NIAB

National Institute of Animal Biotechnology

राष्ट्रीय पशु जैव प्रौद्योगिकी संस्थान

National Institute of Animal Biotechnology

(An Autonomous Institute of the Department of Biotechnology,
Ministry of Science & Technology, Government of India)

वार्षिक प्रतिवेदन २०१३-२०१४

ANNUAL REPORT 2013-14

Index

FOREWORD

Animal husbandry plays an important role in the rural economy of India. To catalyse the growth of animal husbandry sector through application of modern biotechnological tools, the Department of Biotechnology, Ministry of Science and Technology, Government of India, established the National Institute of Animal Biotechnology (NIAB). The goal is to address the challenges in providing animal food security to the growing population of India and to harness the growing opportunities for export of animal based products.

It gives me a great pleasure to present the third annual report of NIAB for the year 2013-14.

During the last one year, efforts were made to build the main campus in the 100 acres of land allotted by the Government of Andhra Pradesh within the campus of University of Hyderabad. The master plan and building designs for laboratory complex, animal house, BSL-3 facility, animal farms, hostels, guest house and residential complex have been finalized and tenders have been invited. We are hopeful that the contractor will be identified and the construction work starts very soon. While efforts are being made to develop the main campus, NIAB has established an interim rented facility with state of the art infrastructure and initiated research programs on infectious diseases, bacterial, viral and protozoan diseases of livestock. In addition research programs on reproductive biotechnology and genomic analysis and genome editing have been initiated.

Along with R&D activities, NIAB has initiated steps to start the following academic programmes from the next academic year:

(1) M.Sc. in Quantitative Animal Genetics and Genomics: In collaboration with University of Hyderabad and Roslin Institute, University of Edinburgh, UK;
(2) Ph.D. in Animal Biotechnology: In collaboration with University of Hyderabad;
(3) Ph.D. in Veterinary Sciences: In collaboration with Indian Veterinary Research Institute (Deemed University) and
(4) Summer Training Programs.

In order to facilitate growth of small businesses and promote bio-entrepreneurship, translational research and innovation have been planned as integral part of the corporate strategy of NIAB. Towards this direction NIAB has recently organized an industry interactive meet, which has resulted in industrial collaborations. Another recommendation of the meet is to provide incubation facilities for the start ups at the earliest.

I sincerely acknowledge the support and encouragement received from the Department of Biotechnology, the distinguished members of the NIAB Society, Governing Body, Scientific Advisory Committee, Finance Committee and Building Committee. The immense support received from Centre for DNA Fingerprinting and Diagnostics (CDFD), University of Hyderabad, C.R. Rao Advanced Institute of Mathematics, Statistics and Computer Science (AIMSCS), Center for Cellular and Molecular Biology (CCMB) and Agri Biotech Foundation is deeply acknowledged. The support received from a number of ICAR institutions, Sri Venkateswara Veterinary University, AP State Animal Husbandry Directorate, Veterinary Biological Research Institute (VBRI) and Veterinary Council of India-AP Chapter is also acknowledged.

The progress made so far is because of the contributions of highly dedicated scientific, technical and administrative staff of NIAB for their untiring efforts in meeting the challenges with limited resources. I sincerely hope and wish continued support and dedication in the years to come in achieving excellence in all the endeavours of NIAB.

Prof. P. Reddanna
Director

OVERVIEW

National Institute of Animal Biotechnology (NIAB) has been set up by Department of Biotechnology (DBT), Govt. of India, on the recommendations of Scientific Advisory Council to the Prime Minister (SAC-PM) to address the challenges in providing animal food security in India.

The goal of NIAB is to harness the technological advances in biology and biotechnology to improve animal health and productivity and at the same time effectively utilize the vast genetic resources established in various Universities and Indian Council of Agricultural Research (ICAR) system. The idea is not to duplicate resources but effectively utilize the abundant resources on a mutually complementary basis. In addition to collaborations with institutions in India, NIAB is committed to establish linkages with leading veterinary schools/research institutions globally. The focus of NIAB is to promote innovation and entrepreneurship and build a strong platform for translational research and promote animal biotechnology based industry. The strategy will be to involve industry in strategic planning and execution of R&D activities throughout the program life cycle. The NIAB is a new generation institution that will adopt a multi-disciplinary approach and provide a frontline demonstration of Academy-Industry Collaborations.

MISSION AND VISION

Mission:

Development of sustainable and globally competitive livestock resources through innovative technologies.

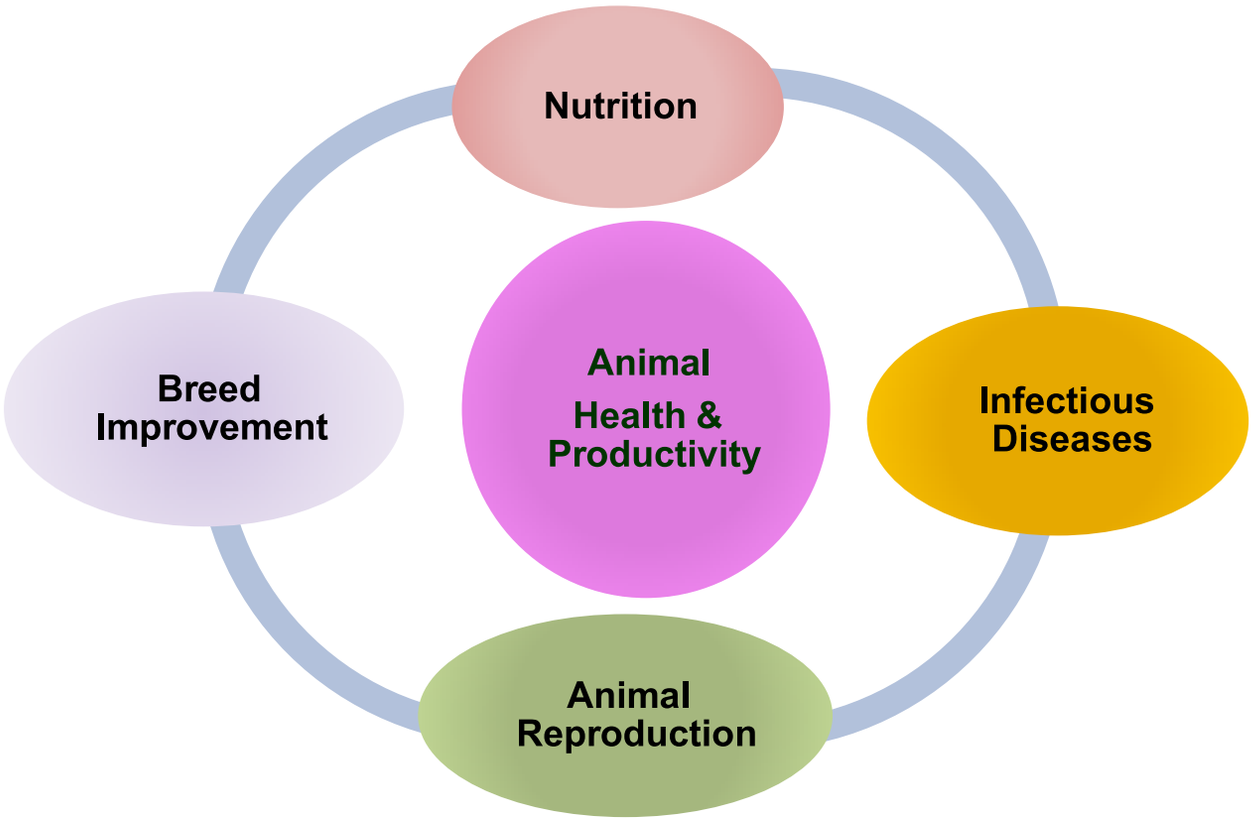
Vision:

To demonstrate excellence in promoting and commercializing leads in Biotechnology to improve animal health and productivity and thus produce globally competitive Livestock products, Pharmaceuticals and Biologicals.

Focus:

- To take up research in the cutting edge areas for improving animal health and productivity.
- The institute aims at translational research leading to the development of novel diagnostics, vaccines and improved therapeutic molecules for farm animals.
- The key feature is that it will function as an incubator for start-up companies and promote bio-entrepreneurship.

PRIORITY RESEARCH AREAS



- Infectious Diseases of Livestock: Host–pathogen Interactions
- New generation vaccines, diagnostics and therapeutic molecules against selected infectious diseases of livestock.
- Genetic Epidemiology- Genetics of disease resistance in farm animals
- Genetic Architecture and genome enabled selection in Indian livestock breeds.
- Reproductive Biotechnology to address infertility problems in livestock
- Stem cells and cell reprogramming for multiplication of elite livestock.
- Nutritional Enrichment in improving Animal health and productivity.

FIVE YEAR STRATEGIC PLAN

- Establishing frontline laboratory infrastructure that supports research in animal biology and biotechnology
- Recruitment of competent scientists and creating an environment that promotes intellectual curiosity, team spirit and scientific excellence
- Initiating collaborations with leading institutions engaged in animal biology and biotechnology research in India and abroad
- Industrial linkages for development and commercialization of technological breakthroughs and promotion of Bio-entreprenuership.
- Humon Resource Development in neglected areas where expertise is lacking in the country e.g. Quantitative Genetics and Genomics
- Extension and outreach activities: Diagnostics for emerging infectious diseases

INTERIM FACILITY

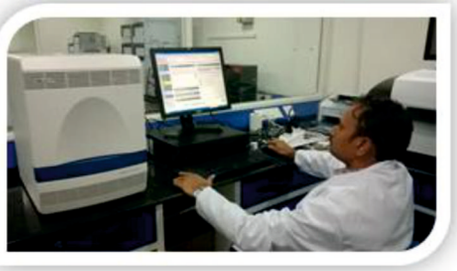
Front view of New Interim Facility

NIAB currently is located in an interim facility at Miyapur, Hyderabad. This interim facility, with state of the art infrastructure, was inaugurated on 2nd September 2014 by Dr. VijayRaghavan, Secretary, Department of Biotechnology, Government of India. The facilities include two main R&D laboratories, Bioinformatics facility, stem cell, mammalian cell and microbial culture facilities, BSL2+ laboratory, genome manipulation facility, centralised instrumentation facility that includes , Flow cytometer, confocal microscope, ultra and high-speed centrifuges, HPLC, microplate reader, RT PCR, 2D Gel electrophoresis system, spectrophotometer, oxygraph and other instruments required for any biochemistry and molecular biology laboratory.

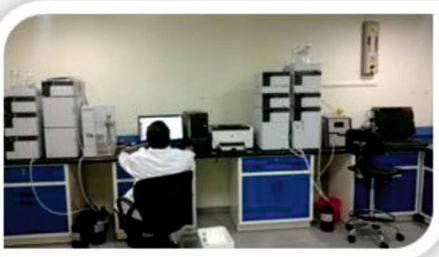
RESEARCH FACILITY

Confocal Microscope

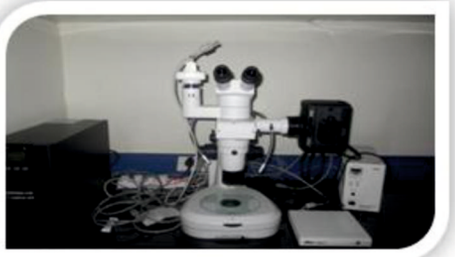
Real Time PCR



HPLC

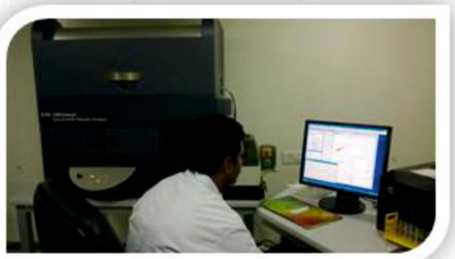


Stereo Fluorescence Zoom Microscope



Flow Cytometer (Sorter)

Flow Cytometer (Analyser)



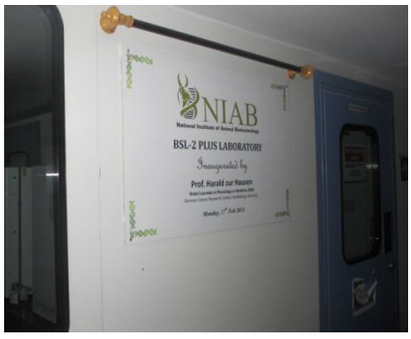
Micromanipulation System

Ultra Centrifuge

SETTING UP BSL-2+ LABORATORY

An essential facility to handle the aerosol transmitted infectious pathogens especially causing zoonotic disease, A BSL 2+ laboratory was established this year. The BSL-2+ involves moderate to high - risk agents and therefore requires a strict adherence to BSL-2+ containment with BSL-3 work practices and procedures. This BSL-2 Plus laboratory constitutes four anterooms (entry and exit), 2 research labs, dirty corridor, dynamic pass box and an autoclave room. Research laboratories are under negative pressure compared to anterooms and corridors. Air quality is Class 10,000 (ISO-7) and air pressure differential is 15 Pa.

The BSL-2+ facility was inaugurated by the Nobel Laureate **Prof. Harald Zur Hausen**, professor emeritus at German Cancer Research Centre, Heidelberg, Germany on 17th February 2014.



FACULTY RESEARCH REPORTS

Pallu Reddanna

Eicosanoids and Inflammation: Role in Regulation of Physiological and Pathological Processess

Inflammation is a key component in host's defense against pathogen's invasion and can be defined as the reaction of vascularized tissues to local injury/infection. Uncontrolled inflammation, however, is associated with cardiovascular, respiratory, neurological and many lifestyle diseases. The inflammatory diseases in livestock include bovine respiratory disease (BRD), endotoxemia resulting out of infection of the mammary gland (mastitis), the reproductive tract (metritis), the lung (pneumonia) etc. The key mediators of inflammation include the bioactive lipids such as eicosanoids.

Eicosanoids, the oxygenated metabolites of polyunsaturated fatty acids (PUFAs) such as arachidonic acid, play a key role in physiological (reproduction) and pathological (inflammatory diseases) processes. At cellular level release of arachidonic acid from the membrane phospholipids is oxygenated via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to the formation of eicosanoids such as prostaglandins and leukotrienes. Current focus of our group is to understand the role of eicosanoid signaling in the regulation of physiological as well as pathological processes. Some of the ongoing studies include:

a. Eicosanoid signaling in physiological processes-reproduction

Eicosanoids have been implicated in the regulation of reproductive events such as ovulation, luteolysis, estrus cyclicity, implantation and parturition. Earlier we have demonstrated that sheep uterus is the most abundant source for a 12, 15-dual LOX, which generates a novel 14, 15-series of leukotrienes (Sailesh et al., Arch Biochem Biophys. 1994 Dec; 315(2):362-8). The key enzymes involved in the conversion of leukotriene A_4 (LTA_4) to leukotriene C_4 (LTC_4) have been isolated and characterized from sheep uterus . Recently the metabolism of arachidonic acid was analysed in the non-pregnant sheep uterus *in vitro*, using conventional chromatographic as well as HPLC techniques (Padma et al., Indian J Biochem Biophys. 2007 Aug; 44(4):216-22). These studies provided comprehensive analysis on the expression of enzymes and the levels of eicosanoid metabolites formed in sheep uterus *in vitro* via the LOX and COX pathways. With this background the current focus is on AA metabolism in fertile, infertile and sub-fertile animals so as to identify the derangements in specific eicosanoid pathways and their metabolites in fertile and sub-fertile animals.

Work done so far:

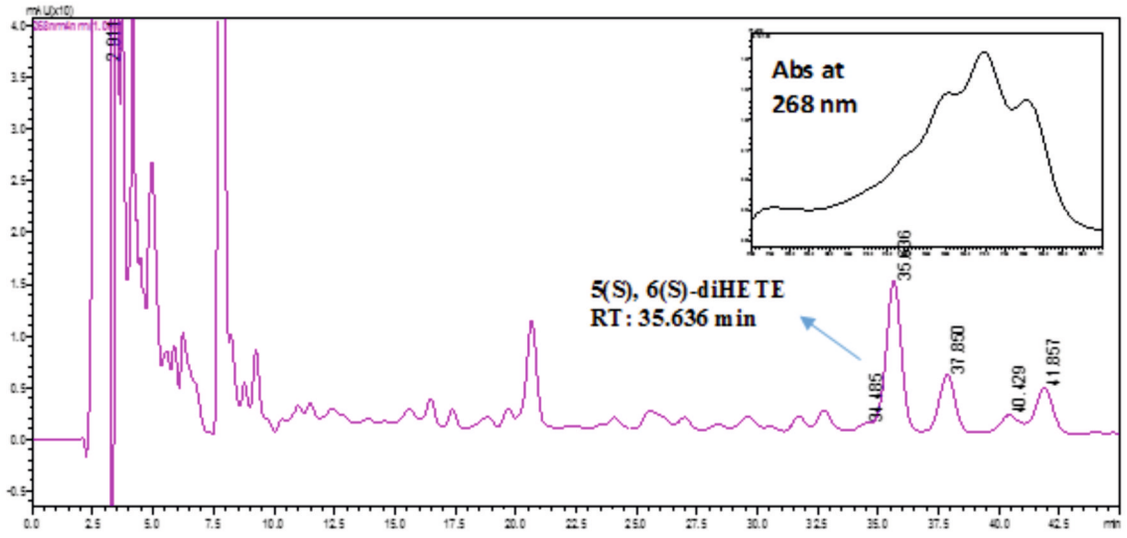


Fig. 1 Separation of 5(S), 6(S)-diHETE from sheep uterus on Reverse phase HPLC. UV-Vis scanning spectrum in the inset.

addition to the primary oxygenated metabolites of AA via the 12, 15- dual LOX and 5-

LOX, analyzed earlier, we have demonstrated the formation of 5(S), 6(S)- diHETE (Fig.1) and 5(S), 6(S) Leukotriene C4 (Fig.2) in sheep uterus. These metabolites, after extraction and isolation were separated on RP-HPLC and identified based on co-chromatography with the standards and spectral characteristics.

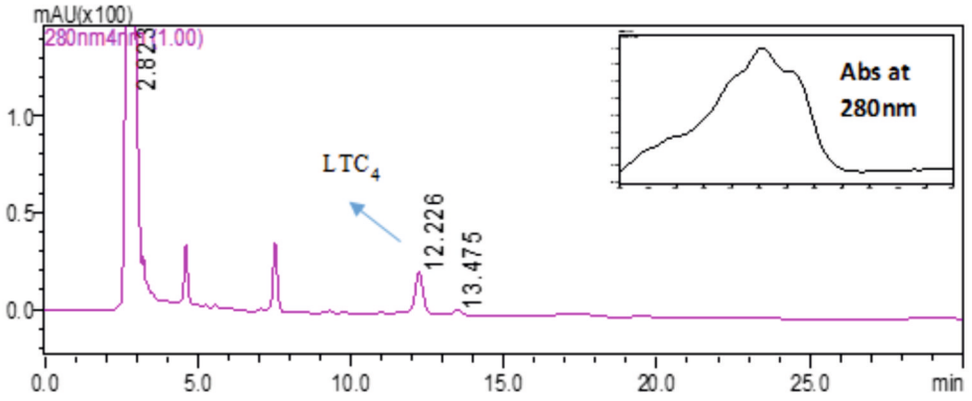


Fig. 2 Separation of 5(S),6(S)-Leukotriene C_4 from sheep uterus on reverse phase HPLC UV-Vis Spectrum in the inset

a. Eicosanoid signaling in pathological processes- inflammation

(i) Eicosanoids in Host-pathogen interactions

There is increasing evidence on the role of eicosanoids in connecting innate and adaptive immunity by acting on cells of both systems. Among these PGE_2, produced at sites of inflammation by the action of inducible isoform of COX, COX-2, and LTB_4 and LTC_4, formed via

the 5-LOX pathway, play a key role in mediating immune and inflammatory responses. While COX-2 and 5-LOX pathways lead to the formation of pro-inflammatory eicosanoids, the 12/15-LOX pathway has been implicated in the biosynthesis of anti-inflammatory resolvins. By acting on various aspects of immune and inflammatory reactions, these lipid mediators emerge as key regulators of the crosstalk between innate and adaptive immunity. The susceptibility or resistance of individuals towards pathogen infection, therefore, depends on the level and type of eicosanoids formed in response to pathogen infection. Recent studies indicate that sequences similar to LOXs exist in bacteria also. However, their role in pathogenesis has not yet been elucidated. The present study, therefore, is aimed at structural and functional characterization of enzymes involved in eicosanoid biosynthesis and analyzing their role in host-pathogen interactions.

Table1. List of mutations performed on various lipoxygenases showing their effect on relative activity and share of HETE's formed by mutants and wild-type in 5-LOX (5-HETE:8/12-HETE), 12S-LOX (12-HETE:15-HETE), and 15-LOX (15-HETE:12-HETE).

Isozyme	Mutant (MT)	Share of different HETE's formed by MT and WT	Relative activity (%)
5-LOX	wild-type	80:20	100
	P570C	95:5	100
	A607Q	99:1	400
	F421S	99:1	5
	A567F	99:1	5
12-LOX	wild-type	95:5	100
	F414S	85:15	33
	A557F	96:4	12
	V661I	99:1	86
	S597G	99:1	99
	T364G	99:1	273
15-LOX	wild-type	85:15	100
	F414S	89:11	36
	A557F	72:28	5
	V661I	91:9	57
	G364T	95:3	127
	G597S	89:4	129

Work done so far:

- Prediction of key amino acid determinants responsible for positional, substrate and stereo specificities in 5-LOX, 15-LOX-1 and 12S-LOX by employing molecular modeling and sequence analysis.
- Experimental validation of these amino acid determinants by site directed mutagenesis and product analysis on HPLC (Table 1).

- Based on *in silico* prediction and experimental validation (Fig.3) the mechanisms behind structural and functional correlations of LOXs are being elucidated.

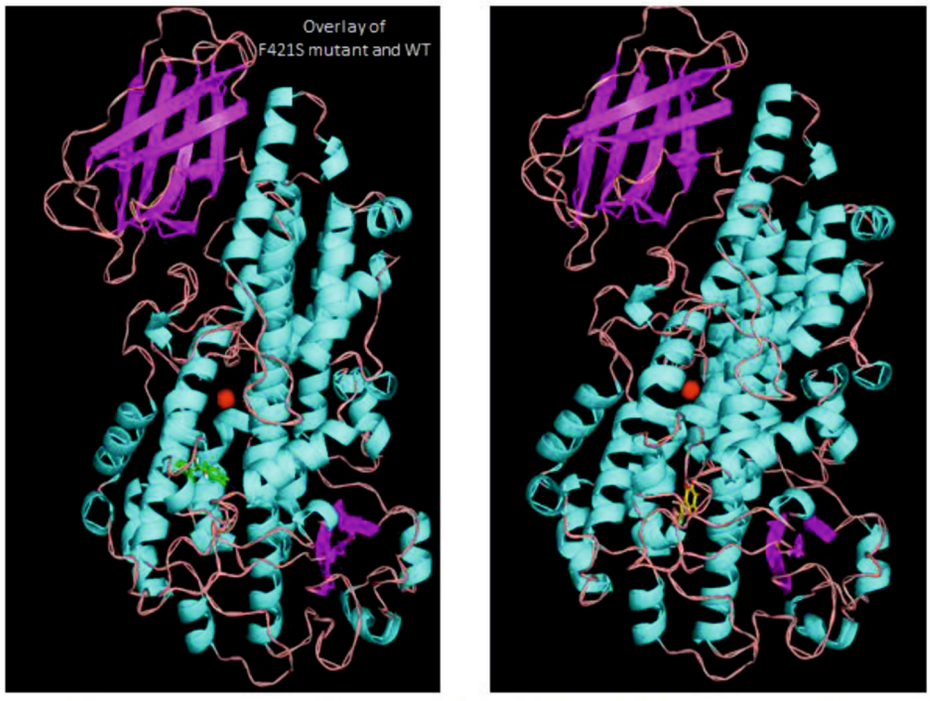


Fig. 3 F421S mutant (phenylalanine is substituted by serine) and A567F mutant (alanine is substituted by phenylalanine) of 5-LOX

(ii) Discovery and Development of natural anti-inflammatory drug candidates.

The present work is aimed at the development of anti-inflammatroy drug candidates from medicinal plant sources. . The goal is towards identification of COX-2/5-LOX dual inhibitors, which could be potential anti-inflammatory drug (CLOXIB) candidates. The candidates thus identified may be taken up for development of next generation anti-inflammatory drugs without gastric and cardiac side effects, which are associated with the conventional non-steroidal anti-inflammatroy drugs (NSAIDs) and COX-2 selective inhibitors (COXIBs) respectively. Recently, we have isolated and identified 4-(Benzyloxy)-1-Phenylbut-2-yn-1-ol, Prenylated Chalcones, Gallic acid and Salvinolone derivative as anti-inflammatory agents targeting either COX-2 and/or 5-LOX (Fig. 4). The hit molecules thus identified are being further optimized for enhancing the specificity and selectivity towards COX/LOX pathways by employing rational drug design approaches.

In addition to above, our efforts on screening of medicinal plants for anti-inflammatory principles resulted in identification of *Gymnema sylvestre* leaves as a potential source for anti-inflammatory compounds. These studies on the evaluation of extracts of *G.sylvestre* showed 54% inhibition against COX-1, 53% inhibition against COX-2 and 24% inhibition

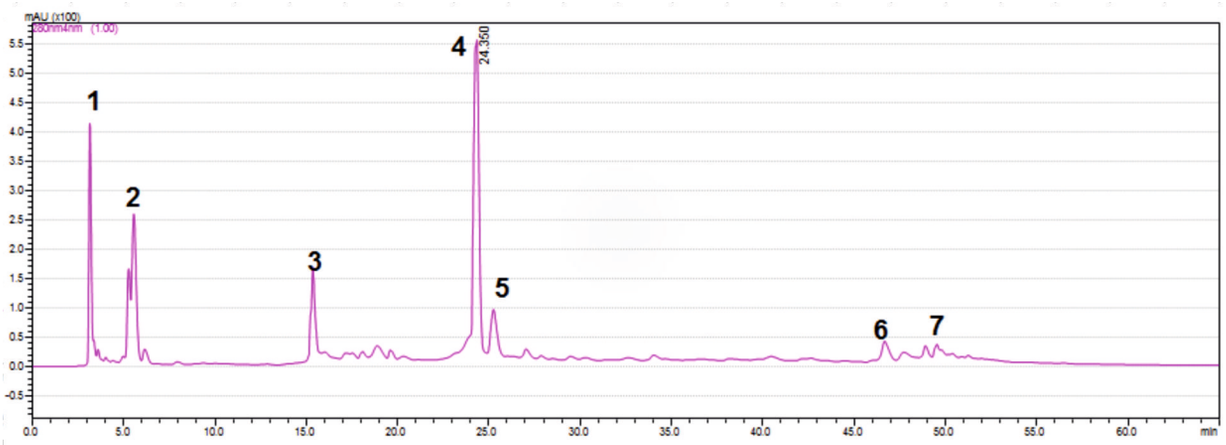


Fig.4. Separation of active principles from hexane extract of G. Sylvestre

against 5-LOX at100 µg/mL of the hexane fraction. Further fractionation of the hexane extracts on straight phase HPLC (Fig. 4) resulted in the identification of the active peak with inhibition of COX-2 and 5-LOX, which is being characterized by LC-MS and NMR analyses.

Research Group

Dr. Rajib Rajhans	-	Research Associate
Dr. Anil Kumar Kotha	-	Research Associate
Ms. Sriravali	-	Project Fellow

Collaborators

Prof. Hartmut Kuhn, Institute of Biochemistry, University Medicine Berlin-Charité,
Charitéplatz 1, D-10117 Berlin, Germany.

Prof. M. Rami Reddy, Rational Laboratories, San Diego, USA

Prof. V. Lakshmipathi, Guest Professor, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad.

Satish Kumar

Biodiversity, genome analysis, genetic mapping and genome editing in laboratory and farm animals

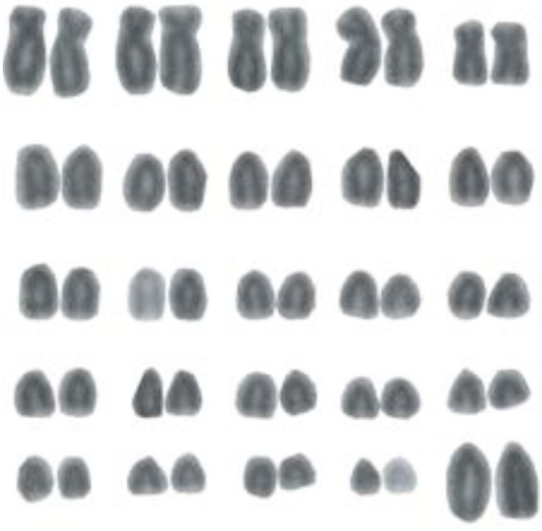
My research interest includes genome analysis and genome editing of livestock species. I am also interested in population genetics of farm animals and some of my seminal contributions in this area include genetic evidence for independent domestication of sheep in India and signatures of atypical domestication of river buffalo.

Program title: Stem Cells and Cell Reprogramming for Multiplication of Elite Livestock
Project title: Differentiation of Pluripotent Stem Cells into Female Germ Cells and Oocytes in Buffalo.

Background: There is a growing need to improve the quality of our livestock for characteristics that are valuable both in terms of the production capabilities and the well being of the animal. *Embryo* technology is a form of reproductive *technology* that has *greatly influenced* the *animal agriculture* industry. In vitro production of embryos is a three-step process involving oocyte maturation, oocyte fertilization and in vitro culture. The quality of the oocyte is crucial in determining the fate of the process. The greatest challenge is in obtaining quality oocytes for transgenesis. In vitro generation of oocytes can revolutionise livestock industry by rapidly enhancing valuable genetic traits. Further, despite several recent advancements, obtaining oocytes for transgenesis remains a challenge due to their complex structure and limited availability. Therefore, differentiation of embryonic stem cells like induced pluripotent stem cells into oocytes can provide an unlimited source of egg cells for the generation of trasngenic livestock. A number of culture systems have so far been tested for use in differentiation of mouse embryonic stem cells and or induced pluripotent stem cells into germ cell lineages. However, it has not been possible to establish an efficient culture system that produces an unlimited number of germ cells with potential to contribute to gametogenesis and offsprings.

Objectives of the project: The main objective of the project is to develop efficient protocols to produce fertile oocytes from pluripotent stem cells. The project proposes to study the establishment of induced pluripotent stem cells and their differentiation into oocytes in buffalo with the following clear objectives:

1: Derivation of buffalo induced pluripotent stem cells
2: Differentiation of buffalo induced pluripotent stem cells into primordial germ cells and further development into oocytes
3: Functional assessment of the derived oocytes from pluripotent stem cells



Karyotype of Female Buffalo Skin

Current Progress:

The first goal of the project is to establish induced pluripotent stem cells from buffalo somatic cells such as skin fibroblasts. To that end we are in the process of optimizing methods to establish standard procedures for the derivation of buffalo iPSCs from buffalo skin biopsies and their successful maintenance in culture. As a first step we have obtained female buffalo skin fibroblasts that serve as the source of adult somatic cells for reprogramming to buffalo iPSCs. Karyotype analysis was performed on these cells to confirm that these cells have a normal chromosome number and a karyotype of female buffalo.

To establish buffalo induced pluripotent stem cells lentiviral approach has been adapted to express transcription factors Oct4, Klf4, Sox2, c-Myc and Nanog for reprogramming. Lentiviruses were produced in 293T packaging cell lines and supernatants containing the viral particles are now used to infect buffalo skin fibroblasts to establish the culture conditions in the lab to generate buffalo iPSCs efficiently for further differentiation in female germ cells and oocytes.

Research Group

Dr. Himabindu Gali	- Research Associate
Dr. Vasundhra Bhandari	- Research Associate
Mr. Akshay Joshi	- Project Fellow

Girish Radhakrishnan

Development of Vaccine for Brucellosis

My research interests include understanding the virulence mechanisms of the infectious intracellular bacterial pathogen, Brucella and development of novel vaccines and diagnostic tools for animal and human brucellosis.

Development of novel live attenuated vaccine for bovine brucellosis using combinatorial gene knockout approach.

Brucellosis is the most frequent zoonotic disease worldwide, with over *500,000* new human infections every *year. Brucellosis is endemic in India and the disease is reported in cattle, buffalo, sheep, goats, pigs, dogs and human.* The prevalence of human brucellosis has been documented from various states of India including Orissa (6.8%), Andhra Pradesh (11.51%) and Punjab (26.6%). Antibiotic treatment of brucellosis remains complex, requiring prolonged administration of more than one antibiotic and the efficacy of treatment is often reduced due to frequent treatment failures and relapses. There is no human vaccine available for brucellosis and the existing animal vaccines have several disadvantages like pathogenicity to human, induction of abortion in vaccinated animals and the secretion of vaccine strains in milk and urine. Control of human brucellosis depends on prevention of the disease in livestock by mass vaccination. Therefore, it is essential to develop safe and efficient *Brucella* vaccines for livestock brucellosis that will eventually reduce the incidence of human brucellosis.

Work done. The project to develop novel live attenuated vaccine for bovine brucellosis using combinatorial gene knockout approach was initiated in collaboration with the Ella Foundation, Genome Valley, Hyderabad. Four virulence genes that are crucial for intracellular survival of *Brucella* were selected for making the attenuated *B.abortus.* Homologous recombination and Cre-Lox technology are employed for target gene deletions in *B.abortus*. Gene knockout cassettes for four virulence genes were prepared and confirmed by restriction digestion and sequencing. Electroporation of *B.abortus* with the knockout constructs is in progress to achieve the multiple gene deletions.

Development of novel serodiagnostic assays for bovine brucellosis: Identification of immunogenic antigens of *Brucella abortus* in the serum of naturally infected cattle.

Early diagnosis of brucellosis in livestock is very crucial for taking effective control measures that will help to reduce the incidence of human brucellosis eventually. Immunoassay for antigens that are secreted into the body fluids during infection is one of the rapid diagnostic tools for early detection of microbial infections. Current serological diagnosis of animal and human brucellosis is primarily based on identification of antibodies to lipopolysaccharide (LPS) of *Brucella* in the patient serum. LPS is the immunodominant antigen but it cross reacts with several other Gram negative bacteria including *Yersinia enterocolitica* 0:9, *E.coli* 0:157, *Francisella tularensis*, *Salmonella urbana*, *Pseudomonas multiphilia* and several others. LPS based serodiagnostic kits cannot detect rough *Brucella* species like *B.canis*. Diagnosis of *Brucella* by culture is difficult because of its fastidious nature, slow growth and

potential hazard to the laboratory personnel. Therefore, it is essential to develop novel and more specific serodiagnostic assays for brucellosis.

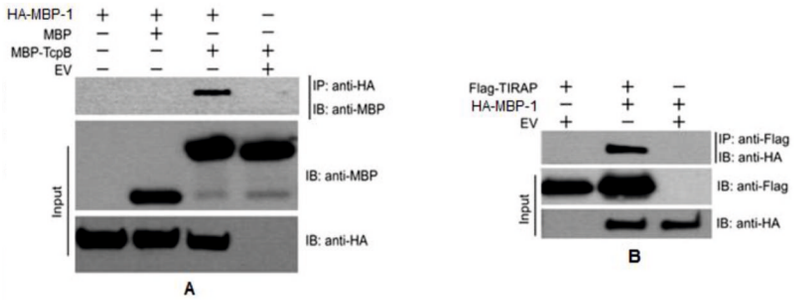
Work Plan. Like any other pathogen, *Brucella* also secrete/shed antigenic proteins into the body fluids of the infected host. These proteins will be sensed by the host immune system followed by the induction of host immune responses including antibody production against the secreted antigens. Therefore, these antigens are ideal candidates for immunodiagnostics development. Towards identification of immunodominant antigens of *B.abortus*, immunoprobing and analysis of *Brucella* protein arrays with serum samples of infected and healthy cattle is in progress.

Whole genome sequencing and comparative analysis of field isolate of *Brucella melitensis* from India.

Work done. We isolated a *B. melitensis* strain from stomach contents of an aborted goat fetus and designated it as Bm IND1. Preliminary analysis of *16S rRNA* sequence of this strain revealed 100% similarity with the Genus *Brucella* and further analyses by PCR for *omp31*confirmed it as *B. melitensis*. The genomic DNA of Bm IND1 was isolated and whole-genome shotgun sequencing was performed using the Illumina HiSeq platform to achieve 100x coverage. The draft whole-genome sequence of the strain Bm IND1 was found to have 3,284,360 bases encoding 3,360 protein coding genes with a GC content of 57.2%. Further, Gene functions were annotated by KEGG, pfam, COG databases. Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession JMKL00000000 and published in *Genome Announcements* journal. Detailed sequence and phylogenetic analysis of Bm IND1 is in progress.

Molecular characterization of the TIR domain- containing protein(Tcp-B) from *Brucella melitensis*

BackgroundFig.1: MBP-1 interacts with TcpB (A) and TIRAP (B)Toll-like receptors (TLRs) are crucial components of the innate immune system that recognize conserved microbial components and trigger antimicrobial responses. The TLR family is characterized by an extracellular domain containing leucine-rich repeats and a cytoplasmic domain, TIR. The leucine-rich repeat domain is involved in the recognition of microbial components, whereas the TIR domain creates a signaling platform to recruit adaptor proteins that ultimately activate NF-kB and cytokine response pathways. Pathogens have evolved many strategies to evade innate immune responses by interfering with TLR signaling to create a replication-permissive environment inside thessss host. *Brucella* encodes a TIR domain-containing protein (TcpB) that inhibits TLR2- and TLR4-mediated innate immune signaling. Our previous studies have



indicated that TcpB targets the TLR adaptor protein TIRAP to inhibit TLR2 & 4. However, the actual mechanism of action of TcpB was unknown. A high-throughput yeast two-hybrid screening identified a eukaryotic microtubule binding protein (MBP-1) that interacted with TcpB and TIRAP (Fig 1).

Work done

Subsequent studies have revealed that MBP-1 is a novel ubiquitin ligase that specifically target TLR adaptor protein, TIRAP. It has been hypothesized that TcpB recruits MBP-1 for targeted ubiquitination and destabilization of TIRAP. *In vivo* ubiquitination assays and mutagenesis studies have performed to analyze the ubiquitin ligase property of MBP-1. Experiments are in progress to analyze the down regulation of TLR 2 and 4 signalling by MBP-1 and its role in chronic persistence of *Brucella* in the host.

Research Group

Dr. Dileep Reddy	-	Research Associate
Dr. Padmaja Jakka	-	Project Fellow
Ms. Bindu Bhargavi	-	Project Fellow

International collaborator

Prof. Satya Parida, Pirbright Institute, UK

Madhuri Subbiah

Vaccines and Diagnostic tools for Viral Diseases of Livestock and Poultry

My research interests are to understand the molecular mechanisms of viral pathogenesis, to comprehend host-pathogen interactions and virus evasion of host immune response. My major focus at NIAB is to apply this knowledge for developing *on-farm* viral diagnostics and novel vaccines.

Establishment of reverse genetics system for rescue of recombinant Newcastle disease virus:

Background:

Newcastle disease virus (NDV) causes one of the most devastating, highly contagious diseases in poultry. NDV has been recorded to have infected more than 236 species of birds worldwide. Four panzootics of NDV are believed to have occurred: from 1926 to the 1960s; from the 1960s to 1973; during the late 1970s; and from the late 1970s to 1981. While most birds show clinical signs of illnesses, some of the wild birds act as carriers of infection. The morbidity (loss in productivity) and mortality (death of the infected birds) depend on the virus strain and susceptibility of the host species. In chickens infected by virulent NDV strains (vNDV) morbidity is up to 100% with 90% mortality. A recent literature has cited that between 2006 and 2009, Newcastle disease had ranked second among all livestock diseases with repect to the number of countries that were affected (*Developmental and Comparative Immunology 2013, Vol 41, pages 447-453*).

The World Organization of Animal Health (WOAH formerly known as OIE) has listed NDV as a notifiable disease. In countries that are NDV-free, huge money is invested for repeated testing to maintain NDV-free status for trade purposes. In developing countries like India, NDV is endemic and has enormous economic impact on both commercial and backyard poultry sectors. Apart from causing direct commercial losses, vNDV indirectly affects the human health by affecting the quantity, quality and availability of the protein food source (chicken egg and meat) especially to the poor sections of the society. The rich genomic diversity of NDV together with the wide avian host range and availability of wild bird reservoirs lead to diagnostic and vaccine failures.

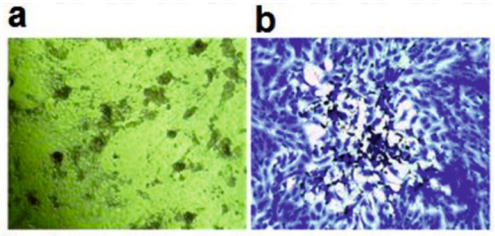


Fig. 1. DF1 (Chicken Embryonated Fibroblast cell line) infected with NDV strain Komarov (a) Rounding of infected cells 3 days post infection (b) Crystal violet stained plaque

Molecular characterization and genotyping of the circulating NDV strains is very important for both epidemiological mapping and for developing effective vaccines and rapid diagnostics for controlling the disease. The Newcastle disease research laboratory at NIAB is focused on developing *on farm* diagnostics for differential diagnosis of NDV and generation of recombinant virus by reverse genetics system for developing efficient vaccines against the circulating virulent strains of NDV.

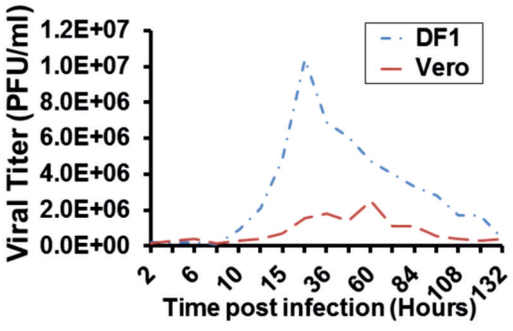


Fig.2. Growth Kinetics of NDV strain Komarov in DF1 (Chicken Embryonated Fibroblast cell line) and (b) Vero cell lines (African Green Monkey Kidney cell line) by multistep growth assay.

Currently, the NDV reference strain and vaccine strain, NDV strain Komarov, is biologically characterized (measuring growth in cell lines: DF1 and Vero; Mean death time, MDT, in embryonated SPF chicken eggs). MDT is the mean time in hours for the minimum lethal dose to kill all the inoculated embryos. The MDT has been used to classify NDV strains into the following groups: velogenic strains (taking less than 60 h to kill); mesogenic strains (taking 60 -90 h to kill); and lentogenic strains (taking more than 90 h to kill). MDT for NDV strain Komarov was 60 h showing that it falls within the mesogenic catergory. We are also sequencing the complete genome of this strain. The information from complete genome sequence will enable construction of full length clone to rescue recombinant NDV. Reverse genetics system is a powerful tool to manipulate the viral genome to understand the molecular biology of the virus as well as to genetically engineer an effective vaccine.

Research Group

Mr. Naveen Gujjar - Project Fellow

Mr. Hanuma Kumar Kranti - Project Fellow

Anand Srivastava

Host-Parasite-Vector interactions, molecular epidemiology of parasitic diseases

My research interest is to understand molecular interactions involved in host pathogen cross talk and identify the potential targets for development of vaccine and diagnostics.

Elucidation of receptor ligand interactions involved in invasion of erythrocyte by *Theileria annulata"*

Background

Theileria spp. infects ruminants, including cattle and sheep, and causes theileriosis. The *theileria* parasites are obligate intracellular apicomplexan hemoprotozoans and are transmitted by ticks. In context of Indian bovines, theileriosis is mainly caused by *Theileria annulata* and the disease is known as "bovine tropical theileriosis". It is highly common in exotic breeds, their cross breeds and young indigenous calves. The main vector responsible for transmission is *Hyalomma anatolicum anatolicum* and other tick species belonging to the same genera. These parasites are responsible for significant economic impact on the livestock industry throughout the world, especially in tropical and subtropical regions. Economic losses are due to the drop in milk production of cattle, and working and breeding capacity of bulls. Also huge economic burden is incurred due to heavy cost of medication of sick animals. Despite huge economic losses due to theileriosis, only limited effort has been made to understand the biology of this parasite. Understanding of molecular events in the life cycle of this parasite will provide clues for intervention and to develop effective, safe, inexpensive and easily deployable vaccine.

Objectives:

1. Establishment of *in vitro* culture for erythrocytic stage of *Theileria annulata*.
2. Identification of proteins on the surface of merozoites which are essential for invasion.
3. Understanding the role of selected surface proteins of merozoites in invasion of erythrocytes.
4. Assessing ability of antibodies against surface proteins for invasion blocking property.

Work done so far

1. Field samples collected from Ananthpur and Karim Nagar district of Andhra Pradesh (table 1)
2. *Theileria annulata* transformed lymphocyte cell line adopted for in vitro conditions.
3. *In vitro* culture for erythrocytic stage of *Theileria annulata* in process.
4. Cloning and expression of *Theileria annulata* merozoite-piroplasm surface antigen (TAMS1) from field isolate (figure1)

Table 1

Prevalence of *Theileria annulata* in Karimnagar district of Andhra Pradesh by polymerase chain reaction (PCR).

District	Samples tested	Positive for *Theileria annulata*
KarimNagar,Andhra Pradesh	70	9 (12.85%)

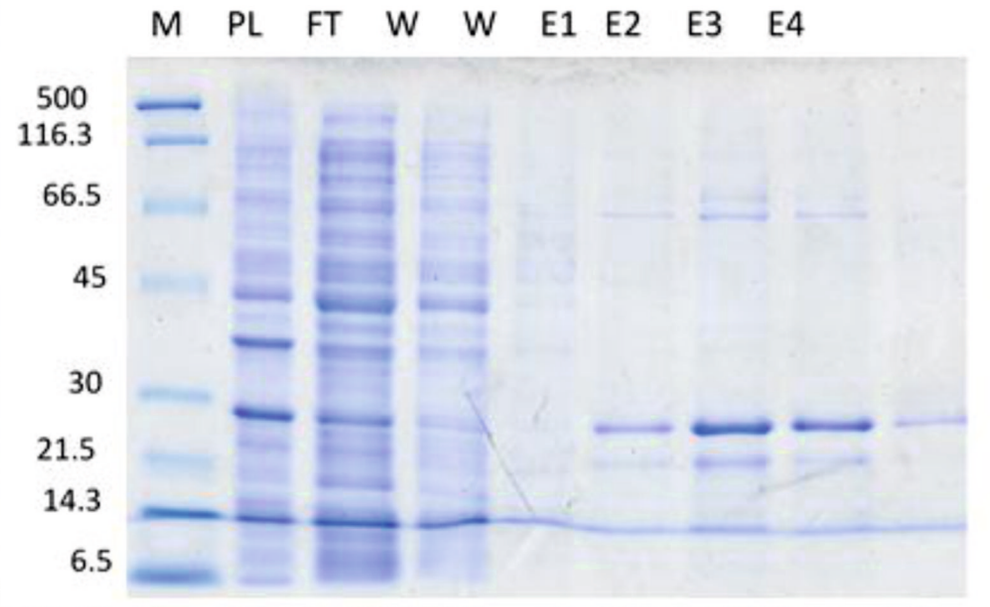


Fig. 1. Expression of *Theileria annulata* merozoite periplasm surface antigen (TAMS1) from field isolate (Ananthpur district of AP). TAMS1, 27kd expressed as His tagged marker protein. (M : Marker.; PL: Preload; FL: Flow through,;W: Wash; E1: Elute1; E2: Elute 2; E3: Elute 3 ; E4: Elute 4)

Research Group

Dr. Rinky Sharma Mukherjee - Project Fellow

Ms. Rakhi Harne - Project Fellow

Paresh Sharma

Functional genomics and genetic basis of disease resistance

My research interest is in understanding the livestock genome that will discern the genetic basis of disease and help in increased production of meat and milk while reducing the producer dependence on antibiotics.

Identification of Signature Markers for the Diagnosis of Pregnancy in Indian buffaloes

Background

Pregnancy diagnosis relies heavily on rectal palpation and Ultrasound, whichrequire highly professional individuals and associated with the risk of abortion. Thelaboratory tests developed for pregnancy diagnosis in buffaloes mostly involve methods like qualitative or quantitative measurement of reproductive hormones or pregnancy associated proteins at specific stages after AI or mating. Unfortunately, none of the methods developed so far in animals are as accurate as is the detection of hCG in pregnant women. None of these, however, are point of care diagnostics for use by farmers. As a result there is a huge demand for a rapid reliable and sensitive point of care diagnostic test for use in livestock, particularly in buffaloes.

Work done. Towards this project, initiated in August 2013, blood samples were collected from the pregnant and non- pregnant buffaloes. Serum is extracted from the blood samples as per the standardized protocol and stored at -80°C. 2 D Gel Electrophoresis experiments have been standardized with the serum samples obtained from the animals. Briefly impurities were removed from the serum samples and proteins were purified using the standard protocols. 2 D Gel Electrophoresis experiments clearly showed a differential expression pattern of proteins between non-pregnant and pregnant samples (Fig 1). The prominent proteins expressed differentially were analyzed using MALDI-TOF. The final analysis is ongoing for identification of differentially expressed proteins from the 2 D gels.

Identification of Virulence factors associated with *Theileria annulata* infection in Indian Cattle

Background

Tick-transmitted Theileria parasites of cattle are a major constraint to the improvement of the livestock industry in large parts of the World. *Theileria annulata*, an economically important species, is responsible for mortality and production losses in dairy cattle in India. Bovine theileriosis is generally controlled by the use of acaricides to kill ticks, also an attenuated schizont stage vaccine is available in India to control the disease. More sustainable, effective and reliable methods for the control of theileriosis are desirable. There is demand for the new generation vaccines as well as diagnostic tools against *T annulata* parasites. The proposed project will help in understanding the virulence mechanism of the *T annulata* parasites and also will lead to identification of virulence related

antigens/genes during parasite infection. This would help to develop efficient vaccines and diagnostic tools against Bovine theileriosis.

Work done

Blood samples have been collected from the areas nearby Hyderabad from the bovines for identifying the prevalent parasite strains. Blood samples collected from the bovines have shown *T annulata* to be the major strain causing Theileriosis. *In vitro* parasite culture has been established and currently standardization for sub culturing, freezing and reviving of parasites is going on. Future plans include finding out the prevalence of major Theileria parasites in Indian Cattle/buffalo. Further whole genome sequencing and proteomics analysis of the avirulent and virulent strain of Theileria annulata parasites will be carried out to identify the genes involved in virulence and infection.

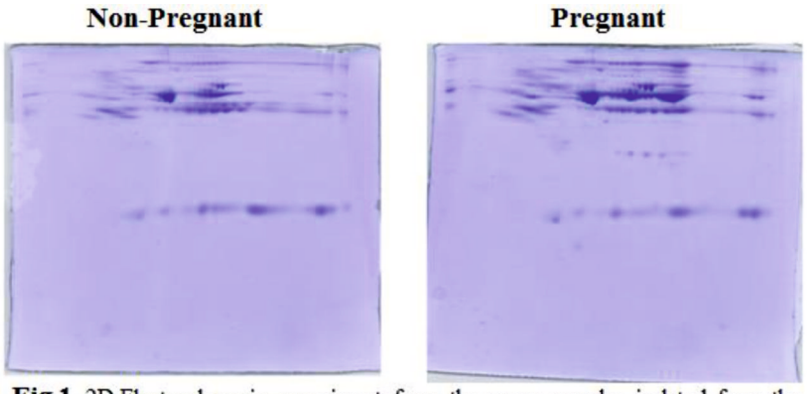


Fig 1. 2D Electrophoresis experiment from the serum samples isolated from the pregnant and non-pregnant buffaloes

Research Group

Mr. Peddi Reddy - Project Fellow

Ms. Neena George - Project Fellow

Sathya Velmurugan

Infertility disorders in Animals, regulation of reproductive axis and ovarian activity: Study of hypothalamic neurons

I am interested in the regulation of reproductive axis and ovarian activity by the newly discovered hypothalamic neuropeptide, kisspeptin. I am also interested in semen quality testing and their impact on male fertility. To delve into basic research, I would like to establish an electrophysiology lab to study the hypothalamic neurons involved in reproduction *in vivo* as well as *in vitro* in brain slices.

Sex-sorting of cattle and buffalo spermatozoa

Background

By 2050, the demand for meat and milk production is expected to double in developing countries where population is expected to double. Increasing production, processing and marketing of meat and milk and their products will be big challenges for livestock producers. Nevertheless, these challenges also present many opportunities. Biotechnology is being harnessed in various aspects of the livestock industry to hasten breed development improve animal health and welfare, address infertility problems, and improve nutritional quality and safety of animal-derived foods. The key challenge is to determine how to intensify livestock productivity in a sustainable manner to meet the increasing demand under the constraints of limited land, water and other natural resources. In reproductive technologies such as artificial insemination and embryo transfer, sexed semen helps in increasing the efficiency of multiplication in breeding permitting pre-determination of sex of the offspring.

Fig. 1. Fluorescent microscopic images of stained sperms. Sperms were stained with Hoechst 33342immobilized with formaldehyde and examined under a fluorescent microscope. Swelling in the tail indicates viable while absence of swelling indicated non-viable spermatozoa.

The availability of sexed semen in dairy cattle has been eagerly anticipated for many years, and recent developments in fluorescence-activated cell sorting (FACS) have brought this technology to commercial application, at least, in the developed countries. Though the demand for sex-sorted semen is huge in India, the availability is extremely meagre. The imported sex-sorted semen, that too of very limited quantity, is from exotic bulls not suitable for our native and crossbred cattle and buffaloes. Hence, there is an imperative need to develop sex-sorting technology for native pure and cross breeds of Indian cattle and buffaloes and to commercialize the same so that sex-sorted semen is readily available for artificial insemination in the field conditions. This project, which is in very early stages, has the following objectives:

Objectives:

1) Standardization and optimization of sex-sorting of *Murrah* (pure) and *Jersey* (crossbred) semen (cryopreserved and fresh) using FACS.
2) Validation of sorted semen by three different methods: Real time PCR, *in vitro* fertilization and *in situ* hybridization
3) Validation of sorted semen in the farm and field animals.
4) Establishment of sexed semen banks for commercialization.

Work done:

1. Standardization of sex-sorting of sperms using FACS:

Sperms from frozen-thawed pretreated and diluted semen is stained with Hoechst 33342 (40 µM; Fig. 1), a nuclear staining fluorescent dye. Sorting is done in a flow cytometer/cell sorter (BD FACS Aria III). The effect of the dye and sorting on sperm morphology and viability are ascertained. Intact viable sperms are flow-cytometrically separated into X and Y populations on the basis of relative DNA content: X sperms have approximately 3% more DNA than Y sperms. Orientation of sperm heads, sorting rate, nozzle size, fluid pressure determine sorting efficiency and yield. Sorting of X and Y populations of sperms is currently being standardized. After a total collection of 1-5 × 10^6 sperms, the sperms are concentrated via centrifugation at 1,500 rpm for 7 minutes and used for validation.

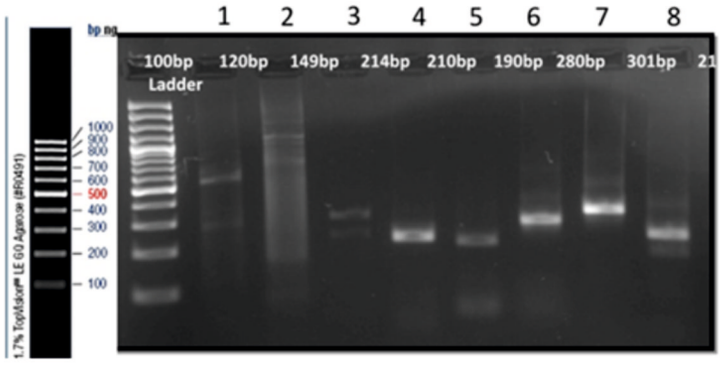


Fig. 4. Agarose gel electrophoresis analysis of PCR products. DNA was extracted from unsorted Murrah serum samples and PCR carried out using eight different primers (lanes 1-8) corresponding to those listed in Table 1. PCR is standardized for first three primers while the signal is clear with rest of the primers.

2. Validation of sorted semen:

After sorting, validation of sorted semen will be done by three different methods:

Real Time PCR, *In Vitro* fertilization (IVF) and Fluorescence *In Situ* Hybridization (FISH).

RT PCR has been standardized and other methods are being standardized currently. Standardization of Real Time PCR: DNA was extracted from unsorted semen and PCR was performed using the following primers targeted at the listed genes (Table 1 and Fig. 2). We are hopeful to complete the first two objectives using Frozen thawed semen in the forthcoming year.

Table 1: The primers and the targets being used to validate sex-sorting of semen.

S. No.	Primer	Length (bp)	Target	Reference
1	y-specific_F	120	GAACGAAGACGAAAGGTGGCT	Malleki *et al*. 2013
	y- specific_R		GCGGGTAAGAAACTCCTCC	
2	X specific PLP_F	149	TAAAGGTGGGTAGGGTCAAGG	Malleki *et al*. 2013
	X specific PLP_R		GCTGTGAATACGACGGTGTAG	
3	AMEL_F		CAG CCA AAC CTC CCT CTG C	Trigal *et al.* 2012
	AMEL_R		CCC GCT TGG TCT TGT CTG TTG C	
4	y-specific_F	210	CCTCCCCTTGTTCAAACG CCCGGAATCATT	Resende *et al.* 2011
	y-specific_R		TGCTTGACTGCAGGGACCGAGAGGTTTGGG	
5	y-specific_F		ATCAGTGCAGGGACCGAGATG	Resende *et al.* 2011
	y-specific_R		AAGCAGCCGATAAACACTCCTT	
6	Autosomal_seq_F	210	AGGTCGCGAGATTGGTCGCTAGGTCATGCA	Resende *et al.* 2011
	Autosomal_seq_R		AAGACCTCGAGAGACCCTCTTCAACACGT	
7	BRY_F	130	ggATCCgAgACACAgAACAgg	Sood *et al.* 1999
	BRY_R		gCTAATCCATCCATCCTATAg	
8	Satellite DNA_F	216	TggAAgCAAAgAACCCCgCT	Sood *et al.* 1999
	Satellite DNA_R		TCgTgAgAAACCgCACACTg	

Research Group

Ms. Swati Merugu - Project Fellow

Mr. NV Siva Kumar - Project Fellow

Sarwar Azam

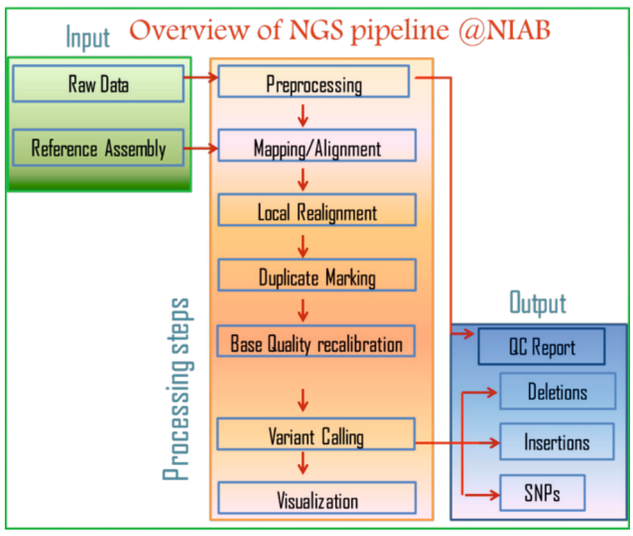
Development and customization of NGS pipeline for variant discovery and expression analysis in livestock

My research interest includes genome sequencing, gene expression studies and genome-wide association analysis to improve the genetic and productive efficiency of livestock. I am also keen to develop fundamental knowledge and bioinformatics tools to facilitate genomic studies in animals.

Development and customization of NGS pipeline for variant discovery and expression analysis in livestock

Background:

With the advent of "Next Generation Sequencing" (NGS) platforms, the sequencing of whole genomes of variety of organisms has resulted in generation of tons of sequencing data.This has revolutionized this field and till date more than 1467 eukaryotic genomes including 124 plants and 353 animals consisting 88 mammalian species have been sequenced. Majority of the livestock, species on which NIAB is focussing, have been sequenced and data are in public domain. NGS has been used in several applications such as Genome Sequencing, Genotyping by Sequencing, Expression studies of protein coding genes, Epigenetics, probing small RNA etc. Recently, it has been used rigorously in population genomics and in the study of effect of domestication on plants and animals. NIAB has also been targeting this new technology and has sequenced bacteria and in the process of re-sequencing of many livestock species. The pipeline will be customized to analyze the data of livestock species.



Work plan:

- Setting of SNP pipeline and further development for expression analysis
- Graphic user Interface (GUI) development for pipeline
- Development of web based application for the pipeline
- Customization of pipeline for livestock species

Satya Parida

Development of vaccine and diagnostic tools for PPR and FMD

My research is focussed on FMD and PPR Vaccines and diagnostics including molecular characterization of PPR and FMD viruses. Currently we are engaged in developing PPR and FMD viral vectored based vaccines using reverse genetics techniques and their immunological evaluations.

Objective: Initiation of translational research on PPR and FMD vaccines and diagnostics

Achievements till date- PPR Work

1. Two workshops (at Hyderabad and Chennai) had been organised between PPR workers all over India. Establishment of collaborations with TANUVAS (Dr Dhinakar Raj and Dr Parimal Roy), IVRI (Dr Muthu Chelvan and Dr R P Singh) and with VBRI, Hyderabad (Dr Krishna Jyothi and Dr Hanumanth Rao), have been established.
2. Successful to win a DBT-BBSRC FADH grant -to study the genetic resistance of PPRV and to develop a PPR DIVA vaccine from a c-DNA clone of Indian vaccine strain
3. NIAB and TANUVAS joint first authored accepted paper in Veterinary Microbiology- Molecular characterization of Lineage IV PPR viruses circulating in Indian.
4. Pirbright and NIAB paper under revision in Emerging and infectious diseases- PPR emergence and molecular evolution of PPRV
5. VBRI, NIAB, Pirbright- manuscript drafting is ongoing- PPR Vaccine efficacy study in undivided Andhra Pradesh

Progress on FMD research

1. Lead in the design of BSL3 laboratory at NIAB in consultation with Dr V.A. Srinivasan and Pirbright Laboratory Biosecurity Head, Dr Uwe Mueller-Doblies.
2. Made various rounds of discussions with Indian Immunologicals (IIL) for initiating work on vaccine development. The work will be initiated after the renovation of the BSL-3 facility at IIL and approval from their Board.
3. Exploring the possibility of doing diagnostics with NDDB group at IIL
4. Coordinating as an investigator for a DBT-BBSRC FADH project with PDFMD, Mukteswar on “Control of FMD in India”
5. Draft for publication between NDDB, IIL, PDFMD, NIAB and Pirbright- Vaccine strain selection for O serotype FMDV is under preparation.
6. Coordinated a brain storming session in the International conference of host-pathogen interaction at NIAB to discuss “how to improve the control of FMD in India” that had been attended h by all the national key researchers, policy makers and FMD vaccine producers .

Future proposed work

- Multivalent PPR and Pox live attenuated vaccine trial- Work initiated in collaboration with VBRI, Hyderabad
- DIVA PPR vaccine from c-DNA clone of Indian vaccine strain
- Making PPR-FMD multivalent live attenuated vaccine to provide life-long immunity

Research Group

Dr. Arvind Babu - Reasearch Assoaciate

Collaborators

Dr Dhinakar Raj and Prof Parimal Roy	-	TANUVAS, Chennai
Dr R.P. Singh and Dr Muthu Chelvan	-	IVRI, Bangalore
Dr Hanumanth Rao and Dr Krishna Jyothi	-	VBRI, Hyderabad
Dr B Pattanaik	-	PDFMD
Dr Girish Radhakrishnan	-	NIAB

Syed Faisal

Development of vaccine for Zoonotic infections

My research interest include understanding the host response and molecular pathogenesis of zoonotic/infectious diseases particularly those caused by *Leptospira interrogans* and *Mycobacterium paratuberculosis* and development of novel vaccines and diagnostic tools. I am also interested in developing novel veterinary adjuvants.

Leptospirosis: Understanding the host response and molecular pathogenesis

Background

Leptospirosis, a zoonotic disease caused by gram negative bacterium called *Leptospira interrogans*is widespread globally. It causes fatal infection in farm and domestic animals as well as in humans (Fig.1). The disease is highly prevalent in the India is of significant importance as the country has a fast growing livestock sector and huge production of animal products. Current vaccines provide limited protection and are unable to prevent the shedding of bacteria in urine of infected animals.

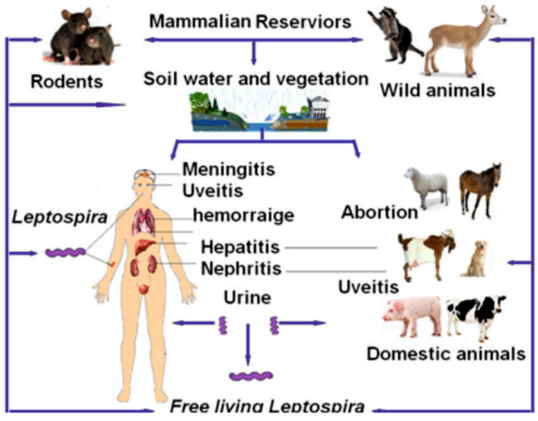


Fig.2 Transmission cycle of *Leptospira*. *Leptospires* are transmitted to domestic/farm animals from rodents (maintenance host) causing hepatitis, nephritis and abortion. Humans get infected from direct contact or water and soil contaminated by urine of these infected animals leading to variety of clinical manifestation and organ failure.

Recent research has shown that *Leptospira* disrupts Toll-like receptor (TLR) signalling by varying Lipopolysaccharide (LPS) expression or down-regulating expression of surface proteins to evade host immune attack and quickly disseminate and establish infection in

various organs. The main focus of my research group is to understand how *leptospira* modulates the host immune response via toll like receptors (TLRs) by exploiting its surface proteins, thereby establishing infection (Fig 2).

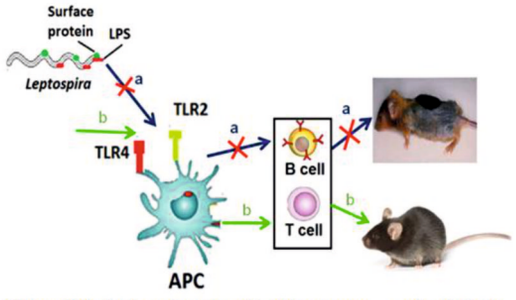


Figure. 2 Mechanism of evasion of host immune response by virulent *Leptospira*. Virulent Leptospira disrupts TLR signaling which blocks the activation of APCs and subsequent failure of adaptive immunity to control infection leading to death (pathway a). TLR activation leads to strong innate and adaptive immunity and clearance of bacteria from the host (pathway b).

Collaborators

Prof. Yung-Fu Chang, Cornell University, USA
Prof. Manjula Sritharan, University of Hyderabad, India
Dr.Ratnagiri Polavarapu, Genomix Biotech, India

Abhijit Deshmukh

Characterization of cell cycle regulators associated with DNA replication machinery

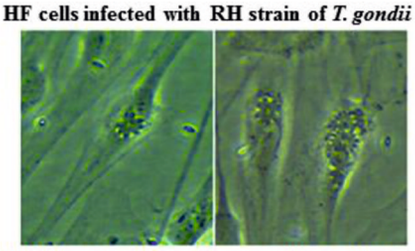
My research is focused on the identification of novel cell cycle regulators associated with DNA replication machinery in *Toxoplasma gondii,* a parasite of medical and veterinary importance. His current project is examining cell cycle regulators and mechanisms involved in endodyogeney of tachyzoite replication and analyzing the consequences of regulators modulation for regulation of cell cycle using genetic tools.

Characterization of cell cycle regulators associated with DNA replication machinery in *Toxoplasma gondii*

Background

Toxoplasma gondii is an obligate, intracellular, protozoan parasite that causes the disease Toxoplasmosis. It is responsible for abortion, stillbirth and neonatal mortality in human and animal alike. *T. gondii* tachyzoites replication cycle differs from the classical animal cell cycle as they divide using a three-phase cycle i.e. G1, S and M phases. G2 phase and major cell cycle checkpoints in tachyzoite replication cycle are absent pointing to potentially novel mechanisms of control over their complex cell cycles. The rapid parasite divisions are critical to virulence and are major cause of overwhelming infections. Therefore, we are interested in identifying the cell cycle regulators which are unique to the parasite cell cycle progression.

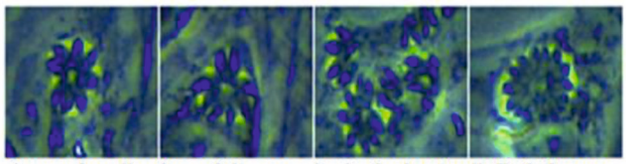
HF cells infected with RH strain of *T. gondii*



Replicating tachyzoites of the parasite in the host cells

Objectives

- Identification of primary cell cycle regulators and core replication proteins in *T. gondii*
- Establishment of *Toxoplasma gondii* culture
- Localization and interaction studies of the identified regulators in parasite progression
- Study of replication proteins for substrates of the identified regulators



Asexual replication of the parasite in the host cell: Endodyogeny

Work done so far

- Several cell cycle regulators and replication proteins are identified using Toxoplasma genome resource and different bioinformatics tools
- Successfully established *T.gondii* culture *in vitro*. Currently RH strain is being propagated on Human Foreskin Fibroblast (HFF) cells.
- Four genes namely TgCdk7, TgMAT1, TgActin and TgPCNA1 from *T. gondii* are cloned and their expression studies are underway.

Collaborator

Dr. Dhanasekaran Shanamugam, Scientist, National Chemical Laboratory, Pune, India

COLLABORATIVE PROJECTS

P1. Antibiotic resistance of bacterial pathogens and cytokines mediated mammary tissue damage in bubaline mastitis: Role of polyphenols and NSAIDs in control

Principal Investigator: **P. Anand Kumar**, NTR College of Veterinary Science, Sri Venkateshwara Veterinary University, Gannavaram

Co-*Principal Investigators*: **G.S. Rao,** NTR College of Veterinary Science, Sri Venkateshwara Veterinary University, Gannavaram

M.K. Arunasree, University of Hyderabad, Hyderabad

Girish K. Radhakrishnan, NIAB, Hyderabad

Background

Bubaline mastitis, usually caused due to bacterial infection, is responsible for heavy economic losses to Indian dairy industry due to its adverse impact on milk production in dairy animals. In addition to bacterial factors, inflammatory cytokines produced during host immune response to the invading bacteria are responsible for damage to the mammary tissue in mastitis. Indiscriminate use of antibiotics for treating the mastitis at field level is a cause of concern as it may lead to development of antibiotic resistance. In addition milk from the animals treated with antibiotics shall be discarded due to excretion of antibiotic residues in the milk. Cortisones administration to treat the mastitis cases poses the risk of cortisone induced immunosuppression. There is dire need for a therapeutic formulation that has antibacterial, anti-inflammatory and immunomodulatory activities, without undesirable effects, so that the mastitis in buffaloes and cows could be controlled.

Objectives:

- Antibacterial activity of polyphenolic compounds (individually or in cocktail or in combination with reduced doses of antibiotics) on mastitis pathogens *in vitro*.
- Sensitivity of mastitis pathogens to antibiotics alone or in combination with NSAIDs *in vitro*.
- Levels of expression of different inflammatory and immunomodulatory cytokines in bovine and bubaline mammary epithelial cells during *in vitro* infection with *S. aureus* and *E. coli.*
- Action of polyphenolic compounds in regulating the levels of inflammatory and immunomodulatory cytokines in bovine and bubaline mammary epithelial cells.

Work done so far

Escherichia coli were isolated from certain mastitic milk samples of buffaloes. The *E. coli* isolates were provisionally confirmed by culture on eosin methylene blue (EMB) agar and also by biochemical tests (IMViC). These isolates were further confirmed by PCR using *E. coli* specific oligonucleotide primers.

Antibiotic resistance pattern in *E. coli* isolates revealed varied degree of sensitivity to the antibiotic enrofloxacin in ABST. Whereas these *E. coli* isolates were found to be resistant to Amoxycilln and ceftriaxone in *in vitro* MIC assays. The polyphenol cinnamic acid exhibited antibacterial activity at 125 µg (well) concentration for half of the isolates, and for the remaining isolates its antibacterial activity was at 250 µg. For majority (75%) of *E. coli* isolates quercitin

exhibited antibacterial activity at 250 µg. and Gallic acid at 500 µg. Cinnamic acid has to be further studied for antibacterial activity in combination with other polyphenols and / conventional antibiotics.

During this year majority of the mastitic milk samples collected from buffaloes, which were processed for detection of *S. aureus*, were found to *Staphylococcus* species other than *S. aureus*. All the samples that were provisionally assumed as Staphylococcus species as per the culture on Mannitol Salt Agar (MSA) and also biochemical tests like catalase, coagulase were subjected to reactivity with oligonucleotide primers specific to Staphylococcus genus and also *S. aureus.*

It is interesting to note that of five isolates *viz.* GDV 5, GV 7, KSP 14, KSP 15 and KSP 16 are coagulase negative variants of *S. aureus* isolated from bubaline mastitis cases. The isolates that were confirmed as *S. aureus* in PCR test were subjected to reactivity with oligonucleotide primers specific to *blaZ* gene in PCR test. (blaZ F AAG AGA TTT GCT TAT GCT TC and blaZ R GCT TGA CCA CTT TTA TCA GC). β-lactamase production of *S. aureus* isolates was detected by starch-iodine agar test.

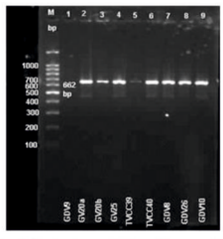


Fig 1: Detection of *E. coli* in mastitic milk samples of buffaloes by PCR test

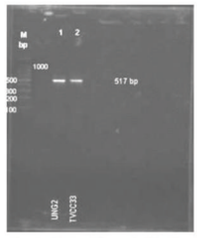


Fig 2: Detection of *blaZ* gene of *S. aureus* in PCR test

Bubaline mammary epithelial cell line (BuMEC) was procured from NDRI, Karnal and it will be used to study levels of expression of different inflammatory and immunomodulatory cytokines in bovine and bubaline mammary epithelial cells during *in vitro* infection with *S. aureus* and *E. coli.* Standardization of QRT-PCR real time assays for different cytokines is in progress.

Characterization of bubaline mastitis S. aureus clinical isolates for mecC gene

Single colony of the each *S. aureus* isolate was grown in BHI medium and total genome was isolated. The genes *mecA*, *mecC* and 16 S RNA were amplified in PCR from the total genome of the isolates. Eight out of 12 (66%) isolates were found to be *mecC* positive indicating a higher prevalence of *mecC* MRSA in bubaline mastitis cases.

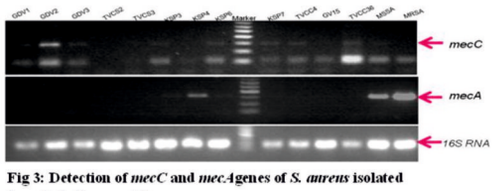
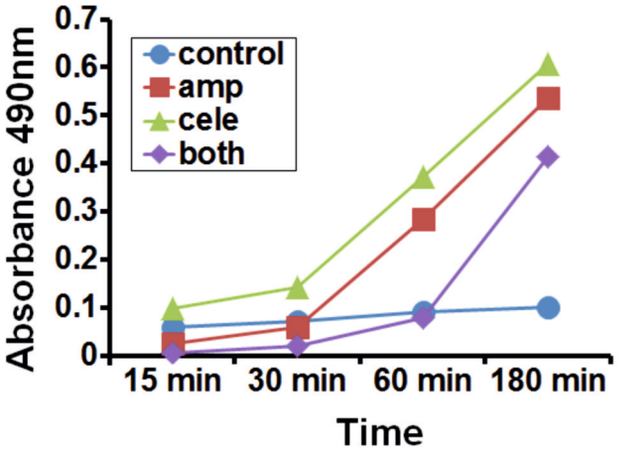


Fig 3: Detection of *mecC* and *mecA* genes of *S. aureus* isolated from bubaline mastitis

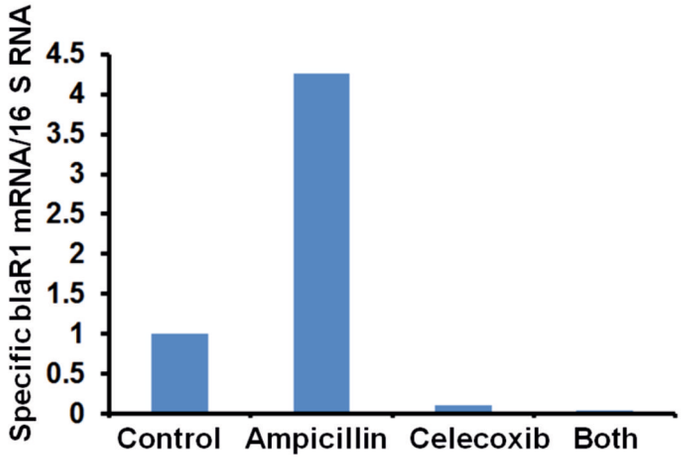
CODE	Methicillin (µg/ml)	% inhibition	Celecoxib (µM)	% inhibition in Combination	Difference in % inhibition
GDV1	0.25	39.7330595	1.625	45.4825462	5.749486653
GDV2	1	33.4592145	3.125	50.98187311	17.52265861
GDV3	1	65.3211009	1.625	90.21406728	24.89296636
TVCS2	1	47.5477972	1.625	68.66167914	21.11388196
TVCS3	0.5	76.1523	1.625	90.2158	14.0635
KSP3	1	75.6734694	3.125	83.59183673	7.918367347
KSP4	1	36.5	6.25	65.234	28.734
KSP6	0.5	21.9056974	6.25	45.18664047	23.28094303
KSP7	2	16.5853659	1.625	22.84552846	6.260162602
TVCC4	0.25	30.9639727	1.625	42.55111977	11.58714703
GV15	2	1	3.25	9.22459893	8.22459893
TVCC36	2	16.7454423	6.25	39.43281567	22.6873734
MSSA ATCC 29213	4	53.5794183	1.625	69.44071588	15.86129754
MRSA ATCC 33591	1	20.3039514	6.25	42.55319149	22.24924012

Study on the combinatorial efficacy of methicillin and celecoxib on S. aureus clinical isolates from bubaline mastitis



Study the effect of Celecoxib on beta-lactamase expression and activity

-lactamase RNA expression levels were determined by real-time PCR. In presence of ampicillin, there was 4-fold induction of -lactamase expression but in combinatorial treatment it decreased to 0.03 fold.



P2. Development and validation of an immunoassay for screening of leptospirosis (NIAB/CP/111/2012)

Principal Investigator : **Manjula Sritharan,** University of Hyderabad, Hyderabad
Co-Investigator : **Anand Srivastava,** NIAB, Hyderabad.

Background

Diagnosis of leptospirosis is the focus of this study. A specific and sensitive diagnostic test for this zoonotic disease will have profound implications both for the welfare of animals and humans, as the disease affects humans, livestock and wild animals. Our ongoing work is focused on the evaluation of the hemin-binding protein HbpA in the serodiagnosis of leptospirosis. HbpA is expressed only by the pathogenic serovars of the spirochaetal bacteria belonging to the genus *Leptospira.* The C-terminal fragment of HbpA, namely HbpA-F3 (aa 470-710) bearing 9 potential B cell epitope sites was expressed as recombinant antigen in the pET28a (+) expression system (Fig. 1).

B cell epitope sites was expressed as recombinant antigen in the pET28a (+) expression system (Fig. 1).
This was used as antigen in the screening of serum samples by ELISA. Significant process was made on the optimisation of expression of the antigen and downstream processing of the expressed recombinant protein. Fig. 1 shows the reproducibility of the purification of the protein. Similarly, sphingomyelinase, expressed as a recombinant protein was purified to homogeneity and used as antigen in ELISA.

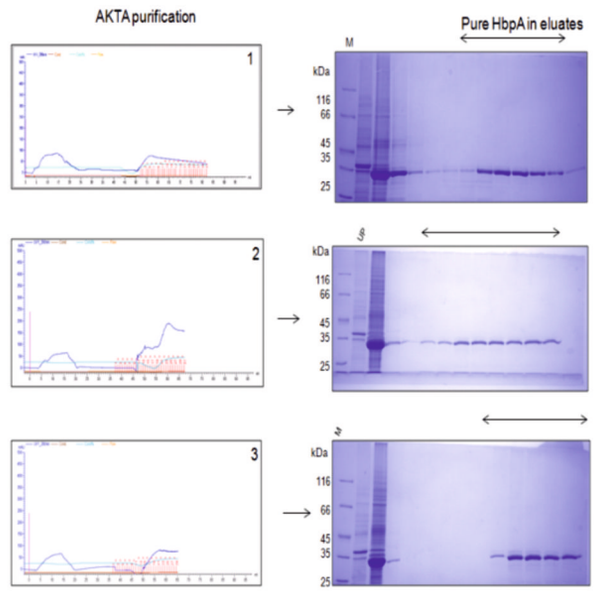


Fig. 1: Purification of rHbpA using AKTA. The left panels show the successive runs and the righ panel shows the purity of the eluted fractions from Ni-NTA column

335 serum samples, including bovine and human samples were screened by MAT and by HbpA-ELISA. The human samples were also screened by the commercial PanBio ELISA. MAT identified Icterohemorrhagiae as the predominant serogroup in the human samples. Both HbpA-ELISA and Sph-ELISA were found to highly promising and studies are ongoing to statistically evaluate the significance of the findings and to assess the feasibility of using the two antigens, either alone or in combination for the screening of leptospirosis. Our ongoing work includes the development and evaluation of a lateral flow device, in addition to ELISA using HbpA and sphingomyelinase as antigens.

In order to identify regions which are immuno-dominant in C- terminal HbpA1 several shorter constructs were prepared as shown in figure 1.

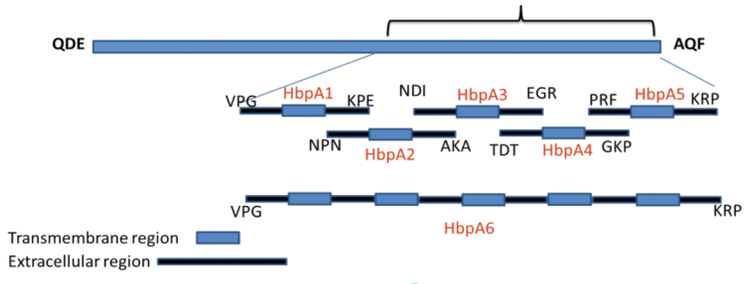


Figure 1. Boundaries of smaller domains of C-terminal HbpA

All these smaller domains of HbpA1 were cloned in pET21a (+) and were verified by sequencing (figure 2).

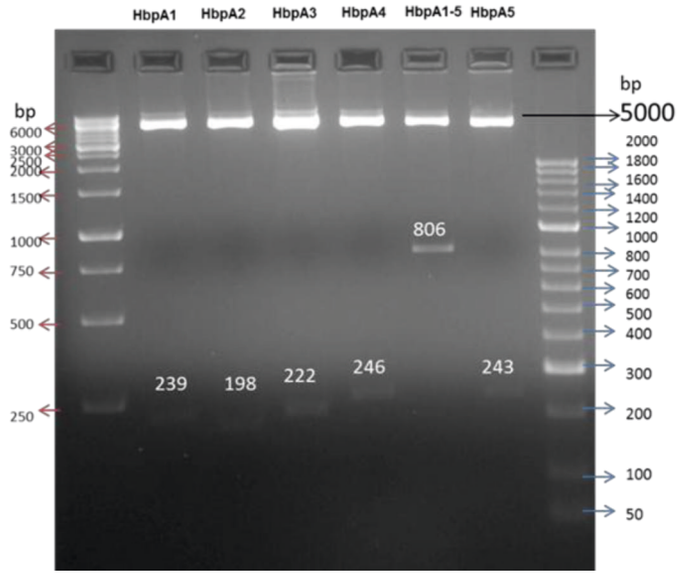


Figure 2. Cloning of smaller domains of C-terminal HbpA in pET21a

The smaller domains were expressed in *E. coli* BL21DE3. All the protein expressed as inclusion bodies. The proteins were solubilized using urea and were purified using Ni-NTA beads. The purification profile of these proteins is shown in figure 3.

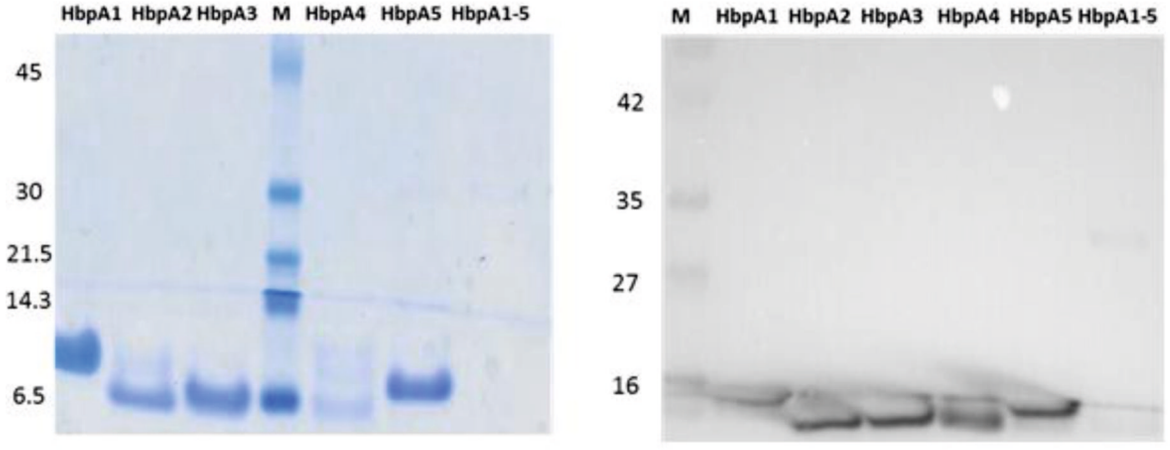


Figure 3. Expression of smaller domains of C-terminal HbpA in bacterial expression system. (A).Coomassie stained SDS-PAGE and (B) western blot with anti-His antibodies

P3. Role of Toll-like Receptor-4 (TLR-4) Signaling Mediated Bacterial Disease Resistance in Indian Poultry

Principal Investigator : **G. Ravi Kumar**, University of Hyderabad, Hyderabad.
Co-Principal Investigator : **Madhuri Subbiah**, NIAB, Hyderabad.
T. R. Kannaki, Directorate of Poultry Research, Rajendranagar, Hyderabad.

Introduction

About 20 different diseases are recorded in poultry including deficiency, the most common diseases are, infectious bursal disease (IBD), Newcastle disease (ND), salmonellosis, mycoplasmosis, vitamin and mineral deficiency disorders, colibacillosis, fowl cholera and coccidiosis. TOLL like receptors (TLRs) sense conserved microbial patterns and endogenous danger signals and are expressed on cells of the immune system, such as monocytes, macrophages, dendritic cells, lymphocytes, neutrophils, natural killer cells. TLRs mediate host-responses to pathogens by promoting cellular activation and the production of cytokines. Interestingly, TLR ligands help prime a robust adaptive immune response by promoting the maturation of professional antigen presenting cells. Indigenous chicken is considered more disease resistant than commercial broiler. The role of TLR4 in bacterial disease resistance has not been fully elucidated in earlier studies. The resistance of Indian chicken to many diseases indicates that they are immunologically superior. However, it is difficult to ascertain whether a single or a set of gene (s) define this property to these chicken breeds. Therefore we hypothesized that TLR4 mRNA expression might vary among different breeds of chicken and comparatively quantify their expression along with other relevant genes by Quantitative Real-time PCR.

Objectives

1. To use a comparative approach to investigate the importance of TLR4 and TLR4-related genes in Indian poultry for bacterial resistance traits using Real-time PCR approach.
   - This objective represents the resistance patterns of different breeds, by comparing the level of expression of the above genes after treatment with LPS.
   - If the levels of expression of above genes are high after the treatment then that particular breed will be more resistant. If the expression level of the genes are low then we can say, that particular breed is more susceptible to the disease.
2. Dissection of the functions of TLR4-MyD88 Dependent and Independent pathways that

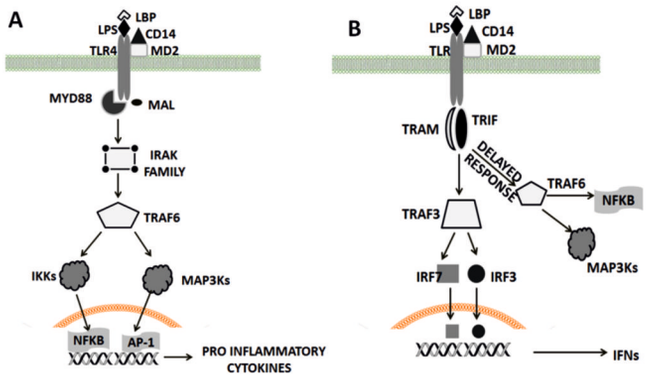


Fig. 1 TLR Signaling Pathway. (A) MyD88 Dependent Pathway, (B)MyD88 Independent Pathway

Poultry innate immune cells activate in distinct signalling pathways.

- This objective reveals whether different breeds follow MyD88 dependent or independent Signalling pathway (Fig.1).
- If the expression levels of MyD88 dependent genes are high when compared to independent genes in a particular breed, we conclude that the particular breed follows MyD88 dependent pathway or vice-versa.

Results and Conclusions

We procured blood samples from indigenous breeds of Indian poultry from PDP Poultry after signing Material Transfer Agreement. As per SAC committee recommendations we are more focused on studying bacterial resistance in various chicken breeds of Indian origin or foreign breeds reared in India. We standardized the techniques associated with Heterophil isolation and treated chicken cells (n=10 per breed) with different doses of LPS to stimulate the TLR4 pathway (Figure 5). Our particular interest is in understanding TLR4 mediated substantial differences in response to Lipopolysaccharide (LPS) in Indian Poultry which will give us detailed picture of TLR4-MyD88 Dependent and Independent pathways due to bacterial infections.

To achieve the above objectives, we have selected 4 breeds namely **Aseel, Ghagus, Dahlem Red and Broiler** (Cobb). **Aseel** being the fighter cock known for its pugnacity are found throughout the nation but abundant in Andhra Pradesh. They are considered excellent sitters but very poor layers. **Ghaghus**on the other hand are majorly maintained for eggs by natives of Karnataka and Andhra Pradesh. **Dahlem Red** is famous for their higher body weight since hatch and hence has been imported and reared in India. Other than the uniqueness in traits of these breeds the resistance to diseases is also equally important before considering them for backyard farming. It was interesting for us to make a comparative study of these breeds to the commercial breeds to understand the difference in underlying mechanism for resistance to different bacterial diseases.

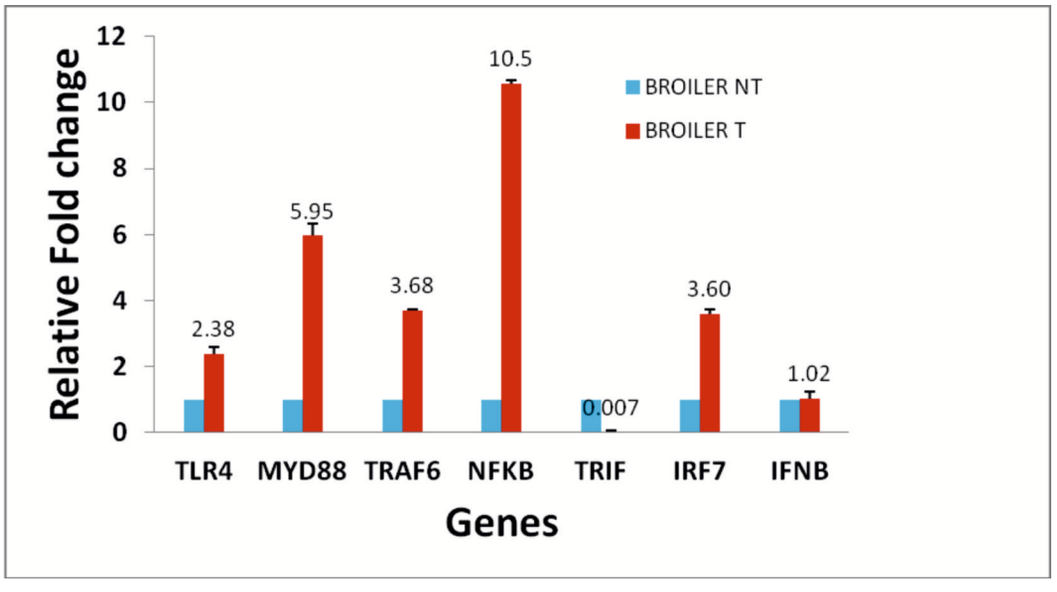


Figure1. In **Broiler (n=10), the** level of expression of MYd88 dependent genes are high when compared to the independent genes. We conclude that **Broiler** is following **Myd88dependent signaling pathway.**

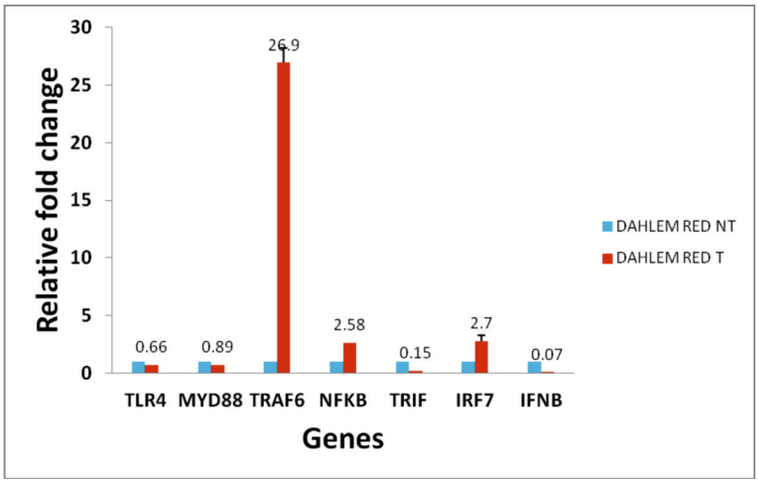


Figure2. In **Dahlemred (n=10),** the level of expression of MyD88 dependent genes are high when compared to the independent genes. We conclude that **Dahlemred** is following **MyD88 dependent signaling pathway.**

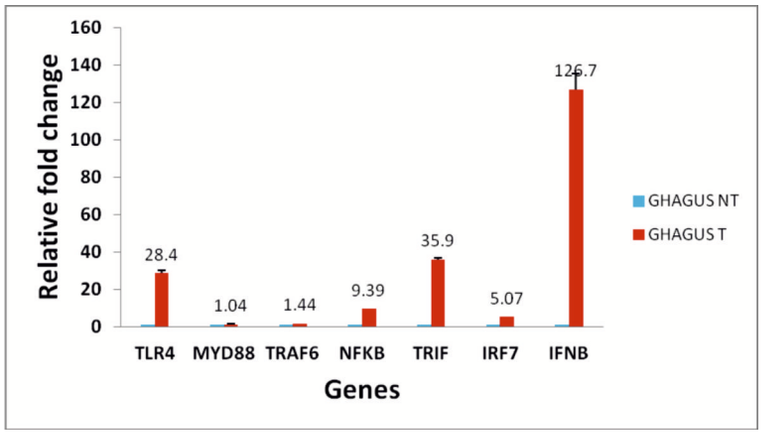


Figure2. In **Dahlemred (n=10),** the level of expression of MyD88 dependent genes are high when compared to the independent genes. We conclude that **Dahlemred** is following **MyD88 dependent signaling pathway.**

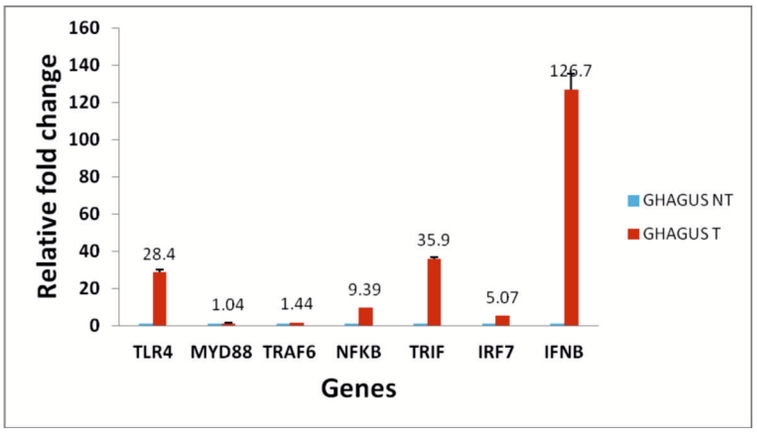


Figure2. In **Dahlemred (n=10),** the level of expression of MyD88 dependent genes are high when compared to the independent genes. We conclude that **Dahlemred** is following **MyD88 dependent signaling pathway.**

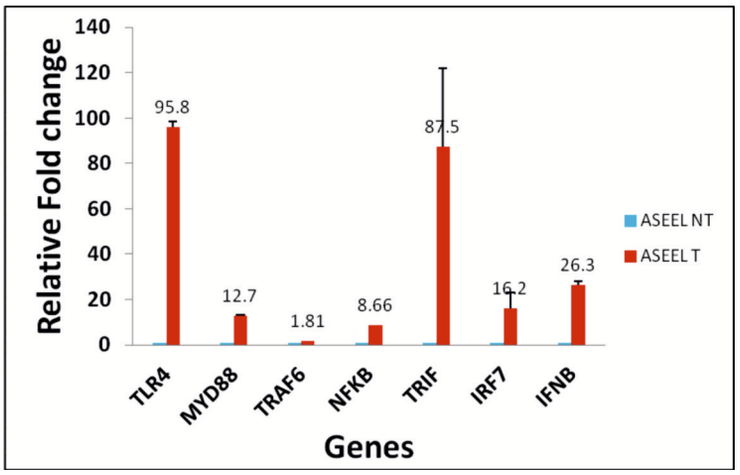


Figure4. In **Aseel(n=10),** the level of expression of MYd88independent genes are high when compared to the dependent genes. We conclude that **Aseel** is following **Myd88 independent signaling pathway**.

By comparing these figures1,2,3,4 the genes that were studied in LPS treatment when compared to non-treated, the level of expression was low in **Dahlemred** breed, high in **Aseel** breed and moderately expressed in **Broiler** and **Ghagus** breeds. This concludes that **Dahlem red** breed is very susceptible, **Aseel** breed is very resistant and the other two breeds are moderately resistant to bacterial diseases. We communicated a manuscript titled "**TLR Pathway: An Overview of Signalling, Polymorphism and Ligands in Chicken"** to International Journal of Inflammation and another manuscript titled "**TLR4 SignalingPathway: MyD88 Independent Pathway Up regulation in Chicken Breeds upon LPS treatment"** is under preparation.

P4. Studies on epigenetic regulation during lactation and its impact on milk biosynthesis

Principal Investigators: **Sreenivasulu Kurukuti**, University of Hyderabad, Hyderabad

Co-Investigators : **Brahmanandam Manavathi**, University of Hyderabad, Hyderabad

Paresh Sharma, NIAB, Hyderabad

Aim of the Project: To understand the epigenetic regulatory mechanisms involved in orchestration of milk biosynthetic pathway gene transcription in mammary epithelial cells in response to Prolactin signaling.

Work carried out so far:

1. Establishment of robust HC11 primary cell culture lactogenic differentiation system.
2. Microarray gene expression profiling during lactogenic differentiation of HC11 primary mammary epithelial cells.
3. Establishment of isolation of primary epithelial cells from mouse mammary gland.
4. Generation of HiC and 3C libraries from normal and Prl induced HC11 cells.
5. Refinement of Bioinfomratic and computational pipeline for the analysis of RNA-seq, ChIP-seq, 4C-seq, HiC and data sets.
6. Protein profiling during lactogenic differentiation of HC11 primary mammary epithelial cells

1. **Establishment of robust HC11 primary cell culture lactogenic differentiation system:** Culturing of HC11 cells in presence of 2%FBS +DIP medium has robustly increased the lactogenic differentiation of primary HC11 cells (Fig1)

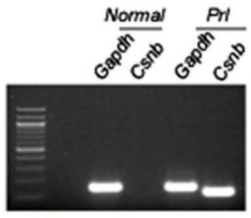


Fig: 1 Agarose gel electrophoresis based semi-quantitative PCRR analysis of *Csnb* expression: Relative expression of *Csnb* compared to *Gapdh*, note robust expression of *Csnb* upon Prlsignalling.

2. **Microarray gene expression profiling during lactogenic differentiation of HC11 primary mammary epithelial cells:** Mammary epithelial cell lactogenic differentiation is accompanied primarily by selective up/down regulation of already transcribed genes and secondarily by selective induction/silencing of fewer genes (Fig2)

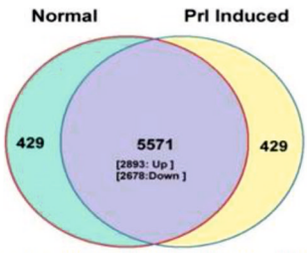


Fig.2 Venn diagram showing differential expression of genes upon Lactogenic differentiation of HC11 cells upon Prolactin signalling. Note substantial numbers of genes are either up/down regulated compared to the genes that are exclusively expressed.

Pathway analysis of microarray expression data sets from Normal and Prl induced HC11 cells for the genes with similar levels of expression by using Path Visio software (http://www.pathvisio.org/) revealed selective initiation following biological pathways during lactogenic differentiation of mammary epithelial cells.

Electron Transport Chain; Cytoplasmic Ribosomal Proteins; Electron Transport Chain; Oxidative phosphorylation; Glycolysis and Gluconeogenesis; Iron Homeostasis; Proteasome Degradation; Arachidonate Epoxygenase Epoxide Hydrolase; DNA Replication; Signaling of Hepatocyte Growth Factor Receptor; TGF Beta Signaling Pathway; Methylation; Translation Factors; Splicing factor NOVA regulated synaptic proteins; Prostaglandin Synthesis and Regulation; Nucleotide Metabolism; Pentose Phosphate Pathway; EBV LMP1 signaling; Non-odorant GPCRs; Glycogen Metabolism; Amino Acid metabolism; Glutathione metabolism; Odorant GPCRs

3. **Establishment of isolation of primary epithelial cells from mouse mammary glands:** Epithelial cells from virgin, pregnant and lactating mouse mammary glands have been isolated by using epithelial cell enrichment kit. Isolated cells were assessed for the purity by analyzing *Csnb* and *Wap* gene expression which suggest the enrichment of epithelial cells from respective stages of mammary gland development.

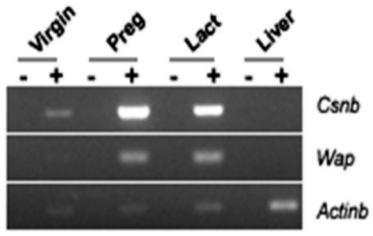


Fig. 4 Agarosse gel electrophoretic RT-PCR analysis for expression of *Csnb*, *Wap* and *Actinb* genes from virgin, pregnant and lactating mouse mammary tissue: Notice selecting abundance of *Casein&Wap* transcripts in late pregnant and lactating mammary tissues.

4. **Generation of improved HiC libraries from normal and Prl induced HC11 cells**: Intra nuclear ligation of HindII digested cross-linked cells were end filled with biotinylated nucleotides following ligation. HiC libraries were prepared from normal and Prl induced HC11 cells were processed for illumine paired end sequencing.

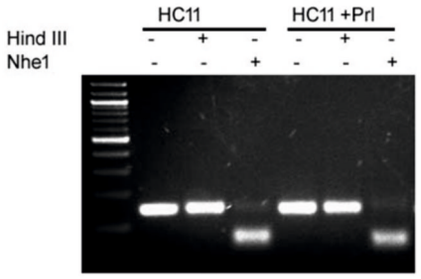


Fig. 5 Internal control Ercc3 3C amplicons were digested with HindIII or NheI. Note the predominant occurrence of NheI digested sample, suggesting proper end filling and ligation of these two remote fragments: nearly 95% digestion of PCR control fragment upon NheI digestion shows the robustness of optimized HiC protocol which is superior to any currently know HiC procedure.

5. **Refinement of Bioinfomratic and computational pipeline for the analysis of RNA-seq, ChIP-seq, 4C-seq, HiC and data sets:** We have automated the tool interface to analyze the complex data (.fastq) of any platform (illumine/solid/solexa) by a user friendly approach by following steps. (i) Pre-Processing (ii) Processing (iii) Post processing (iv) integration (v) visualization as shown in the following picture showing web based interface for analysis of 4C, HiC data analysis.

6. HC11 primary cell culture lactogenic differentiation system has been established. Currently protein has been isolated and purified for profiling studies to identify the differential expression during lactogenic differentiation of HC11 primary mammary epithelial cells. Next 2 D Electrophoresis and MALDI-TOFF analysis will be performed for identifying the significant proteins during lactogenic differentiation.

P5. Integrative approach for identifying host-pathogen interactions: comparative genomics

Principal Investigator: **Sailu Yellaboina,** C.R.Rao Advanced Institute of Mathematics, Statistics and Computer Science (AIMSCS), University of Hyderabad Campus, Prof.CRRao Road, Hyderabad.

Integrating host and pathogen expression data with interaction networks. To identify potential interactions between host and pathogen gene expression signatures, we have identified orthologs between hosts (human, mouse and Cattle) and pathogens (M.*tuberculosis* and M.*Bovis*) (Yellaboinaetal.,2007) using bidirectional best blast hit. We hypothesize that, any common genes (orthologs) that are expressed in host-pathogen environments will be altering the host and pathogen systems. In future, we will be using this information (gene expression and ortholog) and domain interactions to identify the potential interactions (Yellaboina etal., 2012).We have also collected the known interactions of mycobacteria and macrophages to use as gold standard.

Comparative genomics. We hypothesize that the some of the gene expression signatures we identified in mycobacteria species may be conserved across the pathogens which also live in macrophages. Towards this direction, we have collected all the known pathogens which live in macrophages, by taking published literature data as a reference (Table 1).

We have Identified 24 bacteria and 8 parasites which live inside the host macrophages.

Table 1: List of pathogens that interact with macrophages

S.no	Pathogen	Disease	Host	Type_of_Pathogen
1	*Francisellatularensis*	tularemia	Humans	facultative intracellular
2	*Listeria monocytogenes*	listeriosis	Humans	facultative anaerobic
3	*Legionella pneumophilia*	Legionellosis	Humans	facultative intracellular
4	*Yersinia pestis*	plague	Humans	facultative anaerobic,
5	*Bacillus anthracis*	anthrax	Humans and animals	obligate pathogen
6	*Salmonella typhimurium*	typhoid	Humans	facultative intracellular
7	*Salmonella entericaSerovarPullorum*	typhoid	Humans	facultative intracellular
8	*Staphylococcus aureus*	skin infections, Throatinfection and foodpoisoning	Humans	facultative anaerobic
9	*Staphylococcus epidermidis*	Strep throat	Humans	facultative anaerobic
10	*Adherentinvasive Escherichia coli(strain LF82)*	food poisoning	Humans	facultative intracellular
11	*Mycobacterium tuberculosis*	tuberculosis	Humans and animals	Facultative intracellular
12	*Mycobacterium leprae*	leprosy	Humans	Facultative intracellular
13	*Shigelladysenteriae*	shigellosis	Humans	facultative intracellular
14	*Rickettsiae*	spotted fever, typhus andscrub typhus	mice, dogsrabbits,humans(Accidental vectors)	Obligate intracellular parasites
15	*Ehrlichiachaffeensis, Ehrlichiaewingii,*	Human ehrlichiosisis	Humans	Obligate intracellular parasites
16	*Ananplasmaphagocytophilum*	tick-borne fever and pasture fever	Humans	obligate
17	*Chlamydophilapsittaci,*	Chlamydia infection	Humans	
	C.trachomatis C. pneumoniae			
18	*Brucella. pinnipedialis*	brucellosis	Humans and animals	Facultative intracellular
19	*Nocardia*	nocardiosis	Humans	Facultative intracellular
20	*Rhodococcusequi*	severe bronchopneumonia	Wild Boar,domestipigs,Humans	Facultative intracellular
21	*Burkholderiacenocepacia*	cepacia syndrome	plants and humans	opportunistic pathogen
22	*Coxiellaburnetii*	Q fever	Humans, cattle,sheep, goats ,cats and dogs	Obligate intracellular parasites
23	*Neisseria meningitides*	meningitis	Huamns	
24	*Chlamydophilapsittaci*	psittacosis	Birds and humans	Obligate intracellular parasites
25	*Cryptococcus neoformans*	cryptococcosis	Humans	facultative intracellular pathogen.
26	*Theileria annulata*	East Coast fever	Cattle, buffalo,waterbuck, eland,blue wildebees	
27	*Leishmaniaamastigote*	Leishmaniasis	Humans, dogs androdents	Obligate intracellular parasites
28	*Plasmodium berghei*	maleria	humans,mice and rats	
29	*Trypanosomacruzi*	Trypanosomiasis	humans,cattle,dogs,pigs	Obligate intracellular parasites
30	Toxoplasma gondii	Toxoplasmosis	humans,cats	obligate/intracellular
31	Cryptosporidium parvum	cryptosporidiosis	humans,mouse	obligate/intracellular
32	*Pneumocystis jiroveci,*	pneumocystosis	humans	opportunistic infection

WORKSHOP AND EVENTS

Reproductive Biotechnologies for Enhancement of Livestock Productivity

Date: 20 & 21st January 2014

Venue: NIAB Conference Room, Miyapur, Hyderabad.

Workshop Goals:

- To explore the recent advances in reproductive biotechnology at National and global level
- To identify the gaps in the reproduction research that hinder the link between research and its application.
- To identify potential and demanding areas in animal reproduction in which research needs to be undertaken.
- To identify possible collaborations with universities, Institutes and researchers pertaining to reproduction research.

Convenors: Dr. Satish Kumar, Scientist - H, NIAB, Hyderabad
Dr. M. S. Chauhan, Principal Scientist, NDRI, Karnal

Organizing committee: Prof. P. Reddanna, Director, NIAB, Hyderabad
Dr. Sathya Velmurugan, Scientist, NIAB, Hyderabad
Dr. Himabindu Gali, Res. Assoc., NIAB, Hyderabad

Workshop on Reproductive Biotechnologies for Enhancement of Livestock Productivity
20-21 January, 2014
National Institute of Animal Biotechnology, Hyderabad.

Top row (Left to right): Mr. Sashikant Gawai (NIAB), Dr. Sathya Velmurugan (NIAB), Dr. Archana B Siva (CCMB), Dr. S. Sathesh Kumar (TANUVAS), Dr. Umapathy (CCMB), Dr. Prem Singh Yadav (CIRB), Dr. Satish Kumar (NIAB), Mr. Harjit Singh (NIAB), Dr. Manmohan Singh Chauhan (NDRI), Dr. Satish Kumar (CSWRI). Bottom row (Left to right): Dr. Mohan Mondal (NDRI), Dr. Ashok Kumar Mohanty (NDRI), Dr. Ajeet Kumar (GADVASU), Mr. Akshay Joshi (NIAB), Dr. Chandra Shekar (CCMB), Dr. Arindam Dhali (NIANP), Dr. Chaitanya H Pawshe (MAFSU), Dr. Arun Kumar Rawat (DBT), Dr. Ratan Kumar Choudhary (GADVASU), Prof. Pallu Reddanna (NIAB), Prof. Paul J Verma (SARDI), Dr. K Medhamurthy (IISc).

NIAB- Industries Interactive Meet on
"Animal Biotechnology for Improving Livestock Health and Productivity: Challenges and Opportunities"

Date: 27th January 2014
Venue: NIAB Conference Room, Miyapur, Hyderabad.

Convenor: Dr. Anand Srivastava, NIAB, Hyderabad

OBJECTIVES:

- To get feedback from the industries on priority areas of research.
- To identify specific areas of research for collaboration with the industries.
- To discuss on the unmet needs in the field.
- To explore possible biotechnological interventions.
- To provide a platform for NIAB scientists to interact with industries.

EXPECTED OUTCOME:

- Setting up of priority areas for NIAB.
- Identification of common areas of interest for collaboration.
- Setting up of mechanism(s) for funding research projects.

REPRESENTING INDUSTRIES

- Indian Immunologicals Ltd, Hyderabad
- Globion Pvt Ltd, Hyderabad
- Venkateshwara Hatcheries Pvt Ltd, Pune
- Intervet India Pvt Ltd, Pune
- Vivimed labs Ltd, Hyderabad
- GVK Biosciences Pvt Ltd, Hyderabad
- Shanta Biotechnics Ltd, Hyderabad
- VBRI, Hyderabad
- Amar immunodiagnostics, Hyderabad
- DBT-Wellcome trust, Hyderabad

Talk 01: "**Opportunities & Challenges in the Animal Health Market in India**"
Speaker: **Mr. K V Balasubramaniam**, Managing Director, Indian Immunologicals Limited**.**
Mr. Balasubramaniam listed out the opportunities for the animal health market due to the following schemes and developments as follows:

- National Dairy Plan
- 12th Five year plan
- Integration of poultry business
- Increased productivity of livestock with high yielding cows
- Growing ownership of pets

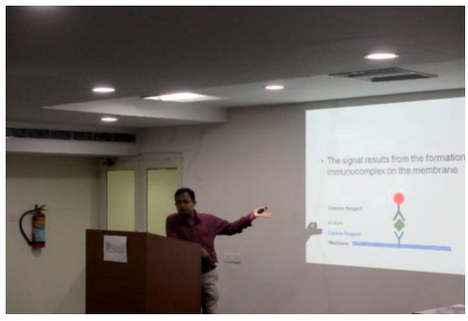
Talk 02: "**Recombinant Vaccine development: Issues & Challenges**"
Speaker: **Dr. Vara Prasad Reddy**, Ex-Managing Director, Shantha Biotech

Dr. Reddy expressed satisfaction over the growing number of companies in developing biotechnological products and also the support which government is providing to research and development in the field of Biotechnology. He emphasized that there is further need to bring more and more number of biotechnological products to the market. He advised young scientists and entrepreneurs that the key to success are hard work and commitment. One need to have a fighting spirit in order to be successful in this competitive world.

Talk 03: "**Current Scenario of vaccines for livestock & poultry and future needs**"
Speaker: **Dr. Sreenivasulu Kilari,** Associate Director (R&D), Intervet India Pvt. Ltd.

Dr. Kilari listed out the current challenges in the vaccine development as follows:

- Expensive in vivo quality control tests
- Need for in vitro potency tests as alternatives
- Requirement for clean cell lines
- Requirement for clean seeds
- Requirement for extraneous agents testing facility (GLP facilities)
- Handling variant strains
- Gaps in technology transfer SOPs
- Difficulties in obtaining regulatory approvals
- High costs of goods
- Emerging diseases
- Commercial viability of Bio units



The future challenges are:

- Developing more preventive concepts
- Economizing livestock and poultry farming
- Increasing per capita productivity
- Producing clean milk, meat and eggs

Talk 4:**"Current Scenario of Diagnostics for Livestock & Poultry and Future Needs – Diagnostic Assays in Animal Health Practice"**
Speaker: **Dr. Ibrahim Bishor**, CEO and MD, ubio Biotechnology Systems Pvt. Ltd

BRAINSTORMING SESSIONS:

Topic 1:**"Setting up of priority areas in the field of vaccine development for livestock and poultry"**
Chairman: Dr. C.B Raju (Brilliant Industries)
Moderator: Dr. Satish S Tongaonkar (Consultant, Veterinary Biologicals)
Panel members: Dr. Sanjay Gavkare (Venky's), Dr. Anand Kumar (IIL), Dr. Piruthiviraj (Globion)

Topic 2: "**Priority areas for disease diagnostics for livestock & poultry**"
Chairman: Dr. Satish Prasad SP (Head R&D team, Vivimed)
Panel members: Dr. Lavleen Kumar (GVK Biosciences), Dr. TVS Rao (Vivimed labs), Dr. Prakash B Reddy (Venky), Dr. Jayant K Bhanushali (Amar Immunodiagnostics)

Topic 3: **"Academy-Industry Collaboration"**
Chairman: Prof. P. Reddanna (Director, NIAB)
Panel members: Dr. Rama Reddy Guntaka (UTHSC), Dr. G. Hanumanth Reddy (VBRI), Dr. C.B. Raju (Brilliant Industries), Dr.SreenivasuluKilari (Intervet), Dr. Satish S Tongaonkar (Consultant,Veterinary Biologicals)

International Symposium on Animal Biotechnology and India-Australia Workshop on "Reproductive Biotechnologies for Agricultural Research"

Date: 11th to 14th February 2014

Venue: South Australian Research & Development Institute, Adelaide, South Australia

Convenors: Prof. Paul Verma (SARDI) & Dr. Satish Kumar (NIAB)

Organizing Committee: Prof. P. Reddanna & Prof. Alan Tilbrook

Event Manager: Belinda Rodda

Workshop Goal: To initiate discussion around building international activity in field of livestock genomics, genome engineering and associated assisted reproduction technologies.

Invited Speakers (TBC)

- Bruce Whitelaw, The Roslin Institute
- Hiroshi Nagashima, Meiji University
- Jim Murray, UC Davis Mark Post,
- Maastricht University
- Troy Ott, Penn State University

Topics discussed

- embryology
- advanced reproductive technologies
- genome-editing & transgenesis
- stem cells
- genomic selection
- proteomics

Funded by Australian Government's Department of Industries, Innovation, Climate Change, Science, Research and Tertiary Industries; Indian Government's Department of Science and Technology, New Delhi and organised by South Australian Research and Development Institute (SARDI), Adelaide Australia, Indian Government's National Institute for Animal Biotechnology (NIAB), Hyderabad, India. This Symposium and subsequent Workshop was coordinated by Prof Paul Verma and Dr Satish Kumar.

Workshop Conclusions:

- There is a large opportunity for applying livestock genetics and genome engineering for both biomedical and agricultural applications in India and Australia.
- Both National and International funding streams are available to support co-operative animal genetics and genome engineering efforts based in India.
- Specific funding opportunities exit through the established Australia-India initiative.
- Initial projects should demonstrate the capability and opportunity – academic and commercial – for both biomedical and agricultural sectors.
- Identified initial projects include (a) animal bioreactors and (b) combating disease in livestock, e.g. FMDV, TB, Para-TB, influenza through genetic engineering
- Dialogue with key Indian organizations should be established, e.g. NIAB, INSTEM, DBT, ICAR.
- Strong international co-operation needed for prospecting the role of genetically engineered livestock for

biomedical and agricultural applications: ethical, social issues and regulatory frame work.

- Strong international support for network to progress animal genetics and genome engineering in India.
- Coordination of this network is required.

Proposal: is to form the International Centre for Animal Genetics and Genetic Engineering (ICAGGE).

ICAGGE aims to:

- Cover transgenic and genome edited agricultural animals; and assisted reproductive technologies (including stem cells) in farm animals;
- Use of genome edited farm animals for biomedical research including incubators for production of proteins of medical and veterinary interests;
- Provide a forum providing international awareness and cooperation;
- Undertake dialogue with appropriate international and national regulatory bodies;
- Coordinate with other relevant activities and professional societies;
- Communicate with commercial entities to determine areas of key focus
- Encourage collaborative projects through joint funding applications;
- Facilitate training and exchange of postgraduate students and scientific staff;
- Promote workshops/symposia and other interactive opportunities;
- Undertake outreach/extension activities including interaction with media and public groups.

INTERIM FACILITY INAUGURATION

Inaugartion ceromany was organized at the new interim facilty of NIAB on 2nd september 2013. **Prof. K. VijayRaghavan**, Secretary, DBT was the chief guest of the event. Dr R. Ramaswamy, vice chancellor , University of Hyderabad;; Dr. Lalji Singh, Vice-chancellor BHU, Dr. Ch Mohan Rao, Director, CCMB, Dr. Gowrishankar, Director, CDFD, Dr. Arun Ninawe, and Dr. A. K. Rawat, from, DBT, New Delhi; and other scientific advisory committee members were the prominent guests in the event. Many faculty members and students from University of Hyderbad, CR RAO Advanced Institute of Mathematics, Statistics and Computer Science, CCMB, CDFD were also present in the ceremony.

BSL-2+ LABORATORY INAUGURATION

The BSL – 2 Plus facility was inaugurated by Nobel Laureate **Prof. Harald Zur Hausen**, Professor Emeritus, German Cancer Research Center, Heidelberg, Germany, in the presence of Dr. Reddanna and other NIAB research staffs on 17th February 2014. Inauguration was followed by his distinguished lecture. The BSL – 2 Plus lab will facilitate the research on aerosol transmitted high risk pathogens

NATIONAL SCIENCE DAY CELEBRATIONS

National Science Day-2014, using main theme of this year's **"Fostering Scientific Temper".** National Institute of Animal Biotechnology (NIAB) organized various events and activities for different schoolchildren present in the vicinity of the institute. The theme **"Fostering Scientific Temper"** was given by Ministry of Science and Technology, Government of India, New Delhi. This was to promote active involvement of students to improve their scientific temper and to encourage the students to interact with researchers and clarifying their doubts in the area of Science and Technology.

The following activities were undertaken on the occasion of National Science Day program: NIAB invited students from Jawahar Navodaya Vidyalaya, Kendriya Vidyalaya and Bharatiya Vidya Bhavan's Public School on the day of National Science Day celebrations (2014) at NIAB premises. These are the activities conducted for the students: On-the-Spot painting competition, scientific exhibits, Quiz competition & Elocution. Topic for Elocution was "Transgenic animals in Biomedicine and Agriculture" and theme of painting competition was also "Transgenic animals". Three prizes (first, second and third) were given for each competition. It was judged by committee of senior scientists and honorable guest. After competition, students from all the three schools were invited to attend the laboratory tours to sophisticated instrument laboratories followed by demo of various small experiments.

Prof. Reddanna welcomed the teachers, students, guests, chief guest and staff of NIAB for award ceremony in the evening. The chief guest of ceremony was Dr. Venkateswarlu, who gave an inspiring speech for the students and distributed prizes to the winners. At the end, Dr. Satish thanked everybody especially organizing committee, Judges, chief guest on behalf of NIAB.

SEMINAR SERIES

1. "Heat Shock Protein 70 a Chaperone? Or a Cytokine?" by **Dr. Ramadevi Nimmanapalli, BHU** on April 9, 2013 and **"Prototype One Medicine Diagnostic laboratory"** on April 10, 2013 at C.R. Rao Auditorium from 11:30 AM to 12:30 PM.
2. **"Insights into the Mechanism of actions of luteinizing hormone and prostaglandin F2 alpha in the regulation of Corpus luteum function in monoovulatory species such as bonnet macaques and buffalo cows** by **Dr. Kunal B Shah, Indian Institute of Science, Bangalore,** Karnataka on 19th April 2014 at NIAB Conference Room, UoH from 11AM to 12 PM.
3. **"Current Trends in Proteomics", by Dr. M. Kameswara Rao, Scientist D, Biochemistry Division, DRDE , Gwalior** on 23rd April 2013 at C. R. Rao Auditorium from 11 AM to 12 PM .
4. **"Avian leukosis viruses (ALVs) in poultry" by Dr. M. R. Reddy, Principal Scientist, PDP, Hyderabad** on 3rd May 2013 at C.R. Rao Auditorium from 3 PM to 4 PM.
5. "**Multiple regulatory roles of the Phosphoproteins of non - segmented - negative sense RNA viruses in Viral Replication" by Dr. Santanu Chattopadhyay, Ph.D, Calcutta** on 10th May 2013 at C.R.Rao Auditorium from 3:30 PM to 4:30 PM.
6. "**Development of Diagnostic kit using Lateral flow assays" by GE Healthcare Pvt. Ltd.,** on 10th May 2014 at C.R.Rao Auditorium from 11 AM to 12 noon.
7. "**G protein Coupled Receptors Signalling and Vascular Wall Remodelling" by Dr. Ravisekhar Gadepalli, Post Doc Fellow, University of Tennessee, USA** on 5th June 2013 at C.R.Rao Auditorium from 11:30 AM to 12:30 PM.
8. "**Early Sex Determination of Fresh Water Prawns" by Dr. Anup Mandal, Central Genetics Lab, Rajiv Gandhi Centre for Aquaculture, Nagapattinum, TN** on 6th June 2013 at NIAB Conference Room from 11:30 AM to 12:30 PM.
9. "**Current Status of Poultry Vaccines in India: Contributions and Scope for the Future Research" by Dr. Prakash B Reddy , Assistance General Manager, Venkateshwara Hatcheries Pvt. Ltd.,** on 7th June 2013 from 11:30 AM to 12:30 PM.
10. "**RNAi Screening for Novel Target Identification" by Dr. Sireesha V Garimella, National Cancer Institute, NIH, USA** on 1st July 2013 from 11:30 AM to 12:30 PM in the NIAB Conference Room, University of Hyderabad.
11. **Recombinant DNA technologies: Bio-safety Rules, Regulations & Guidelines by Dr. T. Venkata Ramanaiah, BioSafety & Regulatory Lead, Advanta India Ltd,** Krishnama House, 3rd Floor, Road # 7, Banjara Hills, Hyderabad on 5th July 2014 from 11:30 AM to 12:30 PM in the C.R.Rao Auditorium, University of Hyderabad.
12. "**Molecular Mechanisms Underlying Innate Host Resistance" by Dr. Suresh Kuchipudi** on 25th July 2014 from 10:30 AM to 11:30 AM in the NIAB Conference Room.
13. "**Antibody Drug Conjugates for Cancer Therapy: Past, Present and Future" by Dr. Jagath Reddy Junutula, Senior Scientist, Genentech Inc., San Francisco, CA-USA** on 13th August 2013 from 11 AM to 12 PM in Sir C.V.Raman Auditorium, Science Complex, University of Hyderabad.
14. "**Death by Design: Influenza VirusPB1-F2 Protein" by Prof. Elankumaran Subbiah, USA** on 20 August 2013 from 11:30 AM to 12:30 PM in the C.R.Rao Auditorium, University of Hyderabad, Hyderabad.

15. "**Putative modifiers of P53 tumor suppressor pathway" by Dr.Rajesh Vyas, Ph. D Belgium** in Cancer Biology on 11th September, 2014 from 11:30 AM to 12:30 PM at Aryabhata Conference Room.
16. "**Role of Stem Cells in Large Animal Genetic Engineering in the TALEN/ CRISPR Era" by Dr. Bhanu Prakash V.L.Telugu** on 11th February 2014 from 11:30 AM to 12:30 PM in the NIAB Auditorium, Miyapur, Hyderabad.
17. "**Computational Approaches to Biological Systems" by Dr. Jan T Kim, Head of Bioinformatics at the Pirbright Institute Guildford, United Kingdom** on 5th March 2014 from 11 AM to 12 PM in the NIAB Auditorium, Miyapur, Hyderabad.
18. "**Genetic determinants of antibody mediated protection in FMD" by Dr. Mana Mahapatra, Senior Scientist, Pirbright Institute, UK** on 20 March 2014 from 11 AM to 12 PM in the NIAB Auditorium, Miyapur, Hyderabad.
19. "**Nuclear Receptors in Biology and Diseases" by Dr. P.S.Suresh, Assistant Professor, Division of Biomedical Sciences, School of Biosciences and Technology, VIT, Vellore, T.N.** on 21st March 2014 from 4 PM to 5 PM in the NIAB auditorium

DISTINGUISHED LECTURE SERIES

"The emergence of mecC methicillin resistant Staphylococcus aureus" by Dr. Mark Holmes, Snr Lecturer in Preventive Veterinary Medicine, University of Cambridge 31st January 2014 from 11:30 AM to 12:30 PM in the NIAB Auditorium, Hyderabad.

"Viewing the Immune System through the Lens of Gene Regulatory Networks" by Dr. Harinder Singh, Professor and Director, Divison of Immunology, The Center for Systems Immunology Cincinnati Children's, Hospital Medical Center, USA on 8th February 2014 from 11 AM to 12 PM in the NIAB Auditorium, Miyapur, Hyderabad.

"Genetically Engineered Livestock Come of Age" by Prof. Bruce Whitelaw, The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Midlothian, U.K. on 18th February 2014 from 4 PM to 5 PM in the NIAB Auditorium, Miyapur, Hyderabad.

UPCOMING EVENTS

INTERNATIONAL CONFERENCE ON HOST-PATHOGEN INTERACTIONS (JULY 12 TO 15, 2014)

The conference includes the following broad sessions:

1. Infection and Immunity (with focus on Infectious Diseases of Livestock and Poultry and Zoonosis)
2. Host-Pathogen Interactions
3. Inflammation and Immunity
4. Antibiotic Resistance
5. Translational Research - Vaccine and diagnostics (including preparedness to epidemics and pandemics).

This conference will allow scientists, post-docs and students to gather under one roof and share their interesting research findings. A limited number of travel grants to attend the conference will be available to students and post-docs submitting abstracts. The selection will be based on merit. Poster awards will be presented for the best posters at the conference.

For more information: <niab.org.in>

PUBLICATIONS 2013-2014

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- Athira, A. P., A. Helen, K. Saja, **P. Reddanna**, and P. R. Sudhakaran. "Inhibition of Angiogenesis In Vitro by Chebulagic Acid: A COX-LOX Dual Inhibitor." *International journal of vascular medicine* (2013).
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- Filosa, Rosanna, Antonella Peduto, Polamarasetty Aparoy, Anja M. Schaible, Susann Luderer, Verena Krauth, Carmen Petronzi, **Reddanna.P** *et al*., "Discovery and biological evaluation of novel 1, 4-benzoquinone and related resorcinol derivatives that inhibit 5-lipoxygenase." *European journal of medicinal chemistry* 67 (2013): 269-279.
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- Reddy, M. R., C. R. Reddy, R. S. Rathore, M. D. Erion, P. Aparoy, R. N. Reddy, and **P. Reddanna**. "Free Energy Calculations to Estimate Ligand-Binding Affinities in Structure-Based Drug Design." *Current pharmaceutical design* 2014;20(20):3323-37.
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- Vadlakonda, Lakshmipathi, Abhinandita Dash, Mukesh Pasupuleti, Kotha Anil Kumar, and **Pallu Reddanna**. "Did we get Pasteur, Warburg, and Crabtree on a right note?." *Frontiers in oncology* 2013 Jul 15;3:186. doi: 10.3389/fonc.2013.00186.
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- Rathore, R. S., M. Sumakanth, M. Siva Reddy, **P. Reddanna**, Allam Appa Rao, Mark D. Erion, and M. R. Reddy. "Advances in Binding Free Energies Calculations: QM/MM-Based Free Energy Perturbation Method for Drug Design." *Current pharmaceutical design* 19, no. 26 (2013): 4674-4686.
- Hofheinz, Katharina, Kumar Reddy Kakularam, Susan Adel, Monika Anton, Aparoy Polymarasetty, **Pallu Reddanna**, Hartmut Kuhn, and Thomas Horn. "Conversion of pro-inflammatory murine Alox5 into an anti-inflammatory 15S-lipoxygenating enzyme by multiple mutations of sequence determinants."*Archives of biochemistry and biophysics* 530, no. 1 (2013): 40-47.
- Vadlakonda, Lakshmipathi, Mukesh Pasupuleti, and **Reddanna Pallu**. "Role of PI3K-AKT-mTOR and Wnt signaling pathways in transition of G1-S phase of cell cycle in cancer cells." *Frontiers in oncology* 2013 Apr 12;3:85. doi: 10.3389/fonc.2013.00085.
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MAIN CAMPUS DEVELOPMENT

NIAB's permanent campus is coming up in about 100 acres of land located within the campus of University of Hyderabad.The neighbouring institutions include the Tata Institute of Fundamental research(TIFR), International Institute of Information Technology (IIIT), Indian Immunologicals (IIL), Indian School of Business (ISB), Institute of Life Sciences (ILS), C. R. Rao Advanced Institute of Mathematics, Statistics and Computer Science (AIMSCS)

In the last academic year, many efforts have been taken to establish the frame work of the permanent campus. As a first activity, the site has been fenced with concrete wall from all sides. By the efforts of Project Maintenance Committee and Building committee of NIAB, “Suresh Goyal and Associates” has been appointedas the architect.. The most suitable master plan, amongst various proposed by the architect, has been approved by the Building Committee as NIAB master plan. Recently, tenders have been invited to identify the contractor. We are hopeful that the contractor will be identified in the next few months.

ORGANIZATIONAL STRUCTURE

MEMBERS OF NIAB SOCIETY

Shri Jaipal Sudini Reddy Hon'ble Minister of Science & Technology & Earth Sciences	President
Prof. K. VijayRaghavan Secretary, DBT, New Delhi.	Member
Dr. S. Ayyappan, Secretary, DARE, New Delhi.	Member
Dr. Amarjeet Singh Nanda, Commissioner, Animal Husbandry, Dairying & Fisheries, Delhi	Member
Ms. Anuradha Mitra, Joint Secretary & Financial Adviser, DBT, Delhi	Member
Dr. Arun S Ninawe, Advisor, DBT, New Delhi	Member
Dr. Lalji Singh, Vice-Chancellor, Banaras Hindu University, Varanasi	Member
Prof. Ramakrishna Ramaswamy, Vice-Chancellor, University of Hyderabad, Hyderabad	Member
Dr. H K Pradhan, WHO Country Office	Member
Dr. C. S. Prasad, Director, NIANP, Bangalore	Member
Dr. V.A. Srinivasan, Advisor, NDDB, Hyderabad	Member
Dr. Satish S. Tongaonkar, Consultant Veterinary Biologicals, Pune	Member
Dr. Vijay Kumar Taneja, Vice Chancellor, GADVASU, Ludhiana	Member
Dr. M.P.G.Kurup, Bangalore	Member
Dr. S. N. Singh, Managing Director, Biovet Private Limited	Member
Dr Satish Kumar, Scientist-H, NIAB	Member
Dr. Girish. K. Radhakrishnan, Scientist-D, NIAB	Member
Prof. P. Reddanna, Director, NIAB, Hyderabad	Member Secretary

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Ms. Anuradha Mitra, Joint Secretary & Financial Adviser, DBT, New Delhi	Member
Dr George John / Dr. Arun S Ninawe Senior Advisor / Advisor, DBT, New Delhi *(upto 31-08-2013) / (w.e.f. 24-09-2013*)	Member
Dr. Lalji Singh, Vice-Chancellor, Banaras Hindu University, Varanasi	Member
Prof. Ramakrishna Ramaswamy, Vice-Chancellor, University of Hyderabad, Hyderabad	Member
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Dr. V.A. Srinivasan, Advisor, NDDB, Hyderabad	Member
Dr. Satish S. Tongaonkar, Consultant Veterinary Biologicals, Pune	Member
Dr. Vijay Kumar Taneja, Vice Chancellor, GADVASU, Ludhiana	Member
Dr. M.P.G.Kurup, Bangalore	Member
Dr. S. N. Singh, Managing Director, Biovet Private Limited	Member
Dr Satish Kumar, Scientist-H, NIAB	Member
Dr. Girish. K. Radhakrishnan, Scientist-D, NIAB	Member
Prof. P. Reddanna, Director, NIAB, Hyderabad	Member Secretary

MEMBERS OF NIAB FINANCE COMMITTEE

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Ms. Anuradha Mitra, Joint Secretary & Financial Adviser, DBT, Delhi	Member
Dr George John / Dr. Arun S Ninawe Senior Advisor / Advisor, DBT, New Delhi *(upto 31-08-2013) / (w.e.f. 24-09-2013)*	Member
Dr. J. Gowrishankar Director, CDFD, Hyderabad	Member
Prof. Ramakrishna Ramaswamy, Vice-Chancellor, University of Hyderabad, Hyderabad	Member
Dr. H K Pradhan, WHO Country Office	Member
Dr. K. T. Sampath Former Director, NIANP, Bangalore	Member
Dr. D. P. Kasbekar Haldane Chair, CDFD, Hyderabad	Member
Prof. P. Reddanna Director, NIAB, Hyderabad	Member Secretary

MEMBERS OF NIAB BUILDING COMMITEE

Dr. J. Gowrishankar Director, CDFD, Hyderabad	Chairman
Dr. A. K. Rawat Director, DBT	Member
Shri P. C. Singh Deputy Secretary, DBT	Member
Shri T. Siddhardha Reddy University Engineer, UoH	Member
Prof. P. Reddanna Director, NIAB	Member
Shri V. H. Rao Sr. Consultant, NIAB, Hyderabad	Member Convener

MEMBERS OF SCIENTIFIC ADVISORY COMMITEE

Dr. Lalji Singh Vice-Chancellor, BHU, Varanasi	Chairman
Prof. David Hume Director, Roslin Institute	Member
Prof. C. Channa Reddy Distinguished Professor Penn State University, USA	Member
Dr. R. K. Singh Director, National Research Centre on Equines	Member
Dr George John / Dr. Arun S Ninawe Senior Advisor / Advisor, DBT, New Delhi *(upto 31-08-2013) / (w.e.f. 24-09-2013)*	Member
Dr. V. A. Srinivasan Advisor, NDDB, Hyderabad	Member
Dr. T. Balganesh Head, OSDD Project, New Delhi	Member
Dr. Satish Kumar, Scientist H, NIAB	Member
Dr. K. M. L. Pathak Dy. Director General, Animal Science, New Delhi	Member
Prof. Jagan Pongubala, Dept. of Animal Biology, UoH	Member
Dr. N. R. Hegde Associate Director, Ella Foundation	Member
Prof. P. Reddanna, Director, NIAB	Member Convener

NIAB STAFF

Scientific

1.	Prof. P. Reddanna, PhD	Director
2.	Dr. Satish Kumar, PhD	Scientist- H
3.	Dr. Girish Radhakrishnan,PhD	Scientist- D
4.	Dr. Madhuri Subbiah,PhD	Scientist- C
5.	Dr. Anand Srivastava,PhD	Scientist- C
6.	Dr. Paresh Sharma,PhD	Scientist- C
7.	Dr. Sathya Velmurugan,PhD	Scientist -C
8.	Mr. Sarwar Azam	Scientist -B
9.	Prof. Satya Parida, PhD	Visting Faculty
10.	Dr. Syed Faisal,PhD	Ramalingaswami Fellow
11.	Dr. Abhijit Deshmukh,PhD	Inspire Faculty

Technical

1.	Ms. G. Rama Devi	Technical Officer
2.	Mr. Shashikant D. Gawai	Technical Officer
3.	Mr. A. Harikrishna	Technical Officer
4.	Mr. Praveen Kumar Poosarla	Technical Officer

Administrative & Support Services

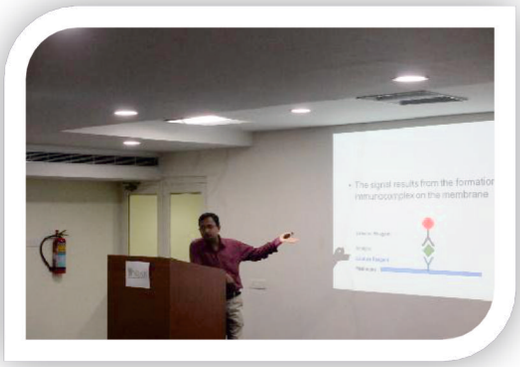
1.	Mr. Harjit Singh	Senior Manager
2.	Mr. B. J. Acharyulu	Finance Officer I/C
3.	Mr. I. Jagadeesh	Manager Office (Accounts)
4.	Mr. Santosh Mhadeswar	Manager (Stores & Purchase)
5.	Mr. Ramesh Babu	Service & Maint. Engineer
6.	Ms. Krishna Priya	PA to Director
7.	Mr. Mohammed Zaheeruddin	Junior Office Assistant
8.	Mr. P.S.G.S. Pavan Kumar	Junior Office Assistant
9.	Mr. Ratnesh Chandra	Junior Office Assistant
10.	Mr. D. Nagesh	Office Attendant
11.	Mr. Ramesh	Office Attendant
12.	Mr. Jahid Hussain	Driver

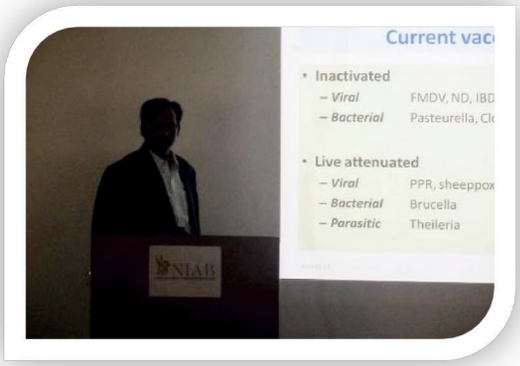
Consultants

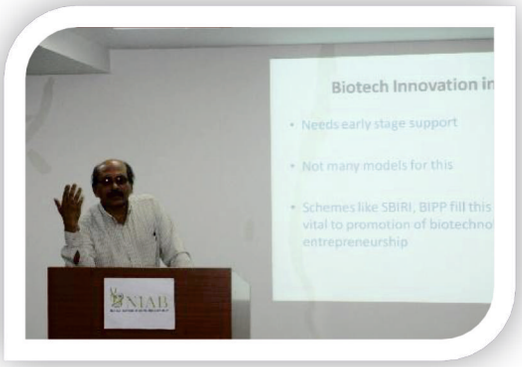
1.	Mr. V. H. Rao	Senior Consultant
2.	Mr N. S. V. Prasad Reddy	Liasion Officer
3.	Mr. V. Lachaiah	Consultant
4.	Mr. C. S. Murthy	Consultant (Instrumentation)
5.	Dr. George John	Consultant Advisor *(w.e.f. 09-11-2013)*

SNAPSHOTS

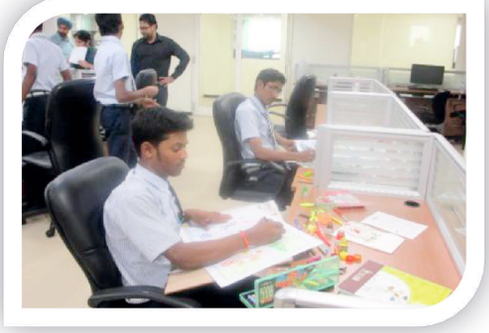
NIAB-Industries Interactive Meet (27th January 2014)







National Science Day Celebrations (28th February 2014)



Group Photo of NIAB Staff

NIAB

National Institute of Animal Biotechnology

AUDITED ACCOUNTS FOR 2013 - 14

National Institute of Animal Biotechnology

(An autonomous Institute of the Department of Biotechnology, Ministry of Science & Technology, Government of India)

D.No.1 -121/1, 4th & 5th Floors, Axis Clinicals Building

Miyapur, Hyderabad — 500 049

AUDITOR'S REPORT

24th April 2014

The Director
National Institute of Animal Biotechnology
D.No. 1-121/1, 4th & 5th Floors, Axis Clinicals Building
Miyapur, Hyderabad – 500 049

We have audited the attached Balance Sheet of **NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY**, Hyderabad, as at 31st March 2014 and also the Income & Expenditure Account for the year ended on that date annexed there to. These financial statements are the responsibility of the organization management. Our responsibility is to express an opinion on these financial statements based on our audit.

We report that:

1. We have obtained all the information and explanations, which are to the best of our knowledge and belief, were necessary for the purpose of our audit.
2. In our opinion, the organization has kept proper books of account as required by law so far, as appears from our examination of those books.
3. The Balance sheet and Income & Expenditure account dealt with by this report is in agreement with the books of account.
4. The Institute has maintained accounts on Accrual basis.
5. In our opinion and to the best of our information and according to the explanations given to us, the said Balance sheet and the Income & Expenditure account read together with the notes thereon gives the required information in the manner so required and give a true and fair view.
   a) In so far it relates to the Balance sheet as at 31st March 2014 and
   b) In so far as it relates to the Income & Expenditure account excess of expenditure over income for the year ended on 31st March 2014.

for B Purushottam & Co
Chartered Accountants
Reg.No. 002808S

[CH SATYANARAYANA]
Partner M No. 019092

Place: Hyderabad
Date: 24th April, 2014

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY,HYDERABAD
BALANCE SHEET AS ON 31st MARCH 2014

(Amount in Rs)

Particulars	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
Corpus / Capital Fund	1	29,03,40,484.00	18,11,03,125.00
Reserves and Surplus	2	23,32,293.22	43,95,564.22
Earmarked / Endowment funds	3	20,04,546.00	6,32,518.00
Secured Loans & Borrowings	4	-	-
Unsecured Loans & Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	23,29,514.00	11,90,534.00
TOTAL		**29,70,06,837.22**	**18,73,21,741.22**
ASSETS			
Fixed Assets	8	27,80,78,152.00	8,80,58,157.00
Investments- From Earmarked / Endowment Funds	9	-	-
Investments - Others	10	-	1,46,087.00
Current Assets, Loans, Advances etc.	11	1,89,28,685.22	9,91,17,497.22
Miscellaneous Expenditure		-	-
Internal & External Electrification		-	-
TOTAL		**29,70,06,837.22**	**18,73,21,741.22**
Significant Accounting Policies	24		
Contingent Liabilities and Notes on Accounts	25		

For B.Purushottam & Co.
CHARTERED ACCOUNTANTS
Reg.No. 002808S

(Ch.Satyanaranaya)
Partner M.No. 019092

DIRECTOR	FINANCE OFFICER	MANAGER OFFICE (ACCOUNTS)
NIAB	NIAB	NIAB

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY, HYDERABAD
Income And Expenditure Statement as on 31st March 2014

(Amount in Rs)

Particulars	Schedule	Current Year		Previous Year	
INCOME					
Income from Sales/Services	12		-		-
Grants/Subsides	13		6,00,00,000.00		1,50,00,000.00
Fees/Subscriptions	14		-		-
Income from Investments	15		23,30,055.00		30,87,735.00
Income from Royalty, Publications etc.	16		-		-
Interest Earned	17		18,32,327.00		-
Other Income	18		4,38,584.00		4,27,384.22
Increase/(decrease) in stock of Finished goods and works-in-progress	19		-		-
TOTAL (A)			**6,46,00,966.00**		**1,85,15,119.22**
EXPENDITURE					
Establishment Expenses	20		1,72,98,727.00		76,39,648.00
Administrative Expenses	21		4,88,08,751.00		1,32,50,395.00
Expenditure on Grants, Subsides etc.	22		-		-
Interest	23		-		-
Depreciation (Net Total at the year-end - corresponding to Schedule 8)		1,07,92,541.00	-	6,77,324.00	-
Less: Transferred to Grants-in-Aid		1,07,92,541.00	-	6,77,324.00	-
Provision For Salaries			5,56,759.00		9,92,699.00
TOTAL (B)			**6,66,64,237.00**		**2,18,82,742.00**
Balance being excess of Expenditure over income (B-A)			**20,63,271.00**		**33,67,622.78**
Transfer to Special Reserve (Specify each)					
Transfer to/from General Reserve					
BALANCE BEING SURPLUS/(DEFICIT) CARRIED TO CORPUS/CAPITAL FUND					
SIGNIFICANT ACCOUNTING POLICIES	24				
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS	25				

For B.Purushottam & Co.
CHARTERED ACCOUNTANTS
Reg.No. 002808S

(Ch.Satyanaranaya)
Partner M.No. 019092

DIRECTOR	FINANCE OFFICER	MANAGER OFFICE (ACCOUNTS)
NIAB	NIAB	NIAB

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY,HYDERABAD

Receipts and Payments Account for the year ended 31st March 2014

(Amount - Rs.)

RECEIPTS	Current Year	Previous Year	Payments	Current Year	Previous Year
1.Opening Balances			1. Expenses		
a) Cash in hand			a) Establishment Expenses (corresponding to Schedule 20)	1,72,98,727.00	76,39,648.00
b) Bank Balances			b) Administrative Expenses (corresponding to Schedule 21)	4,88,08,751.00	1,32,50,395.00
i) In current accounts	-	-			
ii) In deposit accounts	-	-	2. Payments made against funds for various projects		
iii) Savings accounts	49,53,618.22	5,01,82,973.00	(Name of the fund or project have be shown along with the particulars of payments made for each		
2. Grants Received			project) Projects (Annexure F)	5,27,972.00	5,67,482.00
a) From Government of India	18,00,00,000.00	15,00,00,000.00			
b) From State government	-	-	3. Investments and deposits made		
c) From other sources	-	-	a) Out of Earmarked/Endowment funds	-	-
d) Projects (Annexure - C)	19,00,000.00	12,00,000.00	b) Out of Own Funds (Investments-Others)	19,00,00,000.00	4,00,00,000.00
3. Income on Investments from			4. Expenditure on Fixed Assets & Capital Work-in-Progress		
a) Earmarked/Endow. Funds	16,92,798.00	30,87,735.00	a) Purchases of Fixed Assets:		
b) Own Funds (Oth. Investment)			Books & Journals	4,97,459.00	-
Investments Encased	19,00,00,000.00	4,00,00,000.00	Equipment -Lab/Office/Furniture	12,67,49,747.00	16,77,424.00
			b) Expenditure on Capital		
			Work-in-Progress:	7,35,35,430.00	8,26,97,394.00
4. Interest Received					
a) On Bank deposits	6,37,257.00	-	5. Refund of surplus money/Loans		
b) Loans, Advances etc.			a) To the Government of India	-	-
c) Interest on LC	18,32,327.00	-	b) To the State Government	-	-
			c) To other providers of funds	-	-
5. Other Income					
a) Analysis Charges	-	-	6. Finance Charges (Interest)	-	-
6. Any Other Receipts			7. Other Payments (Specify)		
I-Remittances (Annexure-A)	24,32,522.00	6,71,873.00	Advances (Annexure-D)	2,25,53,041.00	9,80,89,157.00
CPF-SUB / GPF, Arrears and adv. Refund	4,74,000.00	1,46,087.00	I-Remittances (Annexure-E)	24,32,522.00	6,71,873.00
Sundry Receipts	1,65,084.00	1,90,260.00	CPF Ac / GPF Ac	4,74,000.00	1,46,087.00
Application Fee	2,73,500.00	2,37,124.22	New Pension Scheme	6,02,500.00	87,778.00
Provident Fund Salvage	-	-			
Free Gifts - Donations	-	-	8. Closing Balances		
Sale OF Tender Forms	-	-	a) Cash in hand	-	-
Leave Salary-Pension Contribution	-	-	b) Bank Balances		
License Fee	-	-	i) In current accounts	-	-
Welfare Fund	-	-	ii) In deposit accounts	-	-
NPS	6,02,500.00	87,778.00	iii) Savings accounts	20,32,060.22	49,53,618.22
Advance/Refunds/Recovery/Adj					
(Annexure-B)	10,05,48,603.00	39,77,026.00			
TOTAL	**48,55,12,209.22**	**24,97,80,856.22**	**TOTAL**	**48,55,12,209.22**	**24,97,80,856.22**

For B.Purushottam & Co.
CHARTERED ACCOUNTANTS
Reg.No. 002808S

DIRECTOR
NIAB

(Ch.Satyanaranaya)
Partner M.No. 019092

FINANCE OFFICER
NIAB

MANAGER OFFICE (ACCOUNTS)
NIAB

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 1 - CORPUS/CAPITAL FUND				
Balance as at the begining of the year		18,11,03,125.00		4,63,12,967.00
Add : Contribution towards Corpus/Capital Fund				
NIAB Core - Plan (Non-Recurring)	12,00,00,000.00		13,50,00,000.00	
Capitalised portion of Capital Expenditure of projects	29,900.00	12,00,29,900.00	4,67,482.00	13,54,67,482.00
Less : Lump Sum Depreciation	-		-	
Less : Depreciation For the Year 2013-2014	1,07,92,541.00	1,07,92,541.00	6,77,324.00	6,77,324.00
Add : Balance of net income/(Expenditure) transferred from the income and Expenditure Account				
BALANCE AS AT THE YEAR - END		**29,03,40,484.00**		**18,11,03,125.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 2 -RESERVES AND SURPLUS :				
1.Capital Reserve :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
2.Revolution Reserve :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
3.Special Reserves :				
As per last Account	-		-	
Addition during the year	-		-	
Less : Deductions during the year	-	-	-	-
4.General Reserve :				
As per last Account	43,95,564.22		77,63,187.00	
Addition during the year	-		-	
Less : Deductions during the year	20,63,271.00	23,32,293.22	33,67,622.78	43,95,564.22
TOTAL		**23,32,293.22**		**43,95,564.22**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 3 - EARMARKED/ENDOWMENT FUNDS :				
(Refer Annexures)				
(a) Opening balance of the Funds		6,32,518.00		-
(b) Additions to the Funds :				
i. Donations /grants	19,00,000.00		12,00,000.00	
ii. Income from investments made on account of funds	-		-	
iii. Other additions	-	19,00,000.00	-	12,00,000.00
TOTAL (a+b)		**25,32,518.00**		**12,00,000.00**
(c) Utilisation/Expenditure towards objective of funds				
(i) Capital Expenditure (Refer Annexures I & II)				
- Fixed Assets	29,900.00		4,67,482.00	
- Others	-		-	
- Total		29,900.00		4,67,482.00
(ii) Revenue Expenditure (Refer Annexures I & II)				
- Salaries, Wages and allowances etc.	-		-	
- Rent	-		-	
- Other Expenses	4,98,072.00		1,00,000.00	
Total		4,98,072.00		1,00,000.00
TOTAL (c)		**5,27,972.00**		**5,67,482.00**
NET BALANCE AS AT THE YEAR-END [(a + b)-c]		**20,04,546.00**		**6,32,518.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 4 - SCHEDULE LOANS AND BORROWINGS :				
1. Central Government		-		-
2. State Government (Specify)		-		-
3. Financial Institutions				
a) Term Loans	-		-	
b) Interest accured and due	-	-	-	-
4. Banks :				
a) Terms Loans	-	-	-	-
- Interest accured and due	-		-	
b) Other Loans	-		-	
- Interest accured and due	-	-	-	-
5. Other Institutions and Agencies		-		-
6. Debentures and Bonds		-		-
7. Others (Specify)		-		-
TOTAL		**-**		**-**
Note: Amount due within one year				

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 5 - UNSECURED LOANS AND BORROWINGS :				
1. Central Government		-		-
2. State Government (Specify)		-		-
3. Financial Institutions		-		-
4. Banks :				
a) Terms Loans	-		-	
b) Other Loans	-	-	-	-
5. Other Institutions and Agencies		-		-
6. Debentures and Bonds		-		-
7. Fixed Deposits		-		-
8. Others (Specify)		-		-
TOTAL		**-**		**-**
Note: Amount due within one year				

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 6 - DEFFERED CREDIT LIABILITIES :				
a) Acceptances secured by hypothecation of capital equipment and other assets		-		-
b) Others		-		-
TOTAL		**-**		**-**
Note: Amount due within one year				

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 7 - CURRENT LIABILITIES AND PROVISIONS :				
A. CURRENT LIABILITIES				
1. Acceptances	-			
2. Sundry Creditors	-			
3. Advances Received	-			
4. Interest accured but not due on:	-			
5. Statutory Liabilities:	-			
6. Other current Liabilities				
NIAB.CP Fund A/C (Annexure-G)	-		1,46,087.00	
EMD	6,42,500.00		5,000.00	
Security Deposit	1,37,556.00	7,80,056.00	46,748.00	1,97,835.00
TOTAL (A)		**7,80,056.00**		**1,97,835.00**
B.PROVISIONS				
1. For Taxation				
2. Gratuity				
3. Superannuation/Pension				
4. Accumulated Leave Encashment				
5. Trade Warranties/Claims				
6. Others (Specify)	15,49,458.00	15,49,458.00	9,92,699.00	9,92,699.00
TOTAL (B)		**15,49,458.00**		**9,92,699.00**
TOTAL (A+B)		**23,29,514.00**		**11,90,534.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	GROSS BLOCK				DEPRECIATION				NET BLOCK	
SCHEDULE 8 - FIXED ASSTES :	Cost/valuation As at beginning c theyear	Addition during the year	Deductions during the year	Cost/valuation a the year end	As at the beginning of the year	On additions during the year	On Deductions during the year	Total up to the year end	As at the Currer year end	As at the Previous year end
A. FIXED ASSETS:										
1. LAND:										
a) Freehold	-	-	-	-	-	-	-	-	-	-
b) Leasehold	-	-	-	-	-	-	-	-	-	-
2. BUILDINGS										
a) On Freehold Land	-	-	-	-	-	-	-	-	-	-
b) On Leasehold Land	-	-	-	-	-	-	-	-	-	-
c) Ownership Flats/Premises	-	-	-	-	-	-	-	-	-	-
d) Superstructures on Land not belongs to the entity	-	-	-	-	-	-	-	-	-	-
3. PLANT MACHINERY & EQUIPMENT	3,368,422.00	122,714,637.00	-	126,083,059.00	929,476.00	9,589,194.00	-	10,518,670.00	115,564,389.00	2,438,946.00
4. VEHICLES	1,256,507.00	984,103.00	-	2,240,610.00	351,173.00	284,350.00	-	635,523.00	1,605,087.00	905,334.00
5. FURNITURE, FIXTURES	293,623.00	-	-	293,623.00	16,113.00	27,751.00	-	43,864.00	249,759.00	277,510.00
6. OFFICE EQUIPMENT	843,293.00	2,115,552.00	-	2,958,845.00	67,595.00	282,561.00	-	350,156.00	2,608,689.00	775,698.00
7. COMPUTER/PERIPHERALS	495,793.00	-	-	495,793.00	-	297,476.00	-	297,476.00	198,317.00	495,793.00
8. ELECTRIC INSTALLATIONS	-	-	-	-	-	-	-	-	-	-
9. LIBRARY BOOKS	-	497,459.00	-	497,459.00	-	250,986.00	-	250,986.00	246,473.00	-
10. TUBEWELLS & WATER SUPPLY	-	-	-	-	-	-	-	-	-	-
11. OTHER FIXED ASSETS	-	428,605.00	-	428,605.00	-	60,223.00	-	60,223.00	368,382.00	-
Airconditioning works	-	-	-	-	-	-	-	-	-	-
Aluminium partition work	-	-	-	-	-	-	-	-	-	-
DG Set	-	-	-	-	-	-	-	-	-	-
Paintings	-	-	-	-	-	-	-	-	-	-
Typewriters	-	-	-	-	-	-	-	-	-	-
Miscellaneous non consumables	-	-	-	-	-	-	-	-	-	-
Other Assets	-	-	-	-	-	-	-	-	-	-
EMB Net	-	-	-	-	-	-	-	-	-	-
TOTAL	**6,257,638.00**	**126,740,356.00**	**-**	**132,997,994.00**	**1,364,357.00**	**10,792,541.00**	**-**	**12,156,898.00**	**120,841,096.00**	**4,893,281.00**
B. CAPITAL WORK-IN-PROGRESS	83,164,876.00	74,072,180.00	-	157,237,056.00	-	-	-	-	157,237,056.00	83,164,876.00
TOTAL	**89,422,514.00**	**200,812,536.00**	**-**	**290,235,050.00**	**1,364,357.00**	**10,792,541.00**	**-**	**12,156,898.00**	**278,078,152.00**	**88,058,157.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 9 - INVESTMENTS FROM EARMARKED/ENDOWMENT FUNDS		
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures and Bonds	-	-
5. Subsidiaries and Joint Ventures	-	-
6. Others (to be specified) - STDRs (Annexure-J)	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 10 - INVESTMENTS - OTHERS (Annexure-K)		
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures and Bonds : UTI Bonds		
5. Subsidiaries and Joint Ventures	-	-
6. Others (to be specified) - STDRs,(CPF), NIAB CP FUND A/C	-	1,46,087.00
TOTAL	**-**	**1,46,087.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 11 - INVESTMENTS - OTHERS :				
A. CURRENT ASSETS				
1. Inventors				
a) Stores and Spares	-		-	
b) Loose Tools	-		-	
c) Stock-in-trade				
Finished Goods		-		-
Work-in-progress		-		-
Raw Materials	-	-	-	-
2. Sundry Debtors:				
a) Debts Outstanding for a period exceeding six months		-		
b) Others-Life Membership Fees	-	-		
3. Cash balances in hand (including cheques/drafts and imprest)				
4. Bank Balances:				
a) With Scheduled Banks:				
-On Current Accounts	-		-	
-On Deposit Accounts (includes margin money)	-		-	
-On Savings Accounts	20,32,060.22	20,32,060.22	49,53,618.22	49,53,618.22
b) With non-Schedules Banks:				
-On Current Accounts	-		-	
-On Deposit Accounts	-		-	
-On Savings Accounts	-	-	-	-
5. Post Office-Savings Accounts				
TOTAL (A)		**20,32,060.22**		**49,53,618.22**
B.LOANS, ADVANCES AND OTHER ASSETS				
1. Loans:				
a) Staff	-		-	
b) Other Entities engaged in activities/ objectives similar to that of the Entity	-	-	-	-
2. Advances and other amounts recoverable in cash or in kind or for value to be received				
a) On Capital Account (Annexure-H)	1,05,80,000.00		9,35,73,732.00	
b) Prepayments - Deposits (Annexure-I)	63,16,625.00		90,147.00	
c) Others	-	1,68,96,625.00	5,00,000.00	9,41,63,879.00
3. Income Accrued:				
a) On Investments from Earmarked/ Endowments Funds	-		-	
b) On Investments - Others	-		-	
c) On Loans and Advances	-		-	
d) Others	-	-	-	-
4. Claims Receivable				-
TOTAL (B)		**1,68,96,625.00**		**9,41,63,879.00**
TOTAL (A+B)		**1,89,28,685.22**		**9,91,17,497.22**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 12 - INCOME FROM SALES/SERVICES		
1) Income from sales		
a) Sale of Finished Goods	-	-
b) Sale of Raw Material	-	-
c) Sale of Scraps	-	-
2) Income from Services		
a) Labour and Processing Charges	-	-
b) Professional/Consultancy Services (Analysis Charges)	-	-
c) Agency Commission and Brokerage	-	-
d) Maintenance Services (Equpiment/Property)	-	-
e) Others (Specify)	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 13 - GRANTS/SUBSIDES		
(Irrevocable Grants & Subsides Received)		
1) Central Government (DBT Plan Grant-in-Aid)	**6,00,00,000.00**	**1,50,00,000.00**
2) State Government(s)	-	-
3) Government Agencies	-	-
4) Institutions/Welfare Bodies	-	-
5) International Organisations	-	-
6) Others (Specify)	-	-
TOTAL	**6,00,00,000.00**	**1,50,00,000.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 14 - FEES/SUBSCRIPTIONS		
1) Entrance Fees	-	-
2) Annual Fees/Subscriptions	-	-
3) Seminar/Program Fees	-	-
4) Consultancy Fees	-	-
5) Others (Specify)	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year		Previous Year	
SCHEDULE 15 - INCOME FROM INVESTMENTS				
(Income on Invest from Earmarked/Endowment Funds transferred to Funds)				
1) Interest:				
a) On Govt. Securities	-		-	
b) Other Bonds/Debentures	-	-	-	-
2) Dividends:				
a) On Shares	-	-	-	-
b) On Mutual Fund Securities	-	-	-	-
3) Rents	-	-	-	-
4) Others (Specify) STDRs	23,30,055.00	-	30,87,735.00	-
TOTAL	23,30,055.00	-	**30,87,735.00**	**-**
TRANSFERRED TO EARMARKED/ENDOWMENT FUNDS				

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 16 - INCOME FROM ROYALITY, PUBLICATION ETC		
1) Income from Royality	-	-
2) Income from Publications	-	-
3) Others (Specify)	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 17 - INTEREST EARNED :		
1) On Term Deposits		
a) With Schedule Banks	18,32,327.00	-
b) With Non-Scheduled Banks	-	-
c) With Institutions	-	-
d) Others	-	-
2) On Saving Accounts		
a) With Schedule Banks	-	
b) With Non-Scheduled Banks	-	-
c) post Office Savings Accounts	-	-
d) Others	-	-
3) On Loans		
a) Employees/Staff		
b) Others	-	-
4) Interest on Debtors and Other Receivables	-	-
TOTAL	**18,32,327.00**	**-**
Note :- Tax deducted at source to be indicated		

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 18 - OTHER INCOME :		
1) Profit on Sale/disposal of Assets:	-	-
a) Owned assets	-	-
b) Assets acquired out of grants, or received free of cost	-	-
2) Export Incentives realized	-	-
3) Fees for Miscellaneous Services	-	-
4) Miscellaneous Receipts	26,997.00	-
5) Other Receipts		
Sundry Receipts	1,38,087.00	1,90,260.00
Application Fee	2,73,500.00	2,37,124.22
Sales Of Tender Forms	-	-
Licence Fee	-	-
Interest On Computer Advance, Conveyance Advance And HBA	-	-
Leave Salary-Pension Contribution	-	-
Provident Fund Salvage	-	-
Free. Gifts-Donations	-	-
TOTAL	**4,38,584.00**	**4,27,384.22**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 19 - INCREASE/(DECREASE) IN STOCK OF FINISHED GOODS & WORK IN PROGRESS :		
a) Closing stock		
-Finished Goods	-	-
-Work-in-progress	-	-
Total (a)	**-**	**-**
b) Less: Opening stock		
-Finished Goods	-	-
-Work-in-progress	-	-
Total (b)	**-**	**-**
NET INCREASE/(DECREASE) [a-b]	-	-

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 20 - ESTABLISHMENT EXPENSES :		
a) Salaries and Wages	94,77,514.00	51,51,201.00
b) Allowances and Bonus	71,04,689.00	23,31,976.00
c) Contribution to Provident Fund	-	-
d) Contribution to Other Fund (NPS)	6,02,500.00	87,778.00
e) Staff Welfare Expenses - Medical charges	1,14,024.00	68,693.00
f) Expenses on Employees Retirement and Terminal Benefits	-	-
g) Others (specify) - Staff leased House	-	-
TOTAL	**1,72,98,727.00**	**76,39,648.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 21 - OTHER ADMINISTRATIVE EXPENSES :		
a) Purchases	1,64,84,227.00	1,09,950.00
b) Electricity and power	86,173.00	-
c) Water charges	32,669.00	5,150.00
d) Insurance	93,412.00	5,020.00
e) Repairs and maintenance	11,17,656.00	5,91,943.00
f) Rent, Rates and Taxes	94,27,941.00	-
g) Vehicles Running and Maintenance	2,06,387.00	10,44,669.00
h) Postage, Telephone and Communication Charges	2,92,403.00	2,85,248.00
i) Printing and Stationary	7,61,935.00	6,73,891.00
j) Travelling and Conveyance Expenses	45,90,802.00	17,69,416.00
k) Expenses on Seminar/Workshops	3,90,186.00	27,41,116.00
l) Subscription Expenses	17,917.00	13,557.00
m) Expenses on Fees	2,500.00	79,250.00
n) Auditors Remuneration	28,090.00	-
o) Hospitality Expenses	2,21,714.00	8,23,047.00
p) Professional Charges	2,000.00	-
q) Advertisement and Publicity	2,78,057.00	3,29,815.00
r) Bank Charges	54,841.00	15,784.00
s) Security & Cleaning Contract Charges	26,55,657.00	2,48,189.00
t) Training Course /Symposia	-	-
u) Other Contingencies	16,04,137.00	13,46,633.00
v) Liveries & Blankets	-	-
w) Other Research Expenses	1,04,57,028.00	31,67,717.00
x)Office Books	3,019.00	-
TOTAL	**4,88,08,751.00**	**1,32,50,395.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 22 - EXPENDITURE ON GRANTS, SUBSIDES ETC. :		
a) Grants given to Institutions/Organisations	-	-
b) Subsidies given to Institutions/Organisations	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MAR 2014

(Amount - Rs.)

	Current Year	Previous Year
SCHEDULE 23 - INTEREST :		
a) On Fixed Loans	-	-
b) On Other Loans (including Bank Charges)	-	-
c) Others	-	-
TOTAL	**-**	**-**

Schedule 24: Significant Accounting Policies & Schedule 25: Contingent Liabilities & Notes On Account For The Period Ended 31/03/2014

1. Method of Accounting:

a. The accounting system adopted by the organization is on "Accrual basis".

b. The organization has been allocating plan grant-in-aid under the "Non-recurring" & "Recurring" heads.

2. Revenue recognition:

Income comprises of Grant-in-Aid, Internal Resources through services and interest from short term deposits. Income accounted on the basis of the Cash/DD/Cheques/Cr notes received.

3. Fixed Assets:

a. Fixed assets are stated at cost. Cost includes freight, duties, and taxes etc.,

b. Depreciation: Based on the recommendation of the Finance Committee and approval of the Governing Body of the Institute, Depreciation Account on Fixed Assets has been prepared at the rate prevailing to the concerned Fixed Assets as specified in the Income Tax Act, 1961 on Written Down Value Method of Depreciation. This has been set off against the Grant in Aid (Non Recurring) in the concerned account.

c. Capital work in progress has been entered to the extent of the last running account bills paid.

d. Realization on sale of obsolete/surplus fixed assets which is not required for the purpose of research activities are adjusted against capital cost.

4. Inventories:

All purchases of chemicals, glassware and other consumables have been charged to consumption at the time of purchase.

5. Foreign Currency transactions:

Foreign Currency transactions are recognized in the books at the exchange rates prevailing on the date of transaction.

6. Investments:

Investments in STDR's are stated at book values.

7. The previous year balances have been regrouped/rearranged, wherever necessary.

for B Purushottam & Co
Chartered Accountants,
Reg.No. 002808S

Director, NIAB **Finance Officer, NIAB** **[CH SATYANARAYANA]**
Partner M. No. 019092

Place: Hyderabad
Date: 24th April 2014

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
Details of Closing balances of various Earmarked / Endowement Funds (Refer Sch-3)
For the Year Ended 31st MAR 2014

Annexure-I (Amount in Rs.)

Previous year	Proj No	Particulars	Current Year
6,32,518.00	SP001	NMMP MODEL NURSERY	6,29,634.00
-	SP002	DST INSPIRE FACULTY	13,74,912.00
6,32,518.00		**Total**	**20,04,546.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
"Details of Fixed Assets fund (Cpitalised portion of Project Grants)for the year ended 31st March 2014"

Annexure-II (Amount in Rs.)

Previous year	Proj No	Particulars	Current Year
4,67,482.00	SP001	NMMP MODEL NURSERY	-
-	SP002	DST INSPIRE FACULTY	29,900.00
4,67,482.00		**Total**	**29,900.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: A Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	I-Remittances	
1,450.00	GSLI	-
3,85,275.00	Income Tax	9,48,420.00
-	LIC	720.00
19,730.00	Professional Tax	35,800.00
44,802.00	Service Tax	2,50,859.00
1,87,372.00	TDS	11,69,885.00
33,244.00	Works Tax	26,838.00
6,71,873.00	**Total**	**24,32,522.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: B Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	Advance refunds/recovery/Adjst.	
5,000.00	EMD	14,47,500.00
-	Equipment [Advance]	9,38,92,447.00
-	GDA [Others]	15,18,188.00
39,25,278.00	General Deposits And Advances	17,79,423.00
-	Insurance [Advance]	52,563.00
-	LTC [Advance]	91,265.00
-	Office Equipment [Advance]	59,140.00
46,748.00	Security Deposit	90,808.00
-	TA India & Abroad [Advance]	1,34,266.00
-	Vehicles [Advance]	9,83,003.00
-	Workshop & Conference [Advance]	5,00,000.00
39,77,026.00	**Total**	**10,05,48,603.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: C Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
12,00,000.00	SP001	-
-	SP002	19,00,000.00
12,00,000.00	**Total**	**19,00,000.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: D Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	Advances	
-	EMD	8,10,000.00
9,28,17,633.00	Equipment [Advance]	1,16,54,814.00
-	GDA [Others]	74,75,357.00
40,15,425.00	General Deposits And Advances	19,41,505.00
-	Insurance [Advance]	52,563.00
-	LTC [Advance]	1,90,360.00
-	Office Equipment [Advance]	59,140.00
-	TA India & Abroad [Advance]	1,37,398.00
-	Telephone [Advance]	5,000.00
7,56,099.00	Vehicles [Advance]	2,26,904.00
5,00,000.00	Workshop & Conference [Advance]	-
9,80,89,157.00	**Total**	**2,25,53,041.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: E Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	I-Remittances	
1,450.00	GSLI	-
3,85,275.00	Income Tax	9,48,420.00
-	LIC	720.00
19,730.00	Professional Tax	35,800.00
44,802.00	Service Tax	2,50,859.00
1,87,372.00	TDS	11,69,885.00
33,244.00	Works Tax	26,838.00
6,71,873.00	**Total**	**24,32,522.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: F Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	Projects - Expenditure	
5,67,482.00	SP001	2,884.00
-	SP002	5,25,088.00
5,67,482.00	**Total**	**5,27,972.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: G Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	NIAB C.P.F ACCOUNT	
	Opening Balance	-
	Add:	
1,46,087.00	Employee subscription/ refunds	-
	Transfer from other departments	-
	Institute contribution (inc. Projects staff)	-
	Interest received	-
	Less Advances/withdrawals/Transfer/Adjst	-
1,46,087.00	**Total**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: H Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	LOANS AND ADVANCES	
9,28,17,633.00	Equipment [Advance]	1,05,80,000.00
7,56,099.00	Vehicles [Advance]	-
9,35,73,732.00	**Total**	**1,05,80,000.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: I Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	DEPOSITS	
-	LTC [Advance]	99,095.00
-	TA India & Abroad [Advance]	3,132.00
-	Telephone [Advance]	5,000.00
90,147.00	General Deposits And Advances	2,52,229.00
-	GDA [Others]	59,57,169.00
90,147.00	**Total**	**63,16,625.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: J Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	INVESTMENT A/C	
-	Investments	-
-	Other Investments	-
-	**Total**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MAR 2014
Annexure: K Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	NIAB C.P.F INVESTMENT A/C	
-	Deposit with Banks	-
-	Employee subscription	-
1,46,087.00	Less Transfer To Bank A/C	-
1,46,087.00	**Total**	**-**

NIAB
Hyderabad
SP001: NMMP MODEL NURSERY
P.I: Prof. P Reddanna
Receipts and Payments Account from 01/04/2013 to 31/03/2014

(Amount in Rs.)

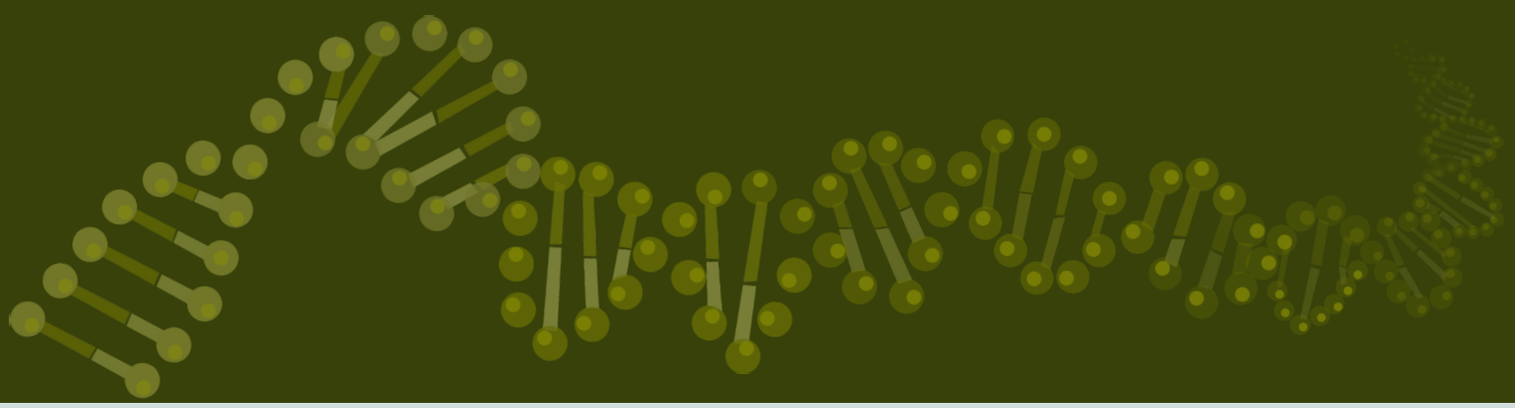
Previous Year Amount	Receipts	Current Year Amount Rs.	Previous Year Amount	Payments	Current Year Amount
-	Opening Balance	6,32,518.00			
12,00,000.00	Grant In Aid	-	-	Salaries - Manpower	1,500.00
		-	-	Consumables	-
-			10,00,00.00	Contingencies	1,384.00
-		-	-	Travel	-
-		-	-	Overheads	-
-		-	4,67,482.00	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
12,00,000.00		**6,32,518.00**	**5,67,482.00**		**2,884.00**
-	Excess of Expenditure over Income	-	6,32,518.00	Closing Balance	6,29,634.00
12,00,000.00		**6,32,518.00**	**12,00,000.00**		**6,32,518.00**

NIAB
Hyderabad
SP002: DST INSPIRE FACULTY
P.I: DR. ABHIJIT S DESHMUKH
Receipts and Payments Account from 01/04/2013 to 31/03/2014

(Amount in Rs.)

Previous Year Amount	Receipts	Current Year Amount Rs.	Previous Year Amount	Payments	Current Year Amount
-	Opening Balance	-			
-	Grant In Aid	19,00,000.00	-	Salaries - Manpower	3,84,516.00
		-	-	Consumables	71,388.00
-			-	Contingencies	6,251.00
-		-	-	Travel	6,036.00
-		-	-	Overheads	2,6997.00
-		-	-	Equipment	29,900.00
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**19,00,0000.00**	**-**		**5,25,088.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	13,74,912.00
-		**19,00,000.00**	**-**		**19,00,000.00**

Animal Health for Human Welfare



National Institute of Animal Biotechnology,
4th and 5th Floors, Axis Clinical Building,
Opp. Talkie Town Theatre
Miyapur, Hyderabad - 500049