9. Livestock Management

Animal nutrition

Livestock feed resource database and forecasting models: Feed resource and livestock database for district, state, agro-eco regions and country was updated and is available in a compact disk with software support for graphical user interface to define query. The feed base version 2012 has special features like district-wise inter census growth rate for different categories of livestock, category-wise requirements of feeds in terms of fodder and concentrates and improved tools for graphical and spatial presentation of results.

Accurate estimation of crop residue production using remote sensing technique for *rabi jowar* showed that estimates arrived by remote sensing for acreage under *rabi jowar* crop compared well with official data available with agriculture department.

Prebiotics production from agricultural waste: Green coconut husks and palm press fibres were evaluated as source of xylan, a precursor for xylo-oligosaccharides (prebiotics). Green coconut husk contained 23.6 % more xylan content than palm press fibre.

Deconstruction of ligno-cellulosic biomass: Lignolytic enzymes harvested from *Coriolus versicolor* and *Ganoderma lucidium* fungi were evaluated in sheep. Enzyme treatment improved average daily gain, dry matter intake, ammonia N; protozoa count and xylanases, proteases and fibrolytic enzymes in rumen.

Newer feed resources: Feeding of detoxified neem seed cake replacing 25 % soybean meal in growing lambs showed no changes in terms of body weight gain, feed efficiency and nutrient digestibilities, N-balance and biochemical parameters and carcass characteristics. Silage was prepared with or without anti-fungal agents to enhance the keeping quality of pineapple fruit residue (PFR). Lactic acid content was highest and fungal count was least in PFR silage after 15 days of anaerobic fermentation. Feeding trial in growing sheep using PFR as total mixed ration along with concentrate mixture at 65:35 ratio for 75 days showed similar growth performance and nutrient utilization as compared to maize silage fed sheep.

Use of 50:50 maize stovers and pineapple waste and ground maize cob and pineapple waste yielded good quality silage after 21 days of anaerobic fermentation. A feeding trial in lambs with 100 and 50% replacement of *ragi* straw with ground maize cobs and pineapple waste showed no adverse effect on intake and digestibility of nutrients.

Moringa oleifera leaves have potential to be used as feed supplement because these leaves increased gas production in buffaloes, dry matter (DM) degradation and microbial biomass production with concomitant reduction in methanogenesis. Precision feeding of female buffalo calves achieved a growth rate of about 750 g/day.

Analysis of leaves of *jungli khajoor* (*Phoenix sylvestris*), *kainth* (*Pyrus pashia*), *akha* (*Rubus ellipticus*) and *meda* (*Litsea monopetala*), revealed that except *jungli khajoor*, addition of all these leaves in wheat straw based diet improved *in-vitro* dry matter and organic matter digestibility, and microbial protein synthesis. On the basis of *in vitro* studies, the nutritive value of these forages can be ranked as *L. monopetala* > *R. ellipticus* > *P. pashia* > *P. sylvestris.*

Prickly pear cactus (*Opuntia*), a fast growing xerophyte drought resistant plant and well-adopted to arid hot environment, contained 12% dry matter, 10.5% crude protein, 57.2% NDF, 25.9% ADF, 2.4% ADL and 3.9 Mcal/kg. With enough water content (88%), requirement of water can be easily met through fresh

Improved calf feeder device

A feeding device/appliance was developed to minimize the feed/fodder wastage. It is much more effective as compared to earlier trough. One device is sufficient for 6-8 calves (up to 6 months of age) at a time for all types of feeding materials. This will eliminate contaminations of feed/fodder through urine and faeces and reduce feed/fodder wastage (no wastage in pelleted feed, <10% wastage in dry and green fodder). Further, the calves can consume feed/fodder easily from the trough.

Opuntia feeding. Water requirement of around 1 litre is met through feeding of 1.20 kg of fresh *Opuntia* without any adverse effect on nutrient intake and utilization.

The supplementation of the combination 3 of herbal products as feed additive apparently induced higher milk production (10.13±0.88 vs. 8.14±0.77 kg/day) in lactating cows. The serum level of triiodothyroxine also improved in the supplemented animals. Detoxified jatropha meal or *karanj* cake effectively replaced 50% of soybean meal protein without any adverse effects on lactation performance and health of dairy cows. Supplementation of specific mineral mixture developed for high altitude of temperate Kumaon hills improved conception rate (28.6%) compared to control (14.3%) anestrous heifers. Feeding of oak leaves *(Quercus semecarpifolia)* along with supplemented mineral mixture further improved conception rate (57.2%). Feeding of oak leaves along with supplemental mineral mixture in concentrate mixture (40:60) improved mineral (Cu, Zn) digestibility showing some synergistic interaction of tannin with minerals improving reproductive performance. Feeding of nitrate, 3% of dry matter intake, to buffaloes reduced 34% methane production, improved growth performance by 15% and feed conversion efficiency by 10%. *Methanobrevibacter* sps. was the most abundant methanogen in buffaloes.

Rumen biotechnology: Gene construct encoding feruloyl acetyl esterase (FAE) was amplified in *Escherichia coli* BL21 stain for improving digestibility of fibrous feeds. Supplementation of FAE enzyme showed improved digestibility and rumen fermentation in crossbred steers fed paddy straw based ration.

A microarray chip was developed, which contains 895 oligonucleotide (17-50 mer) probes and covering 16S ribosomal RNA gene of archaea, mcrA gene of archaea, internal transcribed spacer and 28S ribosomal RNA gene of anaerobic fungi and 18S ribosomal RNA gene of rumen protozoa. Chip successfully tracked dynamics of archaea, fungi and protozoal communities in various *in vitro* and *in vivo* rumen manipulation studies. The chip indicated severe suppression in most of the species of archaea on dosing with garlic oil plus nitrate or saponin plus nitrate to rumen fluid of cow. Similarly, use of the chip for analysis of samples from cows differing in feed efficiency indicated significant difference in some of the archeal genus and species level operational taxonomic units. This is the first comprehensive microarray currently available for studying archeal, fungal and protozoal communities in rumen and is thus useful for providing direct linkages of microbial genes/populations to rumen ecosystem processes and functions, and will help develop effective means to improve rumen function and reduction in emission of methane from buffalo rumen.

Reconstituted milk formulation for lambs: Reconstituted milk formulation consisting of 22–24% crude protein, 30–32% crude fat, 22–25% lactose and 5–10% minerals fed to Malpura lambs @ 10 % of body weight with 3–4 feedings/day resulted in 19% more body weight at 90 days of age.

Poultry

Chicken diet: Solvent extracted *karanj* cake could be fed up to 6% on isocaloric and isonitrogenous basis to layer chickens (26-37 weeks of age) without any adverse effect. In broilers, *karanj* cake at levels beyond 3% significantly depressed performance. These toxic effects of *karanj* cake on broiler chicken could not be alleviated by dietary supplementation of protease (4,000 µ/kg), phytase (400 µ/kg) or liver tonic (0.1%).

Selenium in poultry diet: Organic Se at 0.15 ppm in the diet of broiler breeder pullets (PB-2) during 33-56 weeks of age, resulted in better production performance of breeders and growth of progeny. For hatchability 0.25 ppm Se was found optimum.

Micronutrients/toxic minerals: As inorganic chromium (chromium chloride) is less bio-available, organic chromium in the form of chromium enriched *Azolla* was standardized, which is 10 times less costly than chromium enriched yeast. Supplementation of organic chromium at 400 ppb could reduce cholesterol in yolk and increased chromium in the egg.

AICRP on Improvement of Feed Resources and Nutrient Utilization

Nanotechnology for enhancing bioavailability of minerals: Phosphorus, one of the most limiting minerals in livestock, is less bio-available from inorganic sources leading to environmental pollution. Experiment conducted to evaluate nano phosphorus in rats established that the gut absorption of nano P is higher as compared to conventional source of dicalcium phosphate (DCP).

Database on methane production and mitigation: Based on extensive data on the methane production potential (MPP) of different feed resources, a national catalogue was developed. Legume fodders produced less methane than cereal fodder, while straws produced more methane than green fodder, whereas tree leaves produced least methane. Total mixed ration (TMR) considerably reduced methane production than that of fodder and concentrate alone, and their methane reduction was directly proportional to the level of concentrate in the TMR.

Plant tannins as methane suppressants: Incorporation of tannin containing leaves such as jack fruit, neem and banyan @ 10–15% suppressed methanogenesis without any adverse effect on the fermentation. Jack fruit tree leaves @ 10% in the TMR significantly improved daily weight gain in lambs.

Methane emission: Methane emission from goats under different feeding systems was studied to develop suitable mitigation strategies. The *in-vitro* methane production of cereal crop straws, wheat *(Triticum aestivum)*, barley *(Hordeum vulgare)*, oat *(Avena sativa)*, and sorghum *(Sorghum bicolor)* ranged from 9.92 to 16.6 g/kg. DM CP content had a high correlation with methane production. Legume crop straws, *arhar (Cajnus cajan)*, gram *(Cicer arietinum)* and *guar (Cyamopsis teteragonoloba)* produced methane from 8.7 to 23.4 g/kg DM, which accounted methane energy loss of 482 to 1,299 kJ/kg DM. The major top feed resources produced methane ranging from 7.9 to 20.5 g for each kg digestible DM. Concentrate mixture replacing 50 % conventional protein source with mustard cake reduced methane production as glucosinolate content of mustard cake has antimicrobial properties for several microorganisms.

Physiology and Reproduction

Cattle and buffalo

Bull fertility differentiation kit: In the process of developing suitable fertility diagnostic kit to eliminate sub-fertile bulls, fertility markers like thyroid hormones, sperm functional parameters, tissue inhibitor of metallo proteinases-2 (TIMP- 2) were identified which differ between bulls of different fertility.

Embryo transfer under field condition: Embryo produced from a high yielding buffalo following insemination with the semen of a top ranking bull was flushed out and transferred fresh in a recipient buffalo of inferior type, under field condition resulting in the successful birth of a female calf.

'Mahima' born from cloned buffalo Garima at NDRI

Mahima born a female calf from a cloned buffalo 'Garima' was on 25 January 2013 at the NDRI. This is the first calf born from a cloned buffalo which was produced through hand guided cloning technique. Mahima weighed 32 kg at birth and its birth was normal. 'Garima', born on 22 August 2010, attained early sexual maturity at 19 months of age compared to her contemporaries (around 28 months) and was inseminated with frozen-thawed semen of a progeny tested Murrah bull of NDRI (No. 1875) on 27 March 2012.

'Swarn': A male cloned buffalo calf born NDRI, Karnal

A male cloned buffalo calf named 'Swarn' weighing 55 kg at birth was born on 18 March 2013. The birth of the calf was normal. The cloned buffalo calf is unique and is different from the earlier clones produced from NDRI as the donor somatic cell used was isolated from the seminal plasma of a bull currently being used for donating semen at Animal Breeding Research Centre (ABRC) of NDRI, Karnal. This achievement is of particular interest because, using this approach, it is expected to re-create highly valuable proven sire even after its death.

Sheep

Accelerated lambing system: In present scenario of growing demand of mutton, one lamb per ewe born in a year is not enough. Sheep rearing is shifting from extensive to intensive system, and accelerated mating system to target three lambs in two years was developed. Malpura ewes achieved fourth mating and fourth lambing within 683.33±12.77 and 821.30±8.57 days; 76.7% ewes achieved fourth lambing within a period of 876 days.

AI with 24 hour stored liquid semen: Protocol for use of short-term preservation of semen as an alternative to frozen semen for AI in sheep was developed. Semen of Malpura rams was collected, evaluated and extended 1:2 in egg yolk tris glucose (EYTG) and stored up to 24 h at 5°C. Lambing rate of 42.6% was achieved by fixed-time AI for one cycle with 24 h stored semen.

First test tube yak calf

The first test tube yak calf was born at Nyukmadung farm of National Research Centre on Yak, Dirang, Arunachal Pradesh on 15 July 2013. The male yak calf weighed 19 kg at birth, and was named 'Norgyal'. Transfer of a cryopreserved yak embryo produced through *in vitro* fertilization of oocytes (retrieved through ultrasound guided ovum pick up (OPU) technique from a donor yak resulted in birth of the male calf. The achievement is a breakthrough towards conservation and multiplication of elite yaks in the country.

Goat

Artificial insemination: Using frozen-thawed semen for AI in 32 Jamunapari goats, 17 does became pregnant (53.12%). One doe delivered triplets, nine does delivered twins and seven does produced single kids. Thus 17 does delivered 28 kids that resulted in 1.64 kids/goat. Results indicated that AI using frozen-thawed semen can be successfully used for propagation in goats.

Pig

AI technology in pigs at farmers' field: Artificial insemination technology was used extensively in organized farms and under village conditions. Villages (197) from Kamrup (rural) district, Asom were covered and 1,035 farmers registered as beneficiaries. Using single dose of insemination, a litter size varying from 7 to 18 piglets at birth and conception rate of >80% was obtained. Artificial insemination technique was extended to Kokrajhar district, Asom (265 km distance) on demand and to neighbouring tribal villages of Meghalaya. The artificial insemination technique increased the price of piglets from ` 1,200 to ` 2,000/ piglet due to better growth performance and physical appearance.

Poultry

Egg shell quality: Studies showed that age-associated deterioration in egg shell quality may be related to decrease in absorption of calcium from the duodenum and activity of carbonic anhydrase in egg shell gland.

Stress: Research on molecular mechanism involved in combating stress showed that expression of one of the molecular chaperons, heat shock protein-70, is up-regulated in brain and skeletal muscle of broiler birds exposed to heat stress compared to those reared under thermo neutral environment.

Combating heat stress in chickens: Inclusion of trimethylglycine in diet (300–1,200 mg/kg) of Vanaraja chicks during summer reduced lipid peroxidation and increased activity of super oxide dismutase (SOD) in a dose dependant manner indicating its favourable effects in combating heat stress. The lipid peroxidation reduced significantly in broilers fed with *tulsi* extract

Utilization of equine energy

Deployment of mules in agro-processing is being suggested as an alternative option for their optimum utilization. A study was conducted on use of mule power for chaffing green *bajra* straw with the help of a rotary gear complex, driven by a local mule of 350 kg body weight. The average output capacity of chopped *bajra* straw in rotary mode chaff cutter was 660 kg/h. This mule driven machine may not be as economic as electric chaff-cutter for chaffing operation but because of unavailability/shortage of electric power in rural remote areas, it would be helpful and eco-friendly to utilize mules/ equines in rotary mode operations during idle hours.

(50–200 mg/kg diet, 150mg being the best) as compared to those fed diet without the *tulsi* extract. The activity of glutathione peroxidase (GPx) increased with increase in dietary *amla* extract and maximum response was observed at 150 ppm in broiler chicken. The feed efficiency in Vanaraja chicks fed 0.10% KCl was significantly better than those fed the control diet during summer. A level of 260 meq/kg diet of electrolyte balance supported better performance in broilers. Lipid peroxidation decreased and activities of catalase and glutathione peroxidase increased in broiler chicken fed Zn (40 ppm), Cr (2 ppm) and Se (0.3 ppm). Feeding of organic Cr (400 µg/kg diet) considerably reduced mortality (from 2.22 to 1.61%) during 54 to 68 weeks of age in layers. The activities of SOD and GPx increased in Vanaraja female parents fed organic Se (0.15–0.3 ppm) and vitamin E (100–200 ppm).

Fisheries

Successful farming of Pacific white shrimp in freshwater: *Letopenaeus vannamei* can be cultured in freshwater. The specific pathogen free (SPF) post larvae of *L. vannamei* (PL 10) were stocked in 4 cement cisterns in pre-chlorinated water having a salinity of 2 ppt. Nursery rearing was continued up to 29 days

and the PLs were fed with a commercial pelleted feed during this period. During the nursery period the animals were slowly acclimatized to freshwater by partial water exchange. After nursery rearing, shrimps were shifted to grow out ponds, which were provided with soda mix, magnesium chloride and potash once in a week to avoid nutrient deficiency in addition to feeds. Growth rate of *L. vannamei* in freshwater was almost at par with brackishwater conditions.

Biofloc based shrimp farming technology: Biofloc, a conglomeration of heterotrophic bacteria, algae (dinoflagellates and diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus, acts synergistically to maintain water quality in aquaculture units reducing the need for water exchange and reutilize feed and reduce production cost. The technology is based on the concept of retention of waste and its conversion into biofloc as a natural food using some kind of biomats and supplementing with carbon addition to manipulate C : N ratio within the culture system. A very high survival of 98–99% was achieved in biofloc treatments compared to 91–92% in conventional system. One nursery tank of 100 tonne capacity can generate revenue of ` 50,000–100,000/ year. The shrimp grow-out culture under biofloc based rearing attained a final weight of 22–23g in 110 days, indicating scope for developing a tank based grow-out culture system, which has a potential to produce 20–25 tonne/ha.

Utilization of breweries waste in aquaculture: A feeding experiment was conducted for 90 days with catla fingerlings (average weight 5.0 g) in FRP tanks showed that utilization of breweries solid waste, an end product of beer industry is useful for fish food formation. Breweries waste has crude protein 43.50%, fat 1.05% and ash 8.30%. Study revealed that net weight gain was significantly higher in feed containing 15% breweries solid waste without any adverse effect.

Livestock protection

Genetic resistance to diseases in cattle

Tuberculosis: Out of 37 SNPs identified from candidate genes influencing disease resistance in bovines, seven significantly affected occurrence of

Success story

India's Largest Commercial Sea Cage Farm: Joint Efforts of ICAR and Tribals

The Veraval Centre of CMFRI established Sea Cage farm off Somnath temple in the Arabian Sea in partnership with 20 families of Sidi Tribes living along Gujarat coast. Cage culture technology including hands-on training and collection of seed from nature was transferred to them at Karwar and Mandapam Centres of CMFRI. Twenty cages of 5 m diameter were installed in the sea at a depth of about 7 m and stocked by lobster seed weighing about 50-80 g weight. These were fed well with trash fish and cultured for 110 days resulting in production of about 2,500 kg, which was sold at a price of Rs 1,200/kg valued about Rs 26 lakh being an export item. The farm can raise one more crop after September and get equal production and revenue within a year. Thus CMFRI/ICAR has provided the tribals a permanent livelihood from hunger to an income of about Rs 15,000/ month. In the process they have become – transformed then fully trained in the fabrication of cages, mooring, net handling, feeding and all other requirement for the cage farm management and emerged as very good cage farming entrepreneurs. This is the India's largest commercial sea cage farm.

bovine tuberculosis based on allelic and genotypic frequencies in case and control population.

Genotyping of case: Control population at 22 microsatellite loci revealed that polymorphism at 18 loci was significantly associated with the susceptibility to bovine tuberculosis.

Paratuberculosis: PCR-RFLP at 20 SNPs, located at the peak level of QTLs with significant association to susceptibility to bovine tuberculosis revealed significant association of one SNP i.e. rs41945014 with the susceptibility to bovine MAP infection.

Brucellosis: Five SNPs-2 from TLR1, two from TLR4 and one from Slc11A1, out of 21 SNPS from nine candidate genes showed significant effect on occurrence of brucellosis.

Vaccine development and tissue specific delivery

Avian influenza: A reassortant rgH5N2 virus was generated through plasmid based reverse genetics using mutated HA gene of one of the H5N1 field isolates and NA-N2 gene from H9N2 field isolate, as a non-pathogenic vaccine candidate for developing inactivated DIVA marker vaccine against H5N1 in poultry.

PNA as antisense molecules were transfected using both peptide and AuNPs, and provided better antiviral strategies as this can be easily conjugated with tissue specific ligand peptides. Presently use of brain homing peptides (identified by Phage Display Library) helped to deliver Au-PNA specifically to brain cells.

Diagnostic techniques

Diagnostic techniques were developed and improved for following diseases:

Orf: Loop mediated isothermal amplification (LAMP) assay was optimized for rapid detection of orf virus in clinical samples.

PPR: A lateral flow strip for detection of PPR virus was successfully tested and demonstrated in known samples.

Japanese encephalitis: Standardized indirect IgG ELISA using recombinant E domain III protein of Japanese encephalitis virus for sero-diagnosis of JE in pigs.

Bovine picobirnavirus: RdRp gene based RT-PCR assay was optimized for detection of bovine picobirnavirus.

Avian influenza: Indirect ELISA using recombinant nucleoprotein (rNP) for sero-diagnosis of Type A Influenza virus infection in chickens was optimized.

A liquid-phase immunoelectron microscopy (IEM) protocol was optimized for detection of avian influenza (H5N1) virus using AIV specific polyclonal serum.

Marek's disease: Loop-mediated isothermal amplification (LAMP) test was optimized using in-house synthesized LAMP primers for detection of MDV-1 specific oncogene *meq*.

Q fever: Standardized a novel multiplex PCR targeting *Trans* and c*om1* genes of *Coxiella burnetii* for detection of pathogen in clinical samples.

New castle disease virus: Different pathotypes specific PNAs were prepared and used to detect pathotypes specific RNA by PNA-RNA hybridization. This test was also performed using silver nanoparticles (SNPs). PNAs could differentiate pathotypes of NDV virus and nanoparticles plasmon changes leading to color variations in test solution, which could be visually seen and observed in visible spectrophotometer to have a quantification assay for assessing viral RNA concentration.

Quality control of veterinary biologicals

Vaccines for RD 'F' strain (58,600 doses); RD 'M' strain (600 doses); fowl pox vaccine (20,700 doses); lapinized swine fever (3,03,845 doses); sheep pox (7,96,400 doses); PPR (6,121,600 doses); *Brucella abortus* strain-19 (live) (64,987 doses); enterotoxaemia (2,950 doses); HS adjuvant (3,323 ml); tuberculin PPD (73,640 doses); Johnin PPD (49,800 doses); mallein PPD (22,730 doses); and antigens for *Brucella* agglutination test antigen (51,750 ml); *B. abortus* bang

Potential therapeutic application of stem cells

In vitro differentiation of embryonic mesenchymal stem cells (MSC) to tenocytes (fibroblast like differentiated cells that form matured tendon) was achieved and confirmed by expression of Decorin and Tenomodulin (tenocyte specific markers) by RT-PCR and immuno-cytochemistry. Buffalo and goat MSCs were isolated from Wharton's jelly and amniotic fluid, cultured and characterized using suitable markers. Canine MSCs were experimentally found effective in both allogenic and xenogenic treatment. The cells used were tracked during treatment using fluorescent dye PKH26. Caprine MSCs were transdifferentiated into neuron like cells and were characterized. Transgenic MSCs were transplanted in rats with induced spinal injury.

Molecular characterization of pathogens

- Complete genome sequence of classical swine fever viruses Indian strains (genotypes 1.1 and 2.2) was accomplished. Phylogenetic analysis of CSFV revealed that genotypes 1.1, 2.1 and 2.2 are prevalent in Indian pigs.
- Porcine circovirus 2 detected in pigs with clinical disease was sequenced. Phylogenetic analysis identified it as genotype PCV2a with close homology with viruses circulating in Slovakia, Romania and Serbia in Europe.
- Sequence analysis of bovine and human rotavirus isolates for NSP4 and NSP5 genes revealed possible reassortment event between bovine and human rotaviruses.
- The phylogenetic analysis of HA genes indicated that the 2011-2012 Indian and Bhutan avian influenza viruses belonged to clade 2.3.2.1 and grouped with Bangladesh virus of 2011.
- Determination and analysis of the entire genomic sequence of an Indian BVDV-2 cattle isolate was completed. Its phylogenetic analysis revealed that it is of BVDV-2a subtype and has close genetic similarity with a Chinese cattle BVDV-2 strain.

ring antigen (7,380 ml); rose Bengal plate test (23,320 ml); *Brucella abortus* positive serum (71 ml); *Salmonella*. Pullorum coloured (5,240 ml); *S.* Pullorum plain antigen (4,000 ml); *S.* Pullorum positive serum (38 ml) and *S.* Abortus equi 'H' antigen (1,000 ml); *S.* Pullorum Poly 'O' sera 25 ml, were produced, quality tested and supplied to various organizations. Goatpox vaccine (449,200 doses), 23 PPR c-ELISA and 22 PPR s-ELISA kits were produced and supplied to various institute/state veterinary biological/animal husbandry departments.

Herbal medicine: Poly-herbal formulation, Toxheal, ameliorates arsenic induced hepatotoxic, oxidative and immuno-disruptive injuries in poultry model, and also reduces arsenic deposition in liver and kidney.

Genetic and antigenic differentiation of equine influenza viruses: Analysis of genetic and antigenic differentiation of equine influenza viruses (EIV) showed homology of 98-99.5% to Chinese and Mongolian isolates. The specificity of MAbs-1D12, 1G4, 5A7 and 5F4 raised against EIV by indirect immunoperoxidase technique (IPT) was tested. EI virus-infected DCK cells gave positive immunoperoxidase reactions with all four MAbs and detected accumulation of immunizing antigen. Five EIVs isolates from various parts of the country during 2008-09 epizootic and the isolate of 1987 outbreak were characterized antigenically for HA activity using MAbs 1G4 and 5A7. MAb 1G4 recognized an epitope of 4 EI virus isolates/strains, while strains A/eq/Ludhiana/ 87(H3N8) and A/eq/Ahmedabad/1/09 were not recognised. However, 5A7 MAb recognised an epitope of all six EIV isolates. Ludhiana/87 isolate was found different from 2008-09 epizootic. A TaqMan probe based qRT-PCR for detection of EI targeting nucleoprotein (NP) gene showed specific amplification curve for positive control and for NP gene.

Mice model for pathogenesis of equine influenza was developed in BALB/c mice using EIV [A/eq/Jammu-Katra/08]. Tissues from lungs and nasal turbinates showed positive results by RT-PCR till 5 dpi and 3 dpi, respectively, while virus could be isolated on 1 dpi from lungs. Investigations in BALB/c mice model demonstrated the virus replication in respiratory tract.

Surveillance and monitoring of important equine diseases: During the reported period, 7,462 serum samples were examined for EIA, and one thoroughbred horse was detected positive for EIA. The animal was eliminated as per policy guidelines. No new cases/ outbreaks of EIA were reported during the year. Testing of serum samples (7,601) for glanders revealed seven positive samples from Uttar Pradesh.

Prevalence of bacterial and viral causes of calf scours: Presence of the causative agent – rotavirus - for diarrhoea in organized dairy farms was detected in 18.35% samples with G6 genotype predominating followed by G10. Other infectious agents include bovine carnavirus and *E-coli* (K99 Ag) in Indian calves.

Focal outbreaks of glanders: Suspected cases of glanders as focal outbreaks were detected from Auriaya, Hardoi and Ganjdundwara blocks in Kasganj District in Uttar Pradesh. Affected equines showed respiratory illness and cutaneous lesions. CFT, WB, iELISA and dot-ELISA revealed that seven horses were positive for glanders. Cutaneous and nasal forms of glanders were observed in the affected horses in Hardoi and Ganjdundwara whereas only its respiratory form was observed in Auraiya. The effective surveillance and awareness camps organized by the NCRE, Hisar helped in controlling this dreaded zoonotic disease.

Veterinary Type Culture Collection: During the period under report, 21 viral isolates, 187 pathogenic bacteria, 45 rumen bacteria, 100 dairy microbes, 76 recombinant clones and 138 genomic DNA of bacteria from different animal species were added to VTCC

MASP *in-vitro* cultivation technique for *Theileria equi*

Micro-aerophilus stationary phase system (MASP) was used for *in-vitro* cultivation of *Theileria equi* (Indian strain). MASP cultivation system is a major breakthrough in theileriosis research as it helps the researchers in production of antigen for various purposes, maintenance of parasite in laboratory system and testing the battery of drugs in *in-vitro* culture system.

Success story

Successful treatment and foaling in an Arabian mare

An Arabian mare aged about 12 years which was barren for last 7 years, despite repeated coverings with different stallions was successfully treated. Examination of the uterine swabs showed presence of *Streptococcus* spp., *Staphylococcus* spp., and *Bacillus* spp and the animal was diagnosed as suffering from open pyometra due to chronic bacterial infection. The treatment was successful as ultrasonography after second intrauterine treatment revealed that both uterine body and horns were normal with appreciable uterine folds characteristic of estrus. Pregnancy was confirmed on day 25 of last cover and she delivered a healthy foal.

Success story

Successful diagnosis of neurological trypanosomosis

Many cases of trypanosomosis in horses, manifesting neurological disorder followed by mortality, were reported in India. The episode of neurological disorder was investigated by various national and international labs without success. Thereafter, the first batch of serum samples was sent to NRCE, Hisar. It was subjected to ELISA using whole cell lysate (WCL) and exo-antigen of *T. evansi*, together with reference positive and negative controls. Of them, 8 horses were found sero positive with both antigens for *Trypanosoma evansi* antibodies. These serum samples were subsequently subjected to immunoblot, which further confirmed ELISA positive samples. Immunoblot with exo-antigen also revealed reactivity at 66 kDa region. PCR of the representative samples of blood, brain tissue, CSF revealed *T. evansi* specific amplification. Further, mass spectrometry based protein identification from the buffy coat of the infected horse blood sample revealed presence of variable surface glycoprotein (VSG) and other trypanosomal proteins. The results supported serological findings indicating *T. evansi* infection in horses. Satellitosis and neuronophagia around the necrosed neurons was present along with gliosis and gitter cells indicating neurological trypanosomosis as no concurrent infection of JEV, WNV and EHV-1 viruses was observed in *T. evansi* sero-positive horses.

repository. At present repository has accessioned culture collection (1,630), accessioned veterinary microbes (751, 627 bacterial and 124 viral isolate cultures), accessioned recombinant clones (267) and phage library (27). The VTCC is also maintaining 11 different cell lines along with one primary culture for isolation of different viruses in the repository.

A total of 140 rumen microbes were isolated, characterized, accessioned and deposited in the VTCC repository. During the period, 89 dairy cultures were isolated and characterized, and a total of 490 bacterial cultures are presently available in the dairy microbes repository.

Four new bacteria (*Nocardia otitidiscaviarum, Moraxella ovis, Bordetella bronchiseptica* and *Delftia* spp.) were confirmed by several biochemical tests and by cloning and sequencing of 16S rRNA.

Genome sequence of *Mycobacterium avium*: Whole-genome of Indian bison type biotype of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) strain S5 was sequenced. The MAP was transferred to a commercial firm under PPP mode for preparation of Johne's disease vaccine. Sequencing the genome of 'Indian Bison Type' biotype of *Mycobacterium avium* subspecies *paratuberculosis* strain S5, revealed the genome size of 4.79 Mb. A total of 90 regulator genes were found, indicating the ability of strain S5 to survive in a wide range of environmental conditions.

Skin candidiasis: An effective module was developed for treatment of skin candidiasis in camel: (i) washing the lesions with sodium thiosulphate (10%) solution on first day; (ii) application of 6% sulphur (80% sulphur) and 3% salicylic acid in mustard oil (*Brassica* spp.) on every day for one week. The recovery of hairs on skin was achieved on day 10.

Prevalence of *Amblyomma testudinarium* in mithun: About 9% mithuns (3/33) showed prevalence of *A. testudinarium* infestation in eastern region of Mizoram bordering Myanmar.

A. testudinarium is one of the largest hard ticks, which are usually 1-9 mm long before engorgement and reaches up to 23 mm in length after feeding. This tick usually parasitizes the wild animals and may infest domesticated large animals near forest area. Highest record prevalence in the North Eastern border of India adjacent to Myanmar might be due to favourable ecological and climatological factors. The present report includes Mizoram also in the *A. testudinarium* distribution map of India.

Epidemiology and surveillance of diseases: Analysis of data on mortality revealed that yak calf mortality was more within 0-30 days of age (34.67%) followed by calves aged between 1 and 3 months (30.67%). The least mortality was observed in calves over 3 months of age. Calf mortality was maximum during the rainy season (48%), followed by autumn (44%), winter (6.67%) and spring (1.37%). Causes of mortality were calf scour, chronic debility and weakness, respiratory problems and parasitic infection.

Rapid detection of pig pathogens: PCR protocols for rapid detection of *Streptococcus suis* associated with pathological conditions like polyarthritis, polyserositis and bronchopneumonia etc. in pigs were standardized. Multiplex PCR for detection of prevalent capsular types (in India) of *Pasteurella multocida* from pigs was also standardized.

Success story

Infectious cDNA clone of serotype Asia 1 FMD virus

Full-length genomic cDNA clones provide a valuable platform to modify the virus through reverse genetic techniques for research on functional genomics, elucidating the molecular mechanisms of pathogenicity and developing genetically engineered next generation vaccines with desired attributes. More than 1,000 kb of nucleotide sequence data for Indian strains of FMD virus are available. This database could help navigate the FMD virus genome and select motifs for creating modified genomes to provide mechanistic insights into the intricacies of pathogenesis, virulence attenuation and advanced vaccine designs. A genomic cDNA clone corresponding to Asia1 IND 491/1997 virus was constructed and viable recombinant infectious virus particles could be rescued. The nucleotide sequence, *in vitro* growth characteristics, plaque morphology, tissue culture infectivity titres and antigenic profile of recombinant virus were indistinguishable from those of the wild-type virus suggesting the authenticity of the virus rescued, and its potential application in developing designer virus.

Mycoplasma incidence in chickens: Incidence of *Mycoplasma gallisepticum* was determined among Indian poultry farms using standard culture techniques and PCR. The prevalence of *M.gallisepticum* was 18.6% in central, 1.0% in eastern, 1.76% in northern, and 11.25% in southern regions of India. The prevalence was 12.45% in commercial layers, 9.2% in broiler parents and 7.85% in commercial broilers.

Foot-and-mouth disease: Almost 60% of the 331 FMD outbreaks were recorded in Eastern and North Eastern states, which are not covered under FMD control programme. Maximum outbreaks were recorded in West Bengal and Asom, but no FMD incidence in Punjab and Haryana. The FMD incidence reduced in the Southern and Western regions compared to previous year.

Serotype O caused maximum (79.8%) outbreaks followed by serotypes Asia1 (15.7%) and A (4.5%). Outbreaks due to serotype Asia1 decreased by 1.5 fold compared to the last year and occurrence of serotype A remained almost same. Serotype O was most prevalent in all the geographical regions. Serotype Asia1 has been occurring regularly in Eastern, North Eastern and Western regions of the country. Serotypes O, A and Asia1 occurred in North Eastern and Southern regions. Serotypes O and Asia1 occurred in the Central, Western and Eastern regions. Serotypes O and A occurred in the Northern region. In the Northern region, serotype Asia 1 could not be detected continuously for last three years (since 2010-11), and serotype A appeared after a gap of two years (2010-12). In the North Eastern region, serotype A dominated the scenario followed by serotype O. In Eastern region, incidence of serotypes O and Asia1 increased compared to previous year.

Phylogenetic analysis of serotype O virus revealed that 'Ind2001' strains, which re-emerged in late part of the year 2008, nearly out-competed PanAsia lineage in causing outbreaks in the county. A distinct genetic cluster of Ind2001 lineage (designated here as $Ind2001^{UP\text{-}11}$) responsible for the outbreaks in Uttar Pradesh, Uttarakhand, Himachal Pradesh and Odisha last year became the major cause. Serotype O outbreaks during 2012-13 and the lineage was detected in many states in different regions of the country. The Ind2011 lineage, which appeared during 2011-12 could not be detected in any of the outbreaks this year, probably due to infection immunity or natural extinction. In serotype A, all the isolates were clustered within the genotype 18 in the maximum likelihood tree, and grouped only in the clade 18c of the $VP3^{59}$-deletion lineage. Clade 18c which was first detected in Southern peninsular India during 2007 has disseminated to Central, Eastern, Western and Northern parts of India after 2009. In serotype Asia1, isolates clustered within the lineage C indicating its exclusive prevalence since 2005. Isolates of Western cluster, which were introduced to Southern region during 2011-12, now entered into Eastern region in West Bengal and Odisha. Disease owing to serotype Asia1 in Odisha is very significant as it was not detected during last five years.

Water quality at Triveni Sangam, Allahabad

Central Inland Fisheries Research Institute (CIFRI), Barrackpore collaborated with IITs, Kanpur and Varanasi, Peoples Science Institute, Dehradun and WWF, India to determine environmental flow requirement at Triveni Sangam Allahabad during Mahakumbh 2013.The flow was calculated by building block methodology (BBM). It was recommended to maintain 1.5 m water depth with corresponding estimated flow of 310 cumecs and water surface width of 325 m. Further, analysis of water samples from 7 sampling sites in the river Ganga-Yamuna before, during and after main bathing days showed no significant changes in water quality in River Ganga at Allahabad zone due to maintenance of recommended water flow during Mahakhumbh 2013.

CIFRI is also partnering with Consortium of IITs on biological monitoring and environmental flows in the Ganga river basin under the "Ganga River Basin Management Plan".

Vaccine matching exercise was carried out to evaluate antigenic relationship of field isolates with currently used vaccine strains to monitor antigenic variation, if any, occurring in the field, and also assessing appropriateness of in-use vaccine strains. In serotype O, the vaccine strain INDR2/1975 covered almost all the field isolates showing its appropriateness. A few divergent isolates always emerge and perish. In serotype A, about 60% of the isolates did not show perfect match with the vaccine strain, IND40/2000. As occurrence of serotype A FMD virus is limited in the country, such divergence did not have impact. However, search is on for an alternate candidate strains. In serotype Asia1, the currently used vaccine strain, IND63/1972 covered most of the field isolates.

Under National FMD Serosurveillance, 40,934 bovine serum samples collected at random from various parts of the country were tested for assessing NSP-antibody (NSP-Ab) response, which is an indicator of FMD virus exposure/circulation regardless of vaccination status. The test revealed overall seropositivity in ~ 26.4% samples/animals. The pattern is similar to the previous year. Under FMD control programme, 155,611 pre- and post-vaccinated serum samples were tested and of which, 54,642 serum samples were from first phase FMDCP districts (54) representing XII, XIII and XIV phases of vaccinations, and remaining 100,969 serum samples were from FMD

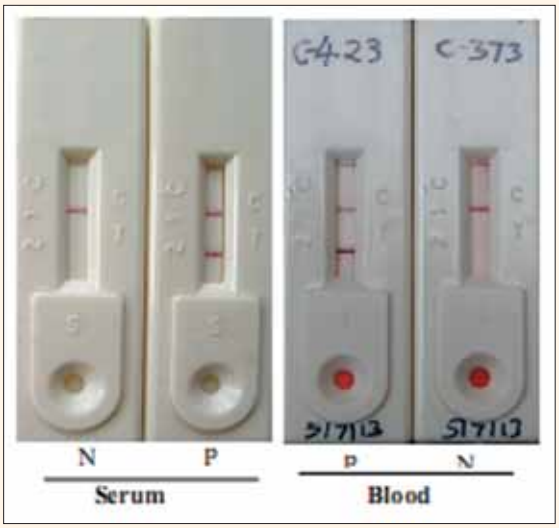
Success story

FMD pen-side diagnostic kit

Two diagnostic kits for detection of non-structural protein (NSP), 3ABC antibodies to foot-and-mouth disease (FMD) were developed at IVRI, Bengaluru. These are useful for differentiation of infected animals in a FMD vaccinated population.

1. **A rapid test:** An immunochromatographic test is intended for use by the farmers and veterinarians at the field level. The test is performed using a drop of blood/serum and gives result in 10 min.
2. **ELISA:** A laboratory based test which takes about 3 h to complete. It needs trained manpower and instrument to perform and interpret the results.

Both the tests detect FMD-NSP antibodies in all species of animals susceptible to FMD. Both the assays are highly cost effective compared to other kits. Rapid test and ELISA will be priced at a cost of Rs 40 and Rs 25, respectively. The kits have been commercialized and an MoU was signed between IVRI (ICAR) and a commercial firm.



LFD strips showing negative (N) and positive (P) results with serum and blood samples

CP districts (167) of 2010-11, representing phases I, II and III. After phase XIII vaccination, 53.6, 41.6 and 42.3% of animals tested were having protective antibody level (log_{10} 1.8 and above) against serotypes O, A and Asia 1, respectively, in post-vaccination serum samples. After phase II vaccination under expanded FMDCP, 67, 43.4 and 34.5% of animals tested were having protective antibody level against serotypes O, A and Asia 1, respectively, in post-vaccination serum samples. There was substantial reduction in occurrence of the disease in first phase FMDCP districts. The extended FMDCP areas are likely to yield positive result soon.

Epidemiology and surveillance

Web based interactive relational software NADRES (National Animal Disease Referral Expert System) developed by PD_ADMAS indicated that foot-and-mouth disease (FMD) and haemorrhagic septicemia (HS) are the top viral and bacterial diseases at national level, respectively. Classical swine fever (CSF) was next in order, of which more outbreaks were reported than *peste des petits ruminants* (PPR). Among the parasitic diseases amphistomiasis still continues to be the major disease affecting livestock. The data indicated that incidence of majority of diseases declined indicating that control measures taken up by the government are effective in controlling these diseases.

A microsoft access based software for storing the data generated at serum bank was developed. The said software was evaluated with incorporation of additional fields this year and currently, holds 14,813 serum samples. At PD_ADMAS screening of serum samples of diseases from 18 states indicated positivity for bovine, ovine, caprine, swine brucellosis; IBR, classical swine fever, bovine viral diarrhea and porcine respiratory reproductive syndrome (PRRS); and some samples were positive for IBR and BVD and IBR and bovine brucellosis; and classical swine fever and swine brucellosis.

Statistical analysis of the PPR outbreaks using data available in the NADRES database (1991 to 2011) revealed PPR as one of the top 10 diseases reported in small ruminants. Even though PPR is endemic in India, in some states especially North Eastern states, few cases were reported. Sheep- and goat-pox continues to be an important viral zoonotic disease of small ruminants. Serological survey for the prevalence of bluetongue indicated that about 80% of sheep population in Karnataka has antibodies against bluetongue virus (BTV) by one year of age. Classical swine fever (CSF) is among the most frequently reported viral diseases in the country. Serological screening of blood samples showed 48.19% prevalence of this disease— highest in North Eastern (NE) states followed by Kerala. Descriptive epidemiology performed during disease outbreaks in Karnataka and Arunachal Pradesh indicated uncontrolled movement of pigs from the neighbouring states. Phylogenetic analysis indicated that all belong to subgroup 2.2. Involvement of viruses of this subgroup in CSF outbreaks indicated a major shift from the past trend, wherein subgroup 1.1 viruses were major ones involved.

Among the bacterial diseases, haemorrhagic septicemia (HS) was the most prevalent, accounting for about 58.77% of the aggregate bovine mortalities in India. As occurrence of FMD precipitates HS, the data on FMD was also included for analysis. Comparison of disease patterns among the states indicated drastic reduction in occurrence of the disease in Andhra Pradesh from 2002-12 compared to Karnataka; the highest case fatality rate in Tamil Nadu for both HS and FMD; high prevalence rate of HS in Karnataka and of FMD in Kerala (2007-12). PD_ADMAS established microscopic agglutination test (MAT), gold standard serological test, for seroprevalence of leptospirosis in bovine and livestock species. The prevalence rate of 32 % was observed in Konkan region. The Hardjo serovar or Sejroe serogroup

was predominantly found.

Host-microbe interaction by genome-wide gene expression profiling in *Staphylococcus* aureus mastitis (Strain SA3, spa t267) was carried out. This is the first report that *S. aureus* subverts the host genome miRNA profile during IMI *in vivo*. The study demonstrated presence of intercellular adhesion genes (icaABCD), its associated genes and consequent biofilm production in *S. epidermidis* isolates of bovine milk.

Recombinant antigens targeting VSG and ISG genes of *T. evansi* were developed both in prokaryotic and eukaryotic host system. For sero surveillance of surra, 1,574 serum samples were collected from cattle and buffaloes from Karnataka, Odisha, Kerala, West Bengal, Maharashtra, Tamil Nadu and Uttarakhand; overall sero-prevalence of surra was 15.43%.

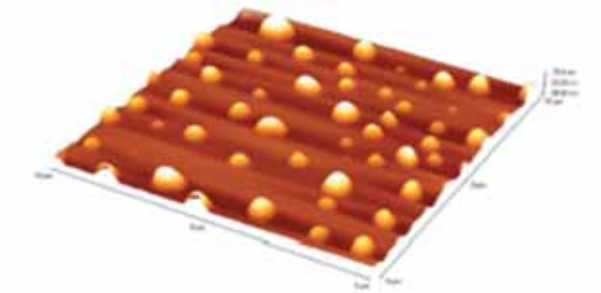
Fish health management

Viral vaccine against Noda virus: The whole cell heat-killed Noda virus vaccine developed under a collaborative project was evaluated with juveniles of Asian seabass *(Lates calcarifer)* under two different temperatures (25±1ºC and 28±1ºC) and observed for 30 days post vaccination for evaluating efficacy of the vaccine in terms of relative percentage of survival (RPS) of the vaccinated and the non-vaccinated. RPS was 80% at 25±1ºC and 60.66% at 28±1ºC. Important antioxidant enzymes like GPX, GST, SOD, GRX, CAT and LPO, and important hematological parameters like total erythrocyte count (TEC), hemoglobin, PCV, MCV, MCH and MCHC were assayed in the fish juveniles infected with Noda virus and also in fishes vaccinated and challenged with Noda virus. The results indicated that activities of enzymes elevated in vaccinated fishes compared to that of non-vaccinated suggesting the improvement of antioxidant defense system. In juveniles infected with Noda virus, haematological parameters count reduced but was at normal level in vaccinated ones. The immune genes expressed in thymus and head kidney of the vaccinated fish were higher in the vaccinated fish indicating that the vaccine is elevating immune related genes expression. These studies indicated that the whole cell heat-killed vaccine is useful for the Asian seabass to protect against the Noda virus infection.

Monoclonal antibody-based marker for monitoring humoral immune response: The monoclonal antibodies (MAbs) were raised against purified serum immunoglobulins of *Catla catla*. These MAbs are crucial for developing sensitive and specific assays for detecting circulating antibodies to important fish pathogens and are also useful tools in evaluating efficacy of vaccines. Quantification of surface Ig+ (sIg+) cells in lymphoid organs and blood revealed that a varying percentage of gated cells from kidney, spleen and blood had sIg. The percentage of sIg+ cells was highest in kidney, followed by blood and spleen. The lowest reactivity of G10/1 MAb was observed in catla thymus. This also implies that majority of thymocytes lack sIg and hence can be presumed to be T-lymphocytes. An increase in Ig+ cells was observed in kidney, spleen and blood following inoculation of killed *Edwardsiella tarda*. Therefore, G10/1 MAb can be a useful tool to study the kinetics of Ig+ cells following vaccination. The study showed that G10/1 MAb can improve understanding of architecture and functioning of immune system in a candidate species.

Diagnostics for transboundary freshwater fish viruses: PCR and RT-PCR-based diagnostics for detection of koi herpes virus (KHV) and spring viraemia of carp (SVC), respectively, were developed at CIFA. These diseases are of transboundary importance. These diagnostics having high sensitivity and specificity can be used in screening of presence or absence of these pathogens in freshwater culture environments, which has significance from export and surveillance point-of-view.

Chitosan nanoencapsulated trypsin biomimics zymogen like enzyme: Chitosan nanoencapsulated exogenous trypsin feed supplement prepared at CIFE, Mumbai first time on fish model releases enzyme in controlled manner and biomimics zymogen-like activity, thus improving the safety of use in addition to production efficiency in fish significantly. Effectiveness



Three dimension image of 0.01% trypsin nanoencapsulated in 0.04% chitosan

and safety of dietary nanoencapsulated trypsin at half the dose rate (0.01%) (D) of bare trypsin (0.02%) (C-1 and C-2) is evident from healthier villi with more height and absorptive surface.

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