

9. Livestock Management

Animal Nutrition

Feed database: A structured methodology and software platform was developed to launch the refined livestock feed resources database with national acceptability for cattle and buffaloes. The livestock population and the feed balance (2009-10) were estimated for various states. Indian livestock feed portal is being developed to include the information on feed resources, nutrient requirements, feed standards, feed markets, imports and exports and feed assessment. A matrix of 712 feed resources with composition, mineral topography, rumen degradable and un-degradable protein contents and amino acid profile, was developed.



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Linear time series models were developed to assess and forecast the feed resources availability for better management of livestock. The projected figures of total dry matter (DM), crude protein (CP) and total digestible nutrients (TDN) for 2025 would be 580, 49.5, 300 M tonnes, an increase of 13.5, 10.6 and 13.3%, respectively, over 2011. The share of dry fodder and concentrates to the total DM, CP and TDN availability by 2025 is showing an upward trend.

Feed safety and biosecurity: Feed microscopy was used in cataloguing microscopic structures of wheat and rice bran and usual adulterants (paddy husk, sawdust) were documented. The particle measurements and surface characteristics of some ingredients were documented which can be used in detection of adulterants.

As fluoride toxicity is quite rampant in different parts of the country and water being a major source of the contaminant, a study was conducted to investigate the effect of fluoride in rats. Supplementation of graded levels of fluoride in water to rats beyond six-months, lowered

the antioxidant (superoxide dismutase, ceruloplasmin) and immune status at higher levels of fluoride ingestion (30 and 60 ppm). Feeding of boron, calcium carbonate and silicate binder partially improved the liver antioxidant activity and radiology of femur bone.

Cattle and buffalo

Lignin degradation: White rot fungi *Pycnoporous sangeus*, *Coriolus versicolor*, *Pleurotus flabellatus*, *Pleurotus floridanus*, *Lenzites striata* and *Poria placenta* were promising for lignolytic enzyme production. Immobilized enzymes had greater purity, control over enzymatic reaction and high volumetric productivity with lower residence time. Enzyme treatment of *ragi* straw improved its digestibility as compared to that of enzyme supplementation. Fungi species *Ganoderma lucidum* and *C. versicolor* yielded the best results.

Mitigation of methane production by livestock

Database on methane production from livestock: Methane production potential (MPP) of several feed samples and diet combinations was determined through *in vitro* gas production technique using rumen liquor. MPP was lowest in rice polish and highest in *jowar kadbi*. The MPP ranged from 1.03 in *kum kum* cake to 7.80 in home-made compound feeds.

On the basis of these data, an equation was developed to estimate methane production based on the nutrient composition of feed ingredients:

$$\text{Methane} = 14.346 + 0.010 * \text{CP} + 0.002 * \text{NDF} - 0.069 * \text{ADF} - 0.001 * \text{ADL} - 0.136 * \text{IVDMD}$$

(CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; IVDMD, *in vitro* dry matter digestibility)

Plant tannins as methane suppressants: The effect of PEG (tannin binder) on *in vitro* incubation established that medicinal and aromatic plants such as *Clerodendrum inerme* (57%), *Gymnema sylvestre* (34.3%) and *Sapindus laurifolia* (30.0%) have the potential to suppress

Copyright applications filed

- Livestock and poultry disease information system (5473/2011-COSW).
- Digital *Pashu swasthya avaum pashupalan prashnottri* (5474/2011-COSW).
- *Pashudhan Avam Kukkut Rog Suchna* (5475/2011-COSW).

Design registrations applied

- Thresher-cum-treatment machine (238547).
- Multi-nutrients feed block making machine (238548).
- Bulk milk feeder for kids (240116).
- Grass cutter (240117).

Patent applications filed

- Novel Sindbis RNA dependent RNA polymerase based Self Replicating DNA vaccine vector for humoral response (160/DEL/2011).
- Attenuated *Pasteurella multocida* with determinant marker (2195/DEL/2011).
- An eco-friendly herbal acaricide to control ticks including acaricide resistant species infesting livestock and pet animals (2196/DEL/2011).
- Pestivirus replicase-based self-replicating RNA-replicon vector for heterologous gene expression in mammalian cells (3805/DEL/2011).
- A novel foot-and-mouth disease viral Asia 1 (Indian vaccine strain) replicon based viral vector for vaccine research and development (3806/DEL/2011).
- A novel ready-to-eat (RTE) salty crisp milk product (Milk Nimiki) (3807/DEL/2011).
- A novel bio-marker based detection of bovine sub-clinical mastitis (3808/DEL/2011).
- Ready-to-cook milk chips (3809/DEL/2011).
- Full-length infectious cDNA clone for Indian vaccine strain of foot-and-mouth disease virus serotype O (IND-R2/75) (626/DEL/2012).
- A rapid, sensitive and user-friendly visual LAMP-based assay for detection of infectious bovine rhinotracheitis (IBR) virus in bovine semen (627/DEL/2012).
- Recombinant antigen based sero-diagnosis of Newcastle disease (628/DEL/2012).
- An essential oil for inhibition of methane emission in buffaloes (methane suppressor) (629/DEL/2012).

methanogenesis. The tannins contained in these plants could be of interest in the development of new additives in ruminant nutrition.

Supplementation of sodium sulphate: Supplementation of sodium sulphate (Na_2SO_4) in the ration of buffaloes reduced methane production. Reductive acetogenesis was higher in the Na_2SO_4 supplemented groups.

Rumen microbial diversity and impact of plant extracts on utilization of fibrous diets: The diversity of fibrolytic bacteria and methanogenic archaea were studied from the metagenomes of buffalo rumen. *Methanobacterium* was observed as the predominant methanogenic archeal genus (79.2%) and the other genera detected were *Methanomicrobium* and *Methanobrevibacter*. Among the fibrolytic bacteria, *Prevotella* was the predominant bacterial genus (27%) and the other major genera detected were *Fibrobacter*, *Clostridium* and *Ruminococcus*.

Plants containing secondary metabolites appeared to have the potential to improve rumen fermentation in positive direction by reducing methane production (29 - 94%) and increasing propionate production (15 - 25%). Extracts of leaves of siris (*Albizia lebbek*), guava (*Psidium guajava*), lasoda (*Cordia dichotoma*), sahjan (*Moringa oleifera*), and fruits of clove (*Syzygium aromaticum*) showed anti methanogenic activity to varying extent in an *in vitro* rumen fermentation system. The reduction potential was highest (94%) in aqueous

extracts of siris leaves and lasoda ripe fruit. Suitable dose level of these need to be decided before use in the diet of ruminants to get maximum reduction in methane production without affecting the feed digestibility.

Sheanut by-products contained about 7 MJ/kg ME and around 12 % CP. The rate of degradation of Sheanut cake (SNC), expeller (SN-E) and solvent extracted (SN-SE) suppressed methane production by 62, 44 and 66 % *in vitro*. There is a great potential for SNC to be incorporated in ruminant feeding not only as a source of energy and protein, but also as an anti-methanogenic compound.

Inclusion of deoiled mustard cake (DMSC) and guar meal @ 5% in complete compressed feed block (CCFB) showed improvement in performance of crossbred calves and reduced methane production (13.75 to 17.57%).

Enhancing shelf life of agro-industrial by-products: Application of mechanical pressure on high moisture content spent grain has positive effect on drying. Greenhouse can also be used for drying high moisture content feeds. The relative drying capacity of milling by-products was graded as de-oiled rice bran > rice bran > wheat bran at different ratio. Microbial mould was inhibited in tomato pomace with urea (0.03 % level; shelf life 3 days) and propionic acid (0.05 % level; shelf life 2 days). Mould growth in spent brewery grain was arrested with urea (0.03 % level; shelf life 4 days) and propionic acid (0.05 % level; shelf life 2.5 days).

Rumen biotechnology

Feruloyl esterase enzyme was purified from modified rumen microbes pet32-FT3-7 BL21 strain. The dialyzed enzyme enhanced the *in vitro* digestibility of finger millet straw.

Sheep

Economic reconstituted milk for lamb feeding: The cost of reconstituted milk to be fed to lambs born to prolific sheep was reduced to nearly half by replacing skimmed milk powder by 3% soy oil and coconut oil by 2% linseed oil.

Feed detoxification trials: Feeding of detoxified neem (*Azadirachta indica*) seed cake replacing soybean meal for five months in growing lambs had no adverse effect on daily gain, feed conversion efficiency, nutrient digestibility, N- balance as compared to soybean meal fed group.

Goat

In goats, studies on detoxified Karanj cake revealed that after removal of karanjin, pongamol and trypsin inhibitors, it can safely replace soybean meal protein moiety up to 50% without any adverse effect on DM intake, nutrient utilization, growth rate, FCE (feed conversion efficiency), metabolic profile, immune response, carcass characteristics and meat quality in kids.

Yak

Vitamin E-selenium: A study carried out to observe the effect of antepartum administration of vitamin E - selenium on reproductive and productive efficiency

revealed — a significantly higher birth weight of the calves; no calf mortality up to 1 month of age; and enhanced milk yield.

Poultry

Antifungal effects of herbal supplementation: Work undertaken to identify herbal products that can prevent the growth of *Aspergillus parasiticus* (NRRL 2999) fungi and aflatoxin production in poultry feed revealed that supplementation of leucas (plant leaves) and *Hemidesmus indicus* (roots) significantly protected against aflatoxin in broilers.

Nutritional value of quality protein maize: Supplementation of the quality protein maize (QPM) in poultry feed proved more beneficial to poultry farmers compared to the conventional maize (CM). The protein, lysine and tryptophan contents were 15.44, 44 and 33% higher, respectively, in QPM than CM. Lysine and threonine digestibilities were significantly higher in QPM compared to CM. Replacement of CM by QPM in the layer chicken diet resulted in 3.3% higher egg production and 4.9% improvement in feed conversion (g egg produced/g feed consumed) compared to normal maize. Eggs laid by hen fed QPM based diet, had darker yolk colour compared to conventional maize.

Physiology and Reproduction

Stem cell research: Bone marrow derived mesenchymal stem cells (BM-MSCs) from bubaline and caprine bone marrow were isolated and characterized according to morphology, growth dynamics, cell surface antigen profile, and differentiation repertoire *in vitro*. Transgenic mesenchymal stem cells expressing green fluorescence protein were generated, which could be further propagated through passaging.

When caprine mesenchymal stem cells after tagging with tracking dye, were transplanted in myocardial infarction in rabbit, it was observed that these cells stayed in the rabbit heart and helped in regeneration.

Cattle and Buffalo

Potential effect on induction of estrus and cyclicity in delayed pubertal heifers was demonstrated by feeding *Aegle marmelos* and *Murraya koenigii* leaves and area specific mineral mixture. Herbal plants showed beneficial effect on follicular development and corpus luteum formation.

Sheep

Egg yolk tris glycerol (EYTG; pH 7.0) is a relatively better semen diluter for ram semen in comparison to egg yolk citrate glycerol (EYCG; pH 6.3) for short term semen preservation (24 hr) as evident by percentage motility, percentage of rapid motile spermatozoa, sperm velocity, sperm head area and beat frequency.

Antioxidants (catalase 100 units/ml and reduced glutathione 10 mM) on fortification of EYCG semen diluter improved the preservability of ram semen in terms of sperm proteins integrity, sperm motion characteristics like percentage motility, percentage of rapidly motile

spermatozoa, sperm velocity, beat frequency and sperm head area.

Goat

Standardized frozen semen technology for AI: The AI procedure is difficult in goats due to complex anatomy of the reproductive tract. The technology of freezing goat semen for AI application was standardized. On the basis of actual kidding, conception rate was 23.33 and 25.92% in Sirohi and Barbari, respectively.

Production of superior germplasm: Using embryo transfer technology, morphologically normal, good quality embryos were flushed from a superior Sirohi goat and transferred to estrus synchronized recipients (2 embryos/recipient) of non-descript goats. On completion of gestation, three surrogate mothers produced four kids (2 male and 2 female) from this Sirohi donor. Earlier kids of Jakhrana and Jamnapari breed were successfully produced using MOET technology; present findings, therefore confirmed and validated MOET technology for use in other breeds as well for multiplication of superior germplasm.

Pig

Use of glucose edate potassium sodium tartarate and sodium citrate dihydrate (GEPS) semen extender that is capable of preserving boar semen for 7 days in liquid state, has popularized use of AI in pigs. Scientists from NRC on Pig have also innovated the technique of AI in sows by sitting on loin simulating the back pressure at service.

Mithun

Birth of first ever mithun calf using ETT: Superovulation in mithun cows was carried out after synchronizing estrus using CIDR and PGF₂α. All the mithun cows were inseminated with cryopreserved mithun semen. Flushing was carried out on day 6 of following estrous cycle followed by searching and evaluation of embryos. Five embryos were recovered and transferred to synchronized recipient mithun cows. Out of the four embryos transferred to the recipient cows, one cow delivered a male calf, the first ever mithun calf produced using ETT.



First Mithun calf born through embryo transfer technology with mother

Technologies Ready for Commercial Transfer

Biotechnology products

- A novel peptide as transfection reagent for protein and nucleic acids
- A novel transfer vector for transferring genes into sheep-pox virus; useful for developing vectored vaccines
- 3 AB protein of foot-and-mouth disease virus expressed in *Pichia pastoris* as a diagnostic tool to differentiate infected animals from the vaccinated (Complete)
- A process for expression of variable surface glycoprotein of *Trypanosoma evansi* in *Pichia pastoris*
- Hybridoma clones for monoclonal antibodies against PPR virus (H and N proteins)
- Live attenuated homologous *Peste des petits ruminants* (PPR) vaccine for small ruminants
- A low-volume saponified haemorrhagic septicaemia (HS) vaccine
- A vero cell based live attenuated vaccine for control of goat pox in goats
- Vero cell based sheep pox vaccine
- Swine fever virus cell culture vaccine
- Aluminum hydroxide gel-concentrated, oil adjuvanted vaccine for FMD

Diagnostics and diagnostic kits

- Monoclonal antibody based sandwich ELISA kit for detection of *Peste des petits ruminants* virus antigen
- Monoclonal antibody based competitive ELISA kit for detection of *Peste des petits ruminants* virus antibodies
- Recombinant antigen based ELISA for diagnosis of animal leptospirosis
- Recombinant yeast expressed VP2 antigen based latex agglutination test for the sero-diagnosis of Infectious bursal disease virus infection
- Diagnostic kit for caprine pleuropneumonia for field use

Herbal drug formulations

- Development of post milking teat dip based on a novel herbal formulation for the prevention of bovine sub clinical mastitis
- Herbo-mineral acaricide formulations against *Boophilus* ticks in cattle
- A process of preparing a bio-organo-mineral formulation for the therapy of skin ailments in animals

Yak

Progesterone estimation in yak blood plasma:

Progesterone hormone is a marker for onset of the cyclic activities of the ovaries in postpartum animals. Antibody microplate coating based competitive enzyme immunoassay was standardized for estimation of progesterone in blood plasma of yak. The detection limit of the assay was 0.4 ng/ml that could measure the minimal blood progesterone levels as found on the commencement of estrus. The assay could also measure up to 10 ng/ml blood progesterone concentrations as exhibited during the luteal phase of estrous and in pregnancy.

Poultry

Providing artificial lighting at the poultry houses using longer wavelength lights (red spectrum- 675 nm) than

incandescent (425 nm) lights improved egg production in commercial layer flocks by about 8%.

Enhancing egg production also warrants improved egg shell quality since increased ovulations should not interfere with the time spent on deposition of egg shell in the uterine shell gland. Zinc oxide fed at 2% level to layer hens of 70 weeks of age for 10 days resulted in molting due to a reduction in feed intake and duodenal calcium uptake.

Tolerance to tropical stress: Naked neck broiler CARIBRO- Mritunjai exhibited better tolerance to tropical stress than CARIBRO-Vishal (normal plumaged), and CARIBRO-Tropicana (naked neck and frizzle plumaged).



CARIBRO-Mritunjai (Naked neck) exhibited better tolerance to tropical stress

Livestock Protection

Vaccines and RNA interference

Pox vaccines: Long-term immunity trial of sheep pox vaccine revealed that the vaccine was protective against challenge with virulent virus even after 2 years of vaccination in sheep.

Haemorrhagic septicaemia (HS): New generation mutant vaccine for HS, an *aroA* deletion mutant of *Pasteurella multocida* was developed as potential vaccine candidate.

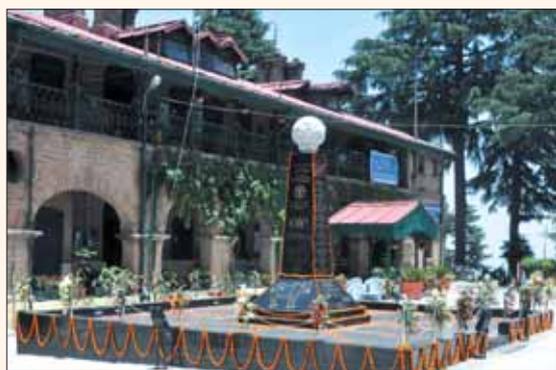
Rabies: RNA targeting rabies virus (RABV) nucleoprotein (N) gene delivered using replication-defective adenoviruses and lentiviruses effectively inhibited RABV multiplication *in vitro* in BHK-21 cells and *in vivo* in mice.

Disease diagnostics

IBR: Loop-mediated isothermal amplification (LAMP) test for the rapid detection of IBR virus in bovine semen was developed. The test had the sensitivity of 10 fg viral DNA or 0.2 TCID₅₀/0.4 infective virus particles per reaction.

Freedom from rinderpest

The freedom from rinderpest globally is a tribute to the skill and efforts of the veterinarians and researchers who fought rinderpest in remote and difficult terrain in various parts of the world. Eradication of rinderpest in India has increased livestock productivity and enhanced the food security and livelihood of livestock owners. The voluminous research work of scientists of IVRI, Mukteswar, over several decades has helped eradication of this deadly disease from India and globally also. A commemorative ceremony of Rinderpest global eradication was organized at IVRI, Mukteswar, Uttarakhand on 2 June 2012 by unveiling the memorial pillar at IVRI Mukteswar Campus. The OIE certification was awarded, which is recognized as a prestigious international honour for notable contribution in achieving zero Rinderpest status for India (2011).



PPR: LAMP assay for rapid detection of PPR virus from clinical sample was standardized.

Pox viruses: A TaqMan hydrolysis probe based real time PCR (rt-PCR) assay targeting the ankyrin repeat protein (C18L) gene sequences was developed for the detection and quantification of camelpox virus (CMLV) nucleic acid and compared with conventional methods. The assay was specific with an efficiency of 99.4%.

Bluetongue: A sandwich ELISA was developed for detection of BTV antigen in blood, tissue materials and culture fluid.

Newcastle disease: Recombinant nucleoprotein based single serum dilution ELISA kit for serological profiling of Newcastle disease virus was developed.

Salmonellosis: LAMP test was standardized for specific detection of *Salmonella* Enterica subsp. enterica serovar typhimurium.

Coccidiosis: Multiplex PCR based on SCAR markers and ITS-1 based nested PCR for species level identification of *Eimeria* oocysts of poultry were standardized.

Greasy pig disease: This disease caused by *Staphylococcus hyei* is responsible for considerable degree of piglet mortality. A PCR method for its rapid detection in pigs was developed.

Pasteurellosis: A PCR method for the rapid detection of *Pasteurella multocida* from pigs was standardized for screening of pigs against pasteurellosis.

Exotic diseases

Avian influenza: High Security Animal Disease Laboratory, Bhopal is designated as OIE Reference Laboratory for avian influenza. Highly pathogenic avian influenza virus (H5N1) was recorded in crows (Jharkhand, Maharashtra, Odisha, Bihar, Uttar Pradesh and Tripura) in addition to chicken and ducks. A total of 86 H5N1 viruses from nine states (Assam, West Bengal, Bihar, Jharkhand, Maharashtra, Meghalaya, Odisha, Uttar Pradesh, and Tripura), six H9N2 from Odisha, Gujarat and Maharashtra and one each of H11N6 and H4N6 viruses (Jharkhand) were isolated. The phylogenetic analysis of HA genes indicated that the 2011-2012 H5N1 viruses belonged to clade 2.3.2.1 whereas 2008 viruses belonged to clade 2.2 H5N1 viruses.

Malignant catarrhal fever: Malignant catarrhal fever was diagnosed in a Wild bison from Zoo at Bengaluru and cattle from State Veterinary Hospital at Bengaluru.

Crimean-Congo hemorrhagic fever virus (CCHFV): Molecular Beacon based real-time PCR assay for detection of Crimean-Congo hemorrhagic fever was developed. CCHFV was detected in ticks collected from a cattle calf from Vadnagar Taluq, Mehsana District of Gujarat. Phylogenetic analysis of 'S' segment sequence of CCHFV revealed that the Indian isolate is closest to Tajikistan virus (AY049083/TAJ/HU8966) falling in Asia 2 group of the CCHFV.

Veterinary biologicals

Doses of vaccines, antigens and serum were produced, quality tested and supplied to various organizations. Details are as follows.

Vaccines — Ranikhet disease 'F' strain (159,000); R₂B (3,200); fowl pox (4,200); lapinized swine fever (352,565); cell culture sheep pox (966,000); PPR (8,186,100); *Brucella abortus* strain-19 (59,983); enterotoxaemia (1,000); HS adjuvant (6,000 ml); tuberculin PPD (79,290); Johnin PPD (44,120); mallein PPD (27,500) were produced.

Antigens— 98,000 ml of *Brucella* agglutination test; 67,240 ml of *Brucella abortus* Bang ring; 22,620 ml of rose Bengal plate test; and 4,560 ml of *Salmonella* Pullorum coloured and 3,000 ml of plain were produced.

Serum— 2,000 ml of *Salmonella* Abortus equi 'H'; 86 ml of *Brucella abortus* positive serum; 7 ml of *Salmonella* Pullorum positive serum, were produced.

Herbal medicine

Sheep: EO-S1 and EO-S2, herbal formulations, showed marked efficacy against clinical infection of *Haemonchus contortus* in sheep.

Diarrhoea in goats: An anti-diarrhoea powder (Herbodoin) was developed by mixing the extract of three native plants on the basis of chemical compatibility and without antagonistic effect. This product was effective in management of diarrhoea @ 10mg/kg body weight. This herbal product helped in reducing the dependence of antibiotic in management of diarrhoea and helped to overcome the problem of drug resistance.

Success story

Validation of recombinant protein (p26) based AGID/ELISA for equine infectious anaemia (EIA)

Control of EIA is based on identification of in-apparent carriers by detection of antibodies to EIA virus (EIAV) by internationally accepted standard serologic tests, generally the agar gel immunodiffusion (AGID) test. To ensure the continuous availability of diagnostic reagents and to serve as a national resource for its availability to disease diagnostic laboratories for sero-monitoring of EIA, recombinant p26 protein based ELISA and AGID assay were developed. Under OIE Training Collaborative programmes at NRC Equines, three proposals, viz. Equine influenza, Glanders and Equine piroplasmiasis were initiated. The OIE has approved these projects to strengthen the infrastructural capabilities at the institute and also enhance the technical accumen of the persons engaged in research in these areas. The collaborative research initiative will help in designation of the said laboratories as Reference laboratory in equine influenza, glanders and equine piroplasmiasis. One workshop under the programme was organized in December 2012 for SAARC personnel.

Surgical and clinical interventions

Venereal tumour in street dogs, regressed with use of recombinant VP3 gene construct of chicken infectious anaemia virus.

Four unique horn cancer specific ligand sequences were identified using phage display method for developing horn cancer specific peptide nano-delivery vehicle.

Epidemiology and surveillance of diseases

Livestock disease database: The livestock disease database at PD_ADMAS has 90,104 records at present. As per the analysis of cumulative disease data HS, FMD and babesiosis were ranked as the top reported diseases, while earlier HS, PPR and ET were the most reported diseases. During the year under report anthrax and haemorrhagic septicaemia were selected for detailed analysis.

Anthrax disease records available in National Animal Diseases Referral Expert System (NADRES) from 1991 to 2010 were subjected for epidemiological analysis. During this period anthrax was reported in eighteen states, viz. Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamil Nadu, and West Bengal. Although there is a constant variation in the percentage of states reporting anthrax (calculated as percentage of total states reporting disease data), overall increase was seen 1999 onwards which might be due to the gradual spread of the disease. The occurrence of anthrax, however, showed a gradual increase in the number of outbreaks with a peak between 2000 and 2002. From 2002 onwards, there is a gradual decline in the occurrence of anthrax. This could be attributed to

awareness amongst the farming community and the control measures taken up by the authorities.

Haemorrhagic septicaemia (HS) occurred in all the six zones of the country. Though there was a lot of variation in the number of outbreaks reported across the years in different zones, a peak was seen every three years and trend of HS occurrence seemed to follow a similar pattern in south, west and central zones. Among the states of south zone, Tamil Nadu and Kerala have effectively contained the disease with proper vaccination and most of their districts fall under medium or low HS pathozone. There was an overall decrease in the HS outbreak reports in the different states of the country during 2001–10 compared to 1991–2000.

Serological studies conducted to determine the prevalence of *Peste des petits ruminants* (PPR) in cattle and buffaloes indicated 4.58% prevalence of PPRV antibody in different parts of Southern peninsular India.

Phylogenetic analysis of 52 *Leptospira* isolates based on *rpoB* gene sequences revealed that 31 isolates belong to either *L. borgpetersenii* or *L. interrogans* species, 13 to *L. inadai* subgroup or subspecies and 8 to *L. kirschneri* species. The overall observed prevalence of *Leptospira* species, viz. *L. borgpetersenii*/*L. interrogans*, *L. inadai* and *L. kirschneri* in animals and human are 59.62, 25 and 15.38%, respectively.

Brucellosis screening of random sera samples from cattle (1,056), buffaloes (332), sheep (180), goats (828) and swine (307), received from 10 AICRP centers, revealed that 12.46% samples were positive in ELISA.

The database of the Directorate has large number of data on outbreaks/cases of parasitic diseases of livestock. Among cattle, buffaloes, sheep and goats, ascariasis appeared as the highest occurring parasitic disease followed by strongylosis, fascioliasis, babesiosis, amphistomiasis, trypanosomiasis, theileriosis; and trypanosomiasis caused highest mortality.

Under the bovine mastitis project (NAIP funded) of the 117 different samples, a total of 174 staphylococci were isolated with predominant being coagulase negative methicillin resistant staphylococci. Of these, the various *Staphylococcus* spp. including *S. sciuri* (n=61), *S. epidermidis* (n=47) followed by *S. aureus* (n=40) were identified.

Molecular archiving of *Brucella melitensis* from goats: Molecular archiving of *Brucella melitensis* strains was done using phylogenetic tree, genetic relatedness and amino acid sequence studies. Molecular archiving was based on seven *Brucella melitensis* genes, viz. omp31, omp16, omp25, omp2b, BP26, L7/L12 and 16s rRNA.

Cutaneous papillomatosis in yaks: Papillomatosis, caused by BPV-1 and BPV-2, is reported in cattle and buffaloes from India. Cutaneous warts were observed in field and farm reared yaks. Papillomatosis was confirmed by histopathology, and presence of BPV-1 and BPV-2 was confirmed by PCR. BPV-1 and BPV-2 were detected for the first time in Indian yaks.

First detection of zoonotic parapoxvirus (pseudocowpox) in cattle: Parapoxvirus was detected by semi-nested PCR and PCR-RFLP targeting B2L

envelop gene in samples collected from cattle and a human from Modipuram area in Meerut in Uttar Pradesh. Lesions were observed only in milking cows with vesicles, scab and ulcers on udder and teats. Clinical samples were collected from the affected animal and human cases.

Whole genome sequencing

Japanese encephalitis virus: To decipher the genetic characteristics, the complete genome of the Japanese encephalitis virus (JEV) strain JEV/eq/India/H225/2009(H225)/ isolated from an infected horse in India was sequenced and compared to previously published JEV genomes. The comparison of amino acids associated with neurovirulence in E protein and non-structural protein, with known virulent and attenuated JEV strains, suggested H225 to be highly virulent strain. This is the first report of whole genome sequencing of genotype III JEV genome isolated from equines. The study confirmed that JEV genotype III is circulating among equines and is associated with clinical cases in equines in India.

***Pasteurella multocida* sub spp. multocida B:2 serotype:** Many isolates of *Pasteurella multocida* are available in the VTCC, Hisar repository. To strengthen the passport information of the isolates, the genome of a novel pathogenic B:2 serotype of *Pasteurella multocida* isolated from a buffalo in an outbreak of haemorrhagic septicaemia was sequenced. Previously, 3 isolates of *Pasteurella multocida* were isolated from that outbreak, and confirmed biochemically as well as by PCR. The isolates were accessioned in repository with the accession nos. VTCCBAA264, VTCCBAA265 and VTCCBAA266. The sequence analysis revealed that the genome of *Pasteurella multocida* (VTCCBAA264) consisted of 207,386 bp nucleotide sequence distributed in 953 contigs.

Veterinary type culture collection: The VTCC repository presently has 546 accessioned veterinary microbes including 440 bacterial and 106 viral cultures along with 180 accessioned recombinant clones.

Foot-and-mouth disease

Foot-and-mouth disease (FMD) research in the country is being carried out at the PD-FMD, Mukteswar which includes epidemiology, diagnosis and surveillance. PD-FMD has been designated as FAO Reference laboratory for FMD. It is a leading diagnostic laboratory in the region; and has imparted four trainers' training programmes for SAARC participants as part of FAO Reference Laboratory functioning. During this period, 335 outbreaks were recorded. Maximum outbreaks were reported from Southern region followed by Eastern, Western, North Eastern and Central regions. Serotype O caused maximum outbreaks (69.6%) followed by serotypes Asia 1 (25.4%) and A (5%). The outbreaks occurred round the year with maximum occurrence from October to March. Serotype O was the most prevalent in all the geographical regions except western region where, serotype Asia 1 dominated the scenario with almost 83% prevalence.

Landscaping of FMD virus: Phylogenetic analysis of serotype O virus showed that 'Ind2001' strains, which re-emerged in later part of the year 2008, out-competed PanAsia lineage in causing outbreaks in the country. During the year, a new group of virus named 'Ind2011' with more than 11% mean nt. divergence from rest of the lineages emerged in the southern region. These virus strains first emerged probably in September 2011 and rapidly spread to Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. Antigenic evaluation relative to vaccine strain indicated that the new lineage is antigenically close to vaccine strain. In serotype A, the isolates clustered within the genotype 18, but grouped both in the non-deletion and the Clade 18c of VP3⁵⁹-deletion lineage. The Asia 1 field isolates were of lineage C reiterating the supremacy of this lineage since 2005. This has been a very stable serotype.

Some of the isolates in VP3⁵⁹-deletion group of genotype 18 showed low r-value in routine vaccine matching exercise. In the initial screening, antigenic analysis of 19 serotype A field isolates with the alternate vaccine candidates and currently used vaccine strain, IND40/2000, and previous vaccine strain, IND17/1982, was carried out. The study identified a panel of 2 candidate vaccine strains from genotype 18 as potential alternate vaccine candidates for use in case of necessity/emergency.

Under National FMD Serosurveillance, 39,434 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 regardless of vaccination status. The test revealed overall DIVA positivity of ~ 27% in the country during 2011-12, similar to the previous year.

FMD control programme: Under this programme 47,510 pre- and post-vaccinated serum samples were tested and of these 24,970 serum samples were from first phase FMDCP districts representing XI, X, XI and XII phases of vaccinations. Remaining 22,540 serum samples were from expanded FMD CP districts representing Phases I and II of vaccinations.

Under collaborative programme entitled "Antigenic and genetic characterization of foot-and-mouth disease viruses in India: Application to effective molecular vaccines", replication defective human adenovirus serotype 5 (hAd5) based FMD vaccine was produced using Indian vaccine strains. Such vaccines are thought to be safe to produce, also stable and can elicit both humoral and cellular arms of immune response.

Fish

Replacement of fish meal in fish feed: The study on the effect of replacement of fish meal with chicken waste meal (CWM) indicated that CWM is a potential ingredient in feed for seabass and it can be included up to 5–10% in replacing fish meal. Similar studies with corn protein concentrate (CPC) indicated that it can be included up to 15% in the diet without affecting growth of seabass fingerlings. Assessment of effect of replacement of fish meal with corn gluten meal (CGM)

indicated that this ingredient could be included up to 10% in the diet of seabass fingerlings. Incorporation of CGM and meat and bone meal (MBM) @ 7.5% each by replacing 15% of fish meal, along with individual supplementation of lysine @ 0.3% and methionine @ 0.15% and also in combination, resulted in superior growth compared to feeds without any supplementation.

Mullet diet prepared with locally available ingredients (rice bran, mustard oil cake, sunflower cake, sesame cake, leucaena leaf meal, and azolla) was fermented with three potential gut bacteria that had been isolated and characterized. The studies revealed that the fermented ingredients could replace 75% of fish meal in diet of *Mugil cephalus* without affecting growth rate, FCR, PER and survival. Supplementation of live bacterial mixture of the three bacteria also improved growth rate, FCR and PER.

Field testing of improved pearlspot feed: Improved pearlspot feed was evaluated under cage culture. The results revealed that animals attained 21.1 ± 3.6 g at the end of 58 days of rearing with FCR of 1.6. This is an improvement over the growth observed under cage culture practiced by farmers where it takes three to four months to reach a size of 20 g from 2 g.

Managing fishery resources through pop-up satellite tags (PSAT): India joins the elite group of countries engaged in satellite tracking of yellowfin tuna (*Thunnus albacares*) a migratory fish. Movements of yellowfin tuna in oceanic waters near and away from Indian waters remain untested and exchange rates are still unidentified. Tagging was done to study growth and migration of marine fishes. Tuna, mainly the bluefin tunas, are tracked using tags in the Atlantic Mediterranean and Australian waters but not in Indian waters. To know about the telemetry studies on migration patterns of tunas in Indian sea, the tagging programme funded by INCOIS under the project entitled "Satellite Telemetry studies on migration patterns of tunas in Indian sea" (SATTUNA) was taken up by the CMFRI. The tags are attached externally and have a release mechanism that causes the tag to detach from the fish at a predetermined time and "pop-up" to the sea surface where the data can be recovered via the ARGOS system aboard polar-orbiting NOAA satellites, therefore provide fisheries-independent



Tuna with pop up satellite tag

Success story

Feeds for fish growth

Feeds developed by CIFA for rearing of *Pangasius fry* (Pongas Grow I) and fingerlings (Pongas Grow II), were successfully tested in farmers' pond. These feeds resulted in over 80% seed survival when fed to fry and fingerlings, and were found suitable for fish growth.



measure of the straight-line distance traveled from the point of tagging. A total of 15 tags were used in two phases along the Bay of Bengal and the Arabian Sea with the Pop-up time ranging from 4 months to a year. Tagging was done in the Bay of Bengal Region from Visakhapatnam and along the Arabian Sea off Lakshadweep.

Potential bacterial pathogens in rainbow trout: Potential pathogenic bacteria profile of rainbow trout mainly cultured in North western Himalayan states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand, was investigated for better maintenance of the stock. The bacterial samples were collected from diseased or moribund rainbow trout. *Aeromonas hydrophila*, *A. veronii*, *A. soberia*, *Pseudomonas lurida* and *Streptococcus* spp. were isolated and identified. In bacterial infection the most affected organ was kidney, where degradation of kidney tubules and massive infiltration of mononuclear cells were observed. All the other organs were found moderately affected. The isolated bacteria were sensitive to most of the antibiotics used in aquaculture. Total 18 isolates of fish pathogenic bacteria were stored in glycerol stock for long-term preservation, after assigning a laboratory strain name.

Horizontal transmission and infectivity of WSSV: Protocols for concentration and enumeration of white spot syndrome virus (WSSV) in water and sediment were developed to comprehend the horizontal transmission of WSSV in aquaculture ponds and study the duration of infectivity of host-free WSSV virions. Efficacy of recovery from water by tangential flow filtration (TFF) was 100% (at all times WSSV could be recovered) and 7.5 to 51.6% of WSSV was recovered. Virus enumeration by epifluorescence microscopy was standardized. The viral counts ranged from 1.6 to 4.9×10^7 /ml in ponds, 1.3 to 1.5×10^5 /ml in hatcheries and 3.3 to 4.2×10^9 /g in sediments. Role of bivalve mollusks in the removal of WSSV was examined by using *Meretrix meretrix*. The virus could be detected up to 48 h by nested PCR and quantitative real time PCR. Clam faecal matter and tissue were devoid of the virus.



Mass spectral libraries of antibiotics: Mass spectral libraries of commonly used antibiotics used in aquaculture were developed for rapid screening of these antibiotics and its metabolites in muscle, water and sediments. Mass spectral library for histamine was developed for rapid identification and low level screening of this biogenic amine.

m-Krishi[®] fisheries service: Mobile service, m-KRISHI[®]-Fisheries, was developed to disseminate potential fishing zone (PFZ) and wind advisories to fishers in local language in public private partnership (PPP). Satellite data on thermal and wind speed as well

as algal movement were utilized to provide exact location of PFZ areas to reduce excessive fuel consumption in search of fish.

Current services available on the prototype are: Potential fishing zone (PFZ) advisories generated from INCOIS website regularly; Oceanic wind speed and direction advisories generated from INCOIS website four times daily; Improvised early warning advisories of wind speed and direction for a period of 5 days in advance; Regional wind speed and direction advisories generated from IMD website daily; and Early warning system (weather alerts/storm warnings).

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