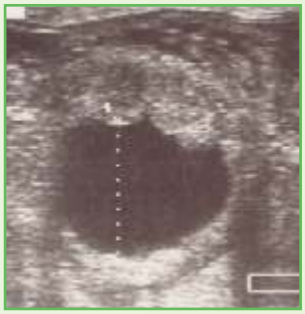


Fig. 2 Pregancy diagnosis by ultrasonography at 21-days of conception

**3. Deworming:** Endo- and ecto-parasites remain a major cause of equine morbidity and mortality in India. A deworming schedule (incorporating Doramectin, Pyrantel pamomate-Fenbendazole, Ivermectin) was provided to the equines of adopted villages (304), equine presented to NRCE (393) and equines at clinical camps (257). Literature on how to control parasitic diseases of equine was also distributed among farmers.

**4. Vaccination:** Following incidence of a clinical case of tetanus in the adopted village, all the equines were vaccinated using tetanus toxoid. A clinical camp was organized where 96 farmers along with their equines participated. In that camp farmers were educated with literature in Hindi and lecture on beneficial effects of vaccination in controlling the disease.

**5. Balanced ration for equines:** Nutritional imbalance is widely prevalent problem of Indian equines that limits its performance and reproduction. Majority of equines are

deficient in some kind of nutrient and it is largely due to ignorance at farmer level. The farmers were provided literature and knowledge about quantity of the feed ingredients required for a balanced ration. Emphasis was on that the balance ration could be prepared from the local farm produce of the area. The feedback from the farmers indicates the improvement in the health, reproduction and performance of their animals due to adoption of balanced feed recommended by the scientists of NRCE.

**6. Post-mortem examination:** Disease investigations were carried out on the necropsies conducted on equines and on morbid material received from the field. In all twelve carcasses of equines were examined for gross and histopathological changes to ascertain the cause of death. Causes of death included Toxaemia (2), Alkaloid intoxication (2), Bronchopneuminia (1), Gastroenteritis (1), Brain haemorrhages and injuries to internal organs (1), still birth/ abortions due to non-infectious causes (3). All the three cases of abortions investigated were negative for EHV-1 as confirmed by immunohistochemical diagnosis as well as PCR. In two cases, severe hyperplasia of bile duct (Fig. 3) and associated changes were strong indicative of accidental grazing on wild plants such as those belonging to species Heliotropeum/ Crotolaria/ Senecio/ Amscinkia/ Trichoderma etc.

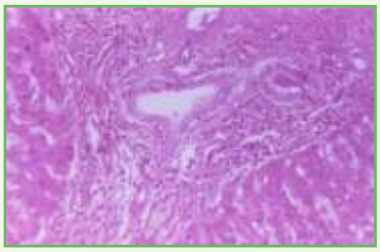


Fig. 3 Bile duct hyperplasia observed in an equine

7. **Bacterial isolations:** For bacteriological examination, 155 vaginal/prepucial swabs received for testing of CEM gave negative results. Other bacteriological studies conducted on 96 equine samples, including nasal swabs, eye discharge, uterine swabs, blood, faecal samples, tissues from carcasses, swabs from other lesions, vaginal swabs, pus samples and aborted foetus yielded 28 isolates belonging to genera rhodococcus, streptococci, staphylococci and *E. coli*. Five *Acholeplasma laidlawii* and one *A.oculi* were isolated from 23 vaginal swabs. One *A. oculi* was obtained from two eye swabs.

| **Bacterial isolates from clinical cases of equines (number of isolates)** |
|---|
| *Rhodococcus equi (1)* |
| *Streptococcus equi subsp. equi (3)* |
| *Streptococcus equi subsp. zooepidemicus (1)* |
| B group Streptococci (2) |
| Streptococci (non-group 'C') (1) |
| beta-hemolytic Streptococci (6) |
| Staphylococci (2) |
| *Escherichia coli (6)* |
| *alfa*-hemolytic Streptococci (3) |
| Diplococci (non-pathogenic) (1) |
| *Acholeplasma laidlawii (5)* |
| *Acholeplasma oculi (2)* |
| Unidentified Gram-negative bacilli (2) |

## Patents

The Centre has filed two patent applications in the area of disease diagnosis:

i) A patent application entitled *A method for preparing an immuno-stick ELISA for detecting antibodies to equine herpes virus-1* as notified in the Gazette of India, New Delhi, No. 46, P1517 dated 15th November 1997.

ii) Another patent for *Complement fixation test based diagnostic (COFEB-Kit) for detection of Babesia equi antibodies* was filed in January 2001.

## Commercialization of Technology

The Centre is providing diagnostic services to the equine industry on payment basis. Through these services, the centre generated revenue to the tune of Rs. 14.23 lacs during the year. In addition, the improved germplasm of equines was provided to the farmers in different parts of the country and under this head, the centre generated an income of Rs. 5.92 lacs during the year.

# RAC, Management Committee and SRC meetings with significant decisions

**Annual SRC Meeting:** SRC meeting of the centre was held on 7th May 2002 under the chairmanship of Dr. S.K. Dwivedi. Principal investigators of different research projects presented the progress made during the year and plan of work for the following year. Two new projects entitled *Development of equine chorionic gonadotropin (ecg) based ELISA based test for pregnancy diagnosis in equines* and *Cryopreservation of stallion semen and perfection of AI in Marwari horses* were discussed and approved by the SRC. The Chairman emphasised on the need of technology-oriented projects to be taken up. He also stressed on the regular monitoring of project milestones

**Research Advisory Committee Meeting:** 4th RAC meeting was held on 14th May 2003 under the chairmanship of Dr. V.Gnanaprakasam, Ex-Vice Chancellor, TNUVAS, Chennai. The Committee approved the proposal for development of animal experimentation facility in view of the suggestions of CPCSEA. The following proposals were also approved: upgradation of NRCE to the status of National Institute; provision of extra funds for the development of NRCE as national referral centre for equine diseases; and new projects as recommended by the SRC.

**Management Committee Meeting:** 22nd Meeting of the Institute Management Committee was held on 8th October, 2002. Important decisions regarding the purchase of equipments for the current financial years, construction works: both at main campus and sub campus at Bikaner were made in the meeting.

**IAEC Meeting:** Meeting of the Institute Animal Ethics Committee was held on 16th October 2002. The Committee under the chairmanship of Dr. A.S. Panisup approved the use of small animals in five different research projects. Two projects involving the use of large animals were considered and referred to the CPCSEA for approval.

### Members of Research Advisory Committee

| | |
|---|---|
| Dr. V.Gnanaprakasam, Ex-Vice Chancellor, TNUVAS, Chennai, | Chairman |
| Dr. S. K. Dwivedi, Director, National Research Centre on Equines, 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-Additional Director of Research, CCS HAU, 514, Sector 15-A Hisar | Member |
| Dr. O.P.Dhanda, Prof. Animal Produciton Physiology, CCS HAU, Hisar | Member |
| Dr.Lal Krishna ADG(AH), ICAR, Krishi Bhawan, New Delhi | Member |
| Sh.Paramvir Singh, Paramvir Stud & Dairy Farm, Tohana, Fatehabad(Haryana) | Member |
| Sh.Amarjit SinghJatana, Vasant Kunj, New Delhi | Member |
| Dr. A.S.Panisup, Principal Scientist &I/C PME Cell, NRCE, Hisar | Member Secretary |

## Members of SRC (2002-03)

| | |
|---|---|
| Dr.S.K.Dwivedi, Director NRCE., Hisar. | Chairman |
| Dr.Lal Krishna, ADG(AH), ICAR, New Delhi. | Member |
| All scientific staff of NRC on Equines | Members |
| Dr.A.S.Panisup, Pr.Scientist | 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. Rajendra Singh, AAO, NRCE, Hisar | Member Secretary |

# Participation of scientists in conferences, meeitngs, workshops, symposia, etc.

1. Bansal R.S, Pareek P.K. and Pal Y. 2002. Ultrasonographic evaluation of mares in reproductive status. In: National Seminar on Standardization of Breed Characteristics of Marwari Horse organized by Marwari Horse Society, Jodhpur, held at Jodhpur October 20-22.
2. Dey S., Dwivedi S.K. and Panisup A.S. 2003. Evaluation of *L. inermis* leaf extract for its antitrypanosomal activity. Proc. *Symp.*Focusing on need to develop new diagnostic, therapeutic and preventive approaches to deal with diseases of farm and companion animals, Anand. February 7-9, .
3. Dey, S and Dwivedi, S.K. 2002. Trace Mineral Concentration in Infertile Jennies. *In:* International conference of Asian Australian Association of Animal Physiology New Delhi. Sept 23-27.
4. Dwivedi S.K. and Dey S. 2002. Fluoride concentration in Indian Thoroughbred Race Horses *In:* International conference of Asian Australian Association of Animal Physiology New Delhi. Sept 23-27.
5. Gupta, A. K., Singh, R., Mamta, Singh, M. K. and Pal, Yash. 2002. Physical and biochemical evaluation of Jack's semen. In: X International Congress of Asian Austral Asian Association of Animal Production held in New Delhi, September 23 to 29.
6. Gupta, A. K. 2002. Molecular characterization of Indigenous breeds of horses - A need. In: National Seminar on Standardization of Breed Characteristics of Marwari Horse organized by Marwari Horse Society, Jodhpur, held at Jodhpur October 20-22, pp 31-40.
7. Gupta, A. K., Pal Yash, Tandon, S. N., Dwivedi, S. K. 2003. Haematological and biochemical profiles in healthy Indian Spiti horses. In: 13th Animal Physiology Conference and National Symposia on Constraints and Strategies for Augumneting Lactation in Dairy Animals, held at Karnal February 6-7.
8. Khurana S.K. 2003. International conference on alternatives to the use of animals in research and education organized by Department of Biotechnology and Ministry of Environment at New Delhi from February 18-20.
9. Kumar, S., Gupta, A. K., Pal, Y. and Dwivedi, S. K. 2002. *In vitro* therapeutic efficacy of different drugs against experimentally produced *Babesia equi* infection in donkeys. In: 4th International Conference on Ticks and Tick-borne pathogens held at The Banff Centre, Banff, Alberta, Canada. July 21 26.
10. Legha, R.A. and Tandon. S.N. 2002. Studies on Cryo-preservation of Jack semen and its use for artificial insemination in equines. In: IX International congress on biotechnology in animal reproduction, Madras Veterinary college, Chennai. December 2-4.
11. Pal, Yash, Gupta, A.K. and Kumar, S. 2001. Draughtability studies in donkey as pack animal. In: X International Congress of Asian Austral Asian Association of Animal Production held in New Delhi, September 23-27.
12. Singh, B.K. and Ahuja S. 2002. Monoclonal antibody based blocking ELISA for detection of equine herpes virus-1 antibodies. In: 72nd Annual Session of National Academy of Sciences, India, NEHU, Shillong October 25-27.
13. Srivastava, S.K, Malik, P, Khurana, S.K, Nandal, A, Yadav, M.P and Panisup, A.S. 2002. Prevalence of

important bacterial diseases of equines in India. In: Xth International Cong. of Asian-Australian Association of Anim. Prod. Societies, Delhi. September 23-27.

14. Tandon, S.N. and Legha. R.A. 2002. Breed characteristics of indigenous horses, their population trend and conservation. In: National Seminar on Standardization of Breed Characteristics of Marwari Horse organized by Marwari Horse Society, Jodhpur, held at Jodhpur October 20-22.

15. Virmani, N., Panisup, A.S., Gupta, A.K., Sengupta, P.P. 2002. Clinicopathological, ultramicroscopic and biochemical investigations on canker (Chronica Progressiva Verrucosa) in horses. In: XIXth Annual Conference of Indian Association of Veterinary Pathologists, held at CSKHPKVV, Palampur (H.P.), September 26-28.

16. Virmani, Nitin, Panisup, A.S. and Malik, Praveen. 2002. Exfoliative cytological investigation of the cases of infertility in mares. Xth International Congress of Asian-Australasian Association of Animal Production Societies on Animal Production for food and environment security, New Delhi, September 23-27.

# Workshops, seminars, summer institutes, farmers' day, etc.

During the year, a number of equine health camps, kisan-goshthis and exhibitions were organized in various villages of Haryana and Rajasthan to provide health services at farmers' door and create awareness amongst farmers about the ongoing developments in the area of equine health and production.

## Clinical Health Camps:

A ***Clinical Camp*** was organized on 23rd August 2002 at Poli Village (District Jind, Haryana) where a total of 102 equines including 3 stallion, 27 mare, 40 mules, 14 foal, 18 donkeys were treated for different ailments It was found that 41.18 % (42 out of 102 cases) equines suffers from lameness of which 16.66% (7 out of 42) of animals have clinical osteoskeletal abnormalities. Reproductive problems were recorded in 13.79% (4 out of 29) mares. General debility was present in 59.80 % (61 out of 102) equines and is particularly predominant among mules besides these bursitis and colic were also recorded in a few

cases. The farmers showed great interest in artificial insemination using semen of exotic French Donkey for mule production. Pregnancy diagnosis was performed in 6 mares and artificial insemination was performed in two mares (for mule production) and one Jenny. All the equines of the area were dewormed using Parental pamonate. Samples of blood, Vaginal swabs from equines and abiotic samples of feed forage and water from the area were collected for laboratory analysis, which could help in taking appropriate preventive measures to save equines of this locality.

**A *Clinical Health-cum-Deworming Camp*** was organized at village Rajli (Haryana) on 17th December 2002. Deworming of 78 equines of the village was done using ivermectin. Farmers were educated about the common health problems and their management, use of artificial insemination in equines and literature about management of equines was distributed.

A thematic equine health camp on ***Balanced Feed for Healthy Horse***s was organized at Barwala (Hisar, Haryana) on 19th December 2002. Fifty-two farmers along with their animals participated the camp. The Scientists demonstrated the preparation of balanced feed for equines from their available farm produce and apprised the importance of

various ingredients for equine health and performance. The feed mixture containing Crusted Oat 45%, Wheat bran 20%, Gram 30%, Mineral mixture 5% that can meet major nutritional requirements of an equine was recommended to the farmers. Leaflets and literature on feeding and management of equines were distributed to the farmers. Besides these clinical cases like lameness (11), infertility (8), hoof deformity (7) and general debility (12) were treated and 4 mares were examined for pregnancy diagnosis in this camp.

**An *Equine Health cum Reproductive Camp*** at Peer Kamadia (Hanumangarh, Rajasthan) was organized on 28th December 2002, where about 40 sick equids were treated for different ailments and pregnancy diagnosis was done for female animals brought at the camp. The veterinarians at the camp were given training on equine pregnancy diagnosis and passing of stomach tube, etc.

**A *Vaccination-cum-Health Camp*** was organized at Rajli (Haryana) on 7th February 2003 for vaccination against tetanus to all the animals of the adopted village. This was done following a reported clinical case of tetanus in the village. In this camp 98 animals were provided tetanus toxoid and farmers were informed about fatality and control measures for the disease.

## Exhibitions/Goshthis:

The Centre arranged a NRCE Exhibition on the occasion of National Seminar on Marwari Horses at Umaid Bhawan, Jodhpur during October 20 and 22, 2002 in which different activities and achievements of the centre were highlighted. The stall attracted equine breeders, researchers, and equine lovers. The presentation was highly appreciated by all the dignitaries including Dr Panjab Singh then DG, ICAR, Hon'ble minister of state for Industry and Commerce GOI, Sh. Rajiv Pratap Rudy, His Highness Maharaja Gaj Singh Ji of Jodhpur as well as by the foreign dignitaries including Ms Kelly, USA and Ms Heidi of Germany.

The Centre participated in an Exhibition organized by Ministry of Agriculture Govt. of India at IARI, Delhi between December 21 and 23, 2002. NRCE Exhibition Stall depicted the services provided by the centre to the farmers. Farmers from various states visited the stall and enriched their knowledge on equine Husbandry. High dignitaries including Hon'ble minister of state Sh.Hukum Narayan Singh Yadav visited the stall and appreciated the work of this Centre. Delhi Doordarsan gave coverage of NRCE stall.

A state level ***"Ashwa Pradarshini" cum "Kisan Gosthi"*** at Equine Production Centre, Bikaner was organized on 17th February 2003 in which 46 horses from various parts of the state participated. On this occasion, the *Kisan Gosthi* was also held in which about 40 farmers attended and exchanged their views and experiences with the scientists. The faculty and students from college of veterinary sciences, Bikaner participated in the event and the N.C.C. Wing from veterinary college performed horse show including jumps and tag pegging.

**Feed back from Kishan Gosthis:** During the year 2002-2003 seven Kishan gosthis were organized in different regions to know the problems of the farmers and the feed back of our extension services of the centre. The following feedback from the farmers were received in these goshthis:

* Farmers are concerned about insurance of their horses and sought help from NRCE in this issue;
* Artificial insemination and Pregnancy diagnosis facilities should be made available to Equine farmers;
* Celebration of farmers day on regular basis where progressive farmers sould be acquainted with the standard practices of feeding and management;
* Clinical Camps should be organized more frequently.

# Distinguished Visitors

| Date of visit | Visitor |
|---|---|
| 18-07-02 | **Mr. Chris Baldock**<br>Director Animal Health Servies, Austvet, Australia |
| 28-08-02 | **Dr.O.S.Tomer**<br>Ex Director, NDRI, Karnal<br>**Dr. V.D. Mudgil**<br>Ex Director CIRB, Hisar<br>**Dr. T.K.Walli**<br>Head, Division of Nutrition, NDRI, Karnal |
| 15-09-02 | **Ch. Ajit Singh**<br>Former Union Agriculture Minister, Govt. Of India |
| 06-11-02 | **Smt. Binoo Sen**<br>Secretary, Department of Animal Husbandry and Dairying, Ministry Of Agriculture, New Delhi |
| 23-12-03 | **Dr.V.K.Taneja**<br>Deputy Director General (Animal Sciences), ICAR, New Delhi |

Smt. Binoo Sen "It was very gratifying to learn how much scientific work is being done in the country. Very impressive! Please keep it up!"

Chris Baldock, Epidemiologist, Australia: 18-07-02: "I surely enjoyed seeing the wonderful achievements on NRCE in which the scientists take the pride"

Former Minister of Agriculture Sh. Ajit Singh: 05-09-2002: "Unique centre for equine development and disease control. I wish them well"

# Personnel

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

| *Scientific* | | |
|---|---|---|
| 1. | Dr. S.K. Dwivedi, MVSc, Ph.D. | Director |
| 2. | Dr. S.N. Tandon, M.V.Sc., Ph.D. | Principal Scientist |
| 3. | Dr. A.K. Gupta, M.Sc., Ph.D. | Principal Scientist |
| 4. | Dr. A.S. Panisup, M.V.Sc., Ph.D. | Principal Scientist |
| 5. | Dr. B.K. Singh, M.V.Sc., Ph.D. | Principal Scientist |
| 6. | Dr. S. Dey, M.V.Sc., Ph.D. | Senior Scientist |
| 7. | Dr. R.C. Sharma, M.V.Sc., Ph.D. (Joined 22.03.2003) | Senior Scientist |
| 8. | Dr. B. R. Gulati, M.V.Sc., Ph.D. (Joined 31.03.2003) | Senior Scientist |
| 9. | Dr. S.K. Khurana, M.V.Sc., Ph.D. | Senior Scientist |
| 10. | Dr. Yash Pal, M.Sc., Ph.D. | Scientist |
| 11. | Dr. P.P. Sengupta, M.V.Sc., Ph.D. (Up to 15.05.2002) | Scientist |
| 12. | Dr. Rajender Kumar, MVSc, Ph.D. (Joined 17.02.2003) | Scientist |
| 13. | Dr. Nitin Virmani, M.V.Sc., Ph.D. | Scientist |
| 14. | Dr. Praveen Malik, M.V.Sc. | Scientist |
| 15. | Dr. Sanjay Kumar, M.V.Sc., Ph.D. | Scientist |
| 16. | Dr. Deepinder Kaur, M.Sc., Ph.D. | Scientist |
| 17. | Ms. Mamta, M.Sc. | Scientist |
| 18. | Dr. R.A. Legha, M.Sc., Ph.D. | Scientist |
| 19. | Mr. Pramod Singh, M.Sc. | Scientist |

| *Administrative* | | |
|---|---|---|
| 1. | Sh. R.A. Parashar | AFAO |
| 2. | Sh. Rajinder Singh | AAO |
| 3. | (Up to 11.02.2003) | |
| 4. | Sh. Hawa Singh | Assistant |
| 5. | Sh. Ram Pal | Assistant |
| 6. | Sh. S.P. Kaushik | Assistant |
| 7. | Sh. Ashok Arora | Jr. Stenographer |
| 8. | Sh. Subhash Chander | Sr. Clerk |
| 9. | Sh. Pratap Singh | Jr. Clerk |
| 10. | Sh. D.D. Sharma | Jr. Clerk |
| 11. | Sh. Om Prakash | Jr. Clerk |
| 12. | Sh. Mohinder Singh | Jr. Clerk |

| *Supporting* | | |
|---|---|---|
| 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. Sajjan Singh | SSGr. II |
| 7. | Sh. Mardan | SSGr. II |
| 8. | Sh. Balwan Sigh | SSGr. II |
| 9. | Sh. Desh Raj | SSGr. II |
| 10. | Sh. Raghubir Singh | SSGr. II |
| 11. | Sh. Ishwar Chander | SSGr. II |
| 12. | Sh. Om Prakash | SSGr. II |
| 13. | Sh. Deepak Kumar | SSGr. II |
| 14. | Sh. Gopal Nath | SSGr. II |
| 15. | Sh. Satbir Singh | SSGr. I |
| 16. | Sh. Hanuman Singh | SSGr. I |
| 17. | Sh. Subhash Chander | SSGr. I |
| 18. | Sh. Ishwar Singh | SSGr. I |
| 19. | Sh. Ram Singh | SSGr. I |
| 20. | Sh. Dalsher | SSGr. I |
| 21. | Sh. Raju Ram | SSGr. I |
| 22. | Sh. Mahabir Prasad | SSGr. I |

| *Technical* | | |
|---|---|---|
| 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-3 | Veterinary Officer |
| 6. | Sh. Ajmer Singh, T-3 | Stock Assistant |
| 7. | Sh. Brij Lal, T-3 | Stock Assistant |
| 8. | Sh. P.P. Chaudhary, T-3 | Lab. Technician |
| 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. | Smt. Indu Jyoti, T-3 | Hindi Translator |
| 14. | Sh. Mukesh Chand, T-2 | Lab. Assistant |
| 15. | Sh. Sajjan Kumar, T-2 | Staff Car Driver |
| 16. | Sh. Arun Chand, T-2 | Tractor Driver |
| 17. | Sh. Khiraj Singh, T-2 | Jeep driver |
| 18. | Sh. Suresh Kumar, T-2 | Vehicle driver |
| 19. | Sh. Joginder Singh, T-2 | |
| 20. | Sh. Shankar Lal, T-2 | Jeep-cum-tractor driver |
| 21. | Sh. Rajendra Singh, T-1 | Lab. Technician |
| 22. | Sh. S.N. Paswan, T-1 | Livestock assitant |
| 23. | Sh. Om Prakash, T-1 | Tractor driver |

## Personnel Milestones:

**Awards:**

Dr. S.K. Khurana was awarded *Certificate of Excellence* for attaining first position in 'Patra-lekhan pratiyogita' organized by 'Nagar Rajbhasha Karyanavayan Samity' under Ministry of Home, Government of India on 5th April 2002.

**Promotions:**

Dr. S.K. Khurana, Scientist has been promoted to Senior Scientist with effect from 21st March 2002.

**Appointments:**

1. Dr. R.C. Sharma as Senior Scientist (Animal Breeding) at Equine Production Campus, Bikaner with effect from 22nd March 2003.
2. Dr. Baldev R. Gulati as Senior Scientist (Microbiology) with effect from 31st March 2003.
3. Smt. Indu Jyoti T-3 as Hindi Translator with effect from 31st January 2003.

**Joined:**

Sh. Joginder Singh, T-2 (Laboratory Technician) joined on November 1, 2002 on transfer from National Dairy Research Institute, Karnal.

**Transfers:**

Sh. Rajinder Singh, AAO to Indian Institute of Pulses Research, Kanpur.

Dr. P.P. Sengupta, Scientist to Project Directorate, Animal Disease Monitoring & Surveillance, Hebbal, Bangalore.

**Staff on Study Leave:**

1. Ms. Mamta, Scientist w.e.f. 23rd March 2000 to 22nd March 2003.
2. Dr. R.S. Bansal, T-9 (Farm Manager) w.e.f. 3rd April 2000 to 2nd April 2003.
3. Dr. Pramod Singh, Scientist, w.e.f. 1st August 2000 to 31st July 2003.

**Visits abroad:**

Dr. Sanjay Kumar, Scientist (Veterinary Medicine) on JSPS post-doctoral fellowship by Japanese Government w.e.f. 23rd November 2001 for two years.

# Infra-structural development and other activities

## Development of NRCE website (http://nrce.nic.in):

To provide the comprehensive and up-to-date information about the Centre and its research activities globally, NRCE website was launched on NIC government server. The website contents include: the background and mandate of NRCE, organizational set up, programmes and activities undertaken and research achievements of NRCE in detail. Scientist's

profile along with research interests and involvement in the ongoing research projects of the Centre is also posted. A brief account of equines in India, their habitat, distribution, breeding tract is also included for the benefit of visitors. Contact information about NRCE and scientists is also available on this website. The site can be visited at URL http://nrce.nic.in.

Sh. Ajit Singh, then Union Minister of Agriculture, Govt. of India formally inaugurated the website on 05.09.2002 during his brief visit to the Centre. He was accompanied by Dr. Panjab Singh, then DG, ICAR, Dr. V. K. Taneja, DDG (AS), ICAR, Dr. J. B. Chaudhary, Former V.C., CCS HAU, Hisar, Dr. R.P.S. Tyagi, Former Member, ASRB, Dr. A. P. Singh, Dean, Vety. College, CCS HAU, Hisar and other dignitaries.

## Experimental Animal Facility:

For undertaking experimentations in small and large animals, the centre constructed an Experimental Animal Facility as per the guidelines and requirements of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This complex was inaugurated by the Deputy Director General (Animal Sciences), Dr. V.K. Taneja, on 23rd December 2002.


**Dr. V.K. Taneja, DDG (Animal Sciences) inaugurating Experimental Animal Facility at the Centre**

## Strengthening of ARIS Cell:

All the scientists and officials at main campus were provided with personal computers for proper maintenance of data as well as smooth flow of information. Local Area Networking of all the computers in the centre was done for

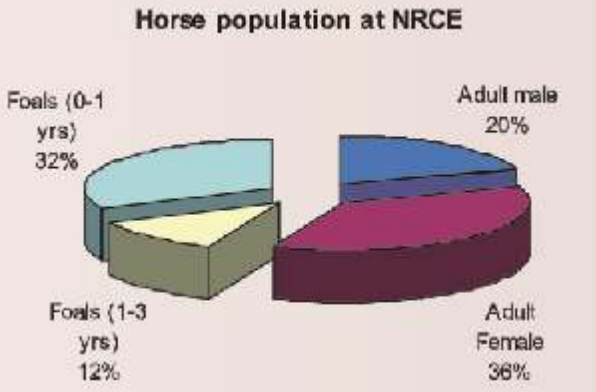
maximum utilization of resources through sharing and cooperation. Presently, there are 18 working client nodes in the local area network (LAN). Twenty four hour internet-connectivity is made available to all the officials through VSAT to make global information accessible for equine research and other related fields. For faster communication in regular scientific and technical matters, internal and external mailing system was developed and the domain ***nrcequine.org*** has been registered and individual mailing identities were provided to each scientist/section.

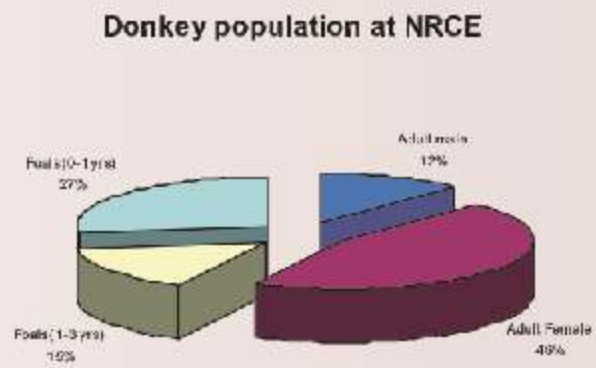

## Support Sections

### Livestock production:

The equine herd-strength comprising of two major indigenous breeds of horses *viz.* Marwari (Malani) and Kathiawari breeds and exotic donkeys (France and Italy origin) is presented in Table 1. During the year, there were six foaling, four in Marwari and two in Kathiawari. One adult Marwari mare died, and 9 animals were sold. There were 17 foaling in donkeys and 29 donkeys were sold or transferred during this period. The Centre has sold nine horses for Rs. 163150/-and 29 donkeys for Rs. 377750/- to the equine breeders.

Breeding performance of farm herd at Bikaner Centre during the year has been presented in the Table-2. The number of breedable horses available were 11 and breedable donkey available were 13, the total number of foaling occurred in horses were 7 and in donkey were 17.




**Total 1. Livestock Strength (Bikaner & Hisar)**

| Age Group | Horses | | Exotic Donkeys | Other Equines |
|---|---|---|---|---|
| | Marwari | Kathiawari | | |
| *Yearlings (0-1 year)* | | | | |
| Male | 2 | - | 4 | 2 |
| Female | 3 | 3 | 5 | |
| (1-3 years) | | | | |
| Male | 1 | - | 3 | - |
| Female | 2 | - | 2 | - |
| *Adults* | | | | |
| Male | 5 | - | 4 | 3 |
| Female | 4 | 5 | 15 | 5 |
| **Total** | **17** | **8** | **33** | **10** |

**Table-2 Reproductive performance of farm herd**

| Parameters | Horses | Donkeys |
|---|---|---|
| Total number of adult female | 11 | 30 |
| Number of animals mated | 9 | 23 |
| Number of animals conceived | 8 | 12 |
| Number of foaling | 7 | 17 |
| Average gestation length (Days) | 336±7 | 362±11 |

## Agricultural Production:

The fodder production during the period 1-4-2002 to 31-3-2003 at Hisar and Bikaner centre is shown in the table 3 below. An income of Rs 74,942/- was generated from the sale of fodder at Hisar centre.

Table 3. Fodder production at NRCE during the year 2002-03

| Type of Fodder | Production in Quintals | |
|---|---|---|
| | Hisar Centre | Bikaner Centre |
| Sudan Grass (*Sorghum Sudanese*) | 1527.8 | - |
| Maize (*Zea mays* L.) | 195.40 | - |
| Berseem (*Trifolium alexandrinum*) | 657.70 | - |
| Lucerne (*Medicago sativa*) | 786.30 | 700 |
| Oats (*Avena sativa*) | - | 100 |
| Millet (*Penesetium typhods*) | - | 70 |

## Managing equine manure for mushroom cultivation

One adult horse produces about 10 tons of farm waste including dung, beddings and urine annually. A study was conducted to use these materials for production of white button mushroom. Mushroom is a nutritious human food having wide acceptability among Indians. The compost was prepared using equine dung, wheat straw, gypsum and equine urine in a compost pit at the required temperature and moisture. The compost was ready after 3 weeks and the seed fungus was shown. The white button mushroom was grown successfully from these organically prepared materials (Fig 1 & 2). Any equine farmer can adopt this technology as an additional income from equine farming.

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**"यह केन्द्र उत्कृष्ट अनुसंधान एवं वैज्ञानिक गतिविधियों द्वारा अश्व स्वास्थ्य एवं उत्पादन में निरंतर सुधार हेतु प्रयत्नशील है।"**

बतौर निदेशक मेरे पिछले दो वर्षो की अवधि में इस अनुसंधान केन्द्र ने घोड़ों के संक्रामक रोगों के उच्चतम अनुसंधान के लिये राष्ट्रीय एंव अन्तर्राष्ट्रीय स्तर की ख्याति अर्जित की। इस अवधि में केन्द्र के वैज्ञानिकों के कठोर परिश्रम एंव समर्पण से संस्थान को अश्व रोगों के निदान के लिये राष्ट्रीय मानक प्रयोगशाला की मान्यता भारत सरकार द्वारा प्रदान की गई। इस केन्द्र के वैज्ञानिकों द्वारा अश्वों में बीमारियों के परीक्षण एंव पहचान, चिकित्सा एंव उपचार तथा दवाओं के विकास के लिये विकसित प्रौद्योगिकी द्वारा देश में अश्वों का संरक्षण सुनिश्चित हो रहा है। इस संदर्भ में इस वर्ष के अन्तराल में कुछ प्रौद्योगिकी विकसित की गयी हैं, जो इस वार्षिक प्रतिवेदन में पाठकों के लिये प्रस्तुत है।

केन्द्र निदेशक के पद पर मेरी नियुक्ति होने के कुछ ही महीनों में मैंनें इस केन्द्र में चल रहे अनुसंधान कार्यो को नयी दिशा देने के लिये इस क्षेत्र में कार्यरत वैज्ञानिकों के सहयोग से अश्व स्वास्थ्य एंव प्रजनन को नयी दिशा प्रदान करने का प्रयास किया। इन प्रयासों का उद्देश्य इस केन्द्र के अनुसंधान कार्यो को अन्तर्राष्ट्रीय चुनौती में खरा उतारना तथा व्यवसायिक महत्व की तकनीकी का विकास करना जो गरीब एंव सीमान्त किसानों एंव अश्व पालको के आर्थिक उत्थान में मददगार हो । मेरा यह प्रयास अब अच्छे परिणाम देने लगा है। इस केन्द्र द्वारा विकसित तीन प्रौद्योगिकियों का फील्ड ट्रायल किया जा रहा है। परिणाम आने के उपरान्त इन्हें अश्व पालकों तथा अश्व प्रजनन में कार्यरत सभी सरकारी एवं गैर सरकारी संस्थाओं के लिये शीघ्र उपलब्ध कराया जायेगा। यह अश्व पालकों एंव गरीब किसानों की वितीय स्थिति के अनुसार उनके पहुंच के अंदर होगी।

आने वाले कुछ वर्षो में हमारी प्राथमिकता है कि यह अनुसंधान केन्द्र घोड़ों में अनुसंधान कार्य के लिये अंतर्राष्ट्रीय स्तर की मान्यता हासिल करे। पशुपालन एंव डेयरी विभाग, भारत सरकार के सहयोग से राष्ट्रव्यापी अश्व रोग सर्वेक्षण का कार्य इस संस्थान को सौंपा जा रहा है। इससे राष्ट्रीय स्तर पर घोड़ों की प्रमुख बीमारियों की वस्तु स्थिति तथा उनके निवारण की दिशा में गति आयेगी तथा भारतीय अश्वों को विश्व में व्याधिमुक्त घोड़ों का दर्जा दिलाने की दिशा में कार्य किया जा सकेगा।

अश्वों में विषाणु–जनित बिमारियों के निरीक्षण एंव पहचान, चिकित्सा, रोकथाम तथा अश्व प्रजनन की दिशा में अन्तर्राष्ट्रीय सहयोग प्राप्त करने की दिशा में हमारा मुख्य प्रयास रहेगा।

सीमान्त एंव गरीबी रेखा से नीचे रहने वाले अश्व पालकों की जीविका का आधार गधे, खच्चर तथा पोनी ( टट्टू ) हैं। उनके लिये कृत्रिम गर्भाधान तकनीकी से उच्च नस्ल के खच्चर उत्पादन करना इस वर्ष हमारा मुख्य लक्ष्य रहा है तथा हमारा प्रयास है कि देश के कई राज्यों में अश्वों में कृत्रिम गर्भाधान तकनीकी का प्रचार एंव प्रसार किया जाय।

वर्ष 2003–2004 वित्तीय वर्ष की अवधि में हमारा लक्ष्य अनुसंधान कार्य के लिये अधिक से अधिक बुनियादी सुविधाएं जुटाने का भी है। इस दिशा में बीकानेर स्थित 'अश्व प्रजनन परिसर' के लिये आधुनिक सुविधाओं से सुसज्जित प्रयोगशाला एवं प्रशासनिक भवन का निर्माण तथा हिसार में प्रयोगशाला भवन का विस्तार जिसमें बायोसेफ्टी लैब–3 सुविधा का विकास करना मुख्य है। इन सुविधाओं का लक्ष्य प्राप्त करने उपरान्त विश्वस्तरीय प्रतिस्पर्धा के अनुरूप अनुसंधान कार्य करने हेतु इस केन्द्र को अन्तर्राष्ट्रीय मानक प्रयोगशाला का मुख्य दर्जा प्राप्त हो सकेगा। हमारे सहायक महानिदेशक पशु स्वास्थ्य का सहयोग इस दिशा में हमें निरन्तर प्राप्त हो रहा है।

अतः यह वर्ष अत्याधिक चुनौतियों से भरा है मैं आपको बताना चाहता हूँ कि इस दिशा में प्रगति का उल्लेख अगले वर्ष की वार्षिक प्रतिवेदन में अवश्य होगा।

मैं इस वर्ष के वार्षिक प्रतिवेदन की नूतन रूपरेखा एंव उत्तम प्रकाशन के लिये प्रकाशन समिति के अध्यक्ष एंव सदस्यों को हार्दिक धन्यवाद देता हूँ तथा भारतीय कृषि अनुसंधान परिषद, नई दिल्ली के सराहनीय सहयोग के लिये विशेषकर डा० वी०के० तनेजा, उप महानिदेशक (पशु विज्ञान) तथा डा० मंगला राय, महा निदेशक भा०कृ०अनु० परिषद का ह्रदय से आभारी हूँ जिनकी प्रेरणा एंव प्रोत्साहन ने मुझे हमेशा उत्साहित किया जिसके फलस्वरूप हम सब राष्ट्रीय अश्व अनुसंधान केन्द्र के सर्वांगीण विकास में लिये कठोर परिश्रम करनें तथा आवश्यकता अनुरूप अनुसंधान करने की दिशा में कार्य करने का संकल्प करते हैं जिससे अश्व पालक कृषक अधिक से अधिक लाभांवित हो सकें।

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# कार्यकारी सारांश

राष्ट्रीय अश्व अनुसंधान केन्द्र के लिये 2002–03 का वर्ष बहुत उपयोगी सिद्ध हुआ है। अश्व रोग टीकाकरण, रोग निदान, दवा–विकास अश्व जाति लक्षण–वर्णन एवं कृत्रिम गर्भाधान में जो अनुसंधान कार्य पिछले कुछ वर्षों में शुरू किये गये थे, उन क्षेत्रों में इस वर्ष सार्थक उन्नति हुई। अश्व हर्पीज़ (ई.एच.वी.–1), एवं सालमोनेलोसिस की पहचान की सुधरी हुई तकनीकों का विकास एवं मूल्यांकन किया गया तथा अश्व ट्राईपेनोसोमा के ईलाज के लिये भारतीय चिकित्सा पद्धति में उपयोगी औषधियों के सक्रिय अंशो की पहचान की गई। राष्ट्रव्यापी अश्व रोग सर्वेक्षण हेतु हमारे प्रयासों को तब एक बड़ी सफलता मिली जब पशुपालन एवं डेयरी विभाग, कृषि मन्त्रालय, भारत सरकार द्वारा इस केन्द्र को राष्ट्रीय स्तर पर अश्व रोग सर्वेक्षण करने की मान्यता प्रदान की। राष्ट्रीय अश्व अनुसंधान केन्द्र की 2002–03 की उपलब्धियों का ब्यौरा संक्षेप में नीचे दिया गया है।

अश्व रोगों के निदान के लिये उतम टीकों के विकास की दिशा में कार्यरत हमने ई.एच.वी.–1 (हिसार–90–7) के निष्क्रिय विषाणुओं के ट्वीन–80 एवं मैन्नाईड मोनोओलिएट समिश्रित टीकों की प्रतिरक्षण क्षमता का अश्वों में विश्लेषण जारी रखा। अश्वों में प्राथमिक टीकाकरण पर दोनों तरह के टीकों ने अच्छा असर दिखाया जो कि कम्पलिमेन्ट फिक्सिंग एवं वायरस न्यूट्रेलाईज़िंग विधि द्वारा मापा गया। इन टीकों की प्रतिरक्षण क्षमता बाजार में बिकने वाले विदेशी टीकों के समान थी। इस वर्ष इन टीकों के बूस्ट्र प्रतिरक्षण के प्रभाव का परीक्षण किया गया और हमारे अनुसंधान से पता चला कि दूसरे टीकों की अपेक्षा मेन्नाईड मोनोओलिएट समिश्रित ई.एच.वी.–1 टीके ने काफी अधिक प्रभावशाली हैं, जो कि कम्पलिमेंट फिक्सिंग एवं वायरस न्यूट्रेलाइजिंग विधि द्वारा मापने पर स्पष्ट हो गया।

इस वर्ष सालमोनेला अर्बोट्स इक्वाई की बाहरी परत के प्रोटीन (ओ.एम.पी.) सब–यूनिट टीके को अकेले एवं ई.एच.वी.–1 के टीकों के साथ मिलाकर इनकी रोग प्रतिरोधक क्षमता का गर्भवती व दूसरे अश्वों में और अधिक परीक्षण किये गये। ओ.एम.पी. टीकाकरण के उपरान्त घोड़ियों में 12 सप्ताह तक उपयुक्त मात्रा में प्रतिरक्षण क्षमता पाई गई जो कि सालमोनेला जीवाणुओं के संक्रमण से बचाने के लिये प्रर्याप्त थी। ओ.एम.पी. और ई.एच.वी.–1 के मिश्रित टीकाकरण से अश्वों में दोनों के प्रति अच्छी प्रतिरोधक क्षमता पाई गई जिससे सिद्ध हुआ कि दोनो टीकाकरण अश्वों में इक्ट्ठे किये जा सकते हैं। हमनें अश्वों में ई. एच.वी.–1 की पहचान के लिये मोनोक्लोनल एन्टीबोडी–प्रयुक्त एक अतिसंवेदनशील एवं सुनिश्चित परीक्षण तकनीकी (बी.एलाईजा) का विकास किया। इस विधि की संवेदनशीलता 100% मापी गई है और इसके परिणाम वायरस न्यूट्रलाईजेशन के परिणामों से अच्छी तरह से मेल खाते हैं (आर.=0.85)। इस वर्ष बी.एलाईजा की ओर अधिक पुष्टता करने के लिये अश्वों के 523 नमूने इस विधि द्वारा जांचे गये। बी. एलाईजा द्वारा इनमें से 271 (51.8 %) नमूने तथा वायरस न्यूट्रेलाइजेशन द्वारा 259 (49.5 %) नमूने ई.एच.वी.–1 संक्रमित पाए गए। दोनों विधियों में बहुत अच्छी सहमति (86.6 %) थी। इससे यह निष्कर्ष निकला कि बी.एलाईजा को वायरस न्यूट्रेलाइजेशन (जो कि एक कठिन विधि है) के विकल्प के रूप में प्रयोग किया जा सकता है।

अश्वों में सालमोनेला अर्बोट्स अक्वाई की पहचान हेतु संशोधित पहचान तकनीकी के विकास में प्रयासरत ओ.एम.पी. का प्रयोग करते हुए लेटेक्स एग्लूटिनेशन विधि का विकास और इसे ट्यूब–एग्लूटिनेशन से तुलना करने पर पाया गया कि दोनों विधियों के परिणामों में 100 % समानता थी।

अश्वों में शीघ्र गर्भाधान पहचान के लिये बायोटेकनोलोजी का प्रयोग करके एक सफल एलाईजा विधि विकसित की गई है। जो कि दूसरी विधियों के समान गर्भाधारण की पहचान कराने में सक्षम है। यह विधि शीघ्र ही कीट के रूप में उपलब्ध होगी।

अश्व ट्राईपेनोसोमा के ईलाज हेतु उत्तम औषधि विकास के अन्तर्गत लासोनिया इनरमिस जड़ी–बूटियों के अंश काफी लाभदायक पाये गये। इस वर्ष के दौरान इस पौधों से एच.पी.एल.सी. और टी.एल. सी. विधियों का प्रयोग करते हुए सक्रिय अंशों की पहचान की गई।

इस केन्द्र ने देश के विभिन्न भागों में फैली पूरी अश्व जाति में रोगों का, विशेषकर ओ.आई.ई. द्वारा नामित का 'क' ओर 'ख' श्रेणी की अश्व रोगों का राष्ट्रीय सर्वेक्षण आरम्भ किया। इस वर्ष के दौरान 16 विभिन्न राज्यों से 1333 रक्त (सीरम) के नमूनों को एकत्रित करके विभिन्न अश्व बीमारियों के लिये परीक्षण किया गया। इस दौरान जाँचे गए 16 राज्यों के नमूनों में से किसी में भी श्लैस्निक ज्वर (इनफलूएंजा), ग्लैण्डरस, सालमोनेला, अश्व एनिमिया (इ0आई0ए0) का संक्रमण नहीं

पाया गया। 606 में से 44 नमूनों में अश्व हर्पिज वायरस (ई.एच.वी–1) एवं 436 में से 19 नमूने माइकोप्लाजमा ग्रसित पाये गये। भारत के विभिन्न राज्यों में अश्व बेबेसिया का संक्रमण 20.9 % (129/598) पाया गया।

अश्वों की स्वदेशी प्रजातियों की कार्यक्षमता के बेहतर प्रयोग की दिशा में मारवाड़ी जाति के अश्वों में लक्षण–वर्णन का कार्य विभिन्न विधियों द्वारा प्रारम्भ किया गया। प्रारम्भिक अनुसंधान से यह निष्कर्ष निकला है कि मारवाड़ी जाति के अश्वों में कुछ वंशानुगत विभिन्नताएं हैं और इस जाति के पशुओं की पहचान के लिये कुछ मोलिक्यूलर चिन्हों की पहचान की गई।

इस वर्ष मारवाड़ी जाति के अश्वों के संरक्षण के लिये और उच्च गुणों वाले हिमीकृत वीर्य की संरक्षणा तथा कृत्रिम गर्भाधान (ए.आई.) के प्रचलन का कार्य शुरू किया गया। इस वर्ष के दौरान मारवाड़ी गर्दभों के वीर्य के विभिन्न भौतिक एवं जैव–रसायनिक गुणों का अध्ययन किया गया। पिछले वर्ष अश्वों के वीर्यों के हिमीकरण संरक्षण विधि का मानकीकरण एवं विकास किया गया तथा वर्ष 2002–03 में इस हिमीकृत वीर्य से 63 मादा अश्वों में कृत्रिम गर्भाधान के प्रयासों द्वारा 47.4–50.0% सफलता मिली जो खच्चर उत्पादन में एक मील का पत्थर साबित हुआ है।

हमारे 'अश्व–रोग–निदान एवं वैज्ञानिक–सलाह–सेवा' कार्यक्रम के अन्तर्गत हरियाणा और राजस्थान के विभिन्न भागों में 393 अश्वों के रोगों का निदान एवं उपचार किया गया, 123 मादा अश्वों की गर्भाधारण एवं दूसरे प्रजनन सम्बन्धी रोगों की अल्ट्रासाऊंड द्वारा पहचान की गई, 954 जानवरों को पेट के कृमि–संक्रमण से बचाव के लिये दवा दी गई। इस अवधि के दौरान 251 बीमार पशुओं के नमूने जीवाणुओं के लिये परीक्षण किये गये और इनमें रोडोकोकस, स्ट्रैप्टोकोकस, स्टेफाईलोकोकस, इकोलाई, एकोलीप्लाजमा इत्यादि वंशों से सम्बन्धित 33 जीवाणुओं को पाया गया। इस दौरान 12 मृत घोड़ों की डाक्टरी परीक्षा की गई।

प्रयोगशाला से किसानों तक वैज्ञानिक सूचनाओं के प्रसार करने के लिये हरियाणा और राजस्थान के विभिन्न क्षेत्रों में कई स्वास्थ्य शिविर, प्रदर्शनियां एवं किसान गोष्ठियों का आयोजन किया गया। इन कार्यक्रमों का उद्देश्य इस क्षेत्र के अश्वों को स्वास्थ्य सुरक्षा–कवच प्रदान करना एवं किसानों से भविष्य में अनुसंधान के लिये आवश्यक प्राथमिकताएं निर्धारित करने के लिये जानकारी प्राप्त करना था।

बुनियादी ढांचे में लगातार सुधार के अन्तर्गत राष्ट्रीय अश्व अनुसंधान केन्द्र ने सी.पी.सी.एस.ई.ए. समिति द्वारा निर्धारित नियम एवं मापदण्ड के अनुसार एक नये प्रयोगात्मक–पशु–सुविधा–गृह का निर्माण किया। इस वर्ष में केन्द्र की एक विस्तृत वैब साईट (http://nrce.nic.in) का भी निर्माण किया गया और वी.सैट के द्वारा 24–घण्टे इन्टरनेट की सुविधा उपलब्ध करवाई गई।

इस दौरान संस्थान ने अपने आंतरिक स्त्रोतों में 23.42 लाख रूप्ये अर्जित किए जिसमें अश्व–रोग पहचान एवं निदान सेवाएं एवं सरकारी एवं गैर सरकारी संस्थानों को उत्तम नसल के अश्वों की बिक्री मुख्य थी।

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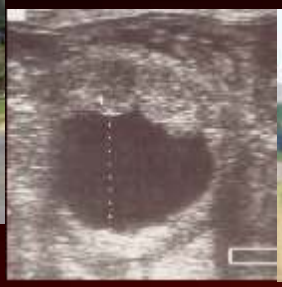



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