

9. Livestock Management

Livestock Production

Areca sheath: Areca (*Areca catechu*) is a commercial crop and the fallen areca sheath is a good source of dry roughage to animals. The nutritional value of areca sheath is similar to paddy straw (CP, NDF, ADF) with an advantage of having less lignin, silica and more calcium, sulphur and copper. Feeding of areca sheath to sheep and dairy animals replacing paddy straw could be an alternate approach.

Lignin degradation: Activities of lignolytic enzymes produced by immobilization on areca sheath recorded temperature and pH stabilities over a wide range, thus making it desirable for supplementation in animals.

Feed additives: Out of the 44 plant products, viz. seed pulp (5), leaves (20), spices (13), citrus fruit peel (3), oils (3), and 23 plant combinations, screened for their anti-methanogenic activity using *in vitro* gas production test, a mixture of plant products and yet another combination of four plants appeared to have good potential as feed additives in the ruminants. Use of nitrate reducing bacteria as probiotic exhibited 80 to 99% reduction of *in vitro* methane emission. There was no accumulation of hydrogen, nitrate or nitrite in the incubation medium, and no adverse effect on *in vitro* true digestibility of feed.

Supplementation of Chicory root powder, *Lactobacillus acidophilus* as symbiotic in the diet of dogs proved beneficial in terms of fibre utilization, calcium and lipid metabolism, hind gut fermentation and cell-mediated and humoral immunity.

Helianthus tuberosus L. *Jerusalem artichoke* *Hathi choke*; a novel phyto-genic-origin probiotic, showed immense potential for use in the diet of dogs for enhancing their digestive health, lipid profile and immunity.

Unconventional feed: Detoxification procedures for jatropha, *karanj* and neem seed cakes were standardized in an industrial process.

In growing lambs, replacement of soybean meal with detoxified jatropha cake up to 37.5% showed no adverse effect on DM intake, nutrient utilization, blood-biochemical profile, growth rate and feed conversion efficiency.

Nutritive value of *Robinia pseudoacacia* in terms of high organic matter, crude protein and low lignin content was comparable to *Grewia optiva*, and showed higher nutritional values than other tanniniferous forages.

Combined treatment with curry and bel leaf powder had positive effect in restoration of cyclicity and fertility in acyclic goats and buffaloes.

Chelated minerals: Cell-mediated immune response was significantly higher in kids given organic zinc (40

SUCCESS STORY

Use of dietary cation anion based mineral mixture

Initiation of lactation causes one of the greatest stresses on Ca homeostasis and is associated with milk fever among high producing dairy cows and buffaloes. Hypocalcemia also increases the incidence of dystocia, retained placenta, metritis, prolapsed uterus and delays uterine involution. During dry period, Ca requirement is 10–12 g/day (fetal and endogenous Ca), which increases to 10 times more than the supply in bloodstream upon parturition. An anionic mineral mixture was fed to pregnant cows few days before parturition so as to create metabolic acidosis and initiate Ca resorption immediately after calving. This anionic diet (12 meq/100g DM) resulted in zero incidence of mastitis and milk fever as compared to 14.3% in cationic diet (15 meq/100g DM) fed cows. The incidence of metritis was also zero against 71.43% cationic diet fed cows. Above all, there was increase of 1.13 kg milk on 4% FCM basis during 90 days postpartum period in anionic diet fed cows resulting in great economic gains.

ppm zinc as zinc methionine) as compared to zinc sulphate.

Nutritional manipulation: Feeding growing Frieswal bull calves with three different levels of rumen degradable protein and rumen undegradable protein for achieving a targeted body weight gain of 700 g and 1,000 g/day, yielded only 608 g and 772 g of average daily gain suggesting that there is a need to develop our own feeding standards for higher body weight gains.

Complete pellets: Loin eye area increased linearly with increased level of concentrate mixture in different supplementation group. Kids fed diet without any concentrate mixture had lower yield of separated lean, fat and bone content. Different levels of supplementation improved meat bone ratio of Barbari kids under intensive system.

Yak

Two complete feed blocks (CFB) were prepared based on tree leaves (50% tree leaves, 43% concentrate and 7% molasses) and paddy straw (50% paddy straw, 43% concentrate and 7% molasses). The result indicated that tree leaves based CFB supplemented with limiting micronutrients can be fed during winter to overcome the weight loss in yaks.

Poultry

Feeding schedules for poultry: In PB-1 females, ME restriction was successful in achieving the desired body weight (83 g/week and 5.75% growth rate) at 20 weeks of age. *Karanj* cake in the diet adversely

affected Krishibro chicks, but feeding of detoxified cake improved the performance.

The production performance of PB-2 line was better with organic Zn supplementation at 60 ppm and hatchability at 80 ppm. Trace mineral retention in tissues of broiler chicks was linearly related to their level in diet.

Herbal antifungal compounds, viz. *Piper longum*, *Ferula assafoetida* and *Cymbopogon citratus* oil were identified as potent antifungal herbal compounds against *Aspergillus parasiticus* in poultry feed under *in vitro* conditions.

Quality protein maize in diet: Dietary replacement (50 to 100% w/w) of normal maize (higher in lysin and tryptophan by 44 and 33%, respectively, over normal maize) resulted in 6.93% higher body weight gain, 7.69% improvement in feed utilization efficiency and 5.15% higher breast meat yield in broiler chicken.

Amelioration of heat stress in broilers: Amelioration of heat stress in broiler chickens was achieved through supplementation of vitamin C and potassium chloride in diet. Heat shock protein 70 (HSP70) was detected in muscle and blood lymphocyte in birds as potential marker of heat stress.

Reproduction

Cattle

Good quality fresh and post-thaw semen of crossbred bulls had significantly higher percentage of HOST positive spermatozoa with low LDH and GOT enzyme leakage as compared to poor quality semen.

Frozen semen doses (166,610) of Frieswal bulls were produced and 73,205 doses were distributed that included 50,725 to military farms, 9,000 to field progeny testing project, and 13,480 to various developmental agencies and farmers for cattle improvement.

Buffalo

Buffalo sperm membrane is highly prone for lipid peroxidation as compared to cattle, and in a study on nutrition and **male fertility**, it was observed that PUFA feeding may reduce lipid peroxidation and improve frozen semen fertility.

Studies on **nutrition-endocrine interactions** in attainment of puberty in female buffaloes showed that supplementation of higher dietary energy (through bypass fat) has a positive effect on early maturity of hypothalamo-pituitary—ovarian axis in terms of early and better LH secretion and ovarian follicular activity in female buffalo calves.

Studies on **physiological reasons for low reproductive efficiency** and early embryonic mortality in buffaloes showed that antioxidant enzymes, Cu and Zn-SOD are induced significantly during early pregnancy and may be important for overcoming oxidative stress that otherwise would lead to early embryonic mortality. LPS and TNF α appear to modulate prostaglandin production in endometrium and corpus luteum and are important for protecting and enhancing

SUCCESS STORY

Enhancing the fertility in buffaloes of Tarai, Uttar Pradesh

- Anoestrus conditions in mature heifers and post partum buffaloes were effectively treated with success rate of more than 70% by de-worming animals with broad-spectrum anthelmintics, gynaecological examination at regular intervals followed by administration of either (i) GnRH + PG + GnRH or (ii) progesterone + PMSG, or (iii) PG using their recommended protocols.
- Immunomodulators such as neem oil, *Escherichia coli* LPS, blood plasma or garlic extract were used to recover most of the endometritic buffaloes using recommended protocols instead of conventional therapy considering economic viability, eco-friendly and easy availability of these agents.
- Under limited resource conditions, the animal feed can be supplemented with 40 to 50 g of mineral mixture daily for 4 to 8 weeks by the farmers for oestrus induction followed by conception in moderate number of buffaloes.

function of corpus luteum to prevent early embryonic mortality. IGF-I also stimulated progesterone secretion from the corpus luteum.

Five proteins, specific to uterine secretions of pregnant buffalo were recognized and it may be possible to use one of these for **early pregnancy diagnosis** to monitor pregnancy and prevent early embryonic mortality.

Goat

Study on effect of dextran, a new **cryoprotectant**, revealed that dextran alone did not prove to be an efficient cryoprotectant, but 3% glycerol dextran in combination with 6% glycerol resulted in 39.33 \pm 1.45% post-thaw recovery.

Hormone delivery system for **synchronization of heat** in goats was standardized by using sponges of different sizes and shapes. The estrus response, onset of estrus and duration of estrus following use of intra vaginal sponge pessaries for induction of multiple birth during peak breeding activity were 100%, 29.14 \pm 4.42 h and 25.71 \pm 0.37 h, respectively, with 57.14% conception rate. The kidding rate following induction of multiple birth was 2.0. During low breeding activity season (summer) the estrus response, onset of estrus and duration of estrus were 71.42%, 60.0 \pm 16.54 h and 26.4 \pm 11.63 h, respectively, following use of intra vaginal pessaries.

Oocytes were recovered by **follicle puncture technique** for *in vitro* maturation, fertilization and culture from goat ovaries. The average recovery of oocytes using follicle puncture was 1.76/ovary. The oocytes were cultured in tissue culture medium (TCM-199) supplemented with EGF (10 ng/ml) and fertilized *in vitro* with a cleavage rate of 41.55%. Embryos were bisected through microtools. Out of 25 bisected cells 3 single cells divided into double cells whereas one bunch of 3 cells developed into 4 cells.

Artificial insemination in camel

Semen collected from camels twice a week and extended immediately after collection using freshly prepared Tris egg yolk citrate extender, was used for single insemination in she camels. The insemination was done at 27–28 h post injection of Pubergen HP, which was used for inducing ovulation. Two camels confirmed as pregnant, this is the first report of successful pregnancy from artificial insemination done with extended semen in Indian dromedary camels.

Adaptability study on goats revealed that water intake of goats kept under asbestos sheet roof sheds was significantly higher compared to the thatch roof shed during both the moderate and cool seasons. Milk production of animals kept inside the thatch roof shed was significantly higher than the animals inside the asbestos roof shed.

Camel

Physiological parameters (rectal temperature, respiration rate, pulse rate) were higher during evening as compared to morning. The morning and evening variation in the THI value was significant for all weeks in different months from October to March. The **Benezara coefficient of adaptability (BCA)** of camel revealed that BCA was significantly higher during evening as compared to morning.

Non-pregnant female camels were divided in groups A, B, C. Each group was tested with poll gland secretion collected in breeding season (PGS-BS), **poll gland secretion** collected during non-breeding season (PGS-NBS) and water. The results indicated that the PGS-BS evoked greater olfactory, contact and flehmen response than PGS-NBS and water.

Nine female camels, which did not conceive during breeding season were given injection of highly purified hCG for **infertility treatment** at the time of mating. Pregnancy was diagnosed in three out of nine camels.

Yak

On application of the vaginal sponge containing 600 mg progesterone for **synchronization of oestrus** followed by AI in yaks resulted in 100% oestrus response with 60% conception rate.

The yaks do not experience cold stress (**environmental stress**) during winter but they are subjected to heat stress (during summer) when the ambient temperature exceeds 10°C at the altitude of 2,750 m above mean sea level. The body weight gain was significantly higher during summer than winter in adult yaks. The lactating yak cows lost about 5.84% of their body weight during winter along with reduced milk yield mainly due to shortage of feed and fodder. Estrous was displayed year round but the highest incidence (around 65%) was restricted to warmer part of the year.

Physiological responses, viz. rectal temperature, respiration and pulse rates of yak calves, bulls and lactating cows were recorded during winter and summer. The physiological responses of calves were significantly

higher than bulls and lactating cows during summer. Standardization for **heat shock protein 70 (Hsp 70)** assay was carried out in yaks.

Mithun

Different protocols for **preservation of mithun semen** indicated that it is possible to preserve mithun spermatozoa at refrigeration temperature in tris-egg yolk diluents till 108 h, and doubling the concentrations of fructose in the diluent buffer increased the progressive motility, live sperm count and decreased total sperm abnormalities. Hence, addition of extra-energy source (fructose) might increase the storage time of mithun sperm at refrigerated temperature up to 5 days.

Dentition patterns of mithun

Dentition pattern of deciduous teeth of calves was studied for determining the age of mithun. In mithun full mouth condition comes only when the animal is six years or more than that as against four years in cattle. The first pair of permanent incisor erupted from 22 months to three years, whereas two pairs of permanent incisors erupted in between three and four years of age. The third pair of permanent incisors erupted between four and six years of age.



Mithun buccal cavity showing four permanent incisors and fifth permanent incisor eruption

Pig

Gloved hand method of semen collection was standardized. Semen preservation was done for Hampshire, Duroc and Ghungroo pigs for first time in Asom. The AI technology was used in the farm and in the nearby villages and highest litter size (15 piglets) at birth was recorded from a Ghungroo sow in the farm.

Poultry

Exposure of birds to 650 nm (red) and 475 nm (blue) wavelengths of **light improved egg production** by 6.55 and 3.05%, respectively, compared to 450 nm (incandescent) light.

Livestock protection

Foot-and-mouth disease: During the period under the report, 799 outbreaks were recorded throughout

Global FMD Research Alliance

The Project Directorate on FMD is designated as FAO Regional Reference Laboratory for South Asia. As member of Global Network of FMD Reference Laboratories, the institute participated in inter-Laboratory Comparative Testing exercise for FMD vaccine matching. The results obtained by the institute are one of the best in the world. Collaborative programme with USDA-ARS on “Effective Molecular Vaccines against Foot-and-Mouth Disease” has been finalized under Global FMD Research Alliance (GFRA). FMD sero-monitoring and sero-surveillance in the SAARC region is being initiated through a FAO programme “Diagnostic Laboratory Network Coordination for FMD surveillance and vaccine evaluation in South Asia”.

the country. Maximum outbreaks were recorded in the eastern region where there was increase in the number of outbreaks compared to last year. Disease incidence in West Bengal directly affects the situation in NE region due to animal migration. Drastic reduction in outbreaks was noticed in southern region. FMD cases were not reported in Tamil Nadu, whereas Himachal and Punjab recorded a single case of FMD each. Maximum incidence of disease was reported in March and from August to November.

Out of 1,624 clinical specimens collected from 799 outbreaks, the virus could be detected in 1,067 samples (serotype O-991, A-38 and Asia 1-38) by using sandwich ELISA and multiplex PCR. Serotype O was responsible for maximum number of outbreaks (568) followed by A (33) and Asia 1 (9). Increase in serotype O incidence was noticed in all regions except northern and western region. Serotype A, which was absent in northern and central region last year appeared this year, making it the second most prevalent serotype affecting geographically all the regions of India. Serotype Asia 1 was completely absent in southern, central and north eastern regions. Incidence of Asia1 serotype increased in western region. Though majority of the outbreaks involved cattle, disease was also reported in buffaloes, pigs, sheep and goats.

A major shift was observed in genetic lineage of serotype O viruses circulating in India. The ‘Ind2001’ lineage viruses gained upper hand after a gap of 8 years and outcompeted PanAsia II lineage. The cases due to PanAsia II, PanAsia 1 and Branch C-II viruses were not completely absent during the year. Branch-C II lineage viruses responsible for outbreaks during 2008 in West Bengal were recorded in Tripura, Assam, West Bengal and Odisha during 2009–10. In serotype A, it was observed that both VP3⁵⁹ deletion and non deletion mutants belonging to genotype VII are co-

circulating in the field in recent times. Precisely, the viruses from Punjab and Uttarakhand shared ancestry and clustered in the same VP3⁵⁹ deletion lineage, VIIf, whereas viruses from two different outbreaks from Odisha and those from Andhra Pradesh clustered together and revealed no deletion in the VP3 coding region. During the period, outbreak due to Asia1 serotype was recorded in Gujarat, Madhya Pradesh, Maharashtra, Uttar Pradesh and West Bengal. The Asia 1 field isolates were grouped with lineage C reiterating the supremacy of this lineage in the field since 2005.

In vaccine matching exercise 33 virus isolates were subjected to one-way antigenic relationship study. All the isolates showed close antigenic match with respective in-use vaccine strains indicating appropriate antigenic coverage.

Virus isolates (133) including 97 type O, 12 type Asia 1 and 24 type A field isolates were added. In National FMD Virus Repository 1,687 (1,096-O, 317-Asia 1, 259-A, 15-C) well characterized field isolates are available.

SUCCESS STORY

Detection of FMD virus in bull semen sample

Standard operating procedure (SOP) was developed and validated for detection and identification of FMD virus in bull semen by multiplex PCR (mPCR). Total RNA was extracted from neat semen by diluting and mixing it thoroughly in RLT buffer (lysis buffer provided in RNAeasy mini kit) in equal quantity and passing through QIAshredder columns.

Under National FMD sero-surveillance testing of 11,560 serum samples from 13 states revealed the presence of protective antibody titre in 42.3%, 40% and 30.5% animal population against serotypes O, A and Asia1 respectively. This baseline response has improved due to application of vaccine. The current in-use vaccine strain IND R2/75 is found still the best and covers all the type O circulating outbreak strains in the country.

Classical swine fever: Classical swine fever (CSF), the topmost diseases in pigs, is one of the OIE listed diseases. The CSF or hog cholera is associated with high mortality, abortion, mummification of fetuses. It is of great concern in areas, where the pig population is in high density. CSF virus isolates maintained at the repository of PD-ADMAS, Bengaluru, were revived and processed for RT-PCR of NS5B genomic region. Phylogenetic tree based on the alignment of 408 nts of NS5B region was constructed. It showed three major branches, each representing three previously described genetic groups with further sub-branches. The majority of the Indian CSFV isolates belong to subgroup 1.1. Clustering of the Indian field isolates away from the vaccine strain and other related strains revealed that the field isolates did not have any vaccine origin. Phylogenetic analysis based on 5’ UTR classified

First AI calf of mithun

The first mithun calf was born through AI technique using cryopreserved semen from genetically superior mithun bulls in field conditions.

subgroup 1.1 Indian isolates into two minor sub-branches, however, no such classification could be seen from the analysis based on NS5b region; nevertheless in the latter analysis grouping of isolates was much more resolved. Lone isolate, IND294/08, is clustered within subgroup 2.2, ak into its grouping based on 5' UTR sequence.

Brucellosis: Serum samples from goat and sheep received from Gujarat, Rajasthan, Uttar Pradesh and Maharashtra revealed that among them 6.35% were positive from brucellosis. Similarly, 1.8% sheep and goat sera samples for Karnataka were positive for brucellosis. Porcine brucellosis is one of the most important emerging zoonoses and currently, no control programme for porcine brucellosis exists and the epidemiological situation is still unknown. Indirect-ELISA for screening brucellosis in swine was standardized using smooth lipopolysaccharide antigen from a standard strain *Brucella abortus* S99; 22.6% were found positive, which is much higher than that found in sheep and goat. An indirect ELISA was developed to detect *Brucella* in any of the susceptible species. Expression of four recombinant *Brucella* proteins, viz. BP26, BP39, superoxide dismutase (SOD) and *Brucella lumazine* synthase (BLS) is being envisaged to overcome the problem of cross reactivity in diagnosis of this disease.

Molecular typing of *Brucella*

PCR-RFLP was used for molecular typing of *Brucella* isolates. The omp 2 gene, which exists as a locus of two nearly homologous repeated copies (omp2 α and omp2 β) that differ slightly among *Brucella* spp. was used as target for differentiation. Because of the existing Pst I site polymorphism between *Brucella* strains, the test may distinguish between different strains of *B. melitensis* Rev1 vaccine strain from *B. melitensis* biovar 3 field strain in less than 12 h. This method can be used in clinical samples directly.

Leptospirosis: PCR based techniques were developed for differentiation of pathogenic and non-pathogenic *Leptospira* as well as for typing. Initially, *Leptospira* genus specific PCR was carried out to differentiate the *Leptospira* from other spirochete organisms, viz. *Borrelia*, *Treponema* and *Leptonema* etc., which amplified 331 bp products from the *Leptospira* organism only.

Sero prevalence of IBR: Serum samples collected from Andhra Pradesh, Gujarat, Jammu and Kashmir, Karnataka, Madhya Pradesh Maharashtra, Meghalaya, Manipur, Orissa, Punjab, Rajasthan, and Tamil Nadu, were subjected to Avidin Biotin ELISA. An overall apparent percentage positive was 34. The highest prevalence of 67% was in Tamil Nadu, and the lowest in Meghalaya. Region-wise positive percentage were as follows: East zone 17%, south zone 24%, west zone 48%, north zone 37%, north-east 39%, and central zone 25%.

Herbal anticoccidial and antidiarrhoeal drugs: The

extracts CIRG-5 and CIRG-6 from two different plants were evaluated *in vitro* against sporulation of coccidian oocysts and found 96 to 100% effective to check the sporulation up to 96 h.

Eight potential plant formulations of herbal extracts were subjected for clinical trials to control diarrhoea caused by *Escherichia coli* in kids of less than 1 month of age. Final product was developed from plant extract prototype CIRG-I and CIRG-2A and final products (formulation) @ of 10 mg/kg bwt for 1–2 days orally was highly efficacious to control EPEC diarrhoea in kid.

Vaccines and therapeutics

- Live attenuated orf, buffalopox and camelpox vaccines met the thermo-stability and potency requirements of the biologicals.
- Bi-valent DNA vaccine containing HN and F genes of Newcastle disease virus was evaluated along with genetic adjuvant comprising IL-2, IL-4, IFN γ , and GMCSF gene constructs.
- An aroA gene knockout mutant of *Pasteurella multocida* serotype B:2 was constructed for the development of HS mutant vaccine.
- Chitosan coated cationic PLG nanoparticles were prepared and when tested for the transfection efficiency with commercial transfecting agent, indicated that these nanoparticles can be safely used for delivery of DNA vaccines.
- *Trypanosoma evansi* beta tubulin protein was expressed in both prokaryotic and eukaryotic expression systems.
- Two new target proteins of *Fasciola gigantica*, viz. leucine aminopeptidase and peroxiredoxin coding genes were cloned and successfully expressed in their native conformation in a prokaryotic expression system.
- Immunomodulators of microbial and herbal origin, i.e. *Mycobacterium phlei*, *Lactobacillus acidophilus* and *Tinospora cordifolia* were effective under field conditions to improve productive performance and mitigate the adverse effect of sub-clinical caecal coccidiosis and thereby improvement of body weight gain in broiler birds.

Herbal medicines

- A herbo-mineral acaricide formulation against ticks (*Boophilus microplus*) in cattle was developed.
- Six herbal extracts NBA/13/B/2, IVRI/1/F/1, NBA/18/D/1, NBA/23/B/1, NBA/21/E/1, NBA/22/F/1, showed anti-tick activity against synthetic acaricide-resistant field collected *Rhipicephalus (Boophilus) microplus*.
- Methanolic extract of *Ocimum sanctum* and *Tinospora cordifolia* showed best immunomodulatory and antioxidant potential, respectively, against canine demodicosis.
- Plant aqueous extracts HNAP2, HNAP6 and HNAP11 exhibited good antioxidant potential against hepato-biliary dysfunction.

- Pharmacokinetics of gatifloxacin revealed that it has therapeutic potential for use in sheep.
- Medetomidine-butorphanol, thiopental and halothane combination provided better clinicophysiological and haemodynamic stability in general anaesthesia in buffaloes.
- Three TLR genes of yak (TLR 5, 7 and 9) and 2 TLR genes of mithun (TLR 4 and 7) were sequenced completely.

Diagnostics

- A recombinant lipoprotein 'P48' of *Mycoplasma agalactiae* was evaluated in an ELISA as a rapid immunodiagnostic tool in sheep and goats.
- A genus-specific PCR assay was standardized for rapid detection of *Arcobacter* sp. targeting 16S rRNA for confirmation of the isolates of this emerging pathogen.
- A 36 kDa protein was identified in different serovars of *Salmonella* during biofilm formation.
- Magnetic nanoparticles (MNPs) were synthesized by two different processes and were characterized for structural, morphological, size and magnetic properties. The MNPs were functionalized for immunoassay applications and evaluated for cellular toxicity in *in-vitro* experiments.
- A specific and sensitive diagnostic marker was identified for early detection of pancreatitis in dog.

Molecular characterization of pathogens/receptors

- Indian H5N1 isolates were characterized genetically and grouped with EMA 3/2.2.3 *sub-clade*, which branched from two distinct nodes in the phylogenetic tree.
- The alignment of deduced amino acid sequences showed that all the Indian H5N1 viruses characterized contained the multiple basic amino acid motif at their HA cleavage site, which is characteristic of highly pathogenic avian influenza viruses.

SUCCESS STORY

Artificially synthesized peptide

Synthetic peptides are useful as transfection reagents for delivering proteins/ peptides and DNA/nucleotides into mammalian cells. The technology developed at the IVRI offers a synthetic peptide to overcome the barriers during cellular transfection by making non-covalent complex of nano meter dimensions with cargo molecules. New technology provides easy to use transfection reagents with simple mixing with cargo molecule, and is devoid of toxicity at the concentration used for maximum transfection (above 70%). The peptide can be used for both protein and nucleic acid transduction, and it is effective for delivery into both primary and established cell lines. Above all the technology is five times cheaper than available commercial reagents.

- Two viruses from 2010 outbreaks in West Bengal contained amino acid asparagine at position 31 of the M2 protein, suggesting that both viruses are resistant to drug amantadine.
- An isolate of buffalo pox from man and animal recovered from an outbreak from Pune was characterized for B5R, H3L, D8L and A27L genes.
- An orf isolate from camel in a sporadic infection was characterized for B2L, GIF and VLTF-4 genes.
- Genotyping and partial sequencing of rotaviruses from man and pigs revealed human-pig reassortants as well as presence of novel combinations in India.
- Complete HN gene of velogenic Newcastle disease virus containing kozak sequence was amplified and cloned into pcDNA3.1 eukaryotic expression for enhanced expression.
- Phylogenetic analysis by AP-PCR, PFGE and ribotyping showed considerable similarity between human and fish isolates of pathogenic and pandemic *Vibrio parahaemolyticus*, which reflected the public health risk potentials of fish.
- Molecular characterization by PCR-SSCP analysis of *Oesophagostomum* spp. revealed two distinct conformers with six nucleotide polymorphisms (SNPs) among male and female parasites.
- Probability of evolutionary changes among prevailing genotypes of *Oesophagostomum* spp. was predicted by analyzing secondary structure of RNA.

SUCCESS STORY

Impact of PPR vaccine and diagnostics kits

Peste des petits ruminants (commonly known as goat plague), a contagious viral disease of small ruminants is highly endemic in India. With 180 million small ruminants in India the estimated annual losses incidental to the disease are over ₹ 1,800 million (US\$ 39 million) every year in the country.

The Indian Veterinary Research Institute, Mukteswar Campus, developed 2 monoclonal antibody based diagnostics, one for antibody detection and the other for detection of the active viral infection in the target hosts i.e. sheep and goat in 2001. Subsequently, a live attenuated PPR vaccine was also developed in 2002. The highly sensitive and specific diagnostics developed by the Institute provided insights on the intensity of the disease prevalence of PPR in sheep and goat in the country and application of vaccination programme paved way to control the disease in the targeted population. The development and use of the potent PPR vaccine in the targeted population from 2003 onward has in turn brought down the number of outbreaks of PPR to nearly 500 in the year 2007 from the country, and thereby significantly reducing the economic losses by 50%.

The projected reduction achieved in terms of the annual losses caused by the PPR disease to the small ruminants in the country is approximately ₹ 900 million, which is primarily due to the technology interventions of the institute.

Repository of Animal (Veterinary/Dairy/Rumen) Microbial Genetic Resources

A repository of characterized isolates of animal pathogens of different livestock species established at Veterinary Type Culture Centre (VTCC) has veterinary, dairy and rumen microbes.

The currently accessioned veterinary microbes consist of 123 bacterial cultures, 89 viral isolates and 110 recombinant clones. In addition, 206 bacterial cultures and 19 viral isolates are under various stages of characterization and authentication before accessioning. Among dairy microbes, VTCC has 296 accessioned bacterial cultures and 62 cultures are undergoing authentication. Under the rumen microbe component, currently, 198 rumen microbial isolates are available with VTCC, which are under characterization process; among them are 142 anaerobic bacteria, 52 anaerobic fungi and 4 methanogenic archaea. These were isolated from rumen of domestic (camel, goat, sheep, buffalo and yak) and wild ruminants (elephant, bison, rhinoceros and deer). Overall, 508 accessioned microbial isolates and 287 microbial cultures under authentication as well as 110 accessioned recombinant clones are available in the centre.

Quality control and production of veterinary biologicals

- 223,500 doses of RD 'F' strain vaccine; 166,000 RD 'Mukteswar' strain vaccine; 132,500 doses of fowl pox vaccine; 184,195 doses of lapinized swine fever vaccine; 423,800 doses of tissue culture sheep pox vaccine; 160,600 doses of PPR vaccine; 57,925 doses of *Brucella abortus* strain-19 (live) vaccine; 3,640 ml HS adjuvant vaccine; 73,500 doses of tuberculin PPD; 50,000 doses of Johnin PPD; 25,250 doses of mallein PPD; 55,250 ml of *Brucella* agglutination test antigen; 6,900 ml of *Brucella abortus* Bang Ring antigen; 19,860 ml of Rose Bengal Plate Test antigen; 219 ml of *Brucella* positive serum; 9,000 ml of *S. Pullorum* coloured antigen; 45 ml *S. Pullorum* positive serum; 87 ml *Salmonella* 'O' serum and 3,750 ml of *S. Abortus equi* 'H' antigen were produced and quality tested.
- 7.32 million monovalent doses of FMD vaccine were produced at Bengaluru campus.
- 435,100 doses of PPR vaccine were produced; similarly, 33 PPR c-ELISA, 22 PPR s-ELISA and 100 doses of goat pox vaccine were supplied by Mukteswar campus.

Sero-monitoring of important equine diseases:

During 2009–10, equine disease sero-survey was conducted in Rajasthan, Haryana, Punjab, Uttar Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh and Sikkim. A total of 176 out of 1,045 equines (16.84%) had antibodies to equine influenza virus while 28 (2.67%) were positive for EHV-1 and 107 (10.43%) were positive for antibodies to Japanese encephalitis virus. Antibodies to *Babesia equi* were detected in 320 (38.09%) of 840 equines, while *T. evansi* antibodies were detected in 49 (5.4%) out of 906 equines tested. One case of equine infectious anemia was detected in

SUCCESS STORY

Updated Vaccine for Equine Influenza

India faced an epizootic of equine influenza (EI) in 2008–09 after a gap of two decades. Molecular and antigenic characterization of current EIV isolates revealed that the EI in India during 2008–09 was due to an antigenically and genetically divergent EIV strain from those of the 1987 isolates. This necessitated the updation of the old vaccine to provide adequate protection. An updated vaccine incorporating EIV isolated from Katra, Jammu was developed. The vaccine has been tested for safety and potency in the small animals as well as in the horses and ponies. The updated vaccine provides effective protective immune response in experimental animals and in horses and ponies at field level. The vaccine is now being tested in large-scale field trials.

Uttarakhand. EIA-positive equine was reported after a gap of 11 years from India. None of the 1,045 equines tested showed antibodies to *Brucella* and *Salmonella* *Abortus equi* 'H' antigen.

Japanese encephalitis (JE) virus isolation: JE, a mosquito-transmitted disease caused by JE virus, is causing disease in equines, pigs and humans. It is prevalent in eastern and southern Asia. Serum samples from clinically affected horses and pooled mosquito vector samples from the surrounding field were found positive for JEV RNA by RT-PCR. Virus was isolated from one of the horses. This is the first report of isolation of JEV from a horse in India. The phylogenetic analysis of the envelope gene (1500 bp) sequence of the isolated virus, JE/eq/India/H225/2009 indicated that the isolate belonged to genogroup III of JEV, and that the equine isolate clustered together with Vellore group of JE isolates from India.

Camelpox virus zoonosis: Camelpox virus (CMLV) causes skin eruptions in addition to severe generalized exanthema mostly in camels and outbreaks occur frequently in camel-rearing north-western region of the country. However, their zoonotic potential has not been proven conclusively. The disease was confirmed on the basis of isolation of the virus from diseased camels, detection of the CMLV antibodies in the serum of diseased camels and human patients by serum neutralization test and amplification of CMLV-specific three full-length envelope protein genes (A27L, H3L and D8L) and one partial gene (C18L) of the isolated viruses from camels and human specimens. The phylogenetic analysis indicated that CMLV isolates cluster closely with variola virus (VARV) and vaccinia virus (VACV). The CMLV zoonosis indicates the changing pattern of host specificity of poxviruses and certainly points towards a declining cohort immunity against Orthopoxviruses in humans, which could be a public health concern. This is the first confirmed report of camelpox virus zoonosis.

Cysteine proteinases from *Trypanosoma evansi*: Parasite derived cysteine proteinases were proposed



SUCCESS STORY

Containment of Glanders outbreak

Glanders, a highly contagious disease of high zoonotic importance, affects primarily the equids. It is notifiable in India and poses a significant human health risk. The disease needs a timely confirmation for applying early mitigation and control strategy. New cases were detected in equines from Raipur city of Chhattisgarh. Two isolates of *Burkholderia mallei* were obtained from nasal swabs of these equines. The adoption of proper sanitary measures after elimination of affected animals in coordination with State Veterinary Services helped in prompt containment of disease in the affected state.

as potential targets for immunotherapeutic, chemotherapeutic and diagnostic reagents for parasitic diseases. The proteinases of camel isolate of *T. evansi* were analyzed by gelatin substrate SDS-PAGE containing collagen antigen confirming the presence of cysteine proteinases in *T. evansi*. Some of these cysteine proteinases were reactive with experimental/field serum sample from equines with *T. evansi* infection. This indicated the potential of these enzymes in serodiagnosis of this economically important disease of livestock.

Fish

Capture fisheries: The marine fish landings of India during the year 2009 has, provisionally, been estimated as 3.16 million tonnes. The pelagic finfishes constituted 52%, demersal fishes 28%, crustaceans 16% and molluscs 4% of the total landings. The sector-wise contributions during the year 2009 were mechanized 74%, motorized 22% and artisanal 4%. The west coast accounted for 56% of the total landings.

The northeast region, comprising West Bengal and Odisha contributed 20% to the total production; southeast region consisting of Andhra Pradesh, Tamil Nadu and Puducherry contributed 24%. On the west coast, the northwest region comprising Maharashtra and Gujarat recorded 26% of the total, and the southwest region comprising Kerala, Karnataka and Goa contributed 30%.

Cruzing for hilsa fisheries in Hooghly: The investigations on distribution of hilsa in Hooghly from Nabadweep to Kakdweep estuary were done through cruising. It established that depth and width of the river stretch affect the fishing effort and hilsa catch. The zones with <3 m depth had rare fishing activity, while fishing intensity was higher in deeper water (10 m depth). Hilsa was evenly distributed over the observed >100 km freshwater stretch. Movement of hilsa in shoals was missing during non-spawning season. Hilsa was available throughout the year with peak in monsoon. The observations may lead to possible occurrence of a resident stock of hilsa in Hooghly in addition to regular seasonal influx of migrant groups

from sea to freshwater zone for breeding.

Impact assessment of fishing ban: To assess the impact of fishing ban on reservoir ecosystem, mass-balanced models were used on different ecological groups before and after introduction of fishing ban in Wyra reservoir of Andhra Pradesh using Ecopath software. It is the first attempt in India on modeling a tropical productive reservoir, which contributes to fishery, particularly the commercially important prawn fishery. The results of the assessment indicated: (i) slight increase in system turnover, (ii) better organization/increased efficiency, (iii) decreased tendency for eutrophication (low sum of all flows into detritus), (iv) superior stability of total biomass/TST, (v) higher total primary production/total respiration, (vi) increased resilience (higher overhead per cent), and (vii) higher maturity and stability (higher primary production/ biomass and lower ascendancy per cent).

Formulation of shrimp feed: Yard trials revealed that 3% Ca with 2% P supplementation resulted in a significantly higher digestibility of Ca and P as well as weight gain compared to other groups receiving lower levels of Ca and P. Feeds for high salinity, indicated that high growth can be achieved at 40 and 33.5% dietary crude protein levels compared to lower dietary protein levels. Based on the better protein efficiency ratio and apparent protein utilization at high salinity (40%), protein utilization efficiency was better at 33.5% dietary protein level.

Reducing fish meal in shrimp feed: One of the bottlenecks to the approach of using plant proteins for reducing the level of costly fish meal in shrimp diet, is the high cellulose content of these feeds. *Bacillus macerans*, a potential cellulolytic bacteria, isolated from gut of *Lates calcarifer*, was identified for testing as feed supplement in plant protein based shrimp feed. After 48 h of incubation, crude protein content, free glucose concentration and microbial count were maximum in the feed and dry matter loss was less. Growth, survival, protein efficiency ratio were significantly higher and feed conversion ratio was significantly lower in shrimps fed with feed supplemented with live cellulolytic bacteria as compared to control feed.

Fish feed for marine ornamental fishes

The CMFRI has launched a dry formulated feeds named Cadalmin™ *Varna* for marine ornamental fish like clown and damsel fish. These feeds contain 40% protein, 9% fat, 39% carbohydrates, 7% ash and less than 2% fibre, colour imparting nutrients like carotenoids from natural sources, nutraceuticals and microbial products. They are slow sinking crumbles available in three particle sizes, 0.25 mm, 0.75 mm and 1 mm produced through twin-screw extrusion technology, which is the state-of-the-art in aquatic feed production. The feed costs only ₹ 400/kg in comparison to imported feed having a price in the range of ₹ 4,000/kg.

Adoption of biosecurity and WSSV incidence:

SUCCESS STORY

Seabass nursery and grow-out culture

A demonstration programme in the farmers' ponds at Andhra Pradesh (Ramudupalem, Gangapatnam, Nellore District), Tamil Nadu (Mahendrapalli, Sirkazhi Taluk, Nagapattinam District) and at Maharashtra (Pancham Aquafarm, Saphale) was taken up by CIBA Chennai, to upgrade and validate the technologies developed for seabass nursery and grow-out pond culture using hatchery produced seed and formulated feed. The major accomplishment was the harvest of uniform sized fish, which were cultured with a formulated sinking pellet feed in pond culture systems. The viability of rearing sea bass fry in the nursery, juveniles in the pre-grow out and marketable sized fish in the growout phase was demonstrated in farmers' ponds. Based on the seabass nursery technology for the hapa-pond system developed by CIBA, a farmer in Bhimavaram was able to raise stockable size seed in a freshwater pond with a survival rate of 95% and a profit of ₹ 1.73 lakh.

Disease surveillance in the two types of culture systems in West Bengal, shrimp monoculture and traditional bheries over a period of three years (2007–10) indicated that white spot disease (WSD), shell associated problems and gill associated problems were statistically significant among the two culture systems. Newly emerging diseases such as monodon slow growth syndrome was observed in both culture systems, whereas, white faecal disease was observed only in shrimp monoculture system. WSD outbreak was significantly high where stocking was done by the seed, which has not been tested by PCR.

Analysis of data regarding adoption rate of bio-security protocols either single or in combination in shrimp farms in Chidambaram and Nagapattinam Districts of Tamil Nadu indicated that white spot disease incidence is at a higher rate when protocols of disinfection of implements and reservoir ponds, were not followed.

Diagnostics for monodon slow growth syndrome: One of the emerging shrimp diseases is the monodon

SUCCESS STORY

Large mesh size purse seine for deep sea waters

With the objective of targeting the underexploited large pelagics in deeper and distant waters, the conventional purse seine net with mesh sizes ranging from 18 to 20 mm in the main body of the netting was redesigned and the mesh sizes replaced with larger mesh size (45 mm) and the modified gear was operated on a participatory mode in association with the purse seine fishermen of the *Manassery Matsyathozhilali Vikasana Kshema Sahakarana Sangam*, Kochi. Experimental fishing operations were carried out on board the vessel Bharat Darshan in the depth range of 50 to 220 m with successful catching of pelagic fishes. The catch mainly comprised large sized mackerels (62.08%), followed by tunas (16.08%), pomfrets (1.93%), carangids (14.43%) and miscellaneous fishes (5.47%). The landing of quality fishes gave an impetus to the purse seine fishermen as it fetched better value for their catch, compared to the conventional purse seine landings. In addition there was a demand for export of large sized pelagic fishes, which further added value to their catch. The success of the large mesh purse seine has encouraged all the purse seine fishermen of Cochin to adapt to the design of CIFT. The good economic returns have led to increased number of purse seiners operating large mesh purse seines at Cochin. This has immensely benefitted the fishermen, stakeholders and the fishing and allied industries by way of employment generation, and income generation and increased exports.

slow growth syndrome (MSGs) with abnormal slow growth and marked size differences among individuals. An improved diagnostic nested RT-PCR with custom designed primer targeting RdRp gene of Laem-Singh virus (LSNV), which has been implicated in monodon slow growth syndrome (MSGs) was developed. Screening of farmed and wild broodstock samples with this improved diagnostic PCR showed a high prevalence of LSNV. High prevalence in broodstock could lead to the spread of this virus by vertical transmission. □