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Livestock Management

Cattle

Fibre digestibility: *Streptococccus bovis*, a numerically predominant bacteria in the rumen of crossbred cattle under different feeding regimes was charaterized. And the cellulase gene obtained from the best fibre degrading fungi isolated from the faecal matter of goat was cloned to *Streptococccus bovis* for enhancing the fibre digestibility of poor quality crop residues.

Suppression of methane production: *In vitro* gas production test revealed that methane suppression among the tree leaves ranged from 4.6 to 82% — minimum recorded in jatropha leaves (4.6%) that contained lowest tannin and the maximum (82%) in *Ficus bengalensis* leaves. Commonly used top feeds like *Sesbania grandiflora*, *Glyricedia maculata*, *Ficus mysorensis* and *Ficus religiosa* leaves showed a methane suppression ranging between 35 and 50%. A mixture of three plant species having anti-methanogenic activity *in vitro*, exhibited 12% reduction in methane emission in crossbred calves, which confirmed that there is a potential in using tree leaves for reducing methane production from enteric fermentation.

Use of fungi for enhanced digestibility of straws: Lignin content of *ragi* straw decreased with all the white rot fungi, viz. *Phanerochate chrysosporium*, *Pleurotus sajorcaju*, *Pleuritous ostreatus* and *Vorialla voloraceae*. High protease activity was observed during the first two days of fermentation after which it declined, and lignolytic enzymes, viz. laccase, manganese peroxidase and lignin peroxidase concomitantly increased up to the fifth day of fermentation. *Phanerochaete chrysosporium* showed the best potential in improving the digestibility of *ragi* straw followed by *P. ostreatus*.

Antifungal property of medicinal/aromatic plants: Melissa and thyme leaves exhibited good anti-fungal activity (>50%) against *Aspergillus parasiticus*. *Pachouli* leaves, curry leaves, rosemary leaves, cinnamon leaves, *sarpagandha* leaves, thumbe leaves, sweet worm wood leaves, *aswagandha* leaves, chicory powder, yellow oleander leaves, *Selastras paniculatus*, *Tinospora cardifolia*, railway creeper and Indian acalypha leaves showed high anti-fungal activity (>50-90%) against *Fusarium moniliforme*.

Energy supplementation for higher milk yield

Strategic supplementation of *ragi* (*Eleucine coracana*) grain to dairy cows during their early to mid lactation period resulted in increased milk production (average 1.9 litre/cow/day) and fat and SNF percentage (average 0.2-0.3%) and also reduced the milk urea content with a net increase in income of about Rs 19/cow a day under field conditions.

Buffalo

Nutrition for the onset of puberty: GnRH challenge studies in the calf attaining maturity at 3 years of age due to nutritional perturbation showed immature status of hypothalamo-hypophyseal-ovarian axis even at the age of 2 years 4 months.

Nutritional modulation of IGF: In sub-fertile male buffaloes nutritional modulation of IGF-I (as a mediator of metabolic hormonal effects) proved beneficial for various sperm functional attributes *in vitro*. The cleavage rate in embryos produced through IVF using such sperms, was also better.

Area specific mineral mixture: Supplementation of area specific mineral mixture (Ca, P, Zn, Mn, Cu) to buffaloes based on the deficiency in the North east zone of Haryana, improved productive and reproductive efficiency

as 70% buffaloes showed normal cyclicity, and 10% increase in milk production. Supplementation of area specific mineral mixture pellets @ 40g daily in cattle and buffaloes during lactation stage increased milk yield by 10-15%, brought cows into estrus within 30-45 days, and reduced the problem of skin keratinization.

Detection of pesticide residues: A rapid multi-residue method for analysis of neonicotinoid pesticides, viz. imidacloprid, acetamiprid and thiacloprid, was developed. The percent recovery from 0.5 to 2.0 ppm concentration varied from 95.6 to 81.17% for imidacloprid, 92.76 to 84.99% for acetamiprid, and 96.96 to 88.50% for thiacloprid with a detection limit of 5ppb, 10ppb and 20ppb, respectively.

Fibre digestibility: Isolates of anaerobic fungi collected from 5 different states and 6 different host species, revealed that isolates from Rajasthan had highest fibrolytic potential and the best isolate improved digestion of wheat straw in buffaloes.

Improving reproductive efficiency

- Buffalo embryos were produced *in vitro* @ 64% cleavage with 20% blastocyst using an improved IVEP protocol.
- A three dimensional (3D) collagen gel culture system for the *in vitro* growth and survival of the buffalo preantral follicles with IGF-I was developed.
- Early diagnosis of pregnancy (by day 20-21 post-breeding) was facilitated by using real time B-mode ultrasonography in goats and buffaloes.
- Bull-biostimulation curtailed the incidence of silent ovulation and service period and increased conception rate in post-partum buffaloes.

Enhancing productivity: Strategic supplementation of protein (<20% CP) during mid-lactation increased productivity of buffaloes. Vitamin E supplementation @300 IU daily was optimum to improve weight gain, and increased its concentration in the muscles of buffaloes.

Early embryonic mortality: Dynamic status of antioxidant enzymes in relation to the stages of oestrous cycle and tissue remodeling was observed. Effective modulation of prostaglandin production by the uterus may rescue corpus luteum and prevent early embryonic mortality.

Improvement of reproductive efficiency: Insemination dose could be reduced from 25 to 15 million spermatozoa without adversely affecting the conception rate. The results are being authenticated with more trials in farmers' herd. Frozen semen samples were evaluated for sperm motility attributes. Bulls with higher field conception rates also had higher sperm total motility, progressive motility, rapid motion and viability.

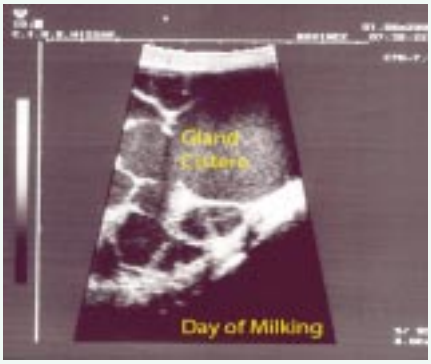
Sheep

Trace element status: Biochemical markers (Cu and Zn-dependent enzymes – ceruloplasmin and Cu/Zn- super oxide dismutase) were evaluated to assess the trace element (copper and zinc) status in sheep at different dietary levels of Cu and Zn. Prediction equations developed by correlating the absorbed Cu and Zn with Cu- and Zn-dependent enzymes; plasma Cu and Zn with Cu and Zn-dependent enzymes; liver tissue concentrations of Cu and Zn with absorbed Cu and Zn, revealed that Cu and Zn status of animals could be assessed by using these enzymes as biochemical markers.

Production performance: Bharat Merino and Gaddi Synthetic sheep under migration to highland pastures gained higher body weights and produced

Induction of lactation

Infertile Murrah buffaloes were treated with subcutaneous injections of estradiol-17 and progesterone for 7 consecutive days, and supplemented with largectil and dexamethasone at specific intervals for induction of lactation. One week of udder massage was given twice daily till the udder was turgid with milk, followed by milking, which started from day 21. Milk from induced-lactating buffaloes became normal within 10-15 days after start of milking. Four of the six treated buffaloes responded successfully and milk became normal between 4-20 days of the start of milking. The fat content stabilized at 7.72±0.3% after about 10 days of first milking. In successful cases, the peak milk yield between 20th and 75th day ranged between 2.5 and 7.0 kg/day. The effect of treatment for induction of lactation — which comprises sex steroids — was also assessed on the ovarian activity of treated



buffaloes. Ultrasonographic scanning revealed that pre-treatment ovarian follicular size of 7-13 mm, regressed to 2-3 mm within 8-10 days of start of the treatment and then no follicular activity was observed for 30 days during treatment. First ovulation took place between 55 and 60 days after treatment. In two buffaloes, follicular cysts of up to 35 mm diameter were detected.

Production and reproduction performance in sheep

The spent sheep after completing productive life constitute more than 30% of meat produced in the country. Pre-slaughter feeding of *ad lib.* concentrate mixture in spent ewes for 45 and 90 days improved body weight gain by 10-11kg. Supplementation of mineral mixture in sheep flocks maintained on degraded rangeland of semi arid Rajasthan brought anoestrus sheep into estrus after 15-20 days of supplementation.

Prototypes of vaginal speculum and plunger of different dimensions, suitable for transcervical insemination of Malpura sheep were fabricated from plastic and nylon material for successful transcervical insemination. Three different protocols for producing synchronized lamb crops were perfected, and the study indicated that ovagen alone and in conjunction with progesterone enhanced lambing in sheep.

more wool in comparison to stationary flocks.

Utilization of fibrous crop residues: Fortification with cellulase, xylanases, pectinase, phytase and protease enzymes enhanced dry matter digestibility by 7.00%, cell wall digestibility by 25.00%, and the end product fermentation by 15.00% of poor quality roughages. Supplementing probiotics of microbial origin like *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and *Kluyveromyces marximanus* and a mixed yeast culture of above three in a ratio of 1:1:1 as microbial probiotics in lambs showed that *Saccharomyces cerevisiae* strain is superior in improving the growth of lambs.

Bioavailability of nutrients: Diets supplemented with condensed tannins improved nutrient utilization, immune response besides protection from GI parasites and fasciolosis in sheep.Supplementation of *Tinospora cordifolia* as a functional food imparted positive influence on the nitrogen metabolism and antioxidant levels in seminal plasma besides significantly improving the erythrocytic antioxidant status and cell-mediated immune response of adult Muzzafarnagari rams. Undecorticated jatropha (*Jatropha curcas*) meal after processing with 1% common salt and 0.5% lime, could replace protein of conventional oil cakes up to 25% in the concentrate mixture of adult sheep and goat for short-term feeding,

Goat

Standardization of* in vitro *fertility test

Hypo osmotic swelling test (HOST): Sperms were evaluated for strongly coiled, weakly coiled and non-coiled under oil immersion lens. The best swelling in terms of strong coiling and total coiling was in 75-mosmol hypo- osmotic solution. There was significant difference in swelling in different

Cylindrical sponges showed maximum retention

strengths of hypo osmotic solution. For frozen sperm 75-mosmol and for fresh diluted semen 100-mosmol hypo-osmotic solution was found to be the best.

Dual staining test: Dual staining technique was standardized for testing viability and acrosomal integrity in frozen and fresh semen. It saved time and chemicals in testing viability and acrosomal integrity thereby avoiding separate tests.

Semen quality: Twice a week semen collection, evaluation and freezing in Jamunapari bucks of 2-5 years of age group, indicated that the semen production was higher under intensively reared bucks compared to the semi-intensively managed bucks.

Rapid estrus detection methods: Sponges of different sizes and shapes were tested for their retention in vagina for 12 days in Sirohi goats for estrus detection. Circular sponges with a diameter of 30 mm and cylindrical shaped sponges with a diameter 25 mm had the highest percentages (>83%) of retention.

Mithun

Feed blocks with locally available feed resources: Feeding of *Lagerstroemia speciosa* tree leaves based complete feed blocks to mithuns showed that the tree leaves could be incorporated in the ration up to 30% for feeding mithuns under semi-intensive or intensive system.

Organic fertilizer: The excreta (faeces and urine) of both mithun and Tho Tho cattle were compared as a source of organic fertilizer. The quantum of faeces voided from mithun was more than that of Tho Tho cattle though faeces of Tho Tho cattle contained less water (more DM) compared to that of mithun. The chemical composition of faeces of mithun and local cattle did not differ significantly. Mithun produced more urine than Tho-Tho cattle. So per animal basis,

mithun supplied more excreta as organic fertilizer than Tho-Tho cattle in Nagaland.

Bakers yeast: Feeding bakers yeast (*Sacharomyces cerevisiae*), a probiotic on roughage based diet, significantly increased average daily gain of mithuns, intake of concentrate and roughage and also improved FCR.

Estrus synchronization protocols: Experiments conducted to synchronize estrus in cyclic and post-partum mithun cows showed more prominent behavioural signs of estrus than spontaneous heat. Application of CIDR on day 45-50 after parturition, induced first postpartum estrus immediately after uterine involution (day 53-58 post parturition). Unlike other bovines, mithun cows exhibit first postpartum estrus at around 97±19.6 days postpartum. Use of CIDR was advantageous in terms of prominent behavioural signs of estrus thereby ease in detection of estrus. The first calf was born from an anoestrus mithun cow synchronized with CIDR.

Hormone-induced maternal behaviour: Mother-neonate bonding was studied using oxytocin intranasal spray. Intranasal administration of oxytocin effectively induced maternal behaviour in primiparous bovine heifers where maternal behaviour was blocked chemically.

Embryo transfer technology: Estrus synchronization was performed by using CIDR protocol and four embryos (compact morula) were recovered successfully from two donors and subsequently transferred into three recipients.

Yak

Trace mineral supplementation: Soil, feeds and fodders of yak rearing zones are deficient in micronutrients, as reflected by the low productive and reproductive performances of yak. Supplementation of trace minerals like Zn, Cu, Co and Mn in the ratio of 40:20:2:1 along with the basal diet significantly increased milk production.

Production performance during winter: Body weight gain was significantly higher during October in calves but from November onwards no significant increase was observed. Bulls gained significantly higher body weight compared to calves up to December. The lactating yak cows lost about 5.84% of their body weight, and milk yield reduced mainly due to shortage of feed and fodder during long winter. Providing adequate nutrition during winter could help in ameliorating winter stress in yaks.

Modified temperature humidity index: Yaks were comfortable at THI of 52 and when THI exceeds 52 yaks experienced heat stress, as expressed through increased physiological responses.

Poultry

Stress related hormone: Under heat stress condition some of the lymphocyte proteins were repressed whereas some others were induced in broiler.

Bioavailability of micronutrients: Se supplementation in broiler chicken diets at 0.15 or 0.30 ppm complemented bioavailability of Zn. In contrast, Se antagonized retention of Mn, Cu and Fe in liver tissues. Se (0.15 ppm) and Zn (80 ppm) improved humoral and cell-mediated immune response in broiler chicks. Zn uptake by tissues was relatively more active during early age (2 week) than at later ages (4 and 6 week), whereas Se retention in tibia and liver was higher at 4 and 6 weeks than that at 2 weeks of age.

Se inclusion in broiler chick diets from 0.15 to 1.35 ppm linearly enhanced its retention in bone and liver tissues and complemented Zn uptake by tissues. Both Cu and Fe responded negatively to Se increases in diets at 5 weeks of age. Vitamin E at 40 IU enhanced Se uptake by bone, but did not influence retention of Zn, Mn, Cu or Fe. Moderate levels of Se (0.15 or 0.45 ppm) and vitamin E at 40 IU produced higher antibody titres, better cell-mediated immune and reduced stress in 5-week-old broilers.

Enhancing utilization of macronutrients: Protease enzyme produced from *Bacillus licheniformis* were supplemented to broiler diets @ 4,000 IU/kg to enhance the feeding value of commercial meat meals and soybean meal low in protein by 3-4% over the recommended level (22%). Meat meal diets responded better to protease supplementation and performance of broilers was equivalent to the control group that was maintained on 22% protein diet. Inclusion of enzyme had

Nutrient requirements of rural poultry

Ca and NPP levels in diet could be reduced to 0.6 and 0.3%, respectively, by maintaining the vitamin D_3 level at 1,200 IU/kg diet in Vanaraja female parent. The effect of interaction between Ca and NPP on performance and bone mineralization of Grampriya chicks during nursery (rearing period of 1 to 42 days) suggested that 0.6% Ca and 0.30% NPP were adequate.

Krishibro chicks responded positively to dietary energy levels (2,900 and 3,000 kcal ME/kg) in starter and finisher phases, respectively. The requirement of Ca, and NPP for dressing yields and bone mineralization in Krishibro chicks appeared to be equal/higher than 0.6 and 0.3%.

Carbohydrate in combination with fat produced better effect on body weight of broiler chickens than combination of fat and protein as chicks could utilize carbohydrate better than fat during initial post hatch period.

significant impact on different production parameters compared to non-supplemented diets, particularly when dietary protein levels were lower than the recommended levels.

Female reproductive system of *desi* fowls: Ovary and oviduct development was noticed clearly around 16 to 18 weeks of age in White Leghorn (WLH), around 20-22 weeks of age in Kadaknath (KN) and around 24 weeks in Aseel peela (AP). At the peak of sexual maturity, around 30 weeks of age, total length of the oviduct was greater in WLH (73 cm) as compared to *desi* fowl (66 cm). The transaminases activity of blood plasma, irrespective of breeds increased linearly with age. An increased pattern of ACP, GOT and GPT activity was found associated with maturation of female reproductive tract and reverse was true with ALP activity among all the breeds.

Enhancing egg production: Using simple feed formulation, egg production could be enhanced markedly over the age of 78 weeks in Aseel peela *desi* fowl. Large-scale replications at institute and field level are being taken up to validate the data.

Supplementation of melatonin: Dietary inclusion of aflatoxin @0.15 ppm level adversely affected body weight, feed intake and FCR and caused lipid peroxidation with simultaneous depletion of antioxidant enzymes (superoxide dismutase and catalase) in broilers. Melatonin supplementation @ 40mg/kg feed alleviated the adverse effect of aflatoxicosis at lower levels (<0.15ppm).

Moulting for enhanced production: Birds were force moulted by feed withdrawal method for 10 days. As the period of fasting progressed from 0 to 10 days a gradual but steady reduction was noticed in the levels of serum triglycerides, which was more pronounced and significant from the fourth day of commencement of feed withdrawal. A similar decline in serum total cholesterol concentration was noticed, which became very apparent from the sixth day after feed withdrawal and a reduction of 30% in total cholesterol concentration was achieved by the tenth day in the moulted hens. In contrast, serum HDL–cholesterol concentration progressively increased during the feed withdrawal period and peaked around the eighth day after withdrawing feed. Accumulation of high lipid in uterus in late laying age results in either shell less or poor shelled eggs. Feed withdrawal for longer period leads to mobilization of lipids from uterus.

Quail semen characterisation: Physical and biochemical characteristics of quail semen showed that birds having larger cloacal gland size ejaculated higher volume of semen and semen production was higher in CARI Uttam than CARI Sweta quails. Mass sperm motility in neat semen was only 50-60% immediately after collection that decreased continuously and reached to zero after 30 min. Sperm abnormalities were higher in CARI Sweta than CARI Uttam. Among the enzymes LDH was exceptionally high in all groups in both the lines, cations sodium and potassium were higher in birds having larger cloacal gland size whereas magnesium and calcium were more in the seminal plasma of birds with smaller cloacal gland. Methylene blue reduction time test revealed that quail spermatozoa are more active than chicken.

LIVESTOCK PROTECTION

A status of freedom from contagious bovine pleuropneumonia infection in cattle and buffalo was obtained from OIE.

Development and improvement of diagnostics and vaccines

Vaccines

- A low volume saponified haemorrhagic septicaemia vaccine was found safe and effective in farm cattle.
- Possibility of DNA vaccine construct against bovine brucellosis was ascertained.
- Chicken cytokine genes (MIP-□, lymphotactin and IFN□) were expressed in mammalian cells thereby opening up the possibility of their use as genetic adjuvant in DNA vaccine.
- LPS and genomic DNA containing CpG from *Salmonella* Gallinarum activated the innate immune system of chickens and gave higher protection after immunization with inactivated NDV in challenged birds.
- Conjugation of Fc with flagellin protein was a good model for efficient antigen delivery resulting in higher immune response than antigen alone.

Diagnostics

- A nested RT-PCR was developed using primers from RNA dependent RNA polymerase region for differentiation of ruminant pestiviruses.

Exotic and emerging diseases

BVDV-1 was identified in yaks of Himalayan region. Phylogenetic analysis established prevalence of BVDV-1b and 1c subtypes in Indian buffaloes and existence of close relationship between cattle and buffalo BVDV-1b viruses.

The phylogenetic analysis of avian influenza virus indicated that the virus might have been introduced into India through migratory birds.

The Indian isolate of PCV1 was found genetically closely related (0.6%) to isolates from China, USA, France and distantly related (1.4%) to the isolates from Taiwan.

- C18L gene based conventional PCR and TaqMan probe based real time PCR were developed for specific detection of buffalo pox virus.
- Duplex PCRs were developed for specific detection and differentiation of buffalo poxvirus from other orthopox viruses, and camel pox from other orthopox viruses.
- C18L gene-based real time PCR was standardized for quantification of camel poxvirus in clinical samples.
- A hybridizing probe based real-time PCR was developed for diagnosis of PMWS and the disease was diagnosed in four private farms in Uttar Pradesh.
- The expressed protein of N gene of PPR virus could be an alternative to whole virus antigen in sandwich ELISA for diagnosis of PPR.
- Developed indirect ELISA for serodiagnosis of Japanese encephalitis in pigs.
- Transformed fibroblast antigen was much superior antigen for detection of anti-avian leucosis virus antibodies in the serum samples of chicken, as compared to gsAg, as determined by an indirect ELISA.
- A highly sensitive PCR targeting new gene of *Mycobacterium a. paratuberculosis* and a quantitative real-time PCR (RT-PCR) were developed for the diagnosis of paratuberculosis in small ruminants.
- Serotype specific PCR was developed for detection of *Salmonella* Typhimurium and *S.* Enteritidis.
- Duplex PCR was developed for simultaneous detection of *Salmonella* genus and Typhimurium serotype.
- Germ tube formation test was developed for detection of chlamydospore in *Candida albicans.*
- Methodology for quick detection of *Echinococcus granulosus* genotypes by polymerase chain reaction coupled with restriction fragment length polymorphism was developed.
- A useful primer was developed and found effective in differentiating cryptic stage of *Echinococcus granulosus* and *Taenia.*

Molecular characterization of pathogens/receptors

- Mutants of *E. granulosus* isolate from Indian cattle and buffalo origin, were detected on the basis of sequence analysis of mitochondrial gene and non-coding spacer gene.
- Molecular characterization of toll-like receptors (TLR2, and TLR4) of *nilgai* revealed higher expression in skin and immune cells of *nilgai*, as compared to buffalo indicating stronger innate immunity.

Herbal medicines

- Immunomodulators prepared with extract of *Tinospora cordifolia* and a probiotic (*Mycobacterium phlei*) showed significant body weight gain in broiler birds and improved their health.
- Significant antidiarrhoeal activity was detected in the seed extract of *Caeslipinea bonducella.*

Surgical and clinical interventions

- The epoxy-pin external skeletal fixation technique was developed, and used to treat a variety of compound fractures of different long bones in small animals.
- A novel design of bilateral external fixator having opposite threadings in the side bars was developed for the management of long bone fractures in large animals.
- Transplantation of autologous bone marrow cells, along with hydroxyapatite induced faster healing of radius fracture in rabbits, as compared to transplantation of hydroxyapatite alone.

SUCCESS STORY

Cell culture vaccine for classical swine fever

Classical swine fever or hog cholera, the most important disease of pigs that causes death in 98 to 100% cases in susceptible populations, result in economic losses of around Rs 500 crore in India. Presently, vaccination is done with a lapinized vaccine that requires killing of rabbits for its manufacture. Since cell culture vaccine is not available in India for protection of pigs against classical swine fever, the Indian Veterinary Research Institute, Izatnagar, developed an effective PK-15 cell line-based live attenuated freeze-dried vaccine. Field validation of the cell culture vaccine on pigs revealed that immune response of the vaccine was highly satisfactory. Vaccinated pigs sero-convert from 21-30 days of vaccination. The vaccine has a shelf life of more than one year and provides immunity for at least a year following a single vaccination after weaning. It is safe, potent and can be applied even during pregnancy and there has been no report of any untoward post-vaccination reactions.

The newly developed vaccine is cell culture based and its production is easier and low cost as compared to the existing lapinized vaccine. Hence there would be a good demand for the product in market. Supply of country's demand of approximately 20 million doses of the vaccine seems possible by use of this cell culture vaccine. This would help in reducing the losses due to mortality and thus improve the economic condition of poor pig farmers in the country.

- Application of autologous bone marrow cells subcutaneously in the periphery of incisional and open cutaneous wounds induced faster healing, as compared to conventional antiseptic dressing of wounds.

Foot-and-mouth disease

Field samples (1,313) received from various states during the year were processed and subjected to sandwich ELISA for type identification. Only 705 samples were typed — 567 samples were typed as O, 58 samples as type Asia1, and 80 samples as A —, and no virus could be detected in rest of the samples. Samples were also processed in BHK 21 cells and virus could be recovered in 119 field samples comprising 24 type Asia 1, 75 type O and 21 type A.

To improve the diagnosis of FMD in suspected clinical samples, a multiplex PCR (m-PCR) was developed and evaluated. Using the test, 42% of the outbreaks that went undiagnosed using ELISA, were identified indicating that mPGR could be used as best supplentary to ELISA to increase the percentage of FMD outbreak diagnosis in the country.

Two-dimensional micro-neutralization test (2D-MNT), a modified form of SNT, was routinely used to test new field isolates to determine the appropriateness of the existing vaccine strains and to select new vaccine strains, if required. In serotype A the most worrying factor, which merits attention is the antigenic heterogeneity of the isolates. In the sense some strains show close antigenic match to the current vaccine strain (17/82) and others to the new strain (IND40/00) in *in-vitro* micro neutralization test. One isolate IND 53/08 from Chhattisgarh, was unique both antigenically and genetically forming a separate cluster with another isolate IND 109/06 from Chhattisgarh.

Among all serotypes prevalent in India, type A virus population is genetically and antigenically most heterogeneous in nature. VP1 coding (1D) region based molecular phylogeny has established circulation of four genotypes of type A so far in India. There is once again an upsurge in incidence of outbreaks due to lineage genotypes VII with amino cicer (aa) deletion at 59th position of VP3. This single aa deletion is at an antigenically critical position in structural protein VP3, which is considered to be a major evolutionary jump probably due to immune selection.

Field isolates (17) of serotype A recovered from outbreaks in Karnataka, Tamil Nadu, Chhattisgarh, West Bengal and Haryana were sequenced at 1D (VP1) region for molecular epidemiological analysis. The determined sequences were aligned with other Indian sequences and some of the retrieved exotic sequences. All the isolates clustered within genotype VII in the N-J tree. Genotype VII is restricted to only India as none of the exotic sequences clustered in this group. Thirteen out of the seventeen isolates sequenced, clustered in the deletion group. 1D region based phylogeny also revealed that this lineage is genetically diverging with time giving rise to three lineages (VII b, f and g) so far.

In serotype O, PanAsia II strains dominated the outbreaks, nevertheless Panasia I and II 2001 also co-circulated. Asia 1 field isolates (19) were subjected to 1D gene sequence analysis. The isolates were grouped with lineage CI that dominated Asia 1 outbreaks. The isolates of 2007 and 2008 showed 15.4 to 16.7% and 12.4 to 14.7% divergence at nucleotide and amino acid level, respectively, from in-use vaccine strain (IND63/72).

The Central laboratory, Mukteswar, contains 1,402 (893-O, 261-Asia 1, 233-A, 15-C) field isolates. Pre- and post-vaccinate serum samples collected up to sixth phase of FMD-CP showed increased levels of protective antibodies against serotypes O, A and Asia 1 over different phases of vaccination.

SUCCESS STORY

Rapid immunoassay kit for serological monitoring of infectious bursal disease of chickens

Infectious bursal disease also known as Gumboro disease, a highly contagious viral disease affecting young chickens, 3 to 6 week-old, has worldwide occurrence. Morbidity of the disease is high with mortality usually of 0-20%, but sometimes up to 60%, thus adversely affecting the economy of poultry industry.

Serological diagnosis of the disease is either labour intensive, slow or require sophisticated equipment. A need was felt to replace these laboratory tests with a simple pen side test. A recombinant protein produced from a heterologous expression system was used as a diagnostic antigen in a simple field assay format. The developed protocol allows the user to rapidly interpret the immune/disease status of the bird by a simple agglutination phenomenon of antigen-coated beads with naked eye within 5 min. This in turn, helps the farmers and poultry breeders in determining the time of vaccination of the chicks. Test results are comparable with agar gel precipitation test and enzyme linked immunosorbent assay under test conditions

The kit has been validated under laboratory conditions from different parts of the country and the technology was transferred to National Research Development Corporation, New Delhi, for further commercialization. The developed kit would help in minimizing the time required for diagnosis and develop judicious vaccination scheduling.

Animal disease monitoring and surveillance

A large databank on the livestock diseases of the country, based on reports submitted to the Government of India by various state governments was developed at the PDADMS. The institution was involved in the sero-monitoring of rinderpest. Large number of sera samples from various parts of the country is maintained in the National Livestock Serum Bank for retrospective studies.

- An offline version of the databank of livestock diseases of the country was developed. Based on the custom queries, various epidemiological analyses are possible e.g., frequency of disease occurrence, top diseases of the country, eco-patho zones. The spatial and temporal analysis of animal disease data is being carried out using this software.
- Molecular diagnosis of brucellosis was standardized that helped in differential diagnosis of *Brucella abortus* and *B. suis*. Based on the results of the serological, biochemical and molecular techniques a rare case of brucellosis in swine due to *B. abortus* was diagnosed. A standardized A-B ELISA kit for the detection of bovine *Brucella* antibodies was developed. An indirect ELISA kit was standardized to identify the magnitude of disease in ovines. Molecular epidemiological studies are being standardized to diagnose and differentiate the brucellosis of cattle, ovine, caprine and humans. Tests were developed to detect the etiological agent directly from the clinical samples such as aborted foetus, placenta and uterine discharges.
- A multiplex PCR was standardized to diagnose the pathogenic leptospira. A repository of the leptospira isolates is being maintained.
- Molecular studies on BHV-1 were carried out. A multiplex PCR for detection of BoHV-1 sequences was standardized. The PCR amplified products of gB (293 bp), gC (173 bp), gD (343 bp) and US 1 (464 bp) were subjected to partial nucleotide sequencing and aligned with different reference sequences of respective genomic regions.
- The PCR amplification of different 'tk'genomic region of BoHV-1 was standardized and the PCR amplicons thus obtained were confirmed using unique restriction enzyme. Multiple PCR using different combinations of primers specific for gB, gC and gD was standardized and was applied for screening of field samples. The partial nucleotide sequencing of gB, gD and US 1 were aligned with the reference sequences, and was analyzed with phylogenetic trees. These results would be of much help in profiling and characterizing BoHV-1 in livestock population.
- The serum samples obtained from Madhya Pradesh, Maharashtra, Andhra Pradesh, Manipur, Kerala, Orissa, West Bengal and Tamil Nadu, were screened for the presence of antibodies against IBR, using AB-ELISA kit and 41.90% of the samples were found positive for IBR antibodies.
- An mPCR for genome detection of leptospira, BoHV 1 and *Brucella* using known standards targeting the LipL32 gene of *Leptospira*, gB gene of BoHV 1 and bcsp31 gene of *Brucella*, was standardized.
- Occurrence of zoonotic bacterial pathogens from the livestock and livestock products was studied. The pathogens were isolated from various sources, and their molecular characterization was completed.
- A computer interface based BHV-1 whole antigen AB ELISA was developed as per the standards of IAEA, standardized and validated. The kit was critically evaluated both in-house and extensive field trials for detection of antibody to IBR virus in bovine serum. This test is highly sensitive, specific, economical and user friendly.
- A kit to detect the antibodies to *Brucella* in swine is being developed and is in the process of standardization.
- A PCR technique was developed to detect the carrier status in domesticated animals. A pair of primers specific to VSG gene of *Trypanosoma evansi* was developed. The PCR technique was standardized and 400 bp amplicon of VSG gene was obtained from the genomic DNA isolated from the blood of *T.evansi* infected experimental animal. Field validation of the technique is in progress.

Intensive expert system on animal diseases

A web based interactive expert system on animal diseases of the country was developed, and it can be accessed at www.nadres.res.in. It depicts national livestock statistics, animal diseases statistics, eco-patho zones of important livestock diseases, epi-reports and the animal disease forecasting. Based on the dynamic factor, frequency of occurrence of diseases, and the static factor, various precipitating factors, an interactive web service was designed. The user can know the probable occurrence of a particular disease in any district of the country by feeding the name of the state and district of his choice. This service is useful to researchers and particularly to the planners to implement the disease control measures well in advance.

- Serum Bank facility has more than 170,000 serum samples from all over the country, which is being used for long-term national surveys on various diseases of economic importance.
  - Development of relational database on Animal Health Information System
  - Development of *India.admasEpitrak* – a relational animal health information database software
  - Development and launching of National Animal Disease Referral Expert System
  - Identification of disease specific Eco-patho zones in the country
  - Providing eco-pathozones and spatial and temporal occurrence of diseases for effective vaccination and control of important diseases in different states e.g. PPR in Andhra Pradesh and Karnataka, Brucellosis in West Bengal and Andhra Pradesh, FMD, PPR, HS and BQ in Maharashtra, Andhra Pradesh and Karnataka.
  - Providing the logistic support to national network projects like bluetongue and HS projects for disease monitoring and surveillance.

Bluetongue

A repository of blue tongue virus isolates BTV-1 (2 isolates), BTV-2 (4 isolates), BTV-9 (3 isolates), BTV-15 (5 isolates), BTV-18 (4 isolates) and BTV-23 (7 isolates) from Izatnagar, Hyderabad, Parbhani, Kolkata, Parbhani, Hisar and Chennai, was made. No outbreak of bluetongue was recorded in the country except Andhra Pradesh, Karnataka and Tamil Nadu. Disease forecasting model was developed. The incidence was as high as 95.5% sheep from Uttarakhand; 88.6% cattle from Panjab; 63.8% goat and 55.6% sheep from Manipur; 50.0% sheep from Jammu and Kashmir; and 18.22% goat from Delhi. A VP7 gene incorporated recombinant antigen based indirect ELISA kit was developed for detection of group specific antibodies in the sera.

Inactivated pentavalent bluetongue vaccine was evaluated at different places particularly in the bluetongue affected states. Vaccine was satisfactory except a nodule formation at the site of inoculation.

Type specific primer designing, VP2, VP5 and VP7 gene cloning and expression, multiplex RT-PCR for BTV, RNA-PAGE and nucleotide sequence studies, were standardized. Confirmation of virus isolates was done by RT-PCR using VP7 gene specific primers.

Haemorrhagic septicaemia

Isolates (93) of *Pasteurella multocida* were characterized and a new serogroup E of *Pasteurella multocida* was identified first time in the country. Most of the *Pasteurella multocida* isolates were sensitive to enrofloxacin, ofloxcin chloramphenicol, doxycycline and resistant to vancomycin, bacitracin, and sulphadiazine. Molecular characterization of different isolates of *Pasteurella multocida* recovered from different species of animals and poultry were carried out by PM-PCR, HSB-PCR, multiplex-PCR, ERIC-PCR and REP-PCR. A low volume saponified HS vaccine was validated successfully in farm cattle, and it was found satisfactory. The OMP vaccine against *P.multocida* type A in ducks provided higher protection as compared to the bacterins. The biofilm vaccine against *P.multocida* type A of sheep origin was prepared and compared with the whole cell vaccine, and it produced higher immune responses on using montanoide oil adjuvant. Economic loss of more than Rs 225 million was estimated due to haemorrhagic septicaemia in cattle and buffalo.

Ethnovetrerinary medicine

Concomitant use of hydromethanolic (1:1) extract of fruit pulp and seeds of tamarind (*Tamarindus indica*) at different doses reduced F concentration in blood and bones and enhanced urinary excretion in rats indicating the ameliorative potential of tamarind fruits in fluoride toxicity. Testing of bio-fractions A, B and C, revealed that fraction B and C reduced the fluoride, whereas fraction A showed antibacterial activities. An indigenous herbal teat dip was developed for effective control of mastitis and the same is being cross validated to facilitate patent**.** *Azadirachta indica* stem extract effectively treated sub clinical mastitis, and for it standardized dose of *A. indica* stem extract was established. An alkaloid isolated from test herb ANAND-EVM-NW-4 revealed antibacterial efficacy against common isolates from field cases of mastitis. Ethanolic extract of AAU-EVM-NW-3 showed potential effects @ 500 and 750 mg/kg p.o. in clinical case of fasciolosis in ovines, and 7.5% ointment of AAU- EVM –NW-2 was effective in *Psoroptic* mange in rabbit (*Oryctologus cuniculus*). Toxicity testing of all the effective herbs established their safety.

Gastrointestinal parasitism

In Rajasthan software 'FROGIN' was evaluated for forecasting of gastrointestinal (GI) nematodosis in semi-arid and arid regions. It gives results as predicted faecal egg counts (FEC) on start of month, intensity of FEC for next 60 day and pasture larval burden for that month. The Garole sheep was not found completely refractory to infection of *Haemonchus contortus*. *Haemonchus, Bunostomum, Nematodirus* and *Oesophagostomum* spp. were found in all the zones in Sikkim. 170 kDa polypeptide of larval antigen of *H. contortus*

was recognized by 4 day sera (prepatent sera) of sheep in western blotting. Zymogram studies revealed that 120 and 170 kDa polypeptide belonged to metalloproteases based upon protease inhibitor studies. In the ES product, cysteine protease and GST (glutathione-S-tranferase) were identified, which are of immunodiagnostic and immunoprophylectic value. In *H.contortus* ES antigen 30-32 kDa polypeptide showed protease activity, which was inhibited by E–64 confirming it to be a cysteine protease. GST was confirmed in western blotting utilizing anti-GST antibody. ES antigen was better than gut antigen of *Ascaris suum.* Dipstic ELISA was comparatively found more efficient than plate ELISA. No correlation could be established between worm burden and antibody titre in naturally infected sera of pig with *A.suum.* Immunodominant polypeptide in *Bunostomum* and *Oesophagostomum* spp. was identified, and a diagnostic kit for serodiagnosis was developed and revalidated. Allele specific PCR was applied to field population of larvae for detection of benzimidazole resistance and was compared to FECRT and EHA. Frequency of BZ-rr (homozygous BZ-resistant) larvae in population ranged from 73.39 to 100% in Northern Rajasthan. Effect of *Fec* B gene on resistance to GIN was conducted in sheep naturally infected with GINs. Lower incidence was observed in Garole sheep. H-11 and H-gal-GP polypeptides of *H. contortus* are being utilized for immunoprophylaxis and studies on H-gal-GP were completed. In H-gal-GP of *H. contortus* MEP-2 fragment showed 94% homology to other international strains.

Equines

Nation-wide active equine disease surveillance, sero-survey was conducted at Rajasthan, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, and Jammu and Kashmir. Antibodies to EHV-1 were detected in 7.1% samples, *Babesia equi* in 24.3% sera tested, and Japanese encephalitis in 5.5% serum samples tested. None of the serum samples tested was positive for equine infectious anemia, African horse sickness, equine influenza and *Salmonella* Abortus-equi. Outbreaks of glanders reported from Uttarakhand, Andhra Pradesh, Himachal Pradesh and Haryana were investigated and diagnosed, and etiological agent *Burkholderia mallei* was islolated. Comparative sero-prevalence of JE in different animal species (equine, cattle, buffalo, pigs) was done in different regions of Haryana and highest incidence was in buffaloes followed by pigs, horses and cattle. EHV-4 virus was isolated from 11 out of 138 samples using equine embryonic lung cells. These results were confirmed by sequencing of PCR products. The centre succeeded in *in vitro* cultivation of bloodstream forms of *Trypanosoma evansi.* The characterization of *T. evansi* antigen by SDS-PAGE of sonicated antigen revealed five major polypeptides in the molecular weight range of 41-81 kDa and proteins of 35-41 kDa exhibited proteolytic activity. Serum neutralization test (SNT) and haemagglutination inhibition (HAI) were standardized for specific differentiation of two related arboviruses i.e., Japanese encephalitis (JE) and West Nile virus (WNV). On comparison sensitivity of HAI was 96.29% and specificity 100% in comparison to SNT. RT-PCR using E-gene (291 bp) was also developed for diagnosis of JE in equines.

An ELISA was developed for detection of *Babesia equi* specific antibodies. The sensitivity and specificity of the ELISA in comparison to commercial CI-ELISA was 94 and 96%, respectively. To study the polymorhphism of the MHC class II gene in Marwari horses, regions of MHC class-II (DRB-2a and 2b) gene fragments of 276 bp and 229 bp were amplified. Restriction analysis revealed that MHC-DRB2 (276 bp fragment) on digestion with *Hin*fI exhibits polymorphism in 48.39% genotypes.

Microsatellite based parentage testing was done using 194 DNA samples collected from blood leukocytes of different horse breeds. Genotyping was performed by analysis of nine microsatellite and selected microsatellites were highly polymorphic as mean number of alleles ranged from 3.78 to 10.78. Total exclusionary power of both parents in all breeds was more than 0.9 and all the foals (67) qualified the offspring-candidate parent compatibility.

Yak

A slide enzyme linked immunosorbent assay (SELISA) was standardized for the detection of *Babesia bigemina* antibodies in yak sera. Serological studies for detection of *B. bigemina* specific antibodies in yak from an organized farm and under field conditions revealed 44.16 and 56.10% seropositivity, respectively. It could be concluded that being more economical and technically simpler, SELISA could be used for seroprevalence studies on babesiosis in yak.

Keratoconjunctivitis was noticed in yaks, and *Moraxella bovis* and *Neisseria* were recovered from the ocular swabs. Serum samples from the affected animals were analyzed by viral neutralization test. AB-ELISA for the presence of bovine herpes virus -1

Bovine herpes virus-1 associated kerato-conjunctivitis in a yak

Equine welfare

The centre extended equine welfare activities in different parts of the country by organizing equine health camps and farmer meets (*Ashwa Palak Goshthis*) to educate the equine owners on various aspects of disease control and management.

(BHV-1) specific antibody. Nested PCR conducted using glycoprotein B and glycoprotein E specific primers (of BHV-1) revealed the presence of BHV-1 in the ocular swab of the affected yaks. The serological and molecular analyses indicated the possible role of BHV-1 in severe forms of keratoconjunctivitis in yaks.

Yak sera samples collected from different yak tracts of India were screened for the detection of BHV-1 specific antibody. The overall prevalence of BHV-1 specific antibody was alarmingly high (more than 40%) in yaks. Sex and location of different yak tracts did not have any influence over the IBR prevalence. However, the prevalence increased with the age of the animals, and was highest in yaks more than 3-year-old. Conjunctivitis and reproductive abnormalities were predominant symptoms among the seropositive yaks. The common ecological niche for feeding, watering and grazing with other domestic and wild animals is the possible avenue of infection in yaks.

Poultry

Marek's disease virus (MDV) circulating in PDP flocks was low virulent strain. MD incidence could be reduced more effectively with HVT (cell free) double dose or HVT+SB1 cell associated vaccines than single dose of HVT vaccine. Tumour samples could be safely stored in phenol-chloroform-isoamyl alcohol for PCR. Leg weakness associated with osteomyelitis caused by *Staphylococcus* spp. was recorded in young broilers, while aspergillosis was observed in female line of Gramapriya.

FISHERIES

Capture fisheries

Marine fish landings and catch structure: The marine fish landings of India during the year 2007 has been estimated as 2.88 million tonnes with an increase of about 1.7 lakh tonnes (6.5%) against the estimate of the previous year. The pelagic finfishes constituted 57%, demersal fishes 25%, crustaceans 14%, and molluscs 4% of the total landings. The sector-wise contributions were — mechanized landings 68%, motorized landings 28% and the atisanal landings 4%. Among the commercially important groups, the landings of oil sardine (26% increase over the previous year), penaeid prawns (13.4%), Indian mackerel (26%), croakers (41.9%) and other clupeids (55.6%) recorded substantial increase over their previous year's landings. The landings of non-penaeid prawns (18.6% decrease from the previous year), ribbon fishes (44%), Bombay duck (4.8%), thread fin breams (16.3%) and cuttle fish (27.2%) recorded marginal to substantial reduction from their previous year's landings. The estimates of region-wise production in the total production were—north-east region 13.2%, south-east region 22.6%, north-west region 29.3%, and south-west region 34.9%.

Ring seine fishery for oil sardine along the northern coast of Tamil Nadu: Oil sardine (*Sardinella longiceps*), is the most important pelagic resource on the west coast of India, and its occurrence along the east coast was considered sporadic and rare. In July 2008, large shoals of oil sardine appeared in the near shore coastal waters of Devanampattinam, Cuddalore and Puducherry. This supported high catches in the ring seine units newly introduced fishing practice, from the near shore waters for a month.

Inland fisheries

Exotic fish species invasion in West Bengal wetlands – a cause of concern: During fish stock assessment, breeding populations of exotic tropical South American Sailfin catfish, *Pterygoplichthys disjunctivus* and *Pterygoplichthys pardalis*, were recorded in Gomokpota *beel* under East Kolkata Wetlands. Huge biomass of these species, approximately 20 metric tonnes, was caught in a single month. These fishes do not fetch remunerative price as food fish but occasionally find place in aquaria. These species are prolonged breeders and voracious detritivores. A number of other exotic fish species, viz. *Barbonymus gonionotus* (*Puntius gonionotus*), *Pangasianodon hypophthalmus* (*Pangasius sutchi*), *Clarias gariepinnus*, *Oreochromis niloticus niloticus* and *Piaractus brachypomus* were also recorded in some other wetlands in the state. This has serious ecological and economic implications for the wetland fisheries in the state.

Culture fisheries

Reservoir fisheries enhancement: Fisheries enhancement in reservoirs Dahod in Madhya

Revival of *ghol* and *koth* fishery along the coast of Gujarat

Making the end of long period of decline and poor catch of *ghol* (*Protonibea diacanthus*) and *koth* (*Otolithoides biauritus*), bumper catches of large size fishes by multi-day trawlers occurred at Salaya landing center, Jamnagar, Gujarat. They were caught from Bay of Kutch at 25-30 m depth.

Cobia fishery at Kochi

Cobia (*Rachycentron canadum*), highly priced, but previously of a rare occurrence in the catch, landed in large numbers at Kochi fisheries harbour by multi-day gill-netters and hooks and line units operated in distant waters continuously from September this year. Fishery was supported mainly by large adult fishes weighing 10kg to over 30 kg. Fishery and biology of the species are being monitored.

Pradesh and Pahuj in Uttar Pradesh, was attempted through stocking of fish seed and improvement of institutional arrangements for fish catch and marketing. This resulted in improved fish production by over 60% in one year of experimental intervention. The catch/month, total fish catch and per month fishing days increased. The case studies will be helpful in formulating strategies for reservoir development in the Indo-Gangetic basin.

Freshwater aquaculture

***Labeo gonius* in polyculture system:** The compatibility of *Labeo gonius* with other major carps was studied through three combinations catla, silver carp, rohu and gonius; catla, silver carp, mrigal and gonius; and catla, silver carp, rohu and mrigal at combined density of 7,500 fingerlings/ha. Silver carp gave higher overall species survival, while catla showed the lowest level. Survival of rohu, mrigal and gonius, was intermediate and did not differ among them.

Coastal aquaculture

Low fish meal feed for shrimp: A low fish meal shrimp feed was developed by replacing fish meal and other marine protein sources with plant protein sources. Shrimp production after four months of feeding on the low fish meal feed was 1,308 kg shrimp/ha with a feed conversion ratio (FCR) of 1.31:1. This feed can be successfully used for culturing tiger shrimp at low cost of production.

WSSV risks to shrimp farming due to increased culture of crabs: Crabs are known carriers of white spot syndrome virus (WSSV), hence to address apprehensions of shrimp farmers

Fish stock enhancement in beel fisheries

Haribhanga *beel,* covering an effective area of 125 ha, is under the control of Assam Fisheries Development Corporation (AFDC). The pen culture technology intervention in a partnership mode with leaseholder resulted in doubling fish production/unit and recording a benefit cost ratio of 1.89 and 2.01 for consecutive two years.

The fish catch recorded was 131.1 tonnes of which Indian major carps contributed the major share followed by exotic carps, thus registering per unit increase in per unit productivity to 1,050 kg/ha in the *beel*. With an initial investment of Rs 10 lakh, the net income was Rs 53.5 lakh. The benefit-cost ratio of the lessee was 2.40.

to crab culture, studies were carried out to assess WSSV risks to shrimp farming due to enhanced culture of crabs. The prevalence of WSSV in crustaceans in different geographical regions was estimated based on samples from Andhra Pradesh, Tamil Nadu, Maharashtra and West Bengal. The prevalence levels in crabs used for crab fattening or those found in wild crabs indicate that they do not pose any additional WSSV risks.

Coconut wood for canoe construction

The strength properties of coconut wood compare well with those of other structural timbers like teak and jungle jack, and studies showed that wood with a density of more than 600 kg/m^3 can be used for boat building. A plank built type of canoe (length 6.4 m, breadth 0.83 m and depth 0.42 m) for gillnetting in backwaters was constructed out of coconut wood of more than 50 years old. The price of coconut wood is less than half of the conventional boat building timber, viz. *aini* (*Artocarpus hirsuta*).

Canoe structured of coconut wood