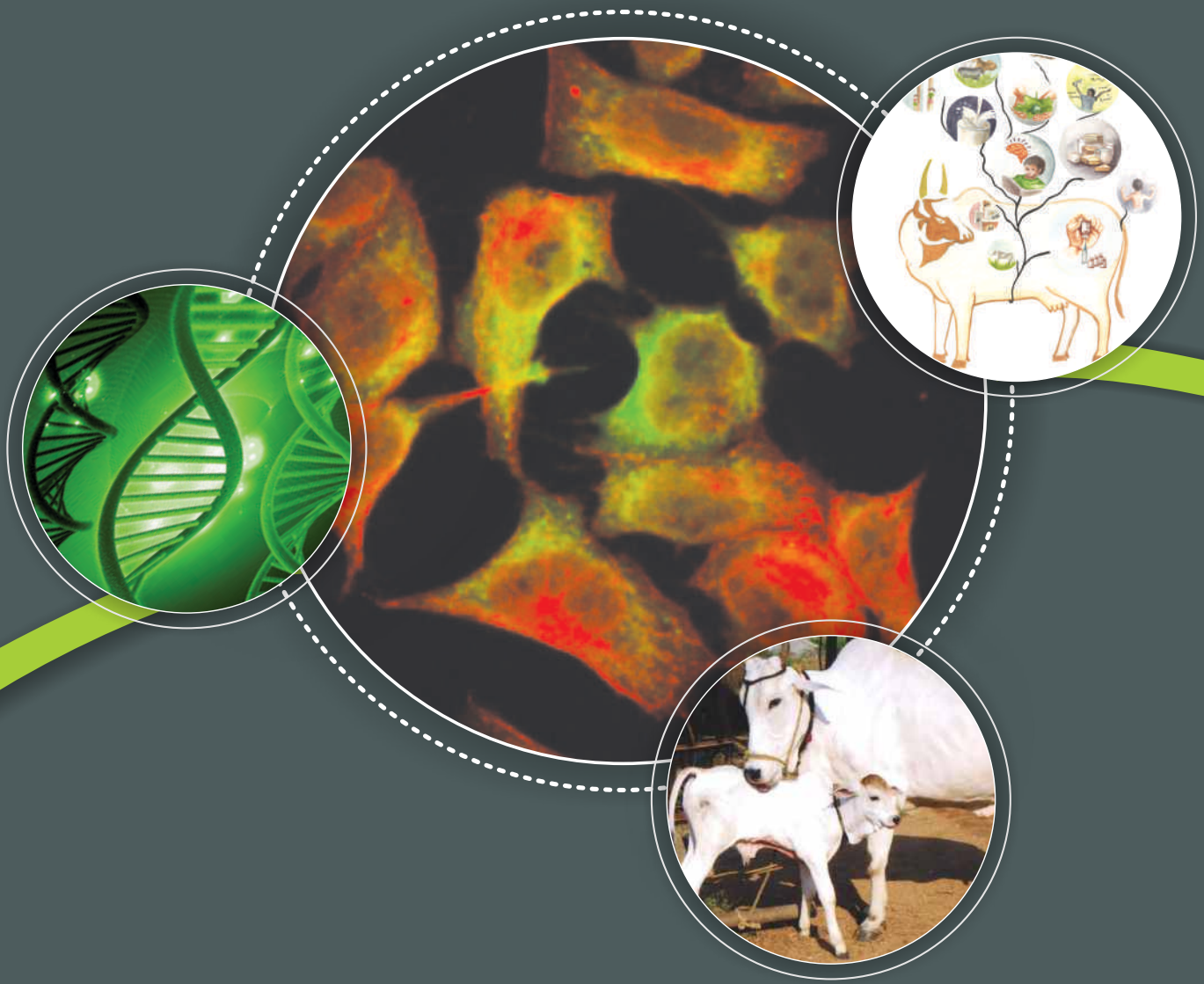
NIAB

National Institute of Animal Biotechnology

राष्ट्रीय पशु जैव प्रौद्योगिकी संस्थान

National Institute of Animal Biotechnology

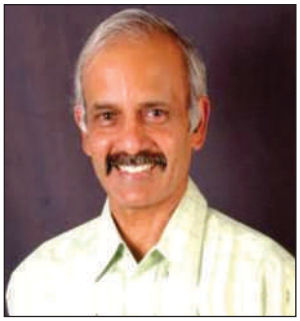
(An Autonomous Institute of the Department of Biotechnology,
Ministry of Science & Technology, Government of India)



वार्षिक प्रतिवेदन २०१४-१५

ANNUAL REPORT 2014-15

From the desk of Director



It gives me great pleasure to present the Annual Report of National Institute of Animal Biotechnology (NIAB), an autonomous Institute of the Department of Biotechnology, Ministry of Science & Technology, Government of India, for the year 2014-15.

Set up as a Registered Society in May 2011, the vision of NIAB is to produce globally competitive livestock products, pharmaceuticals and biologicals for animal health and productivity through innovative technologies. A key feature of NIAB is that it will function as an incubator for start-up companies and will also act as a national repository of biotech products and processes and promote bio-entrepreneurship in the country.

The mandate of the Institute is to harness novel and emerging biotechnologies, and to take up research in cutting edge areas, for improving animal health and productivity. The research focus at NIAB is to be on infectious diseases, animal genetics and genomics, animal reproduction and bioinformatics. During the last one year, research programme was initiated from the interim rented facility with state of the art infrastructure including BSL2+ facility. The faculty at NIAB are working on bacterial (brucellosis and leptospirosis), viral (ND, FMD and PPR) and protozoon (babesiosis, theileriosis and toxoplasmosis) diseases of Indian livestock. Virulence mechanisms, molecular pathogenesis and host-pathogen interactions are being studied with the aim of development of efficient diagnostic tools and novel vaccines. In addition, in the area of animal reproduction, endocrinological studies are being initiated to address infertility in livestock. Sequence analysis for marker discovery and comparative genomics studies are being done in the discipline of bioinformatics. Collaborative research projects, with the focus on important areas such as antibiotic resistance and TLR signaling pathways, are being carried out with the faculty at the University of Hyderabad. The following research programs have beeninitiated during the period of report :

- Development of novel vaccines and diagnostic tools for various livestock diseases including brucellosis, Newcastle disease virus, Peste-des-petits ruminants virus disease, foot and mouth disease, leptospirosis, theileriosis and toxoplasmosis.

- Research projects in reproductive biology to augment fertility and productivity inlivestock.

- Marker discovery, gene space exploration and development of web resources for livestock species using bioinformatics tools.

Along with R&D activities, NIAB has initiated steps to start the Research Scholars Programme (RSP) in collaboration with the Manipal University and University of Hyderabad. In addition, International Conference on Host Pathogen Interactions (ICHPI) was organized by NIAB in association with University of Hyderabad and CR Rao Advanced Institute of Mathematics, Statistics and Computer Science from 12th to 15th July, 2014. This conference focused on the basic and advance studies of host-pathogen interactions with respect to livestock and poultry including zoonotic infections. ICHPI was conducted mainly to create a platform for scientists, post-docs, and students along with leading industries in veterinary health to gather under one roof and share the cutting edge research findings.

Simultaneously, efforts are underway to build the main campus (comprising around 24000 sqm of built up area, along with farms for large animals) in the 100 acres of land alienated to NIAB by the State Government adjacent to the campus of University of Hyderabad. After the identification of contractor, after following the due procedure, the contract has been awarded to M/s Engineering Projects India Limited (EPIL), New Delhi, a Public Sector Enterprises for construction of laboratory complex, animal house, animal farms, hostels and guest house complex. All statutory payments have been made and formalities completed to obtain the building permissions from the civic authorities, which are awaited soon.

I sincerely acknowledge the support,encouragement and advice received from the distinguished members of the NIAB Society, Governing Body, Scientific Advisory Committee, Finance Committee and Building Committee, as well as the support of the Department of Biotechnology, in furthering the activities of NIAB. The immense support received from Centre for DNA Fingerprinting and Diagnostics (CDFD), University of Hyderabad and other Institutes is greatly appreciated. I also acknowledge the contributions of the cohesive team of highly dedicated scientific, technical and administrative staff of NIAB for their untiring efforts in meeting our challenges within the limited resources. And last but not the least, I wish to place on record NIAB's acknowledgement and appreciation of the selfless endeavours and contributions of Prof. P. Reddanna as Officer on Special Duty and then Founder Director of this Institution from inception until 30 September 2014, in building up its activities to the present level.

I sincerely hope and wish for continued support and encouragement in the years to come so that we may strive to achieve excellence in all our endeavours.

J. Gowrishankar
Director-In-Charge

31 March 2015

Objectives of NIAB

1. To undertake directed, basic and applied research towards technology and product innovation. Characterization of breeds and selective breeding to enhance productivity; develop technologies for multiplication of elite genotypes. Development of transgenic animals for producing molecules of pharmaceutical value. Enrichment of crop residues into high value products. Development of new generation vaccines, diagnostics and drugs.

2. To develop human resource across the value chain, primarily for translational research, industrial R&D; facilitate introduction of short term advanced training, new courses like MSc/MVSc-PhD and Ph.D. degree with a focus on interdisciplinary science, innovation and the science of manufacturing.

3. To contribute to national policy formulation related to animal biotechnology, animal bio-safety issues and ethical issues.

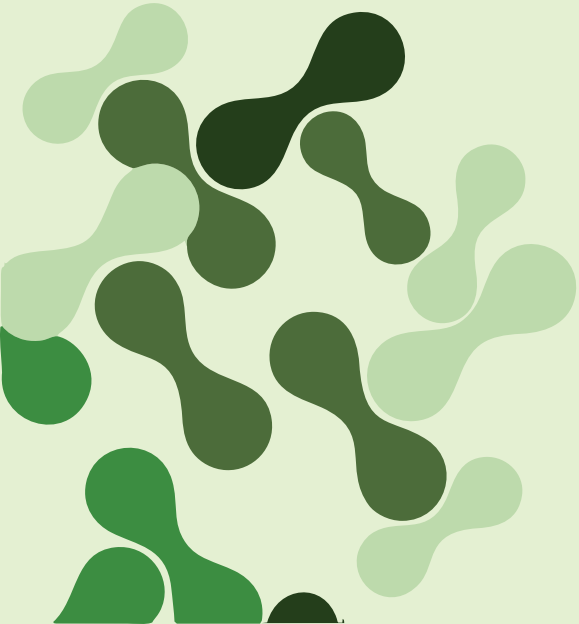
4. To promote intellectual property protection, business development, technology transfer, and academia-industry partnerships.

5. To develop collaborative programmes with national and international partners with focus on translational research and product development.

6. To provide incubation facilities for entrepreneurs/startup companies.

7. To create (i) extramural centers with emphasis on product innovation and translational research (ii) 'not for profit' companies; and (iii) facilitate the creation of 'for profit' companies.

NIAB RESEARCH PROJECTS



Inflammation Biology

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

Principal Investigator	Prof. Pallu Reddanna	
Members	K. Kumar Reddy	Senior Research Fellow
	Dr. Aparna Rachamallu	DST-WOS-A Women Scientist
	Dr. Anilkumar Kotha	NIAB Post-Doctoral Fellow
Collaborators	Dr. Paresh Sharma	NIAB, Hyderabad
	Prof. Hartmut Kuhn	Institute of Biochemistry, Germany
	Prof. M. Rami Reddy	Rational Laboratories, USA
	Prof. V. Lakshmipathi	NIPER, Hyderabad
	Dr. Aparoy	CUHP, Dharmashala

Objectives

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), endotoxaemia resulting out of infection of the mammary gland (mastitis), the reproductive tract (metritis), the lung (pneumonitis), 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 (AA), play a key role in physiological (reproduction) and pathological (inflammatory diseases) processes. At cellular level, release of arachidonic acid (AA) 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:

1) Exploration of binding site pattern in AA metabolizing enzymes, COXs and LOXs.

2) Studying the role of gamma deltaT cells in COX-2 induced inflammation.

3) Evaluation of anti-inflammatory natural compounds for therapeutic use in mastitis of dairy animals.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Exploration of binding site pattern in arachidonic acid metabolizing enzymes, Cyclooxygenases and Lipoxygenases

Non-steroidal anti-inflammatroy drugs (NSAIDs), the non-specific COX inhibitors, and COX-2 specific inhibitors (COXIBs) are the drugs being used for the treatment of inflammatroy disorders in humans as well as livestock. However, both NSAIDs and COXIBs are associated with gastric and cardiac side effects respectively. As AA is the substrate common to variety of COXs and LOXs, inhibition of one pathway results in the diversion of the substrate to other pathways, which often is responsible for undesirable side effects. Hence there is a need for development of not only isozyme specific inhibitors but also dual/multi enzyme inhibitors. Understanding the interactions of AA and characterizing its binding sites in these enzymes therefore is crucial for developing enzyme specific and multi enzyme inhibitors for enhancing therapeutic efficacy and/or overcoming side effects. Hence, AA binding sites in COXs and LOXs are identified and compared by the development of receptor based pharmacophore using MultiBind. Physico chemical properties were compared to understand the details of the binding sites in all the enzymes and elucidate important amino acids that can be targeted for drug design. The alignment of AA binding sites in the seven enzymes COX-1, COX-2, 5-LOX, 12-LOX, 15-LOX and plant soybean LOX-1 and LOX-3 was performed. It has been identified that in the COXs, there are 36 common features at the AA binding site; the only difference at the binding sites was Val substitution in COX-2 for Ile in COX-1 at position 523. Previous studies suggested that this small change in amino acid provides additional space in COX-2 binding pocket. The sulfonamoyl phenyl or methylsulfonyl phenyl moieties of COXIBs, particularly those of the diaryl heterocycles (celecoxib and rofecoxib) can therefore accommodate only in the active site of COX-2 by placing them in the side pocket. This supports that amino acid differences at the common physicochemical parameters studied can contribute for the development of specific/selective inhibitors.

The comparative study of human 5-LOX, 12-LOX and 15-LOX has shown that there are 8 common features, two common ACC, one DAC, one PI and four ALI interaction groups (Fig. 1). This is in correlation with the previous reports on ligand based pharmacophore model of 5-LOX developed by Aparoy *et al.*[Bioorg Med Chem Lett. 2010; 20:1013–8] and Charlier *et al.* [J Med Chem. 2006; 49:186–95], where the significance of these four types of interactions has been explained. Human LOXs have 10–15 common interacting groups (Table 1). 5-LOX has maximum similarity score of 41.92 with 15- LOX and has 14 common features. A major difference that has been observed in human LOXs is that Glu, amino acid with acidic side chain is replaced with Gln in 5-LOX. Rational design of compounds which would interact effectively with Gln than Glu can form potential specific 5-LOX inhibitors. Even bulkier amino acid Ile 593 in 15-LOX is replaced with smaller amino acid Ala in 5-LOX. A bulky hydrophobic group in the ligand can occupy the corresponding space in 5-LOX. These differences can be taken advantage of in the development of specific 5-LOX inhibitors as in the case of COX-2 inhibitors.

In the pair wise comparison of COX-2 with all LOXs individually, it has been observed that COX-2 is more similar to human LOXs than the plant isoforms. COX-2/5-LOX has 15 common features compared to 12 common features in COX-2/15-LOX (Table 2). The less similarity of COX-2 and 5-LOX at binding site and almost equivalent features with 15-LOX makes the design of dual inhibitors concept more challenging. COX-2/5-LOX model has more PI interactions (6) in common compared to COX-2/15-LOX and COX-2/12-LOX, hence the amino acids at 5-LOX binding site Tyr 181, Phe 359, Phe 421, Trp 599 can be targeted for aromatic interactions which may increase specificity towards COX-2/5-LOX.

COX-2/5-LOX model generated was further validated by docking studies with a known COX-2/5-LOX dual inhibitor, licofelone (Fig. 2). Licofelone formed interactions with amino acids that were found to be important. The only charge group, the carboxyl moiety of licofelone interacted with common DAC feature (Tyr 355 in COX-2 and His 372 in 5-LOX). The hydrophobic Cl group aligned at the common ALI feature (Val 523 in COX-2 and Leu 368 in 5-LOX). The aromatic ring of licofelone formed strong PII interactions with Trp 387 in COX-2 and Phe 421 in 5-LOX, in correlation with the common PII feature observed in the model. The dimethyl cyclopentane moiety formed hydrophobic interactions with few common ALI features observed. Hence, the docking results showed that the model generated can be used to elucidate the common features and can be further used in the design of dual inhibitors. The current approach is safer as more effective drugs will result from designing very selective ligands where undesirable and potential side effects have been removed.

Summary

The complete analysis of AA binding sites in the seven enzymes of COX and LOX pathways was performed; 120 combinations for the seven enzymes were studied in detail. All the seven enzymes are structurally quite different, yet they share AA as the common binding partner. Comparisons in various combinations showed how they are similar and dissimilar with each other. This information will be helpful in designing mono-, dual- as well as multi enzyme specific inhibitors for the development of anti-inflammatory drugs without side effects.

Project 2: Role of gamma deltaT cells in COX-2 induced inflammation:

Lymphocyte subsets that are expressing Vγ9Vδ2 T-cell receptor, broadly known as $\gamma\delta$ T cells, accumulate at the sites of inflammation in response to wide variety of pathogens such as bacterial, viral, and parasitic infections and play a key role in the regulation of the host response to inflammatory challenges by secreting various cytokines such as IFN-γ, IL-10, and TNF-α, as well as by direct cytotoxic actions toward target cells. Also these cells are critical in the development of efficient cancer immunotherapy strategies, by the recognition and elimination of transformed cells. Despite the ability of immune cells such as Natural killer cells (NK cells) and $\gamma\delta$ T cells to infiltrate and kill tumor cells, tumor cells and their microenvironment often produce numerous immunomodulatory molecules that can negatively influence the functions of immune cells. Prostaglandin-E2 (PGE_2) is one such major inhibitory factor produced by tumor cells or their surrounding microenvironment. Prostaglandin E_2 is generated by the sequential metabolism of arachidonic acid by a rate limiting enzyme called cyclooxygenase (COX) and prostaglandin E synthase. Recent studies clearly demonstrated that over expression of PGE_2 down regulates the cytotoxic properties of T cells. However the mechanisms by which PGE_2 and COX-2 regulate the immunomodulation of $\gamma\delta$ T cells are not clear. As COX-2 is the main mediator of inflammation, COX-2 selective inhibitors (COXIBs) are used in the treatment of inflammatory disorders. As a result of their cardiac side effects some of the COXIBs have been withdrawn from the market and some are still in use. At present it is not clear how these COXIBs are associated with cardiac side effects. Hence it is critical to know how the COX-2 plays a role on modulation of innate immune system, specifically the cytotoxic properties of $\gamma\delta$ T cells. With this background, the present study is focused on understanding the role of COX-2 on $\gamma\delta$ T cells.

Summary

Naive T cells could not get activated by LPS as indicated by lack of induction of cytokines compared to CD3 as well as CD3 + LPS activated cells. No significant difference was observed in cytokines expression (TNF-α and IFN-γ) between CD3 alone and CD3+LPS treated cells (Fig. 3 and Fig. 4). This might be due to the high concentrations of either CD3 or LPS. These findings indicate the need to standardize the effective concentration of CD3 and LPS to get differential expression of cytokines by activated T cells (Fig. 5). Also further studies will be taken up on how COX-2 regulates the activation and immunological response of T cells. The studies will be taken up by silencing /over expressing COX-2 or incubating with COX-2 metabolites/inhibitors and analysing the T cells and analysing the regulation of immunological responses in these cells.

Project 3: Evaluation of anti-inflammatory natural compounds for therapeutic use in mastitis of dairy animals

Mastitis is an inflammation of mammary gland parenchyma which is characterized by a range of physical and chemical changes of the milk and pathological changes in the udder tissues. Significant changes in the milk that can be observed in bovine mastitis are the presence of clots in milk, milk discolouration and high numbers of leukocytes. Furthermore, apparent clinical signs in bovine mastitis comprise swelling, heat and pain in the udder. Mastitis is usually caused by bacterial pathogens; *S.aureus* is the most common etiological organism responsible, but *E. coli, S. epidermidis* and *Streptococci* are occasionally isolated as well. Mastitis is an endemic disease and happens to be the most frequent and most costly disease affecting dairy herds worldwide. In India, it is the second most important and challenging disease after FMD affecting dairy animals. According to Bhikane & Kawitkar (2000), this most important disease in dairy cattle can cause up to 70% of reduced milk production, 9% of milk discard after treatment, 7% of the cost of veterinary services and 14% of premature culling. Control of mastitis has involved the use of chemical disinfectants and antibiotic therapy. Antibiotic therapy has been used in mastitis control for about 50 years. However, it was suggested that antibiotic therapy was not actually helping to reduce the incidence of mastitis. In fact, several problems arise from the use of antibiotics, for example, developing resistance to antibiotic, questionable drug efficacy and presence of antibiotic residues in the milk. Antibiotic residues in milk during antibiotic therapy render the milk ineligible for consumption and hence such milk has to be discarded. Natural medicines are increasingly becoming important in treating variety of diseases and traditional knowledge provides a starting point in the search for plant-based medicines. The present project is designed to isolate and identify the anti-inflammatory compounds from the leads obtained in the earlier project and evaluate their efficacy, either alone or in combination with antibiotics in the treatment of mastitis. The project involves isolation of natural compounds from the medicinal plants and evaluate their efficacy on (1) isolated enzymes- COX-2 and 5- LOX, and on (2) mouse macrophage cell line, RAW264.7, stimulated with a pro-inflammatroy agent *in vitro.* These studies should lead to the identification of natural anti-inflammatory compounds for potential therapeutic use in mastitis.

Pueraria tuberosa, (Willd.) DC. (Fabaceae) is a large climbing herb that has been used in traditional medicine. Tuberous roots of the plant are being used as spermatogenesis enhancer, immune booster, aphrodisiac, anti-inflammatory agent, cardiotonic and brain tonic. This study was aimed to isolate and identify the cyclooxygenases (COX-1 & COX-2) and/or 5-lipoxygenase (5-LOX) inhibitors from the tubers of P.tuberosa DC. Air dried tubers from P. tuberosa were powdered and extracted with n-hexane, ethyl acetate and methanol, using soxhlet apparatus. These extracts were screened for inhibition of COX and 5-LOX by employing spectroscopic or polorographic methods (Table 3). The methanolic extract, which showed potent inhibition of COX-2 and considerable inhibition of 5-LOX was further purified by silica gel column using n-hexane and n-hexane-ethyl acetate step gradient mixtures. The major peaks thus obtained (PT-1, PT-2 and PT-3) were characterized for their enzyme inhibition (Table 4) and structural determination by mass spectrometry and 2D-NMR techniques including HSQC, HMBC, NOESY and 1H-1H COSY. The three major constituents, PT-1, PT-2 and PT-3, were identified as puerarin, isoorientin and mangiferin, respectively. Among these, Isoorientin showed potent inhibition against COX-2 with an IC_{50} value of 39 µM (Fig 6).We further evaluated the anti-inflammatory effects of Isoorientin on air-pouch murine model *in vivo.* Air cavities were produced by subcutaneous injections of 1.5 ml of sterile air into the intracapsular area on the dorsal side of the animal on three alternate days. Six days after the initial air injection 0.5- 1 ml of 1% (w/v) solution of carrageenan dissolved in saline was injected directly into the pouch, to produce an inflammatory response. Animals were divided into 5 different groups. They are: 1. Saline treated, 2. Carrageenan (0.5-1ml of 1% (w/v) carrageenan in saline), 3. Carrageenan + Control drug (20mg/kg body weight), 4. Carrageenan + Medicinal compound (10 mg/kg body weight) and 5. Carrageenan + Medicinal compound (20 mg/kg body weight). These *in vivo* studies using isoorientin are currently under progress.

Publications:

1. Reddy KK, Vidya Rajan VK, Gupta A, Aparoy P and Reddanna P(2015). Exploration of binding site pattern in arachidonic acid metabolizing enzymes, Cyclooxygenases and Lipoxygenases. BMC Res Notes 8: 152.

2. Latha TS, Reddy MC, Durbaka PV, Rachamallu A, Reddanna P and Lomada D (2014). $\gamma\ \delta$ T Cell-mediated immune responses in disease and therapy. Front Immunol 5: 571.

3. Kallubai M, Rachamallu A, Yeggoni DP and Subramanyam R (2015). Comparative binding mechanism of lupeol compounds with plasma proteins and its pharmacological importance. Mol Biosyst 11: 1172 - 1183.

Table 1: Pair wise alignments of 5-LOX binding site with other LOXs using MultiBind.

Compared proteins	No. of detected features	Score
5-LOX– 12-LOX	10	32.6815
5-LOX– 15-LOX	12	41.8927
5-LOX– LOX-1	8	19.6051
5-LOX– LOX-3	11	35.3228

Table 2: Multi Bind data on pair wise alignments of COX-2 binding site with all six enzymes

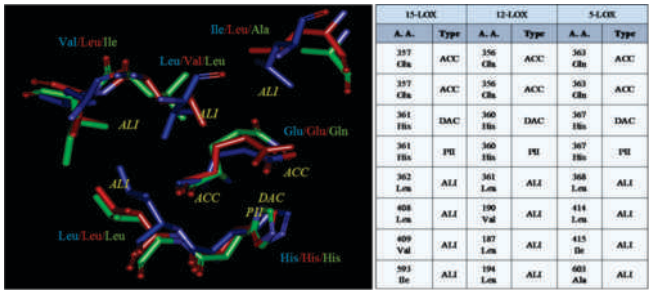
Compared proteins	No. of detected features	Score
COX-2 – COX-1	36	90.4106
COX-2 – 5-LOX	15	33.4303
COX-2 – 12-LOX	10	32.6815
COX-2 – 15-LOX	12	41.8927
COX-2 – LOX-1	8	19.6051
COX-2 – LOX-3	11	35.3228

Table 3 : Percentage inhibition of different extracts of p. tuberosa against COX-1, COX-2 and 5-LOX at 100 µg/ml conc.

Extract	COX-1	COX-2	5-LOX
Hexane	6.43	11.23	-
Ethyl acetate	15.25	87.97	5.25
Methanol	26.79	91.94	22.65

Table 4 : Percentage inhibition of Puerarin (PT-1), Isoorientin (PT-2) and Mangiferin (PT-3) against COX-1, COX-2 and 5-LOX at 100 µM.

Compound	COX-1	COX-2	5-LOX
PT-1	1.55	-	10
PT-2	-	63.96	14
PT-3	79.4	45.94	5
Indomethacin	100	-	-
Celecoxib	-	100	-
NDGA	-	-	100



15-LOX		12-LOX		5-LOX	
A. A.	Type	A. A.	Type	A. A.	Type
357 Gln	ACC	356 Gln	ACC	363 Gln	ACC
357 Gln	ACC	356 Gln	ACC	363 Gln	ACC
361 His	DAC	360 His	DAC	367 His	DAC
361 His	PII	360 His	PII	367 His	PII
362 Leu	ALI	361 Leu	ALI	368 Leu	ALI
408 Leu	ALI	190 Val	ALI	414 Leu	ALI
409 Val	ALI	187 Leu	ALI	415 Ile	ALI
593 Ile	ALI	194 Leu	ALI	603 Ala	ALI

Fig. 1: Common physiochemical parameters identified for 5-LOX, 12-LOX and 15-LOX. The amino acids of 5-LOX (green), 12-LOX (red) and 15-LOX (blue) contributing to the common properties are shown.

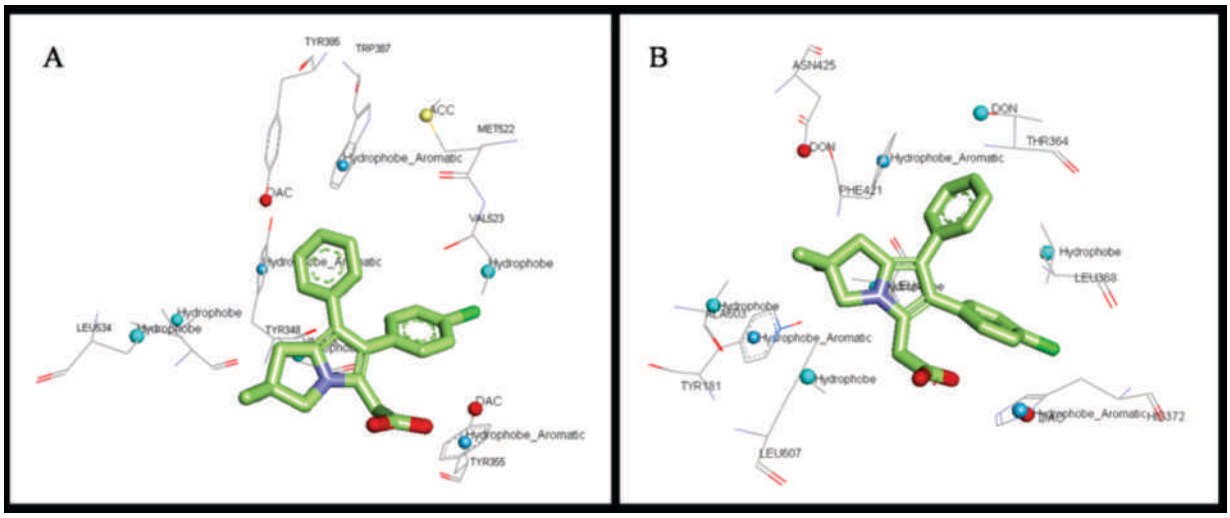


Fig. 2: Licofelone in the binding site of A) COX-2 and B) 5-LOX.

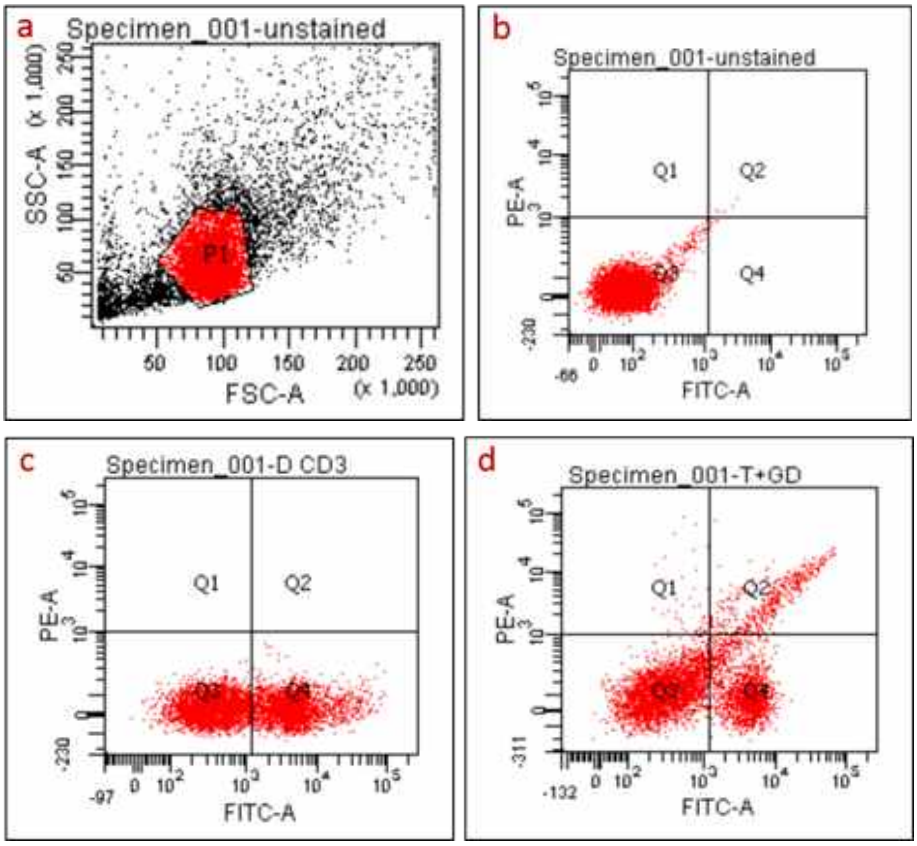


Fig. 3 : Flow cytometric analysis of T cells. a) Gating of spleenocytes, b) Unstained control cells, c) T cells stained with CD3-FITC antibody, d) Double positive cells for CD3 FITC and -PE antibody.

Experiment Name:	Experiment_026
Specimen Name:	Specimen_001
Tube Name:	C+GD
Record Date:	Feb 27, 2015 11:43:23 AM
$OP:	Administrator
GUID:	31fb2aed-52d1-42c7-9c8f-d1bf4bd7783c

Population	#Events	%Parent	%Grand P...	FITC-A Mean	PE-A Mean
P1	6,665	66.6	####	1,223	894
Q1	83	1.2	0.8	579	15,477
Q2	560	8.4	5.6	8,548	7,031
Q3	5,538	83.1	55.4	339	101
Q4	484	7.3	4.8	2,966	371
P2	727	10.9	7.3	7,905	4,846
P3	688	10.3	6.9	7,179	7,653

Fig. 4: Showing the percentage of positive cells in each quadrant for CD3FITC and -PE antibody.
Q3 : Unstained cells. Q4: CD3 FITC positive cells. Q2: CD3-FITC and -PE positive cells (Double positive).

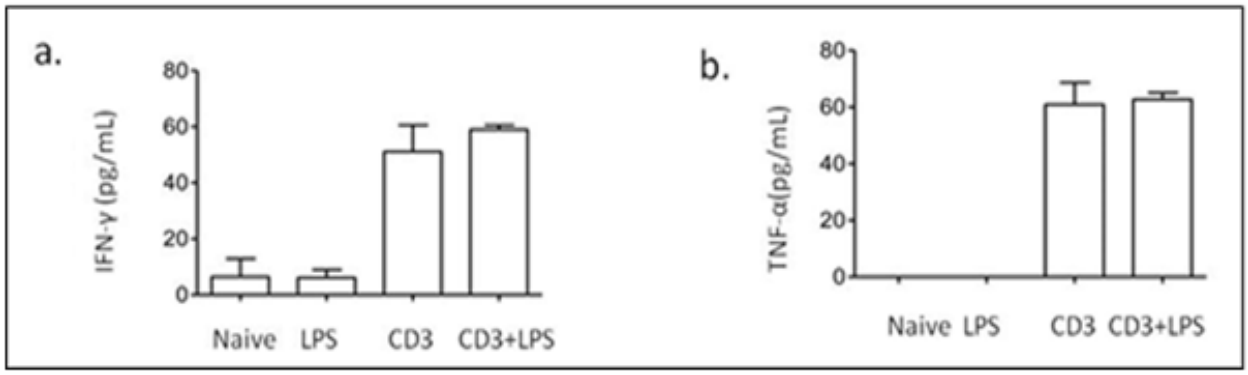


Fig. 5: Quantification of INF-γ and TNF-α released in nad've, CD3 and LPS activated T cells.

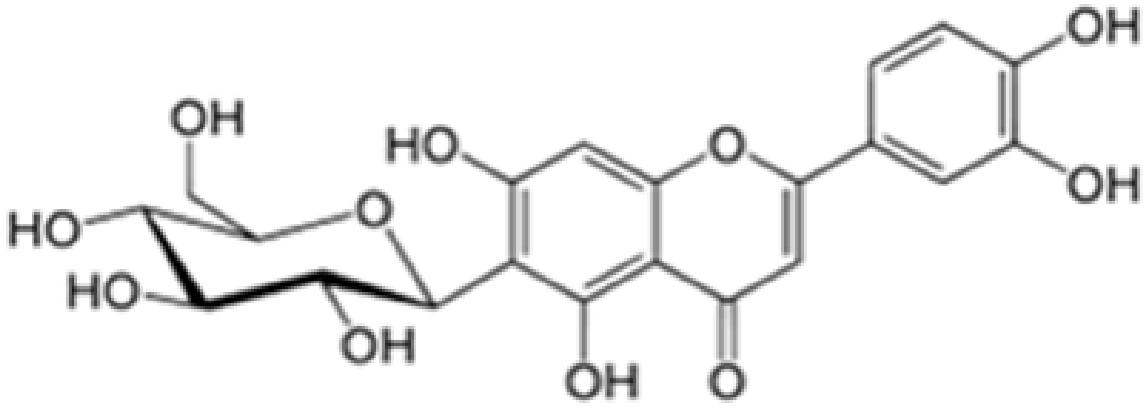


Fig. 6: Molecular structure of Isoorientin.

Animal Genetics and Genomics

Differentiation of Pluripotent Stem Cells into Female Germ Cells and Oocytes in Farm Animals (Sheep and Buffalo)

Principal Investigator	Satish Kumar	Scientist H
Lab Members	Himabindu Gali	Research Associate
	AkshayJoshi	Project Fellow (Apr - Nov 2014)

Objectives

There is a need to improve the quality of our live stock for improvement in their production capabilities and for well being of the animal. Embryo technology is a form of applied reproductive science that has greatly influenced the animal agriculture industry. *In vitro* production of embryosis 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 revolution size live stock industry by rapidly enhancing valuable genetictraits. Further, despite several recent advancements, obtaining oocytes for transgenesis remains a challenge due to their complex structure and limited availability. Stem cells by their potentially unlimited proliferative capacity and the ability to form every cell type of the body provide a unique approach to assist reproduction, embryo manipulation and genomeediting (transgenesis), especially in large farm animals, as it substantially improves genomeediting efficiency and reduces product development time lines. The manipulation of gametes and embryos in farm animals are gaining importance in disease modeling, basic research and generation of transgenic animals. Therefore, differentiation of stem cells, like induced pluripotent stem cells, into oocytes can provide an unlimited source of egg cells for the generation of transgenic animals with desired transgene. However, this area of research in large animals suffers the lack of effective reprogramming protocols and reliable differentiation protocols capable of developing different line ages of stem cells. The main goal of the project is to develop highly efficient methods in farm animals to produce fertile oocytes entirely *in vitro* which can be further fertilized and developed into embryos for use in embryo transfer technologies. Therefore, differentiation of embryonic stem cells like induced pluripotent stem cells (IPSCs) into oocytes can provide an unlimited source of egg cells for the generation of transgenic live stock. A number of culture systems have so far been tested for use in differentiation of mouseem bryonic stem cells and or induced pluripotent stem cells into germ cell line ages. 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 game to genesis and off spring.

The project proposes to study the establishment of iPSCs and their differentiation into oocytes in farm animals (sheep and buffalo) with the following clear objectives:

1. Establishment of fibroblast cell lines from sheep and buffalo.
2. Derivation of sheep and buffalo iPSCs.
3. Differentiation of sheep and buffalo iPSCs into primordial germ cells and further development into oocytes.
4. Functional assessment of the derived oocytes from iPSCs.

Summary of work done until the beginning of this reporting year (up to 31st March, 2014)

Isolation and Culture of Skin Fibroblasts :

Previously isolated sheep and buffalo fibroblast cells were used for generation of iPSCs.

Karyo typing :

Exponentially growing sheep and buffalo skin fibroblasts were arrested in metaphase by treating with 0.02µg/ml colcemid for 3hrs.Cells were try psinized and treated with 8ml of hypotonic solution (75mm Kcl) pre warmed to 37C for 15min followed by addition of few drops of fixative solution (methanol: glacial acetic acid 3:1) and cells were mixed gently. Cells were centrifuged at 1000rpm for 5min and the supernatant was discarded. Cells were re-suspended in another 5ml of fixative solution. This step of washing was repeated in fixative solution thrice. Atleast 20-30µl of cells us pension was added onto a clean glass slide and dried at room temperature. Dried slides were stained with Giemsa (4% in Gurr buffer) for 2h or over night followed by rinsing in distilled water. Slides were mounted in 50% glycerol containing PBS and were analyzed under an Olympus microscope.

Lentiviral transduction and isolation of sheep and buffalo iPSCs :

FUW-OSKM and FUW-Nanog, lentiviral plasmid sex pressing pluripotency factors, mouse Oct4, Sox2, Klf4, c- Myc and mouse Nanog were transfected into HEK 293T cells using Lipofectamine. Viral particle containing supernatants were harvested at 48 h and 72 h after transfection. These supernatants were transferred onto skin fibroblasts of both cells lines in growth media containing 2-10µg/ml polybrene. 48 hafter viral transduction, fibroblasts were plated onto mouse embryonic fibroblast based feeder cells. 24h after plating on feeders, cells were cultured in iPS media containing DMEM, 20%knock-out serum replacement, 2mML- glutamine,1%Non-essential aminoacids,0.1mMβ-mercaptoethanol, 4ng/ml bFGF, 1000U/ml LIF for about 3wks. iPS cells with a ES cell like morphology were isolated and replated on fresh feeder coated dishes. Isolated colonies were expanded into iPSC cell lines, frozen and also maintained for around 20 passages while monitoring their ES cell like morphology.

Details of progress made in the current reporting year (April 1, 2014-November 20, 2014)

The first goal of the project was to establish cell lines from sheep and buffalo skin fibroblasts and to produce induced pluripotent stem from these cells. Earlier we established adult somatic cell lines from sheep and buffalo for reprogramming of these cells into iPSCs. Karyo type analysis was performed on these cells to confirm if these cells have a normal chromosome number and a karyotype.

To establish induced pluripotent stem cell lines from both sheep and buffalo cell lines lentiviral approach been adapted to express transcription factors for reprogramming. Lentiviruses were produced in 293T packaging cell lines and supernatants containing the viral particles were used to infect skin fibroblasts to establish the culture conditions in the lab to generate iPSCs efficiently for further differentiation into female germ cell lines and oocytes.We were able to isolate putative iPSCs from sheep and buffalo and the following (Fig. 1) is a representation of iPSCs derived by cell reprogramming. Further characterization of these colonies will be necessary to ascertain their pluripotency.

Publications:

1. Singh VP, Gurunathan C, Singh S, Singh B, Jyothi Lakshmi B, Mishra AP, Kumar S(2015). Genetic deletion of Wdr 13 improves metabolic pheno type of Leprdb/dbmiceby modulating AP1 and PPARγ target genes. Diabetologia 58: 384 - 392.

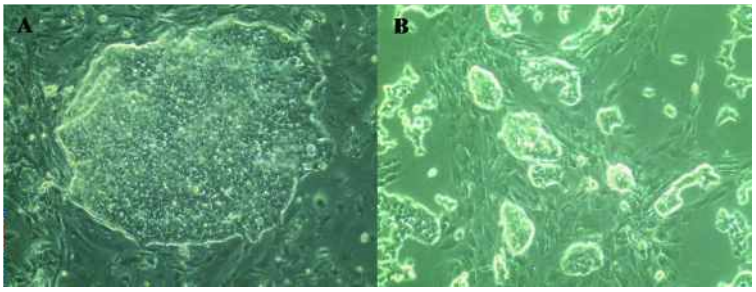


Fig 1. Undifferentiated iPSCs growing on feeder cells (A) Sheep iPS cells and (B) Buffalo iPS cells.

Infectious Diseases

Bacterial Disease

Understanding the Virulence Mechanisms of the Zoonotic Pathogen, *Brucella,* and Development of Novel Vaccines and Diagnostic Assays for Animal and Human Brucellosis

Principal Investigator	Girish K Radhakrishnan	Scientist D
Lab Members	Bindu Bhargavi	Research Fellow
	Padmaja Jakka	Research Fellow
Others Members	Sarwar Azam	Scientist B, NIAB
	Dileep Reddy	DBT-RA, NIAB

Objectives

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that affects humans as well as domestic and wild animals, leading to significant impact on public health and animal industry. Brucellosis is endemic in India and the disease is reported in cattle, buffalo, sheep, goats, pigs, dogs and human. India has experienced a sharply increasing rate of human brucellosis in recent years and the species of main concern are *B. melitensis* and *B. abortus.* 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. Minimal information is available on the basis of *Brucella* host specificity and the virulence factors that enable *Brucella* to survive and replicate in the host. Given the limitations of current vaccines and therapies for brucellosis, novel therapeutic and preventive strategies are needed, but the progress has been hampered by a lack of knowledge on the essential mechanisms and virulence factors enabling *Brucella* to survive and replicate in the host. Overall objectives of my research projects are as follows:-

1) To develop novel vaccines and diagnostics assays for animal and human brucellosis.
2) To analyze the genetic diversity of *Brucella melitensis* and *Brucella abortus* strains in India.
3) To study the *Brucella*-host interaction.

Summary of work done until the beginning of this reporting year (up to March 31, 2014)

Control of human brucellosis depends on prevention of the disease in livestock by mass vaccination. Towards developing novel live attenuated vaccines for livestock brucellosis, we plan to make *B. abortus* deficient in multiple virulence genes using homologous recombination and Cre-Lox technology. Gene knockout cassettes for four virulence genes were prepared and confirmed by restriction digestion and sequencing.

To develop novel sero-diagnostic assays for bovine brucellosis, we initiated a project to identify immunodominant antigens of *Brucella* in naturally infected livestock by immunoprobing of *Brucella* protein arrays. Towards this objective, we collected serum from healthy and *B. abortus* infected cattle and analyzed the serum samples by ELISA to confirm the infection.

To analyze the genetic diversity of *Brucella* isolates in India, we performed whole genome sequencing of *B. melitensis* IND1 strain, isolated from naturally infected goat. The genome was annotated and deposited at DDBJ/EMBL/GenBank under the accession JMKL00000000.

Brucella encodes a TIR domain-containing protein (TcpB) that inhibits TLR2- and TLR4-mediated innate immune signaling. 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. Using a high throughput yeast-two hybrid screening, we identified that TcpB interacts with a microtubule binding protein (MBP-1). Subsequent analysis revealed that MBP-1 functions as ubiquitin ligase that specifically target the TLR adaptor protein TIRAP.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015).

Project 1:To analyze the genetic diversity of *Brucella melitensis* strains in India.

Though brucellosis is an endemic disease in India, the genetic diversity and population structure of *Brucella* spp. remain unknown. *B. melitensis* is the common cause of human brucellosis in India and the disease is mainly acquired by ingestion of raw milk and milk products. We analyzed the genetic diversity of five *B. melitensis* isolates from India by Multi Locus Sequence Typing (MLST). We amplified and sequenced nine loci that included seven housekeeping genes, one locus from the outer membrane protein 25, and one locus from an intergenic region (Fig. 1). Subsequently, we compared the allelic profiles of the four *B. melitensis* IND isolates with each other and with other reported *Brucella* species.

For detailed genetic characterization, we carried out whole genome sequencing and comparative genome analysis of one of the *B. melitensis* isolates, Bm IND1. Genome details of Bm IND1 have been published (Rao et al. Genome Announcements, 2014). Comparative genome analysis identified 141 unique SNPs, 78 VNTRs, 27 Indels, and 2 putative prophage integrations in the Bm IND1 genome (Fig. 2). These genetic markers could be employed for developing high resolution epidemiological typing tools to understand the structure of *Brucella* population and for outbreak analysis. Information on prophage integration events and Indels in the virulence associated genes will provide important leads for further experimental characterisation of virulence properties of Bm IND1. This may ultimately lead to the development of efficient therapeutic and preventive strategies to control animal and human brucellosis.

Project 2 : Identification of immunodominant antigens of *B. abortus* to develop novel diagnostics assays for brucellosis.

Early diagnosis of brucellosis in livestock is very crucial for taking effective control measures that will help to reduce the incidence of human brucellosis. Immuno assay 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 the identification of antibodies to lipopolysaccharide (LPS) of *Brucella* in the patient serum. LPS is the immunodominant antigen but it cross react with several other Gram negative bacteria including *Yersinia enterocolitica* 0:9, *E. coli* 0:157, *Francisella tularensis*, *Salmonella urbana*, *Pseudomonas multiphilia* and several others. Anti-LPS antibody based serodiagnosis also suffer from the inability to differentiate whether the LPS antibodies are due to vaccination or new infection. 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.

We performed immunoprobing of *Brucella* protein microarrays with infected/healthy cattle serum, which identified 15 potential immunodominant antigens of *B. abortus.* Five high-scoring antigens were cloned and over expressed in *E. coli* (Fig. 3). Purification of immunodominant antigens of *B. abortus* is in progress for the assay development.

Project 3 : Molecular characterization of TIR domain containing protein from *B. melitensis.*

Brucella spp. encodes a TIR domain containing protein (TcpB) to subvert host innate immune responses to create a less hostile environment for its survival and replication. TcpB inhibits NF-κB activation and pro-inflammatory cytokine secretion mediated by Toll-like receptor (TLR) 2 and 4. TcpB deficient *B. melitensis* presented an attenuated phenotype in mouse model indicating that TcpB acts as an important virulent factor of *Brucella.* TcpB is a cell permeable protein and targets the TLR adaptor protein TIRAP to inhibit TLR2 & 4 signaling. Our preliminary studies indicated that TcpB recruits the microtubule binding protein (MBP-1) for enhanced ubiquitination of TIRAP. We performed luciferase reporter assay to analyze whether MBP-1 suppresses TLR4 induced NF-κB activation. The analysis indicated that MBP-1 suppressed the TLR4 signaling in a dose dependent manner (Fig. 4). Silencing of endogenous MBP-1 in macrophages using shRNAs potentiated TLR4 mediated secretion of pro-inflammatory cytokines (Fig. 5). We are currently performing the silencing of MBP-1 in mice using gene specific siRNAs to confirm the suppression of TLR4 signaling. It appears that TcpB exploits the MBP-1 mediated negative regulation of TLR4 signaling to subvert the host innate immunity.

Project 4: Analysis of the Single Nucleotide Polymorphisms in SLAM and Nectin-4 receptors in PPRV susceptible and resistant goat breeds.

Peste des petits ruminant (PPR) is an acute, highly contagious transboundary viral disease. PPRV is associated with high morbidity and mortality rates causing a loss of 1800 million Rupees annually to small ruminant population of the country. Goats are more susceptible to the disease and the symptoms are characterized by

pyrexia, oculo-nasal discharges, gastroenteritis and bronchopneumonia followed by death.

Signal lymphocyte activating molecule (SLAM) is a membrane glycoprotein, which functions as a putative co-receptor for PPRV infection. Apart from SLAM, Nectin-4, an epithelial receptor was also identified as binding site for PPRV. Barbari and Tellicherry goat breeds are susceptible to PPRV where as Kanni and Selam Black are resistant to PPRV infection. This project aims to examine the SNPs in SLAM and Nectin-4 receptors in the PPRV susceptible and resistant goat breeds. The SNPs data may help in marker assisted breeding for increased PPRV resistant traits.

Towards this objective, genomic DNA was isolated from peripheral blood mononuclear cells of Tellicherry goat breed. PCR primers were designed to amplify the exons of SLAM and Nectin-4 genes. Amplification of SLAM gene exons from Tellicherry goat breed has been shown in Fig. 6. Sequencing and analysis of PCR amplicons are in progress.

Summary

We performed whole genome sequencing and comparative genome analysis of *B. melitensis* IND1 from India. Data generated from our studies may help to develop new diagnostic assays based on stable markers such as SNPs and VNTRs for molecular epidemiological studies. Identification of SNPs, Indels and novel phage integration sites will provide insights into the virulence mechanisms of this stealthy pathogen which could ultimately lead to development of novel therapeutic and preventative strategies to control brucellosis. Towards developing novel diagnostic assays for brucellosis, we identified immunodominant antigens of *B. abortus* in naturally infected cattle. Molecular characterization of TcpB of *B. melitensis* provided novel insights into the mechanism by which *Brucella* suppresses the host innate immune responses. We are analyzing the SNPs in SLAM and Nectin-4 receptors in PPRV susceptible and resistant goat breeds that may provide important leads for understanding the mechanism of host susceptibility/resistance to PPRV infection.

Publications:

1. Rao SB, Gupta VK, Kumar M, Hegde NR, Splitter GA, Reddanna P and Radhakrishnan GK (2014). Draft genome sequence of the field isolate *Brucella melitensis* strain Bm IND-1 from India. Genome Announcements 2(3): e00497 - 14.

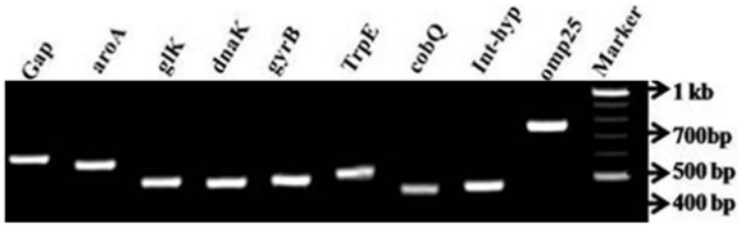


Fig. 1: Gel photo showing the amplification of nine loci from *B. melitensis* IND1 strain.

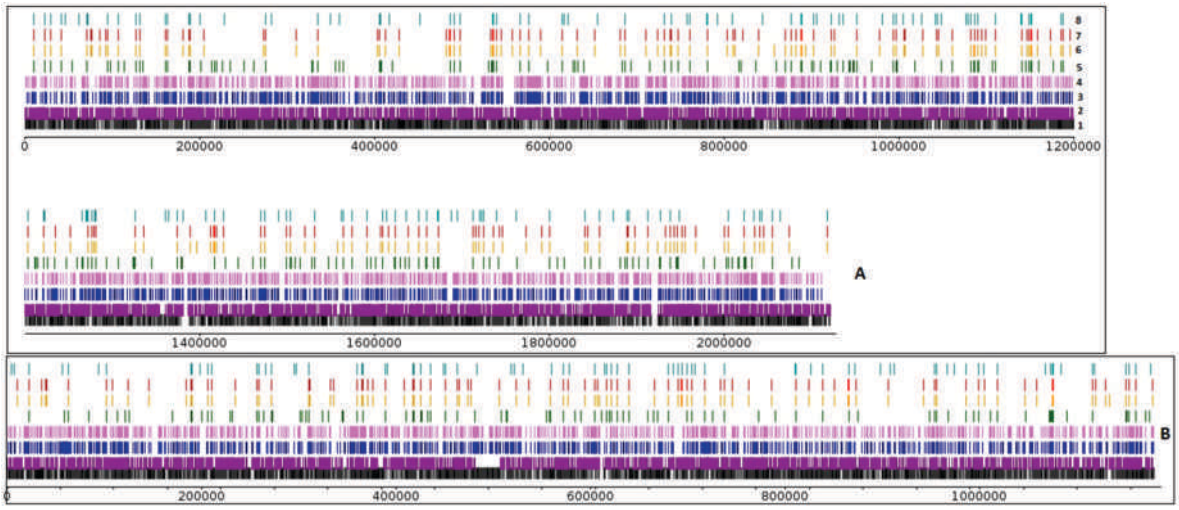


Fig. 2 : **Single Nucleotide Polymorphisms in** B.melitensis **IND1 Chromosome I (A) and Chromosome II (B).** Line 1, CDS (black); line 2, SNPs against *B. abortus* 2308 (purple); line 3, SNPs against *B. melitensis* 16M (blue); line 4, SNPs against *B. melitensis* ADMAS-G1(pink); line 5, SNPs against *B. melitensis* NI (green); line 6, SNPs against *B. melitensis* M5-90(gold); line 7, SNPs against *B. melitensis* M28 (red);line 8,SNPs against *B. melitensis* ATCC 23457(turquoise).

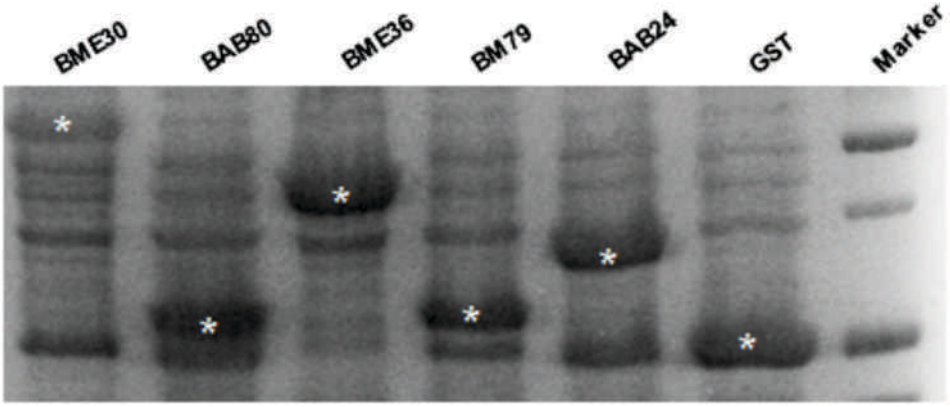


Fig. 3: SDS PAGE analysis of over expressed *Brucella abortus* antigens.

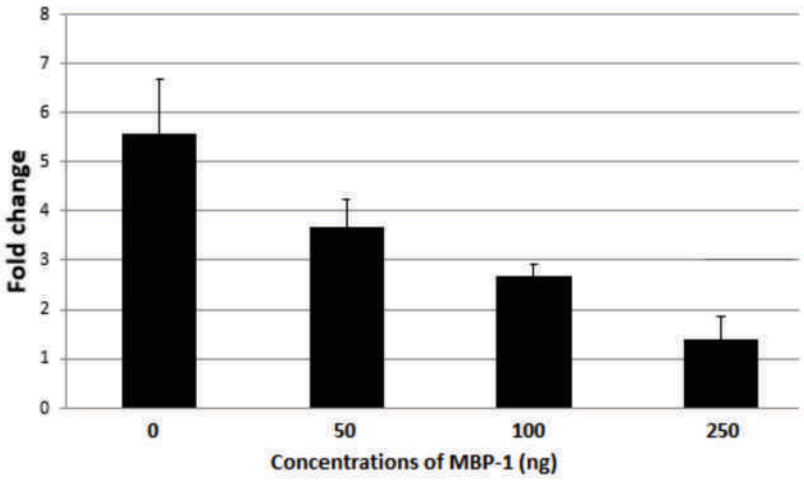


Fig. 4: MBP-1 inhibitsTLR4-induced NF-κB activation. HEK293 cells were co-transfected with plasmids encoding TLR4, CD14 and MD2 (200 ng each) with increasing concentrations of MBP-1 (50, 100, and 250 ng), pNF-κB -Luc reporter plasmid (100 ng), and PRL-TK (50 ng). The total amount of DNA was made constant by adding empty vector. 24 h post-transfection, the cells were induced with LPS (300 ng/ml), and luciferase activity was assayed after 12 h using the Dual-Luciferase reporter assay system.

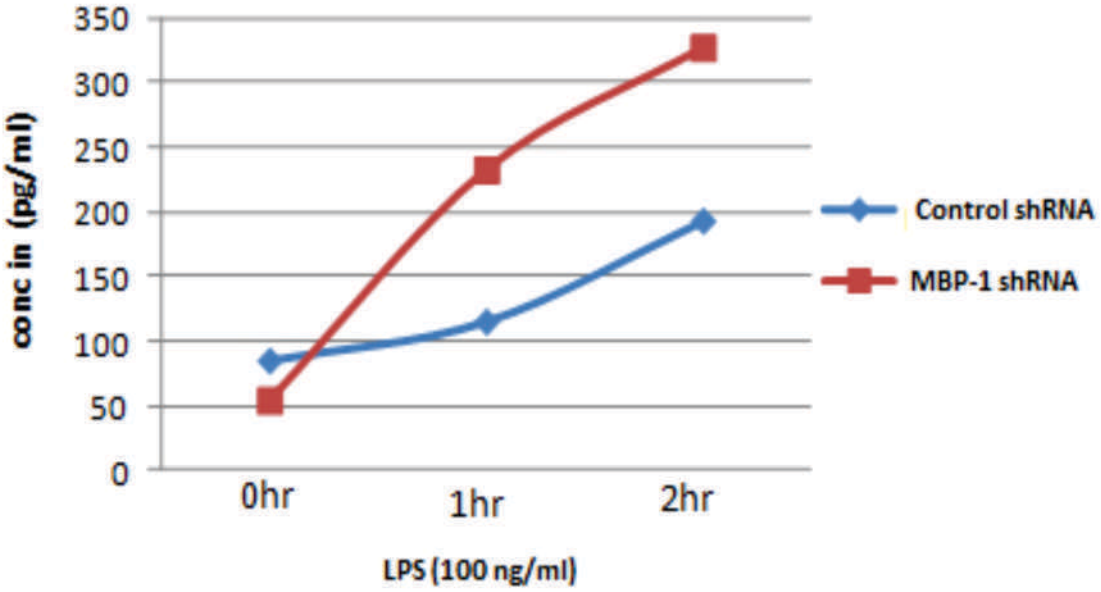


Fig 5 : **Silencing of MBP-1 potentiated secretion of TNF-α by macrophages.** J774 cells were transduced with lentivirus harboring control or MBP-1 specific shRNA followed by induction with LPS for indicated time points. Secretion of TNF-α was quantified by ELISA.

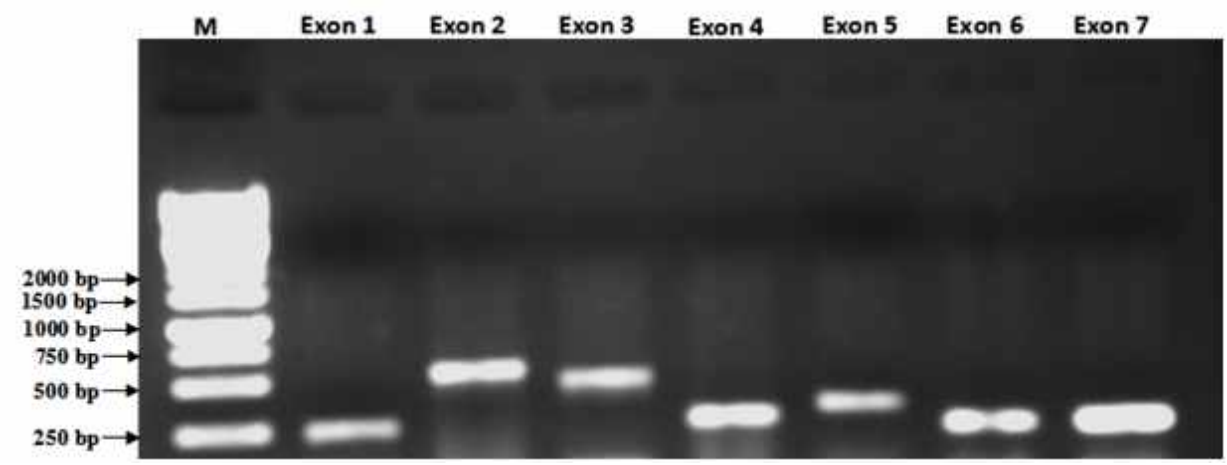


Fig 5 : **PCR amplification of SLAM gene exons from the genomic DNA of Tellicherry goat breed.**

Understanding the Host Response and Molecular Pathogenesis of *Leptospira* Infection

Principal Investigator	Syed Faisal	Ramalingaswami Fellow
Lab Members	Subathra Murugan	Senior Research Fellow
Collaborators	Prof. Yung-Fu Chang	Cornell University, USA
	Prof. Senthilkumar	TANUVAS, Chennai
	Prof. Manjula Sritharan	HCU, Hyderabad
	Dr. Ratnagiri Polavarapu	Genomix Biotech, India

Objectives

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. 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.

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 TLRs by exploiting its surface proteins, thereby establishing infection. Our research is focused on following objectives.

1. To determine whether targeting TLR2/4 via surface proteins leads to activation of innate response.
2. To identify the pathways involved in the associated inflammatory response.
3. To analyze the TLR dependent immune response and protective efficacy of best surface protein/MPLA formulation in animal model.

Summary of work done until the beginning of this reporting year (up to March 31, 2015)

We cloned and expressed few *Leptospira* outer membrane proteins (OMPs)/surface proteins. These proteins (LipL32, Lsa21) were purified as GST fusion proteins. Since Lig proteins are most promising vaccine candidate identified till date it was our main target. We cloned, expressed and purified various domains of conserved and variable regions of LigA and LigB proteins. For example, conserved region of LigA corresponding to domain 1-3 (Ligcon1-3), 4-7.5 (Ligcon 4-7.5), variable region of LigA (LigAvar), and various domains of LigB (B1, B2, B3, B4, B5, B6, B7, B8, B9 and B10) were purified (Fig. 1 and Fig. 2).

These proteins and domains were then tested for TLR2 activity on HEK293 cell line transfected with TLR2 plasmid. Cells were stimulated with proteins and tested for TLR activity using Dual luciferase assay kit. Each protein showed TLR2 activity in dose dependent manner and with variability in response (Fig. 3). Conserved region of Lig protein (Ligcon) or their domain (Ligcon 1-3, Ligcon 4-7.5) did not show TLR activity, however, variable region showed some activity. To test which part is mediating interaction with TLR we tested TLR activity of individual domain of LigB (B1, B2, B3, B4, B5, B6, B7, B8, B9 and B10). The variation in TLR activity of these domains was observed (Fig. 4). We are in process of repeating these experiments to identify potent TLR2 activating protein/domain. They will be further tested to understand inflammatory response by using J774 and THP1 cell lines. We have target of screening around 200 OMPs of *Leptospira.*

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Testing of TLR2 activity of various surface proteins of *Leptospira*

Having purified several OMPs of Leptospira and testing of TLR2 activity on HEK293 cell lines we are in process of identifying protein/s with most potent TLR2 activity (Fig. 1-4). We have mapped various domains of Lig protein for evaluating activation/inhibition of TLR activity. We are also conducting assays for identifying inflammatory pathway by using inhibitors of various mediators of signaling pathways like MAPK, JUNK and NFkb. These proteins

will be further tested for their ability to activate APCs which will be determined by ability of these cells to secrete proinflammatory cytokines (IL-6, IL-12, TNF-a) and expression of costimulatory molecules (CD80, CD86) and maturation markers (MHCII).

Project 2: Host response against *Leptospira* infection.

In this project, we are planning to infect WT or TLR KO mice and Syrian hamsters with various serovars of *Leptospira* and analyse the immune response in vital organs like kidney, liver and lung at various time points. The data may help in understanding the *Leptsopira* immune evasion strategy and mechanism by which the infection leads to organ failure.

Summary

We have cloned and expressed several outer membrane proteins of *Leptospira* and tested their TLR2 activity on HEK293 cells. Of these Lsa21 has shown strong TLR activity. We are analyzing this protein for its ability to activate TLR dependent pathway in THP-1, RAW 264.7 cell lines and mouse DCs/Macrophages in terms of cytokine production (IL-6, TNF-a, IL1b) and expression of costimulatory molecules (CD80, CD86, MHCII).

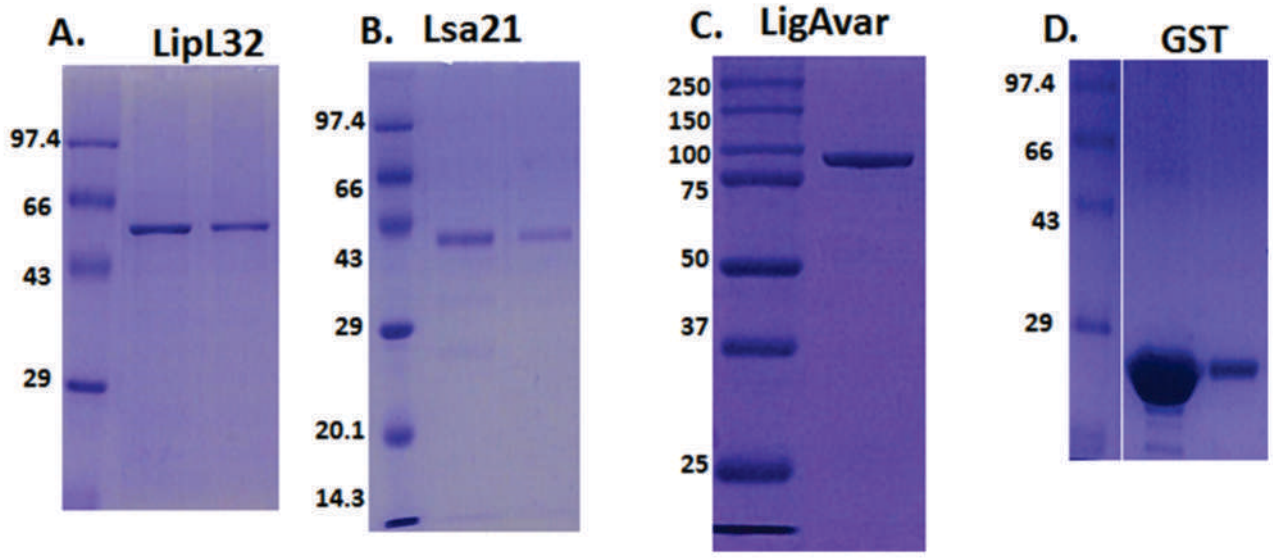


Fig. 1. Purification of GST fused outer membrane proteins/surface proteins of *Leptospira.* OMPs genes were cloned in pGEX4T2 vector and expressed in *E. coli*, purified through GST agarose columns and subjected to SDS-PAGE analysis. (A). LipL32 (B) Lsa21 (C) variable region of LigA (LigAvar) (D) GST. Molecular weight markers are indicated at left.

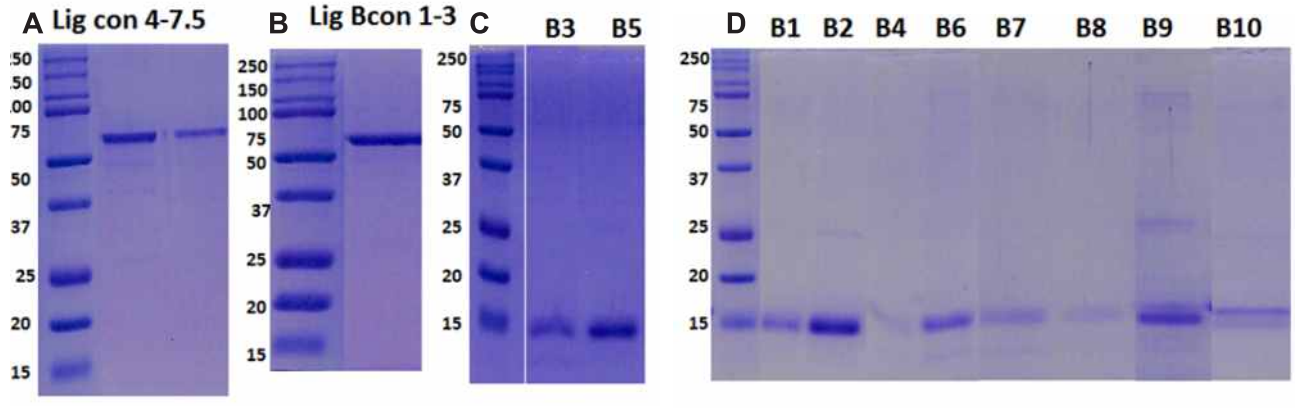


Fig. 2. Purification of various domains of *Leptospira* immunoglobulin like protein (LigA and LigB). Various domains of LigA and LigB were cloned in pGEX4T2 vector and expressed in *E. coli,* purified through GST agarose columns and subjected to SDS-PAGE analysis. (A) Ligcon 4-7.5, (B) LigB con1-3, (C) domain 3 and 5 of LigB and (D) Domains of LigB (B1, B2, B4, B5, B6, B7, B8, B9 and B10). Molecular weight markers are indicated at left.

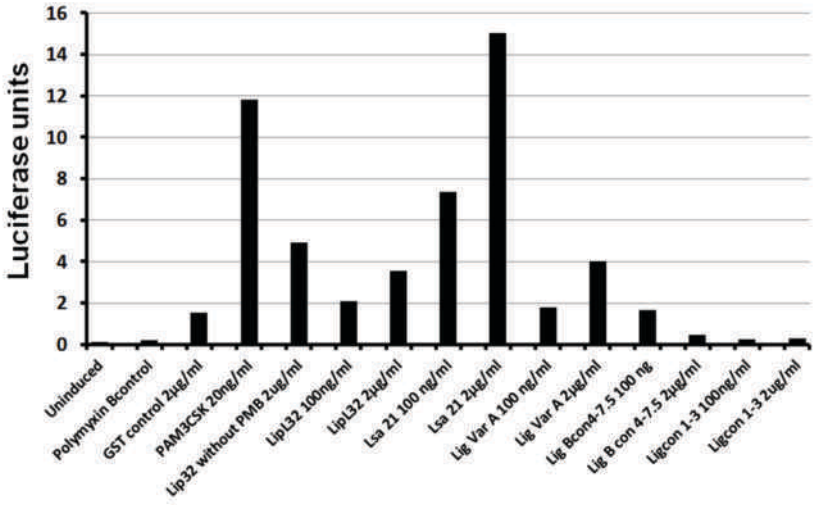


Fig. 3. TLR2 activity of various *Leptospira* outer membrane /surface proteins on HEK293 cell lines tested by luciferase assay. HEK 293 cells were transfected with TLR2and NFkb reporter plasmid using Lipofectamine. Cells were stimulated with various proteins (LipL32, LigAvar, Lsa21, Ligcon1-3 and Ligcon4-7.5) at concentration of 100ng/ml and 2ug/ml. Pam3CSK was used as positive control and GST as negative control. Polymixin B was used to inhibit activity of contaminating LPS.

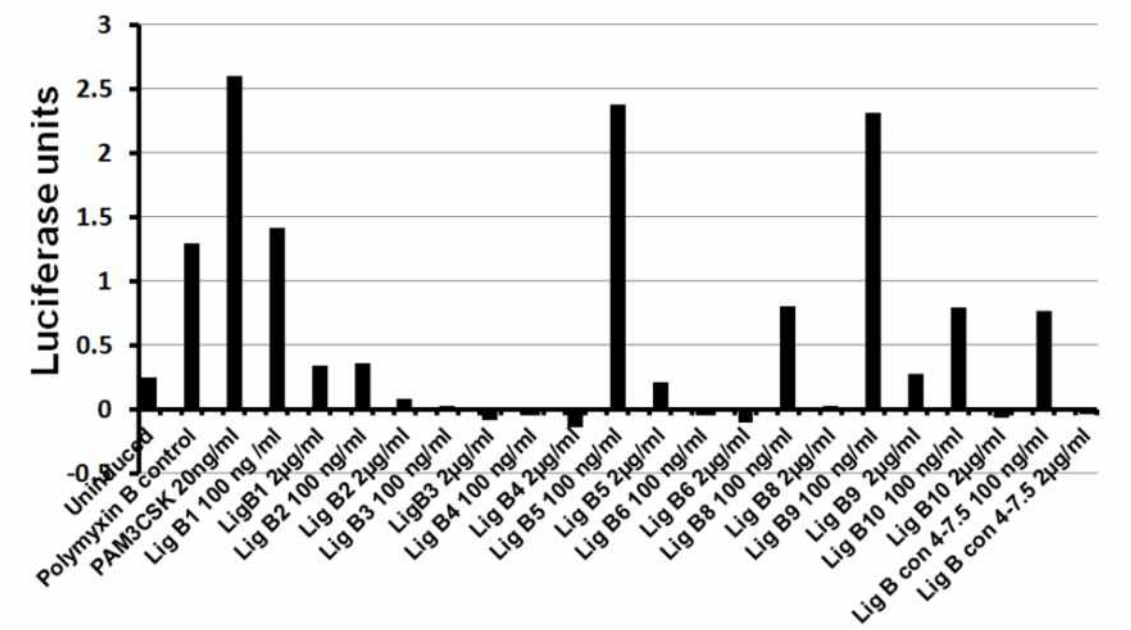


Fig. 4. TLR2 activity of various domains of *Leptospira* immunoglobulin like proteins (Lig proteins) on HEK293 cell lines tested by luciferase assay. HEK 293 cells were transfected with TLR2 Cells were stimulated with various domains of Lig proteins (LigB1, LigB2, LigB3, LigB4, LigB5, LigB6, LigB8, LigB9, LigB10) at concentration of 100ng/ml and 2ug/ml. Pam3CSK was used as positive control and GST as negative control. Polymixin B was used to inhibit activity of contaminating LPS.

Host Pathogen Interaction Studies on Newcastle Disease Virus for Developing Effective Avian Vaccines

Principal Investigator	Madhuri Subbiah	Scientist C
Lab Members	Naveen Gujjar	Project Fellow
	Hanuma Kumar Karnati	Project Fellow (until Aug 2014)
	Saraswathy Iyer	Project Fellow (Nov 2014 to Present)
	Bhuvan Cherukupalle	PhD Student, SV University (Jan-Dec 2014)
Collaborators	Dr. Ravi Kumar Gutti	UoH, Hyderabad
	Dr. Kumanan Kathiaperumal	MVC, TANUVAS, Chennai
	Dr. Suresh Kuchipudi	University of Nottingham, UK
	Dr. Piruthiviraj	Globion Pvt. Ltd, Hyderabad
	Dr. TR Kannaki	DPR, Hyderabad
	Dr. MR Reddy	DPR, Hyderabad

Objectives

The genus Avulavirus under family Paramyxoviridae consists of avian paramyxoviruses (APMV). The prototype APMV-1 or Newcastle disease virus (NDV) causes a highly contagious respiratory, neurological, and/or enteric disease in chickens. NDV outbreak has huge economic impact on poultry industries. 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, morbidity is up to 100% with 90% mortality. Between 2006 and 2009, ND had ranked second among all livestock diseases with respect to the number of countries that were affected (Dev. & Comp. Immunol. 2013, 41, 447-453). The control of NDV largely depends on regular use of safe and effective vaccines. In India, the vaccine market for NDV alone is more than US $ 44 million a year (GALVmed report, 2011). Currently, live avirulent and mesogenic strains of NDV are used for vaccination. Despite the availability of live vaccines, vaccination failure is a common occurrence. One of major reasons is the non-protective nature of the vaccine strain against the virulent field strain. To address this issue, the research work at my lab is focused towards establishing a reverse genetics system for NDV. This system will be used as a tool to understand the viral biology and host-pathogen interactions with an ultimate goal to develop an effective thermostable and multivalent *in ovo* deliverable vaccine against avian pathogens. We are currently working on the mesogenic vaccinal strain Komarov. Depending on the pathogenicity, NDV strains are classified as lentogenic or avirulent, mesogenic or mildly virulent and velogenic or virulent strains. Though majority of live vaccines are derived from lentogenic strains, some mesogenic strains are used as vaccines. Komarov is an example of a mesogenic NDV strain used as live, secondary vaccine after a first vaccination with a lentogenic vaccine and in birds over 4 weeks of age. Komarov strain is a field isolate (from Palestine, 1946) modified by serial intracerebral passages in ducklings. Although, this strain has been used as a vaccine for a long period, until now only partial genome sequence is available with limited reports about its biology. The research work is being carried out with the following major objectives:

(i) Characterization of NDV strain Kumarov

(ii) Establishment of reverse genetics system for NDV strain Komarov for developing a vector system for potential multivalent vaccines

(iii) Understanding the role of nonstructural viral protein (W) in viral biology and host-pathogen interactions

Summary of work done until the beginning of this reporting year (up to March 31, 2015)

Project 1: Complete genome sequencing of NDV strain Komarov

NDV vaccinal strain, Komarov, obtained from VBRI, Hyderabad, has been characterized biologically and using molecular methods. The viral genome has been completely sequenced by classical method and the same has been validated using RNAseq data. Briefly, cDNA was synthesized from the viral RNA using reverse transcriptase and the genes were amplified by PCR using consensus primers. The sequenced regions were aligned with reference NDV genome. The gaps between the contigs were filled using overlapping primers designed to cover the gaps. The genomic terminii were sequenced by 3'RACE and 5'RACE method. For the next generation sequencing method, viral RNA was isolated from purified virus obtained from ultracentrifugation and the RNA was subjected to sequencing using Illumina NextSeq 500. We observed that the complete viral genome length is 15,186

nucleotide (nt) and follows the 'rule of six'. Phylogenetic analysis reveals clustering of strain Komarov with Genotype II NDV strains.

Project 2: Construction of full length clone for reverse genetics system

The sequence data has been utilized to construct the full length clone for establishing the reverse genetics system.

Project 3:

Viruses are known to effectively use their compact genomes. For example, paramyxoviruses express nonstructural (NS) proteins by co-transcriptional editing. These NS proteins are neither coded by unique gene sequences, nor packaged in the virion but are expressed when the virus is actively replicating in the host cell. This implies their possible role in viral replication, host immune evasion and/or pathogenesis. The RNA genome of NDV consists of six genes coding for six structural proteins. Additionally NDV expresses two NS proteins, V and W, by co-transcriptional (mRNA) editing of P gene by polymerase stuttering mechanism. A single G residue insertion results in V protein and insertion of two G residues leads to W protein. These two NS proteins share common N-terminal region with P protein and vary at their C-terminal ends. NDV mutants lacking both V and W proteins or lacking only the carboxyl terminal of V protein have been shown to yield less progeny viruses in cell culture, failed to propagate in 9 - 10 day-old embryonated chicken eggs and were attenuated in vivo. However, these studies have not addressed the function of W protein. This is largely because of lack of an approach to specifically target W protein. We are currently looking at using RNAi technique to elucidate the possible role(s) of W protein and to answer the following questions:

(i) Why does NDV express W protein only during viral infection? and

(ii) Could W mRNA and/or W protein be a key factor for the viral replication and transcription, to evade host immune response or for pathogenesis?

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Complete genome sequencing of NDV strain Komarov

NDV strain Komarov was sequenced completely using classical RT-PCR and RACE techniques. The virus was purified using sucrose gradient centrifugation and the RNA was sent for RNAseq. The data collected by RNAseq was compared with the classical sequence data and sequence was further confirmed. The total length of NDV strain Komarov genome is 15,186 nt. This follows the 'rule of six', six genes are arranged in tandem with conserved 'Gene Start' and 'Gene End' sequences on either side of the gene cassettes. The genes are separated from each other by unique intergenic sequences. The length of leader sequence is 54 nt while that of trailer is 114 nt. The following salient features were noted:

- Amino acid sequence motif for N-N self-assembly: ^{322}FAPAEYAQLYSFAMG^{336}
- P gene edit sequence:^{2280}AAAAAGGG^{2287}
- V protein: 7 cysteine residues in the C terminal portion that resemble the zinc-finger like motif ……TTISWCNPSCSPIKAEPRQYPCICGSCPATCRLCASDDVYDGGNITESK
- F cleavage site ^{112}RRQKRF^{117} -multibasic amino acid residues and phenylalanine at the beginning of the F1 subunit.
- Sialic acid binding site: ^{234}NRKSCS^{239} and potential predicted N-linked glycosylation sites: N119, N341, N433, N481 and N538
- The conserved transcription activity motif within L domain III :^{750}GDNQ^{753}

Project 2 : Construction of Full Length clone for reverse genetics system

The sequence data is being used to construct full length clone of NDV strain Komarov by ApE and DNASTAR software. Also, the support plasmids, N, P and L, are being cloned into mammalian expression vectors. The full length clone and the support plasmids will be used to recover the recombinant virus by reverse genetics.

Project 3: Elucidation of the role of nonstructural viral protein, W, in viral biology and host-pathogen interaction

Based on the sequence data analysis, P, V and W gene and protein sequences were annotated. The ORFs of these proteins are being cloned into pEYFP/ GFP for expression studies. As an initial step, the localization of these proteins within cellular compartments will be studied before proceeding with RNAi studies.

Summary

We have completed the whole genome sequencing of NDV strain Komarov which is valuable for establishing reverse genetics system to rescue recombinant virus. This system will be used to develop effective thermostable multivalent *in ovo* deliverable poultry vaccines. Also studies are ongoing to understand the role of nonstructural viral protein W in host-virus interactions.

Publications:

1. Karnati HK, Pasupuleti SR, Kandi R, Undi RB, Sahu I, Kannaki TR, Subbiah M, Gutti RK (2015). TLR-4 signalling pathway: MyD88 independent pathway up-regulation in chicken breeds upon LPS treatment. Vet Res Commun 39: 73 - 78.

PPR and FMD Research

Principal Investigator	Satya Parida	NIAB Visiting Faculty
Collaborators	Dr. Dhinakar Raj	TANUVAS, Chennai
	Prof. Parimal Roy	TANUVAS, Chennai
	Dr. R.P. Singh	IVRI, Bareilly
	Dr. Muthu Chelvan	IVRI, Mukteswar
	Dr. Hanumanth Rao	VBRI, Hyderabad
	Dr. Krishna Jyothi	VBRI, Hyderabad
	Dr. Girish K. Radhakrishnan	NIAB, Hyderabad
	Dr. Aparna Rachamallu	NIAB, Hyderabad
	Dr. B. Pattnaik	PDFMD, Mukteswar
	Dr. Madhan Mohan	IIL, Hyderabad

Objectives

Our research is focussed on the development and validation of marker vaccines and associated diagnostics including molecular characterization for FMD and PPR, with three lines of investigation: (1) improving our understanding of the aspects of the immune response that are important in the protection of vaccinated animals against acute and persistent infection; (2) developing alternative means of detecting infection in vaccinated animals and molecular characterization of the causative agents; and (3) developing and evaluating improved marker vaccines (i.e. Differentiating Infected from Vaccinated Animals (DIVA) vaccines) for FMD and PPR. Marker vaccines allow differentiation between infected and vaccinated subjects, which is particularly important for the control of disease epidemics affecting livestock.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Emergence and molecular evolution of PPRV

Despite safe and efficacious vaccines against peste des petits ruminants virus (PPRV), this virus has emerged as the cause of a highly contagious disease with serious economic consequences for small ruminant agriculture across Asia, the Middle East, and Africa. For the first time, we sequenced the complete genomes of lineage III PPRV isolates from Ethiopia 1994, Oman 1983, UAE 1986, and Uganda 2012, so that we could use complete genome sequences of all 4 lineages of the virus to investigate evolutionary and epidemiologic dynamics of PPRV. A Bayesian phylogenetic analysis of all PPRV lineages mapped the time to most recent common ancestor and initial divergence of PPRV to a lineage III isolate at the beginning of 20th century (Fig. 1). A phylogeographic approach estimated the probability for root location of an ancestral PPRV and individual lineages as being Nigeria for PPRV, Senegal for lineage I, Nigeria/Ghana for lineage II, Sudan for lineage III, and India for lineage IV (Fig 2). Substitution rates are critical parameters for understanding virus evolution because restrictions in genetic variation can lead to lower adaptability and pathogenicity. The mean evolutionary substitution rate of the PPRV complete genome was estimated to be 9.09 ×10–4. The manuscript has been published in *Emerging and Infectious Diseases.*

Project 2 : Improving immunogenicity of FMD vaccine by adding new generation adjuvants

Eight new adjuvants; Abisco300, CPG, ISA206, Poly I:C, Imiquimod, MPLA, liposome and ISA70, were tested with half of the normal dose of FMD antigen + ISA206 (oil) in cattle at the BSL3+ facility of Indian Immunologicals Ltd., Hyderabad. One of them improved the immunogenicity (both humoral and cell-mediated) of the existing vaccine and provided complete protection upon challenge with virulent FMD virus (Fig. 3). The manuscript has been drafted and will be submitted soon to the Antiviral Research journal.

Project 3 : Molecular characterisation of PPRV isolated from an outbreak in the Indo-Bangladesh border of Tripura state of north east India.

Joining in a serosurvey led by our collaborator at IVRI, Mukteswar, in the Indo-Bangladesh border of Tripura state of North-East India, we sequenced the outbreak viruses and by doing phylogenetic analysis, we demonstrated the transboundary movement of PPRV between Bangladesh and India. This work has been published in Veterinary Microbiology.

Project 4: Study the genetic resistance of PPRV in different breeds of goats and other species : Toll-like receptor responses to peste des petits ruminants virus in goats and water buffalo

This is part of a DBT-BBSRC funded project between TANUVAS, NIAB and UK partners. Differences in susceptibility to PPR among different breeds and water buffalo exist. The host innate immune system discriminates between pathogen associated molecular patterns and self-antigens through surveillance receptors known as Toll like receptors (TLR). We investigated the role of TLR and cytokines in differential susceptibility of goat breeds and water buffalo to PPRV. We examined the replication of PPRV in peripheral blood mononuclear cells (PBMC) of Indian domestic goats and water buffalo and demonstrated that the levels of TLR3 and TLR7 and downstream signalling molecules correlation with susceptibility vs resistance (Fig 4). Naturally susceptible goat breeds, Barbari and Tellichery, had dampened innate immune responses to PPRV and increased viral loads with lower basal expression levels of TLR 3/7. Upon stimulation of PBMC with synthetic TLR3 and TLR7 agonists or PPRV, the levels of proinflammatory cytokines were found to be significantly higher while immunosuppressive interleukin (IL) 10 levels were lower in PPRV resistant Kanni and Salem Black breeds and water buffalo at transcriptional level, correlating with reduced viral loads in infected PBMC. Water buffalo produced higher levels of interferon (IFN) α in comparison with goats at transcriptional and translational levels. Pre-treatment of Vero cells with human IFNα resulted in reduction of PPRV replication, confirming the role of IFNα in limiting PPRV replication. Treatment with IRS66, a TLR7 antagonist, resulted in the reduction of IFNα levels, with increased PPRV replication confirming the role of TLR7. Single nucleotide polymorphism analysis of TLR7 of these goat breeds did not show any marked nucleotide differences that might account for susceptibility vs resistance to PPRV. Analyzing other host genetic factors might provide further insights on susceptibility to PPRV and genetic polymorphisms in the host.

Project 5: Establishment of the reverse genetics techniques for Indian PPRV vaccine strain Sungri 96

This is part of a DBT-BBSRC funded project between Indian and UK partners. The whole genome of Sungri 96 PPRV have been assembled and ready to set the rescue experiment soon.

Project 6 : Development and evaluation of Real Time RT-PCR for PPR virus detection and whole genome sequencing.

Real time RT-PCR has been developed at NIAB for PPR diagnosis and many field samples were evaluated in collaboration with VBRI. Similarly field viruses were provided by Dr. Parimal Roy, TANUVAS, and were processed at NIAB for outsourcing the complete genome sequencing using NGS.

Summary

We have established a PPR laboratory at NIAB for gel based PCR, RT-PCR, ELISA and library preparations for NGS. Collaborating with the Pirbright Institute, TANUVAS and IVRI, we have managed to study for the first time the molecular evolution of PPRV using full genome sequences from all the 4 lineages of PPR viruses. Collaborating with TANUVAS, we have initiated to investigate the disease resistance factors in different breeds of goats and large ruminants and we were able to show that the basal level of TLR3 is playing a potential role in addition to basal level of PPRV specific receptors as shown in the previous studies. Analyzing other host genetic factors in the DBT-BBSRC project in future might provide further insights on susceptibility to PPRV and genetic polymorphisms in the host. To improve the immunogenicity of FMD vaccine we have added various new generation adjuvants to the existing vaccine formulation and were able to demonstrate that one of them immensely helped to improve the immunogenicity of the existing vaccine and provided complete protection upon challenge with virulent FMD virus.

Publications:

1. Kumar KS*, Babu A*, Sundarapandian G, Roy P, Thangavelu A, Kumar KS, Arumugam R, Chandran ND, Muniraju M, Mahapatra M, Banyard AC, Manohar BM and Parida S (2014). Molecular characterisation of Lineage IV Peste des petits ruminants virus using multi gene sequence data. Veterinary Microbiology 174: 39 - 49. (* Equal contribution)

2. Muthuchelvan D, De A, Debnath B, Choudhary D, Venkatesan G, Rajak KK, Sudhakar SB, Himadri D, Pandey AB and Parida S (2014). Molecular characterisation of peste-des-petits ruminants virus (PPRV) isolated from an outbreak in the Indo-Bangladesh border of Tripura state of North-East India. Veterinary Microbiology 174: 591 - 595.

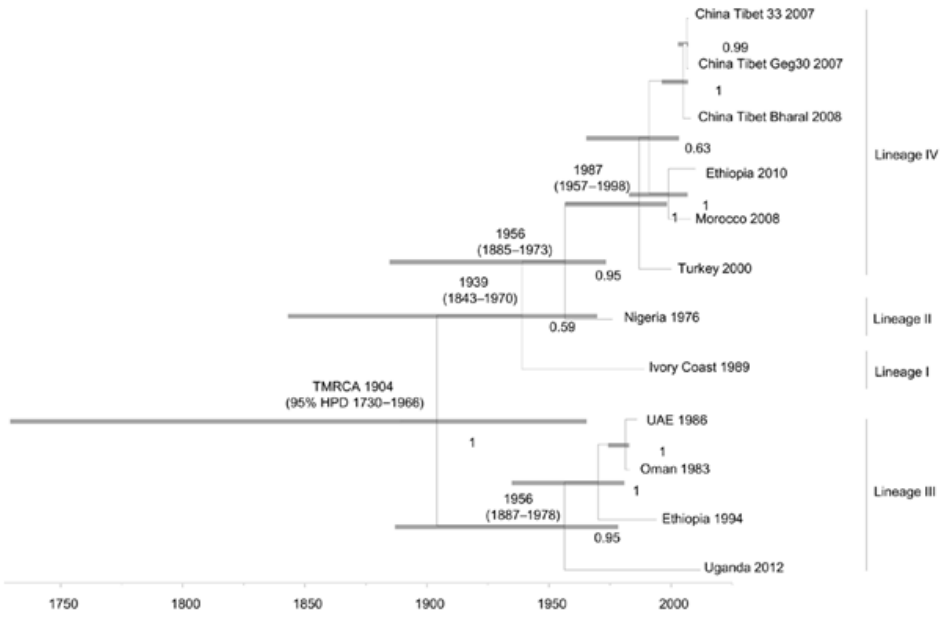


Fig. 1. Time-scaled Bayesian maximum clade credibility phylogeny tree based on peste des petits ruminants virus complete genome sequences. The tree was constructed by using the uncorrelated exponential distribution model and exponential tree prior. Branch tips correspond to date of collection and branch lengths reflect elapsed time. Tree nodes were annotated with posterior probability values and estimated median dates of time to most recent common ancestor (TMRCA). Corresponding 95% highest posterior density (HPD) interval values of TMRCA are indicated as gray bars. Horizontal axis indicates time in years. UAE, United Arab Emirates.

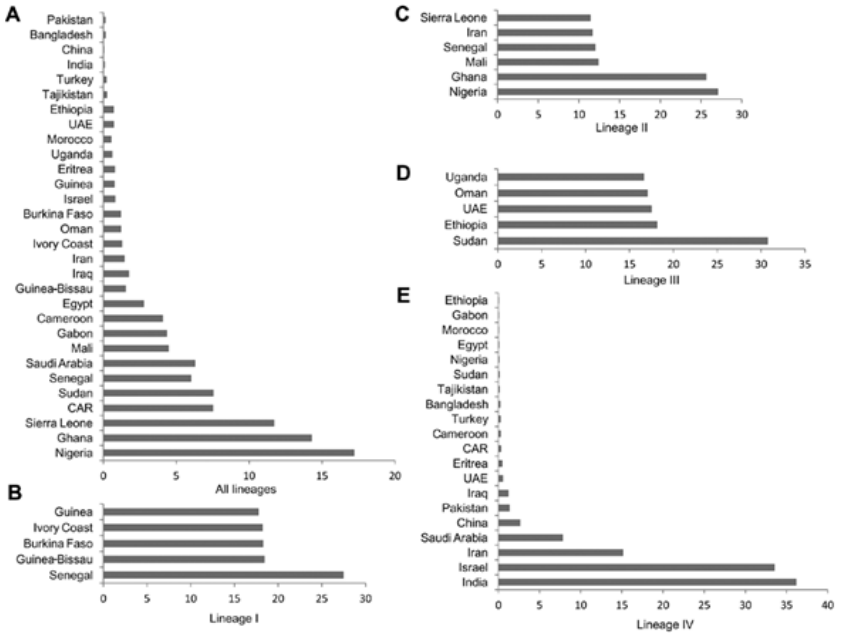


Fig. 2. Probability of root locations of the most recent common ancestral peste des petits ruminants virus (PPRV). MCC trees were obtained by using the continuous time Markov chain and Bayesian stochastic search variable selection procedures. Root location probabilities of the most recent common ancestor using global PPRV isolates (panel A) are shown graphically alongside lineages I–IV (panels B–E) and were estimated by using a complete dataset of PPRV partial nucleoprotein gene data and individual lineages separately. Probabilities of root locations are shown as percentages along the x-axes. UAE, United Arab Emirates; CAR, Central African Republic.

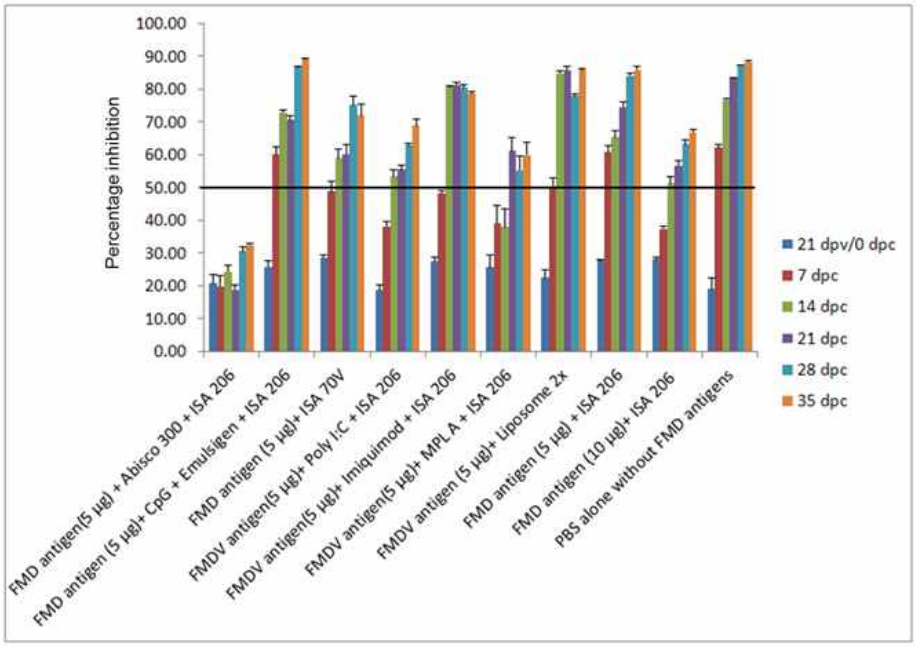


Fig. 3. FMD virus non-structural antibody responses in vaccinated challenged cattle. Percentage inhibition 50 is considered as cut off. In Abisco 300 group the non-structural antibody level is seen lower than the cut off level providing evidence of complete protection of vaccinated animals with half dose of antigen pay load.

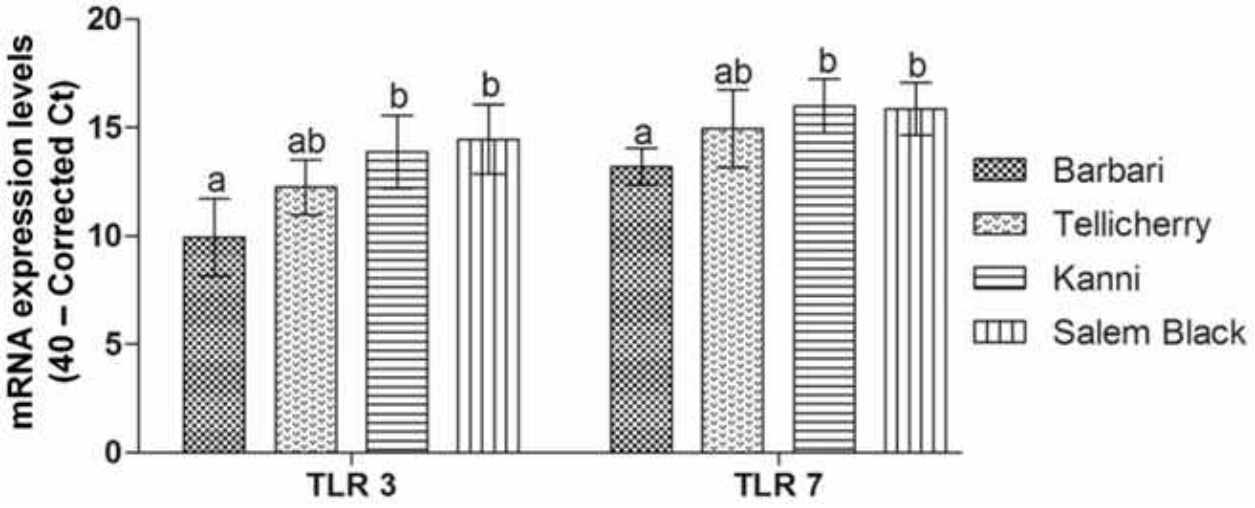


Fig. 4. Basal expression levels of TLR3 and 7 mRNA in Barbari, Tellicherry, Kanni and Salem Black goat breeds. Significantly higher basal TLR3 ($p<0.001$) and TLR7 ($p<0.001$) mRNA expression levels observed in Kanni and Salem Black breeds (resistant breeds), compared to Barbari (susceptible breed). Bars with the same superscript do not differ significantly. Values represent mean ± SD of 40-corrected CT of TLR3 and 7 in nine individual animals per breed (n = 9).

Protozoan Disease

Understanding Host-Parasite-Vector Interactions at Molecular Level

Principal Investigator	Anand Srivastava	Scientist C
Lab Members	Shweta Nori	Project SRF (since Dec 2014)
	Anil Kumar Kotha	PDF (since March 2015)
	Rakhi Harne	Project JRF (Mar-Dec 2014)
	Rinky Sharma Mukherjee	Project JRF (Until June 2014)
	Bala Pradeeba	Summer trainee (May-July 2014)

Objectives

My research interests are to understand molecular interactions involved in host-parasite-vector cross talk and to identify potential targets for development of vaccine(s) and diagnostics.

My research group works on "Ticks and Tick Borne diseases" (TTBDs) that are responsible for the high economic losses especially in developing countries like India. Ticks, apart from sucking blood, act as a carrier for various disease causing bacteria, viruses and parasites. In context of India, the important parasites which are transmitted by ticks are *Theileria* and *Babesia.* These parasites have complex interactions with the host(s) and the vector (tick). Molecular understanding of these interactions will provide key information about important pathways for the survival of both parasites and vector. Furthermore, disruption of these important interactions will be lethal for parasites and vectors.

Theileria spp. infects ruminants, including cattle and sheep, and causes theileriosis. The *theileria* parasites are obligate intracellular apicomplexan hemoprotozoans. In India, bovine theileriosis is mainly caused by Theileria annulata and the disease is known as "bovine tropical theileriosis". This disease is highly common in exotic breeds, their cross breeds and young indigenous calves.

Our aim is to understand the basic metabolic pathway(s) important for survival for parasites and vector. We utilize tools of molecular biology, imaging, *in vitro* parasite culture techniques, to investigate the basic metabolic pathway. Furthermore, we perform epidemiological studies to understand disease burden at the farm level. In particular, we have been focussing on the following objectives:

1) To understand the molecular mechanism(s) involved in transformation of host cell by *Theileria* parasite.
2) To understand the epidemiology of parasitic (*Theileria* and *Babesia*) infections.
3) To understand haemoglobin uptake by tick during blood meal.

Summary of work done until the beginning of this reporting year (upto March 31, 2014)

We successfully adopted *Theileria* infected lymphocytes, isolated from the field, to the laboratory conditions. The presence of *Theileria* was detected using PCR for *Theileria* specific genes. We also started epidemiological studies to find out presence of *Theileria* and *Babesia* infections at the farm level. In random sampling, we estimated approximately 12% infection of *Theileria* in the field.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Understanding the molecular mechanism(s) involved in transformation of host cell by *Theileria* parasite.

A few *Theileria* spp. possess the ability to transform host cells. These are the only eukaryotes which can transform another eukaryotic cells. Our group is interested in understanding the molecular mechanisms underlying the process of transformation of host cell by the *Theileria* parasites.

Theileria parasites are injected into the blood stream of host by the infected tick during the blood meal. The parasites (sporozoites) invade lymphocytes and transform it. Parasite multiplies along with the host cells. The intercellular parasite manipulates the molecular pathway(s) of the host cell. A number of research groups have shown modulation of various host pathways by the parasites. However, limited studies have been done in order to understand parasite factors that subvert these pathways. With the availability of the genome sequence of *Theileria annulata* and *Theileria parva* (transforming parasites), it is easier to predict proteins which could play role in the process of transformation.

We have identified a few potential candidates which could be involved in the process, such as:

1. **Prohibition:** These are evolutionarily conserved genes, ubiquitously expressed and mainly localized in mitochondria and nucleus. The functions of these proteins include anti-proliferative, tumor suppressor, regulator of cell cycle and apoptosis. Furthermore, some studies have also shown role in cellular immortalization and loss of the senescent phenotype. Genome search of prohibitin in the *T. annulata* database results in three putative genes (TA04375, TA19320, and TA08975). The multiple alignment of these putative proteins show significant similarity between TA04375 and TA19320. The gene encoding putative prohibitin (TA19320) was cloned in pET21a (Fig. 1A) and expressed in BL21DE3 cells which was confirmed by western blot analysis using anti-his antibody (Fig. 1B and 1C). However, it is expressed as inclusion bodies. Efforts are being carried out to express this protein in soluble form by refolding or by expression in other vectors such as pGEX. The protein will be used for raising antibodies and pull down assays will be performed with these antibodies.
2. **Cyclophilins:** They belong to the family of proteins known as peptidyl prolyl-isomerase. These proteins interconvert the cis and trans isomers of peptide bonds with the amino acid proline. These proteins have been linked to cancer. Genome search of cyclophilins in the *T. annulata* database results in nineteen putative genes (TA14055, TA13975, TA13185, TA19340, TA05805, TA03410, TA04070, TA19600, TA16315, TA16335, TA12765, TA13570, TA17965, TA07835, TA18945, TA03050, TA06050, and TA06205). Of these at least three proteins: TA13185, TA19600, and TA18945 are predicted to be released in the host cytoplasm. The gene encoding cyclophilin (TA13185) was cloned in pET21a (Fig. 1A) and expressed in BL21DE3 cells which was confirmed by western blot analysis using anti-his antibody (data not shown). However both proteins are expressed as inclusion bodies. Efforts are being carried out to express these proteins in soluble form by refolding or by expression in other vectors. Both these proteins will be used for raising antibodies and pull down assays will be performed with these antibodies.
3. **Actin:** The gene encoding actin protein TA15750 was cloned in pET21a (Fig. 1A) and expressed in BL21De3 cells (date not shown).

Project 2 : Understanding haemoglobin uptake by ticks during blood meals.

Blood ingestion and digestion is one of the highly important metabolic activities of ticks. In the midgut of ticks, erythrocytes are lysed and hemoglobin is taken up by the digestive cells. However, the mechanism of selective uptake of hemoglobin by the digestive cells remains largely unknown. It has been hypothesized that hemoglobin is taken up by digestive cells through receptor mediated endocytosis. Identification of these receptors and elucidation of mechanisms will open up avenues in tick biology.

This is a recent activity in our lab. We have cloned house-keeping genes namely: Actin, and Glutathione S-transferase. Along with this we have cloned surface gene coding for Asparatic protease (Fig. 2A). We have cloned these genes in pET21a vector and expressed in BL21DE3. The Glutathione S-transferase gene was expressed as a soluble protein which was confirmed by western blot analysis using anti-his antibody (Fig. 2B and C).

The antibodies against this protein have been raised in mice. Furthermore, we have identified putative genes with role in clathrin mediated endocytosis namely: Clathrin adaptor protein (BOC00464), vesicle coat protein (BOC924), clathrin light chain (BOC01377). Presently we are in the process of cloning these putative genes. The recombinant proteins will be used for raising antibodies which will be further used for localization and pull down assays.

Summary

We have started work towards understanding the role of *Theileria* parasite molecules in transformation of host cells. Also we have started research work to understand molecular mechanisms involved in digestion of blood by the ticks.

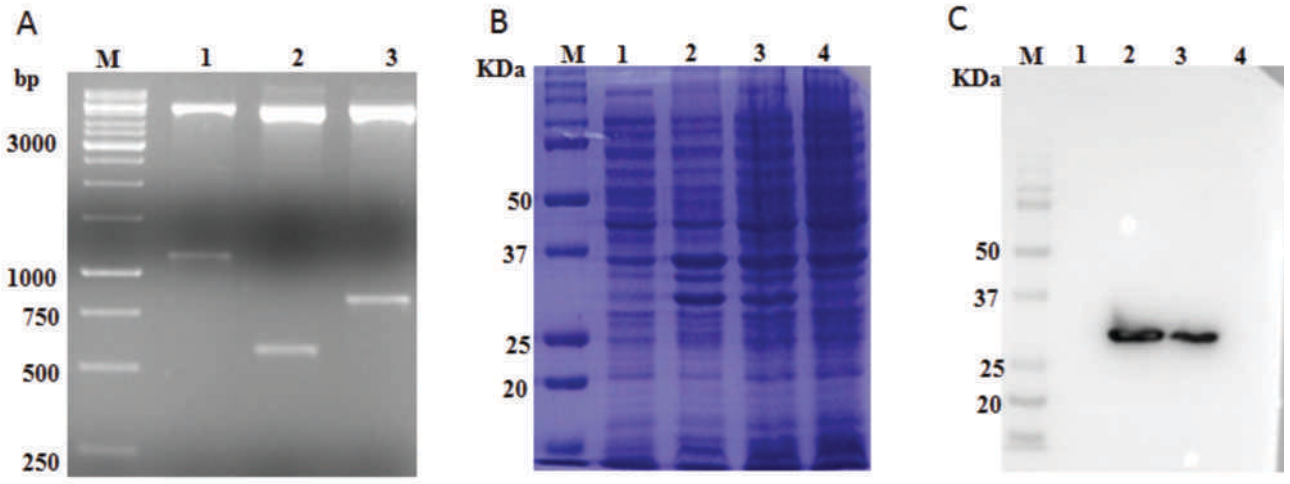


Fig. 1. Cloning and expression of various genes of *Theileria.* (A) Cloning of putative genes in pET21a vector. 1: Actin (TA15750) 1131bp, 2: Cyclophilin (TA13185) 591bp, Lane 3: Prohibitin (TA19320) 834bp, (B) Prohibitin (TA19320) gene expressed in BL21DE3 and induced with 1mM IPTG, resolved by SDS-PAGE, M, marker 1. Uninduced, 2. Induced lysate, 3. Induced pellet, 4. Induced soluble fraction, (C) transferred to PVDF membranes, followed by immunoblotting with anti-his antibodies, a tag-specific antibody. M: marker; 1. Uninduced lysate, 2. Induced lysate, 3. Induced pellet, 4. Induced soluble fraction.

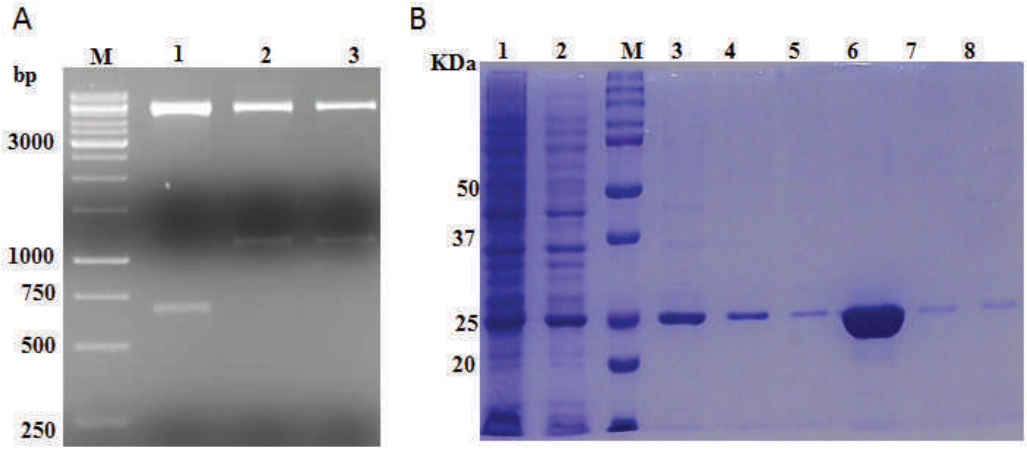


Fig. 2. Cloning and expression of various genes of ticks. (A) Cloning of putative genes in pET21a vector. 1: Actin (669 bp), 2: GST (1127 bp), Lane 3: Aspartic protease (1134 bp), (B) GST 25.6 kDa protein expressed in BL21De3 and induced with 1mM IPTG, purified by Ni-NTA beads, and resolved by SDS-PAGE, M, marker 1. Lysate, 2. Flow through, 3. Wash 1, 4. Wash 2, 5. Elute 1, 6. Elute 4, 7. Elute 8, 8. Elute 10. M, marker; 1, purified protein.

Pathogenesis and Host-parasite Interactions in Bovine Theileriosis

Principal Investigator	Paresh Sharma	Scientist C
Lab members	Neena George	Project Fellow
	Peddi Reddy	Project Fellow
Collaborators	Dr. Anand Kumar	SV Veterinary University, Tirupati
	Dr. Vasundhra Bhandari	NIAB, Hyderabad

Project 1: Understanding disease pathogenesis and host parasite interactions in bovine theileriosis

Apicomplexan parasite of the genera *Theileria* cause serious infection and economic loss in bovine animals worldwide. The parasites invades the blood and the infection may lead to death. *T. annulata* and *T. parva*, the most economically important species, are responsible for mortality and losses in production. In India, *T. annulata* is the most prevalent parasite and is diagnosed by traditional methods using microscopic observation of parasites in stained blood smears. Epidemiological surveys and monitoring is needed for understanding disease pathogenesis and to identify common prevalent species of *Theileria* to formulate effective control strategies against parasitic infections. Current chemotherapy using theilericidal drugs such as buparvaquone as well as tick control using acaricides have side effects and drug resistance has been reported from the field. These shortcomings can be overcome by vaccinating bovines with attenuated *T. annulata* schizont stage vaccine available by the name of Rakshavac-T in India. However, the mechanism of action of vaccine and disease pathogenesis is unknown. The main practical disadvantage of the cell culture vaccine is the requirement for a cold chain for distribution to remote sites. Better understanding of disease pathogenesis, host-parasite interaction and properties that determine virulence of parasitized cell lines could provide markers that would allow more rapid selection of attenuated lines, in order to devise in vitro monitoring of attenuation before testing in susceptible animals. The goal of our project is to collect extensive data on the prevalence, to study the mechanism of action of current vaccine and to identify genes differentially expressed in virulent parasites which will help in deciphering the virulence factors and mechanism involved during the disease process. The identified genes can also play an important role in host-parasite interactions.

Objectives

1. Epidemiology of *Theileria* parasites in Andhra Pradesh and Telangana states.
2. Identification of genes involved in host parasite interactions and virulence during bovine theileriosis.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

A total of 862 blood samples was collected from a random population of cattle and buffaloes from different places of Andhra Pradesh and Telangana for identifying prevalence of *T. annulata* parasites. Clinical isolates of the *T. annulata* parasites was isolated from the infected animals and further protocol for parasite culture and propagation was standardized. DNA was isolated from the 862 blood samples followed by PCR with 18S rRNA and TAMS genes, specific to *T. annulata* parasites. Our results estimated overall 32.40 % prevalence of *T. annulata* infections in cattle and buffalo combined in the states of Telangana and Andhra Pradesh (Fig. 1). Further studies are going on to identify the virulence markers and to understand disease pathogenesis which will help in the identification of new vaccines and drug targets.

Project 2 : Identification of disease related markers against *S. aureus* - a major pathogen causing Mastitis

Mastitis, or inflammation of the mammary gland, is one of the most complex and devastating diseases affecting bovines. In India, it is the second most important and challenging disease after FMD affecting dairy animals. *S. aureus* is an important pathogenic bacterium with a broad host range and the most common cause of clinical or subclinical mastitis worldwide. To cause mastitis, *S. aureus* must gain access to the mammary gland through the teat canal and overcome local humoral and cellular immune defenses. *S. aureus* isolates produce several virulence factors including surface-associated secretory products, leukotoxins and enterotoxins. The infection pattern for *S. aureus* intramammary infections is often different from herd to herd, and these differences could be related to strain differences even if other studies suggest that only a few clones are involved in bovine mastitis development. Most of the studies on *S. aureus* epidemiology in dairy herds have been focused on the presence and role of the different virulence factors or on different genotype. However, complete genomic and proteomic information of the common strains of pathogen is not available till date for Indian strains. The pursuit of biomarkers for use as clinical screening tools, measures for early detection, disease monitoring, and as a means for assessing therapeutic responses, has

steadily evolved in human and veterinary medicine over the past two decades. The project is aimed at characterizing the common strains of *S. aureus* in India using genomic and proteomic tools for identification of differences and similarities between the strains. Identification of these genes is important to define biomarkers that can be used for development of new diagnostic tools and to identify potential vaccine targets. The findings from the project will help in understanding of disease pathogenesis and therefore can be of great help to the livestock industry.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

We have collected milk samples from the subclinical and clinical cases of mastitis infected animals like cattle, buffalo and goat from different states of Andhra Pradesh and Telangana. *S aureus* was isolated and maintained in vitro in brain heart infusion broth at 37C. Antibiotic sensitivity assay was done against all isolates for screening minimum inhibitory concentration (MIC) using microbroth dilution assay. The bacterial strains were screened using colorimetric assay. The MIC was defined as the lowest concentration of the drug that prevented a change in colour or calculated using regression analysis using Origin 6.0 (Table 1). Further molecular and biochemical characterization of *S. aureus* isolates was done using PCR and gene specific primers for the presence or absence of antibiotic resistance and virulence genes such as mec A, mec C, Van A, Van B, Van X, VanY, Van Z, PVL gene other than 16S rRNA and BlaZ gene (Fig. 2) . Also SPA typing and MLST is underway using standard primers to identify the lineages of the *S. aureus* strains. Next genomic and proteomics analysis will be done for in-depth characterization of the isolated strains based on antibiotic sensitivity, virulence and novel spa types.

Table 1 : Antibiotic Susceptibility of *S. aureus* clinical isolates from bovine mastitis cases

S.No.	Strain ID	Clinical sample/ Origin	MIC Oxacillin (µg/ml)	MIC Vancomycin (µg/ml)
1	ATCC 29213	Standard Strain -ATCC	0.5	0.5
2	ATCC 50	Standard Strain -ATCC	>10	8
3	S-1	Milk sample/cow	1.25	0.5
4	S-2	Milk sample/Buffalo	10	1
5	S-3	Milk sample/Cow	0.625	1
6	S-4	Milk sample/Cow	2.5	1
7	S-5	Milk sample/Cow	1	0.5
8	S-6	Milk sample/Cow	10	1
9	S-7	Milk sample/Cow	1.25	1
10	S-8	Milk sample/Cow	0.625	1
11	S-11	Milk sample/Goat	0.039	0.5

Micro-broth dilution assays was done using CLSI guidelines and including the standard ATCC strain for quality control. All experiments were performed thrice with duplicates.

Fig. 1. Agarose gel electrophoresis of amplified DNA from different bovine blood DNA samples by using 18S rRNA. Lanes: 1-6 & Lane 8- 11 bovine DNA samples, Lane 7- 50 base pairs DNA ladder; Lane 12 – Positive control: T. annulata culture DNA; Lane 13- Negative control distilled water. Expected size of the band 150bp.

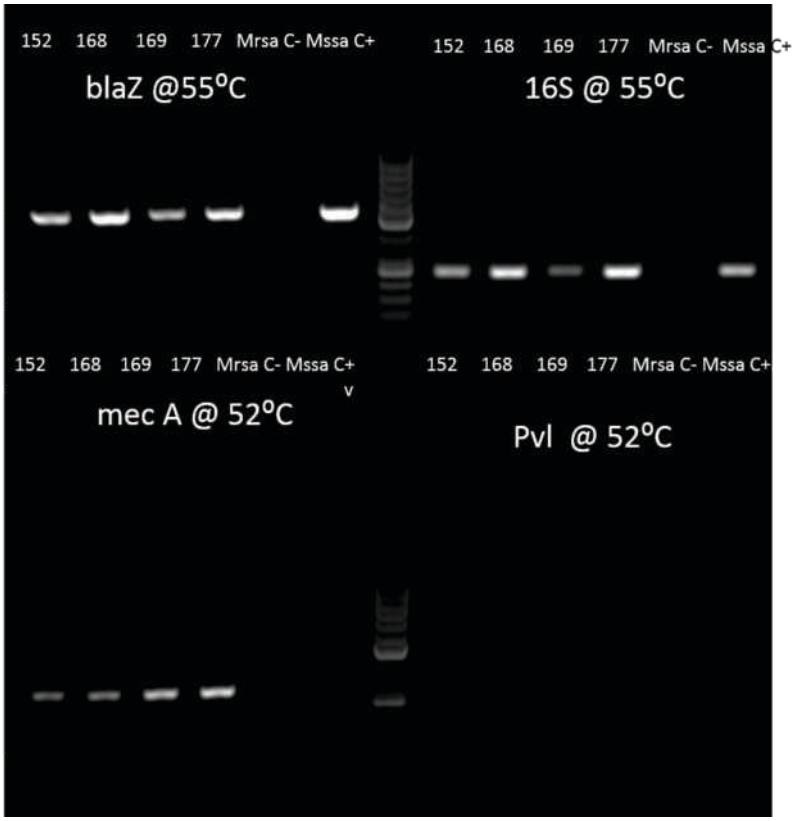


Fig. 2. PCR results with *S. aureus* clinical isolates from the field with four different primers Blaz, 16S r RNA, mec A and PVL gene specific to S. aureus **at different annealing temperatures.** Lane 1 to 4 are four different isolates and last two lanes are MRSA positive and negative control.

Characterization of Cell Cycle Regulators Associated With DNA Replication Machinery in *Toxoplasma Gondii*

Principal Investigator Abhijit S. Deshmukh DST-INSPIRE Faculty

Objectives

Toxoplasma 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. Initial work using DNA synthesis inhibitors suggested that Toxoplasma lack classical cell cycle check points and pointing to potentially novel mechanisms of control over their complex cell cycles. Cyclin-dependent kinases (CDKs) play pivotal role in cell cycle control. Progression through the cell cycle is driven by successive activities of CDK-cyclin pairs. In mammalian cells nine catalytic subunits, CDKs (Cdk1-9) and at least 16 regulators cyclins have been identified which regulate the cell cycle by different mechanisms one being its interaction with replication proteins. In *Toxoplasma gondii* complete list of CDK-cyclins and their substrate are not known and thus the downstream events of CDK activation are not clear. The CDK Activating Kinase (CAK) activates CDKs through phosphorylation at their T-loop. CAK is composed of three subunits: CDK7, CyclinH and MAT1 (ménage a trios). Together with other six subunits, CAK is also part of general transcription factor TFIIH where it is involved in promoter clearance and progression of transcription from the preinitiation to the initiation stage. CAK varies dramatically in different species. In vertebrates and Drosophila, CAK is a trimeric complex whereas in budding and fission yeast it is a monomeric and dimeric protein kinase respectively. Keeping these facts in mind I planned my study with the following major objectives.

i. To identify the CAK subunits in the *Toxoplasma gondii*

ii. To study the presence of monomeric/dimeric/trimeric CAK and their subunits

iii. To study the role of Toxoplasma CAK in parasite cell cycle and transcription

Summary of work done until the beginning of this reporting year (October 7, 2013 - March 31, 2014)

In vitro culture systems for *Toxoplasma gondii* are fundamental to Toxoplasma research. The production of tachyzoites is essential for all experimental models, genetic studies, biochemical pathway and drug studies and the development of serological tests. In this laboratory, I developed an *in vitro* culture system in human foreskin fibroblasts (HFFs), which could provide regular harvests of fresh viable tachyzoites. *T. gondii* RH strain is maintained by passage through human foreskin fibroblasts (HFFs, culture cells obtained from ATCC-CRL 1634) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal calf serum and 2mM glutamine at 37C and 5% Co_2.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project: Identification and characterization of *T. gondii* CDK Activating Kinase (CAK)

The cell cycle is driven by activation of the CDKs by binding to a cyclin partner and phosphorylation on the activation segment or T-loop by a CAK. CAK is a trimeric complex of CDK7, Cyclin H and MAT1. The same trimeric complex is a part also part of basal transcription factor TFIIH. In Toxoplasma not a single component of CAK is identified. Data mining and homology search using Toxoplasma genome resource identified *T. gondii* genes coding proteins homologous to the known partners of vertebrate CDK7, Cyclin H and MAT1.

TgCdk7 : Cdk7 and cyclin H are essential components of the transcription factor TFIIH and implicated as the kinase that phosphorylates the carboxyl-terminal domain of the largest subunit of eukaryotic RNA polymerase II. The predicted protein sequence of TgCdk7 has homology to the Cdk7 family. The homology is based on ATP binding domain and catalytic domain (Fig. 1).

TgCyclin H: The predicted protein sequence of TgCyclin H has homology to the cyclin H family. The homology is based on cylin box, a region of cyclins responsible for cdc2-related kinase binding. The cyclin box forms the first of two alpha helical repeats of the canonical cyclin fold. The cyclin box of TgCyclin H has its highest homology within the 5 alpha-helical subregions of repeat one (H1-H5) (Fig. 2). All cyclin H homologs identified to date have been shown to have non-conserved N-terminal and C-terminal helices essential for both kinase activity and CRK binding.

TgMAT1: MAT1 is a CAK assembly factor helps in stable interaction of Cdk7 and CyclinH. The predicted TgMAT1 protein contains a conserved RING-finger domain, namely a C3HC3 finger, in the N-terminus (Fig. 3). The RING-finger is a specialised type of Zn-finger of 40 to 60 residues that binds two atoms of zinc and is probably involved in mediating protein-protein interactions.

The three identified CAK components and their bona-fide substrates such as CDK2, CTD of RNA polymerase II and PCNA (DNA replication protein) are cloned and expressed in the E. coli. (Fig. 4). All the mentioned genes are amplified using cDNA as a template and cloned into either pET21a or pET28a vector. The genes such as Cdk7, MAT1, CDK2 and PCNA1 are clones as full length genes. The Cyclin H is a truncated protein with deletion of N-terminal without initial 54 amino acids and RNA Polymerase II CTD contains carboxyl-terminal 323 amino acids.

Summary

I have identified all the three CAK subunits (Cdk7, CyclinH and MAT1) in *Toxoplasma gondii*. All three subunits of CAK and their bona-fide substrates are cloned and expressed in the *E. coli* as recombinant proteins.

Future plans

1) To raise the polyclonal antibodies against all CAK subunits.
2) To study the trimeric CAK in the parasite by *in vitro* and *in vivo* interactions within the CAK subunits.
3) To probe the active CAK in the parasite by kinase assay using bona-fide substrates.
4) To study the role of CAK subunits in parasite cell cycle and transcription.

```
Pf_Cdk7         ----------------------------------------MENNSTERYIFKPNFLGE 18
Tg_Cdk7         MAAPQVKPEVGDSPEATRQGVLLPSASEEAAANGSSSPRLVPIKREGKQRYRQCDAFLGE 60
Hs_Cdk7         ----------------------------------------MALDVKSRAKRYEKLDFLGE 20
                                                          :. :.  :      ****

Pf_Cdk7         GSYGKVYKAYDTILKKEVAIKKMKLNK----------------------ISNYIDDCGIN 56
Tg_Cdk7         GTYGRVEKAEDLRTHQIVAIKKVKASAGSLFASGDSGSVGSGEKTRAQLLRQNVGSVGLH 120
Hs_Cdk7         GQFATVYKARDKNTNQIVAIKKIKLGH----------------------RSEAKDGIN 56
                * :. * ** *   :: *****:* .                          .   . *::

Pf_Cdk7         FVLLREIKIMKELKHKNIMSALDLYCEKDYINLVMEIMDYDLSKIIN-RKIFLTDSQKKC 115
Tg_Cdk7         FTTVRELKVMRELEEENVMGVVDVFVEQDFICLVMELMHGDLKKLVD-SKTRLAIQHVKC 179
Hs_Cdk7         RTALREIKLLQELSHPNIIGLLDAFGHKSNISLVFDFMETDLEVIIKDNSLVLTPSHIKA 116
                 . :**:*:::*:.. *::. :* : .:. * **:::*. **. ::.   .  *: .: *.

Pf_Cdk7         ILLQILNGLNVLHKYYFMHRDLSPANIFINKKGEVKLADFGLCTKYGYDMYSDKLFRDKY 175
Tg_Cdk7         IMLQILRGLHALHKRYIVHRDLAPANVFINDQGICKVADFGLSRCFGCPVVSGTLSKQEQ 239
Hs_Cdk7         YMLMTLQGLEYLHQHWILHRDLKPNNLLLDENGVLKLADFGLAKSFGSPNRA-------- 168
                 :*  *.**. **: :::**** * *:::.:*  *:*****.  :*    :

Pf_Cdk7         KKNLN-----------------------LTSKVVTLWYRAPELLLGSNKYNSSIDMWS 210
Tg_Cdk7         SKGETSQPGKESSVESASKTAPVAISRKELMTSKVVTLWYRPPELLFGADRYGQAVDMWS 299
Hs_Cdk7         ----------------------------YTHQVVTRWYRAPELLFGARMYGVGVDMWA 198
                                            * :*** ***.**** :*:  *. .:***:

Pf_Cdk7         FGCIFAELLLQKALFPGENEIDQLGKIFFLLGTPN---------ENNWPEALCLPLYTEF 261
Tg_Cdk7         VGCIMAELLTGSPLFPGANEIDQLSRIFSLRGTPTTAAALLDEEPSLWPLASSLPSFFPF 359
Hs_Cdk7         VGCILAELLLRVPFLPGDSDLDQLTRIFETLGTPT---------EEQWPDMCSLPDYVTF 249
                .***:****   .::** .::*** :**   ***.          . **   .** :  *

Pf_Cdk7         TKATKKDFKTYFKIDDDCIDLLTSFLKLNAHERISAEDAMKHRYFFNDPLPCDISQLP- 320
Tg_Cdk7         THTKPKSLKSVLPFCCADSLDLLDKLLQLDPSKRITAAEALNHRWFQQNPKPCSPKDLP- 418
Hs_Cdk7         KSFPGIPLHHIFSAAGDDLLDLIQGLFLFNPCARITATQALKMKYFSNRPGPTPGCQLPR 309
                .      ::  :     * :**:  :: ::.  **:* :*:: ::* : * *    :**

Pf_Cdk7         ----FNDL---------------------------- 324
Tg_Cdk7         ----VHLLGKF---------------------- 425
Hs_Cdk7         PNCPVETLKEQSNPALAIKRKRTEALEQGGLPKKLIF 346
                    .. *
```

Fig. 1. Amino acid alignment of *T. gondii* Cdk7 (accession no.TGME49_270330) with *P. falciparum* Cdk7 and Human Cdk7. The catalytic domain is highlighted in the aligned proteins. The TgCdk7 and PfCdk7 share overall 40% identity.

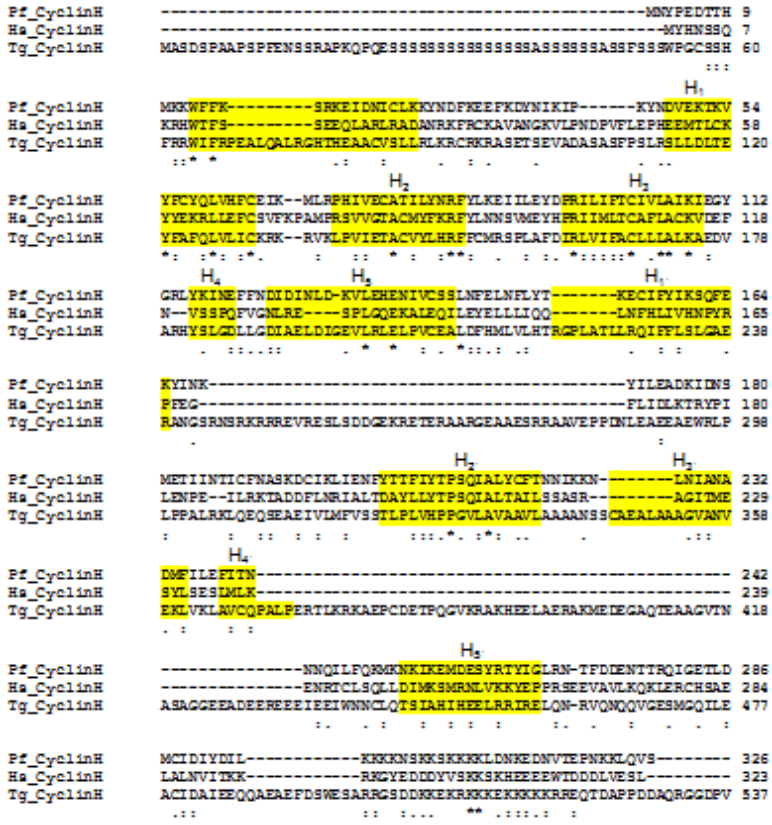


Fig. 2. Amino acid alignment of *T. gondii* CyclinH (accession no.TGME49_260250) with *P. falciparum* CyclinH and Human CyclinH. The cyclin H box region spans H1-H5 and encompasses the region of highest homology between the aligned proteins. TheTgCyclinH and PfCyclin H share 30% identity within the cyclin box.

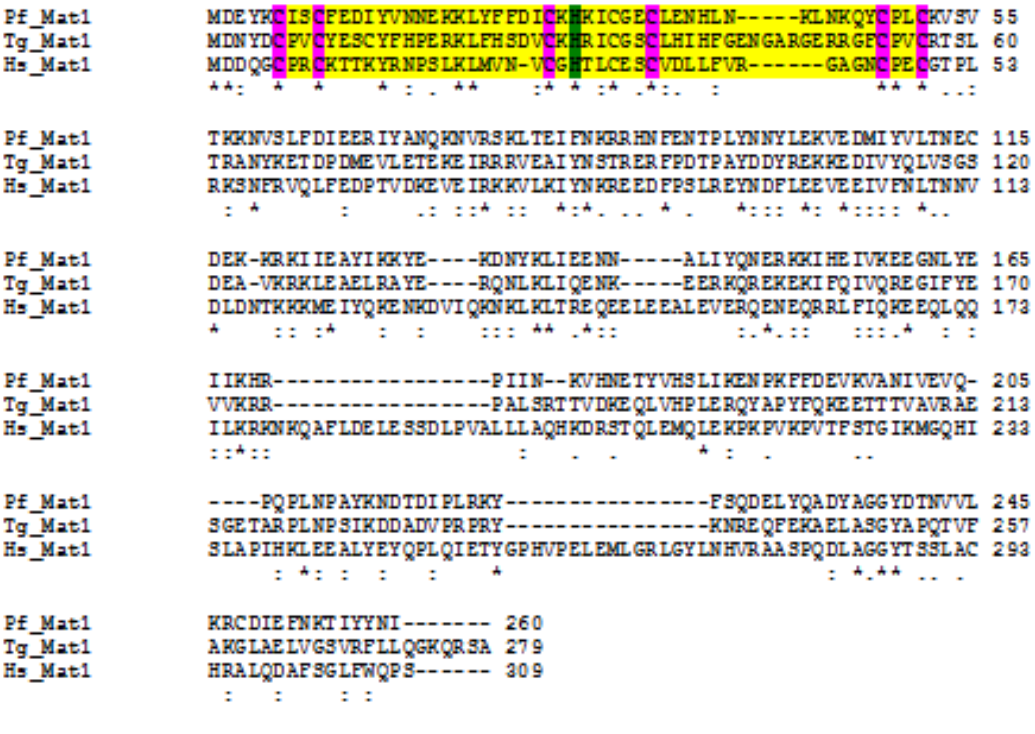


Fig. 3. Amino acid alignment of *T. gondii* MAT1 (accession no.TGME49_320070) with *P. falciparum* MAT1 and Human MAT1. The RING-finger domain (highlighted region) spans between N-terminal 50 amino acids residues of the aligned proteins. TheTgMAT1 and PfMAT1 share 34% overall identity.

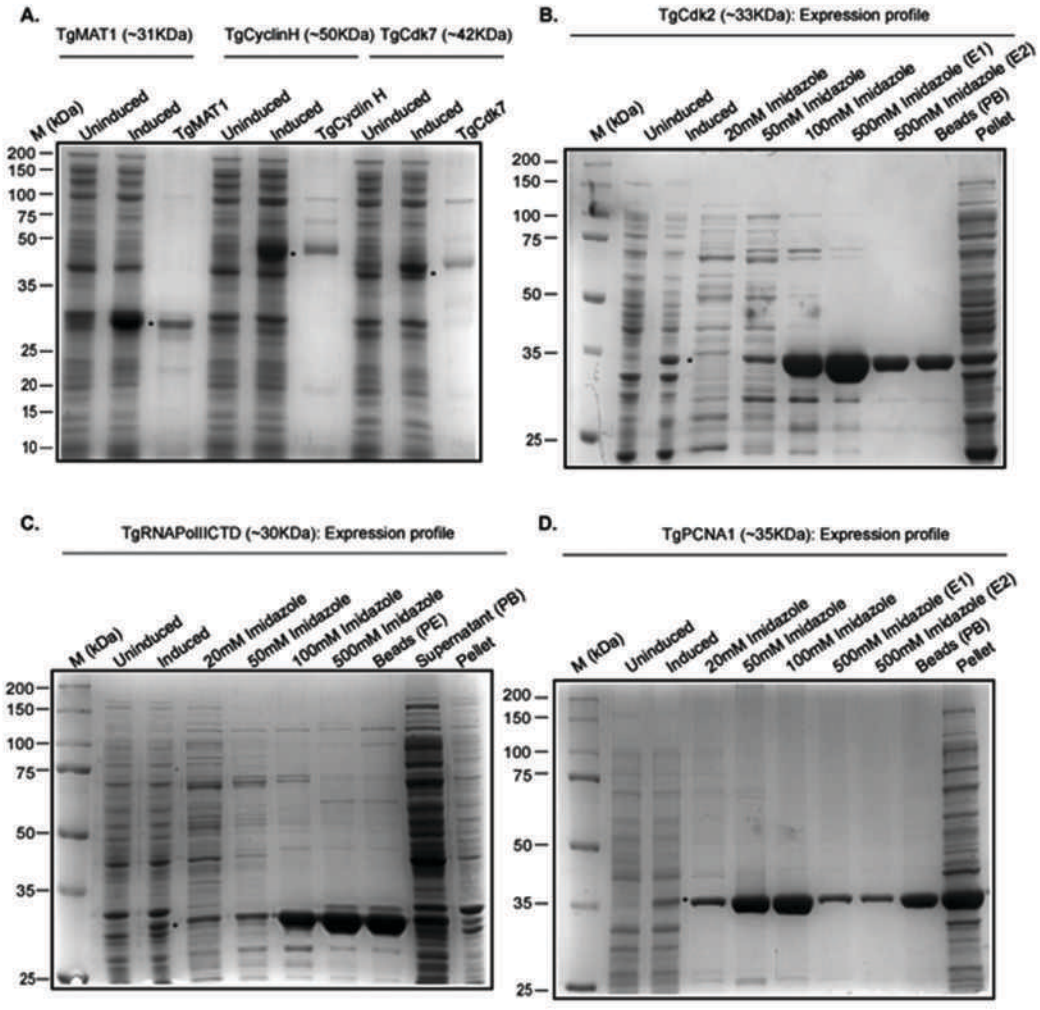


Fig. 4. Protein profiles of *T. gondii* CAK subunits, CDK2, RNAA polymerase II CTD and PCNA1. The corresponding proteins are expressed and purified as His fusion proteins. Coomassie gel showing recombinant T. gondii MAT1, CyclinH, Cdk7 (A); CDK2 (B), RNA Polymerase II CTD (C) and PCNA1 (D). The asterisk in the figure indicates the expected size of the respective proteins.

Animal Reproduction

Management of Infertility in Indian Livestock

Principal Investigator	Sathya Velmurugan	Scientist C
Others members	Swathi Merugu	Project Fellow (Jan - Nov 2014)
	NV Siva Kumar	Project Fellow (Mar - July 2014)
	Rakhi Harne	Project Fellow (Dec 2014 - Present)
	S Sri Ravali	Project Fellow (Feb 2015 - Present)
Collaborators	Dr. G. Aruna Kumari	SPVNRTSUVAFS, Hyderabad
	Dr. T. Raghunandhan	SPVNRTSUVAFS, Hyderabad

Objectives

With the aim of improving productivity and fertility in large animals, the following studies have been undertaken: (1) Sex-sorting of cattle and buffalo sperms: Sex-sorting of sperms for native breeds and validation of sorting efficiency are being standardized which will be followed by studies on the characteristics of sorted semen. (2) Kisspeptin regulation of female reproduction: Kisspeptin, the recently proposed master controller of reproductive axis, governs puberty and fertility. Understanding the physiology of kisspeptin will subsequently enable us to evaluate the therapeutic potential of kisspeptin for infertility disorders.

Summary of work done until the beginning of the reporting year (up to March 31, 2014)

Intact viable sperms can be separated into X and Y chromosome bearing populations by flow cytometry on the basis of relative DNA content: X sperms have approximately 3% more DNA than Y sperms. Cattle (Jersey crossbred) and buffalo (Murrah) sperms from frozen-thawed and diluted semen, stained with Hoechst 33342, a nuclear staining fluorescent dye, were subjected to sex-sorting in a flow cytometer/cell sorter. Standardization of the technique and validation using qPCR was began.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Sex-sorting of cattle and buffalo spermatozoa using flow cytometry

Sperms from frozen-thawed and diluted cattle and buffalo semen are stained with Hoechst 33342 (100µg/ml for 45min at 37 C; Ex/Em: 355/465 nm). Sorting is done in a flow cytometer (BD FACS ARIA III) using a 100µm nozzle with a sheath fluid pressure of 20psi. Two regions of high and low fluorescence intensity, indicating differences in DNA content, are gated and sperms falling in these two regions (X and Y populations, respectively) are separated. Sperms are sorted in single cell mode. Sperm morphology and viability pre- and post-sorting are checked. The two populations identified as X and Y peaks based on the degree of fluorescence intensity are shown (Fig. 1). The sperm cells are viable and tails are intact post-sorting, however, motility is lost.

Validation of sorted sperm (Murrah and Jersey cross) samples using qPCR:

The following protocol has been standardized for optimal yield of sperm DNA, which otherwise is difficult to extract. Sorted or unsorted sperm samples are washed in 70% ethanol (500µl for 100µl sperms) followed by centrifugation at 13,000rpm for 5min. The sperm pellet is resuspended in 500µl lysis buffer; 2.5µl Triton-X100 (0.5%), 21µl DTT (1M), and 40µl proteinase K (10mg/ml) are added to the sample and kept overnight at 50C incubation with moderate shaking. The sample is centrifuged, supernatant and DNA quantified.

For the amplification of X and Y-specific genes, DNA primers and probe sequences were used as reported earlier (Parati et al 2006 Theriogenology 66:2202-9) (Table 1). The X, Y and Amelogenin (AMEL) Taqman probes were labeled with 6-Carboxyfluorescein (FAM) at the 5 end and BHQ-1 quencher (black hole quencher) at the 3' end of all probes to permit fluorescent detection. Primers and probes were synthesized from Bioserve Biotechnologies (India) Pvt. Ltd.

Three recombinant plasmids containing the amplified section of the PLP gene (p-PLP), SRY (p-SRY) gene and AMEL (p-AMEL) gene are constructed (Fig. 2) to be used as reference templates for quantification of the sex-related DNA sequences in qPCR. Isolated recombinant plasmid samples are sequenced (Xcelris Genomics) for matching the correct gene insert sequence. Sequencing results are awaited.

Project 2: Regulation of female reproduction by Kisspeptin

(1) Effect of administration of synthetic kisspeptin on plasma LH concentration in Deoni cattle.

Kisspeptin (Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH2) was synthesized at Auro Peptides, Hyderabad. A preliminary study was conducted at the Instructional Livestock Farm Complex, College of Veterinary Science, SPVNRTSUVAFS, Rajendra Nagar, Hyderabad, to validate the physiological activity of the synthetic peptide.

In three pre-pubertal Deoni heifers (~150kg body weight), a single intravenous bolus injection of synthetic kisspeptin was administered at the rate of 5µg/kg body weight. Blood samples were collected 10min before and 15, 30, 60 and 120min after administration of kisspeptin. Plasma was separated and analyzed for LH concentration by ELISA (Abnova).

From an average basal concentration of 4.5 ± 1.55 ng/ml, LH concentration increased to 14.23 ± 1.95 ng/ml at 15min and 17.77 ± 5.57 ng/ml at 30min after kisspeptin administration (Fig. 3). It returned to 7.06 ± 2.68 ng/ml, comparable to basal concentration, by 2h. From this preliminary study, it can be ascertained that the synthetic kisspeptin, used in this study, is biologically active and elevates plasma LH levels in cattle. Subsequent studies are being planned, in comparison with GnRH, to study endocrine profile and follicular dynamics upon kisspeptin administration.

(2) Effect of peripheral administration of chronic high doses of Kisspeptin on induction of puberty in rats

High doses of peripheral kisspeptin (50nmol/d for 13d) has been reported to cause testicular degeneration in male rats (Thompson et al 2006 Am J Physiol Endocrinol Metab 291: E1074–82). To study whether chronic high doses of kisspeptin affects puberty in female rats, saline or kisspeptin (100nmol/d) was administered from day 25 to day 50 and pubertal onset was observed. On day 66, after all the animals attained puberty, the rats were injected with either saline or kisspeptin, blood samples collected 1h after the injection to analyse endocrine profile, and the animals sacrificed.

Complete canalization of vagina (i.e. vaginal opening; VO), an external indication of puberty, was observed in control rats from day 40 onwards with the average of 50 ± 2.5 days while kisspeptin treated rats exhibited vaginal opening only from day 50 (average: 58.44 ± 1.6 days; P=0.01, t-test). Hence, pubertal onset was significantly delayed in kisspeptin treated rats compared to control rats (Fig. 4; P=0.01, Kaplan-Meier survival analysis). None of the kisspeptin treated rats showed VO when 50% of the control rats have already shown (see inset, Fig. 4). There was no difference in the body weight on the day of VO among both the groups.

Kisspeptin administration significantly reduced feed intake and body weight compared to saline treated controls (Fig.5a and 5b). There was no difference in the organ coefficients of gonads, heart and liver. Plasma LH levels were slightly elevated upon kisspeptin administration on day 66. There were no differences in the histological features of uterus and ovaries among control and treated groups. Tissue mRNA expression of *kiss-1*, *GPR-54* and *GnRH* in uteri and ovaries are being analyzed.

Summary:

(1) Sex-sorting of cattle and buffalo sperms: We could successfully sort X and Y bearing sperms of Jersey cross cattle and Murrah buffaloes. After sorting and its validation, the characteristics of sorted sperms will be studied using a battery of tests in vitro and via artificial insemination.

(2) Kisspeptin regulation of female reproduction: Elevation of plasma LH upon synthetic kisspeptin administration in cattle confirms that it is biologically active. Plasma endocrine profile and follicular dynamics upon kisspeptin treatment will be studied in comparison with GnRH to optimize the dose and route of administration of kisspeptin. Studies in rats show that prolonged kisspeptin treatment at higher doses delays puberty, probably through a negative feedback mechanism.

Table 1 : . Primer and probe sequences.

Primer/probe	Sequence (5' 3')	Product size (bp)
X chromosome specific primers and probe		**96**
PLP-Forward	GTTGTGTTAGTTTCTGCTGTACAATAAAGTG	
PLP-Reverse	GATGGCAGGTGAGGGTAGGA FAM	
PLP Taqman Probe	TGTATACACATAGCCCCTCCCTCTTGGA CC BHQ1	
Y chromosome-specific primers and probe		**66**
SRY-Forward	CCACGTCAAGCGACCCAT	
SRY-Reverse	AGAGCCACCTTTCGTCTTCG FAM	
SRY Taqman Probe	AACGCCTTCATTGTGTGGTCTCGTGA BHQ1	
AMEL specific primers and probe		**150**
AMELF	CCTGTGCACCCCATCCAG	
AMELR	CCCGCTTGGTCTTGTCTGTFAM	
AMEL TaqMan Probe	CCACAGCCACCTCTGCCTCC BHQ1	

PLP: bovine proteo-lipid protein gene; SRY: sex-determining region Y; AMEL: bovine amelogenin sequence.

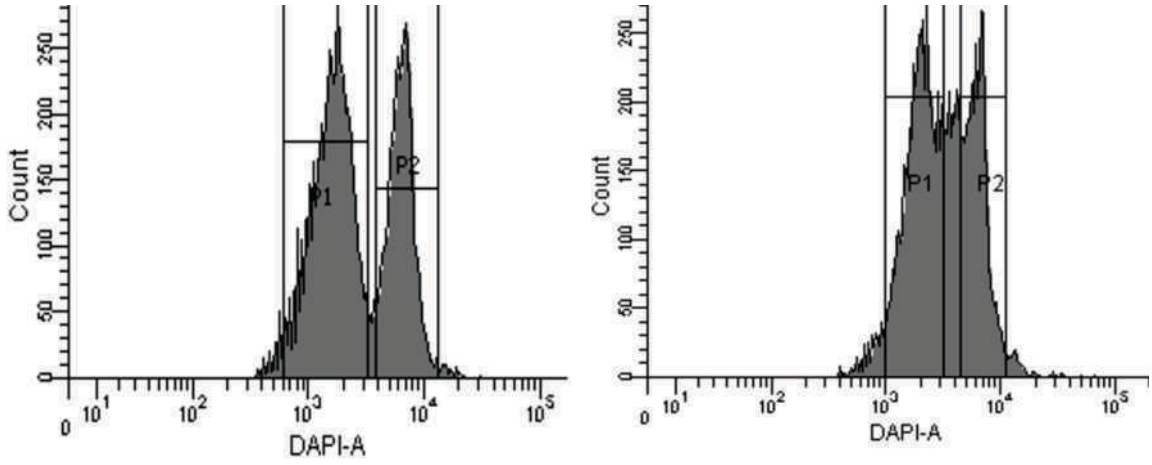


Fig. 1. Representative histograms showing two peaks assumed to correspond to X (P2 region) and Y (P1 region) populations of Murrah sperms. The peaks were gated and two populations were sorted.

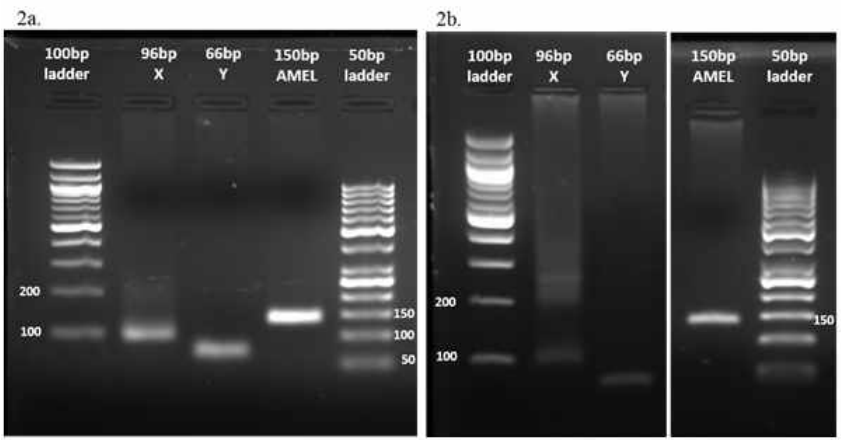


Fig. 2. Amplification of X, Y, AMEL from Jersey cross cattle (a) and Murrah buffalo (b) genomic DNA.

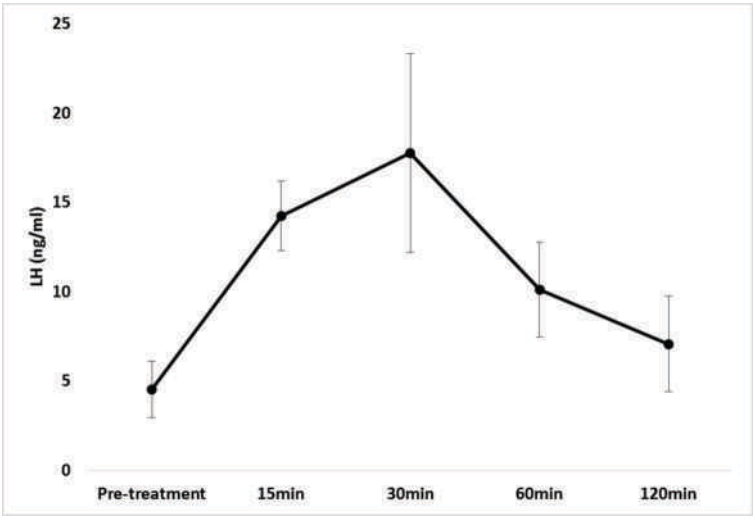


Fig. 3. Effect of intravenous administration of synthetic kisspeptin (5µg/kg) on plasma LH concentration in Deoni cattle (n=3).

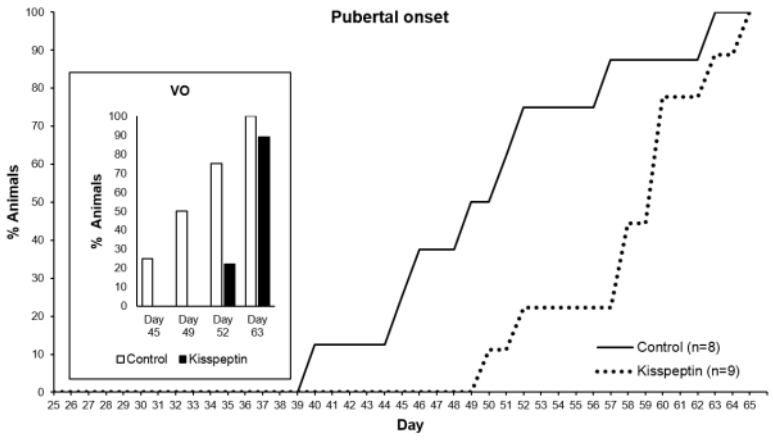


Fig. 4. Effect of peripheral administration of kisspeptin on pubertal onset in female rats. The day of vaginal opening (VO) was noted in rats treated with either saline or kisspeptin day 25 to day 50. Control vs. kisspeptin: P=0.01, Kaplan-Meier survival analysis followed by Gehan-Breslow test. Inset: The percentage of animals that have reached puberty was compared between control and treated rats: at the respective time point when 25, 50, 75 and 100% of control rats have shown VO, only 0, 0, 22 and 89% of treated rats have shown VO.

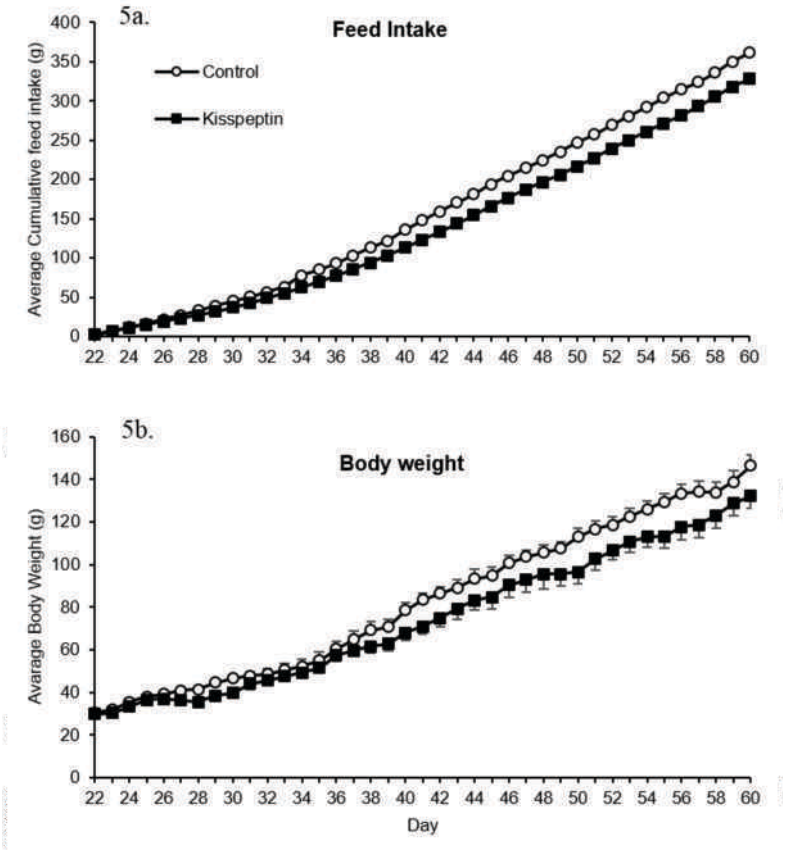


Fig. 5. Effect of peripheral administration of kisspeptin on feed intake and body weight. Saline or kisspeptin was administered from day 25 to day 50. (a) Average cumulative feed intake. Control vs. kisspeptin: $P<0.001$, two-way ANOVA. (b) Average body weight. Control vs. kisspeptin: $P<0.001$, two-way ANOVA.

Bioinformatics

Analysing Sequence Data for Marker Discovery and Comparative Genomics

Principal Investigator	Sarwar Azam	Scientist B
Members	Veera Narasimha Rao	Project Fellow
	Hita Verma	Project Fellow
Collaborators	Dr. Girish K Radhakrishnan	NIAB, Hyderabad
	Dr. Satish Kumar	NIAB/CCMB, Hyderabad

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Marker discovery, gene space exploration and development of web resource for river buffalo (Bubalis bubalis)

***In silico* mining of putative microsatellite markers in river buffalo**

The genome sequences of river buffalo were mined for short tandem repeats (STRs) using MIcroSAtellite (MISA) with a parameter of repeat restriction such as minimum ten repeats for mono-, six repeats for di-, and five repeats for tri-, tetra-, penta- and hexa-nucleotide for simple STRs. A total of 982,593 repeats of which 94,929 are complex STRs (Table 1). Distribution of motifs of annotated STRs and their sizes has been summarized in Table 2 and Fig. 1.

Each annotated STRs were subjected for designing primer pairs using Primer3V2.3.6 with following criteria (i) annealing temperature (Tm) between 50–65 °C with 60 °C as optimum; (ii) product size ranging from 100 bp to 350 bp; (iii) primer length ranging from 18 bp to 24 bp with an optimum of 20 bp; (iv) GC % content in the range of 40–60%. A total of 880528 primer pairs were successfully generated for corresponding STRs which can be used for its validation and genotyping.

Development of a database for buffalo genomic research

India has 105.1 million buffalo and they comprise approximately 59% of the total world buffalo population. To boost the genomics of buffalo, we have initiated the development of a comprehensive database of genomics resources named as "BhainsBase". The database will provide genome wide marker information and variations within and between the breeds of buffalo. This web resource will facilitate visualization, comparisons or performing new evaluations for genes and other genomic structure (Fig. 2A).

Database architecture

BhainsBase has been developed in Java environment using PostgreSQL object-relational database management system (Fig. 2B). J Browse tool, which is fast embedded genome browser in Java has been integrated to Bhainsbase for visualization & exploration of genome sequence and its structure. For searching similar sequences and comparative genomics, blast server has been setup with the database. The graphical user interface (GUI) has been developed in JSP, Jquery and CSS. The Apache web - server is used to deploy this web application.

BhainsBase has been enriched with 7,718 genomics sequences which is scaffolded-level assembly of water buffalo. It has also been enriched 982,593 annotated STRs. All the STRs with their flanking primers can be visualized using Browser tab of Bhainsbase.

Annotation & expression analysis of genes in river buffalo

To explore the gene space and predict accurate gene models, we did in-silico gene prediction which is the primary prediction and resulted with highly inflated numbers of genes (~58489) in the genome of water buffalo. Therefore a comprehensive approach for gene annotation has been taken which involve ab-initio gene prediction, homology based gene prediction and evidence based gene prediction. In this regard, we have collected expression data of 30 different tissues of buffalo generated using Next Generation Sequencing (NGS) from NCBI. We will be using Tuxedo protocol and EvidenceModeler (EVM) for the prediction of all genes and their structure and distribution in the genome. The information will be merged with the database which will help user to directly select the marker which is found within the gene or near the gene of interest.

Project 2: Comparative genomics of *Brucella melitensis* strain Bm IND1

Development of chromosomal level assembly of Bm IND1

The scaffold level assembly of *B. melitensis* strain Bm IND1 was submitted to GenBank under the accession JMKL00000000. To generate chromosome level assembly, the scaffolds were assigned to two Brucella chromosomes with proper order and orientation. To achieve this, we considered genomes of *B. melitensis* 16M and the *B. melitensis* isolate from China, M28; both of these genomes have been used previously as reference genomes for other strains. We aligned the raw data of Bm IND1 on both the genomes using bowtie2 and identified the SNPs. The analysis provided 298 highly confident SNPs between Bm IND1 and *B. melitensis* M28, whereas the analysis between Bm IND1 and *B. melitensis* 16M identified 2806 SNPs. Since the B. melitensis M28 displayed the least number of SNPs, which indicates minimum genetic divergence, we selected the genome of this strain as the reference genome for chromosomal assembly of Bm IND1. Total number of scaffolds assigned on Chromosome I and II are 24 and 6, respectively. Subsequently, assembly was manually curated with a focus on those scaffolds that showed higher physical coverage to fix the duplications. We observed the duplication of scaffold 20 and inversion of a large portion of scaffold 22 on Chromosome I with respect to the *B. melitensis* M28 genome. Next, we aligned the genomes of *B. melitensis* M28, *B. melitensis* 16M and Bm IND1 and observed for macro level synteny and large genomic rearrangements. In fact, they were highly syntenic with each other except one segment of *B. melitensis* 16M on Chromosome II which was in reverse orientation in *B.melitensis* M28 and Bm IND1 (Fig. 3).

Whole genome phylogeny

Genomes of *B. melitensis* 16M, *B. melitensis* M28, *B. melitensis* M5-90, *B. melitensis* ATCC 23457 and *B. abortus* 2308 were downloaded from NCBI (www.ncbi.nlm.nih.gov). To assess the core genome and single copy orthologs, Orthomcl v.1.4 was used to cluster the genes using default parameters. We used 2509 single copy orthologs to construct a maximum likelihood tree following the published approach (Creevy et al 2011 PLoS Comput Biol 7: e1002269).

A total of 15637 genes of *B. melitensis* strains were clustered in 3140 gene families, of which 2760 genes showed single copy orthology in each strain. There were 2781 genes present in all the five strains that could be considered as the core genome of *B. melitensis* clade. We used *B. abortus* 2308 as the out-group for whole genome phylogeny that increased the total cluster of genes to 3250. After including the *B. abortus* strain, the total number of single copy orthologs decreased to 2523 genes. There were 230 unique gene families in *B. melitensis* strains, which might have contributed to the evolution of the *B. melitensis* clade and its host specificity. To infer the phylogeny, we considered 2523 single copy orthologs. In the phylogenetic tree *B. melitensis* M28 and *B. melitensis* M5-90 clustered with Bm IND1 (Fig. 4). While contemplating the branching pattern of the tree, it appears that considerable genetic diversity exists among *B. melitensis* isolates from Asia.

Identification of SNPs

We downloaded the genome sequences of *B. melitensis* 16M, *B. melitensis* M28, *B. melitensis* ATCC 23457, *B. melitensis* M5-90, *B. melitensis* NI, *B. melitensis* ADMAS-G1 and *B. abortus* 2308 for SNP analysis. We established a pipeline for finding SNPs between two reference sequences using Nucmer and show-snps program from the Mummer 3 package. Show-snps provide SNPs only from uniquely aligned regions. SNPs were extracted from each strain against Bm IND1 as reference and further annotated using SnpEff to predict SNP effects in the genome. Distribution of extracted SNPs are represented in Table 3.

INDELs analysis

To find insertions and deletions in genome sequence, VCF file generated against *B. melitensis* M28 using Bm IND1 reads were annotated with SnpEff. INDELs in coding regions and their corresponding function from *B. melitensis* M28 annotations file were extracted using in house perl script. We have identified 119 indels in the Bm IND1 genome with respect to *B. melitensis* M28, of which 92 are located in non-coding regions and 27 are located in coding regions.

Project 3: Identification of immunodominant proteins for Brucella vaccine development

Brucella melitensis is the most virulent species in human, primarily affects goat and the zoonotic transmission occur by ingestion of unpasteurized milk products or through direct contact with fetal tissues. So, we took an effort to identify proteins that are potentially involved in *B. melitensis* pathogenesis, which are good novel targets for candidate vaccines. The workflow has been established to find the protein vaccine candidates (PVCs) (Fig. 5).

Pre-screening of putative vaccine candidate from Bm IND1

Brucella melitensis is a Gram-negative bacteria. In general, the outer membrane proteins (OMPs) and secretory proteins of Gram-negative bacteria play a crucial role in virulence and pathogenesis. They are involved in the integrity and stability of the bacterial envelope, passive and active transport of substrates and nutrients, cell-to-cell communication, adhesion to host cells, and virulence.

To screen all the OMPs and secretory proteins, genes of BM IND1 strain were analyzed by two global programs i.e. P-SORT software v3.0 (http://www.psort.org/psortb/) and CELLO v2.5 Each genes of BM IND1 were characterized for its subcellular localization (Table 4) and comprehensive set of genes characterized by either software as outer membrance or secretory genes were filtered using in-house per script (Fig.6)

Summary

A chromosomal level assembly of *B. melitensis* IND1 strain were achieved and compared with other Brucella strains for phylogeny, SNPs and INDELs. This Analysis is the part of the project entitiled "Analyze the Genetic diversity of Brucella melitensis and *Brucella abortus* strains in India". Moreover, a reverse vaccinology approach has been applied to identify the immunodominant genes of *B. meletensis* IND1 for vaccine development. On the other hand, a database for buffalo genomics has been developed and enriched with STR markers. Further, an effort of gene annotation in buffalo and their expression studies are being carried out.

Table 1 : Summary of STR identification

Total number of sequences examined	7718
Total size of examined sequences (bp)	2615754706
Total number of identified SSRs	982593
Number of SSR containing sequences	4125
Number of sequences containing more than 1 SSR	3410
Number of SSRs present in compound formation	94929

Table 2 : Frequencies of STRs based on their sizes

Size of STRs	Number of STRs
<10	505,129
11-13	265,262
14-16	130,904
17-25	74,398
>25	6,900

Table 3 : Frequencies of STRs based on their sizes

Species	Total number of SNPs
Brucella melitensis 16M	2561
Brucella melitensis M28	280
Brucella melitensis M5-90	300
Brucella melitensis NI	351
Brucella melitensis ATCC 23457	252
Brucella melitensis ADMAS-G1	2366
Brucella abortus 2308	6047

Table 4 : Subcellular localization of Bm IND1 genes

Subcellular localization	Predicted proteins from PSORTb	Predicted proteins from CELLO
Cytoplasmic	1448	2269
PeriplasmicInner	101	406
Membrane	745	521
Extracellular	18	46
Outer membrane	34	71
Unknown	967	-
Total	3313	3313

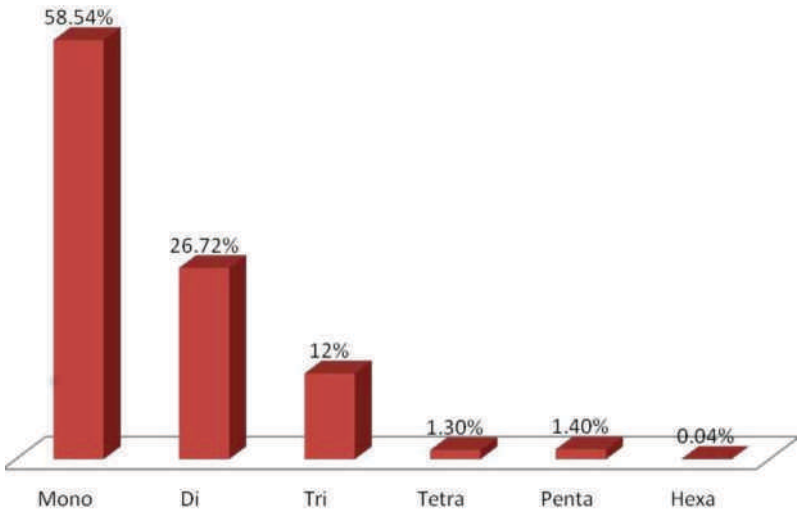


Fig. 1. Distribution of STR motifs in river buffalo

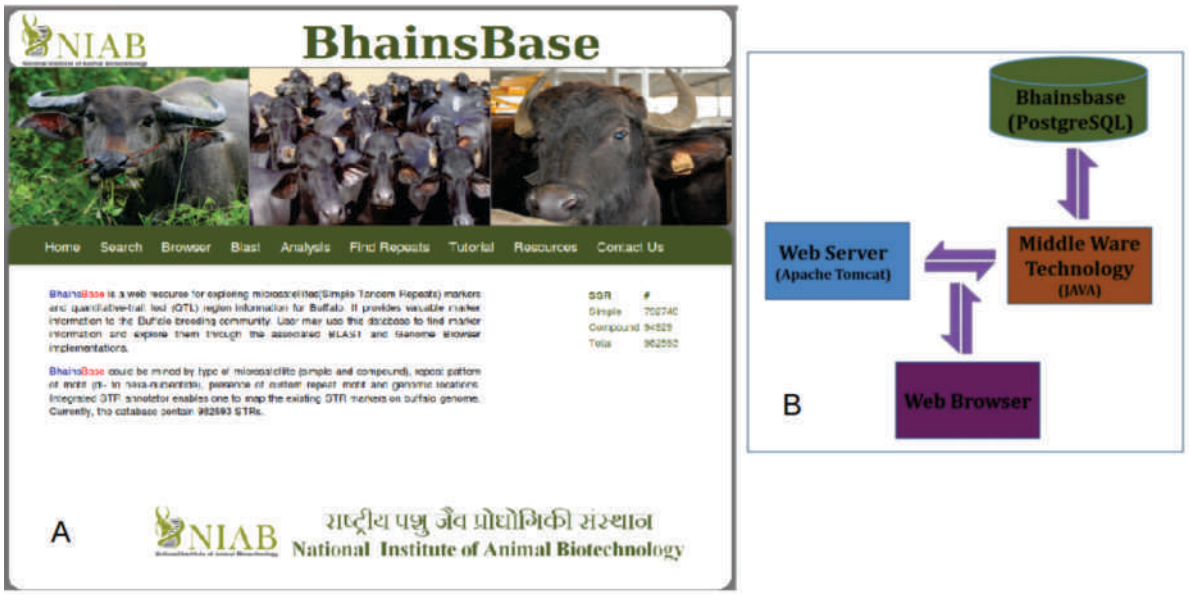


Fig. 2. (A) Snapshot of homepage of BhainsBase, (B)Three-level schema architecture of BhainsBase.

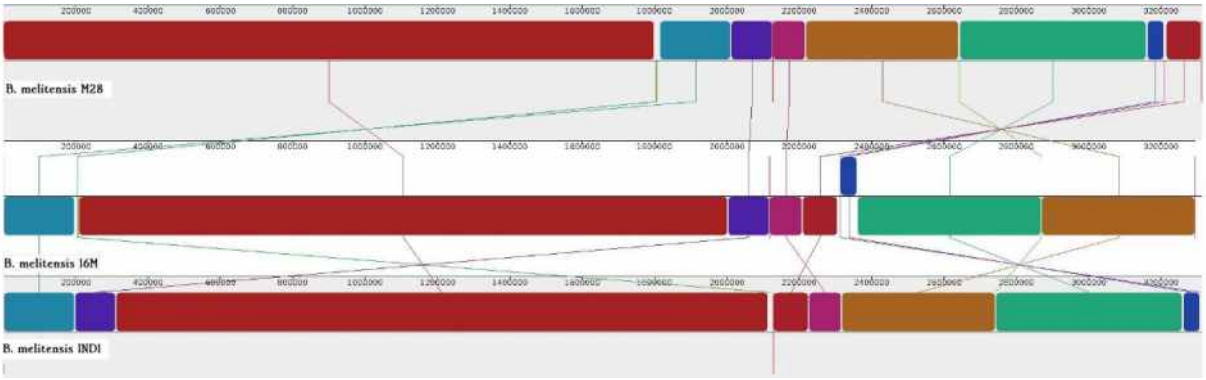


Fig. 3. Alignment of *B. melitensis* 16M, B. melitensis M28 and *B. melitensis* IND1 genome. Bm IND1 and B. melitensis M28 aligned well with each other, however, a segment (blue color block) on Chromosome II of Bm IND1 is in reverse orientation in *B. melitensis* 16M.

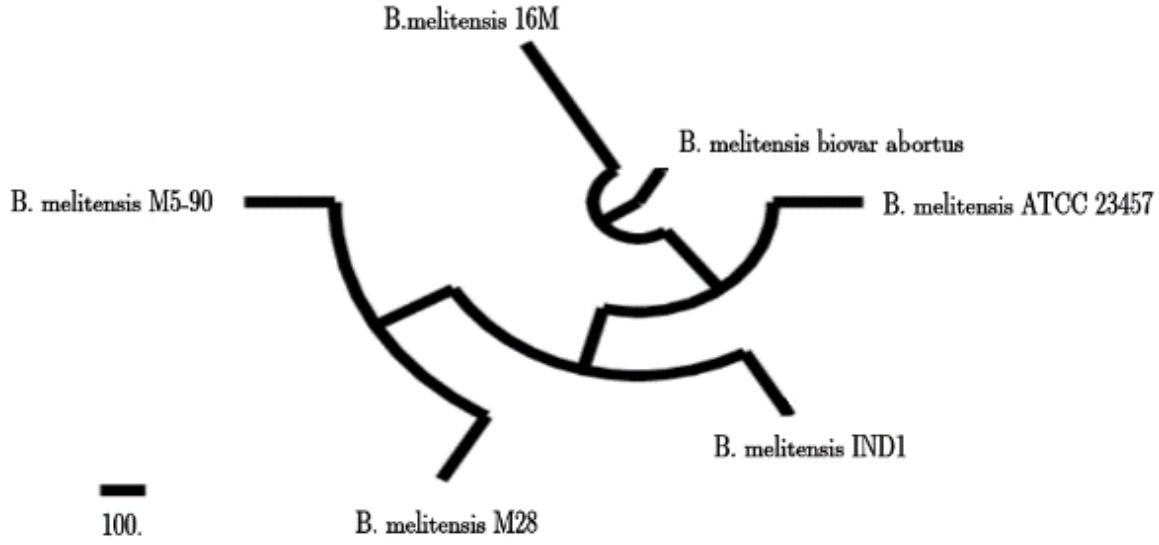


Fig. 4. Phylogenetic tree showing relationship between *B. melitensis* IND1 and other *B. melitensis* strains and *B. abortus* 2308. Maximum-likelihood tree rooted at *B. abortus* 2308, is based on core genome multiple alignment.

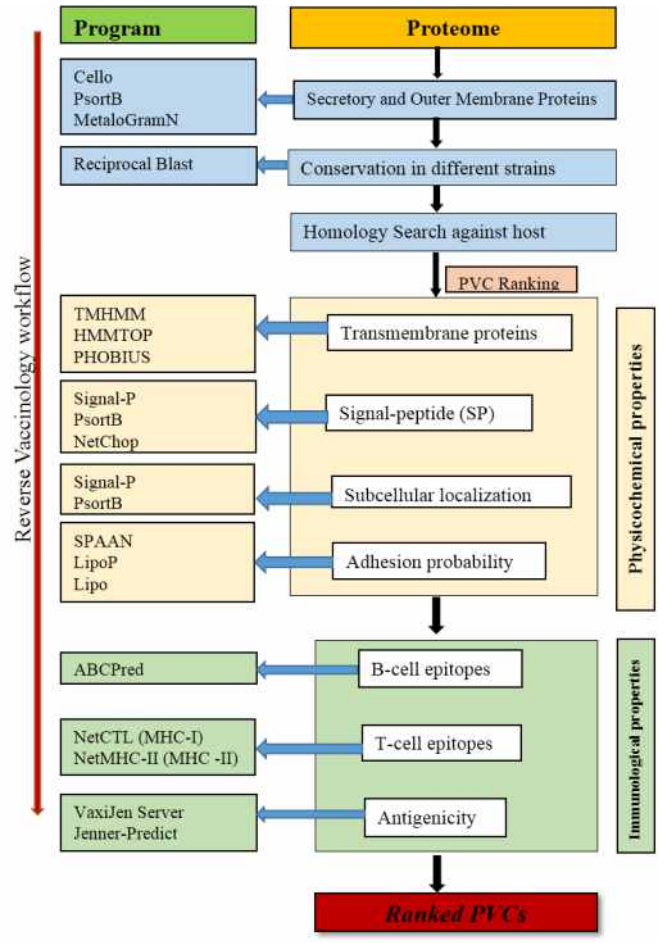


Fig. 5. Representation of workflow to identify and rank the vaccine candidates using comparative genomics and immunoinformatics approaches.

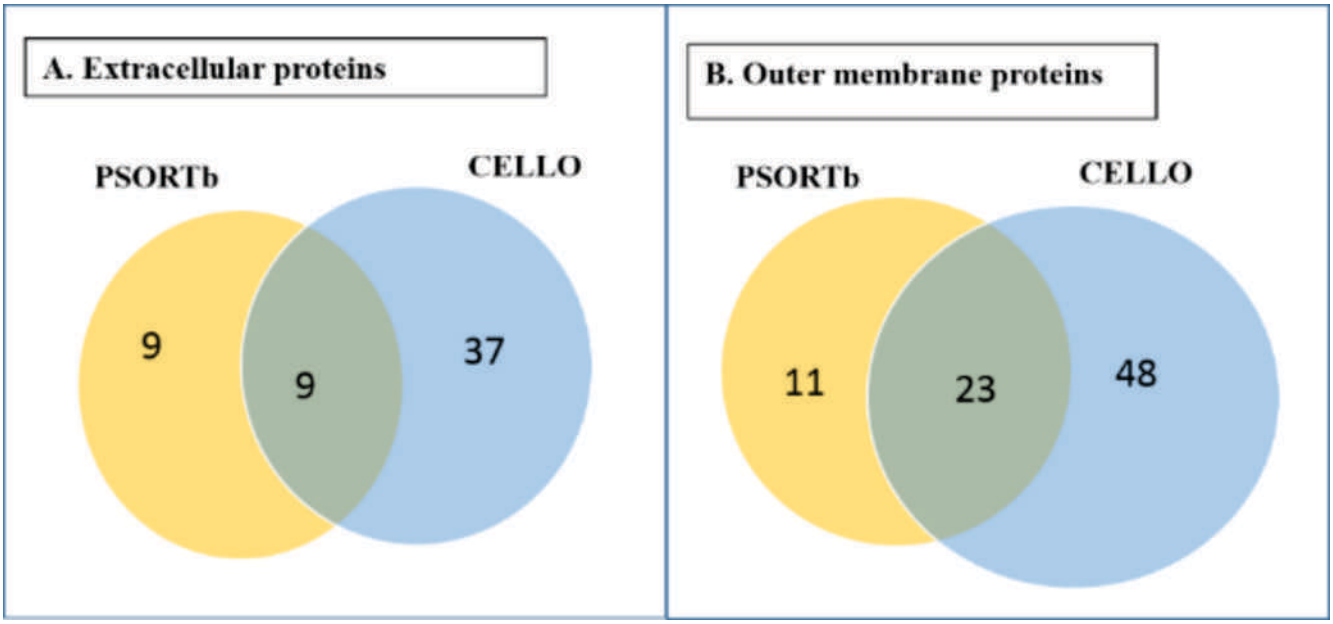


Fig. 6. Venn diagram representing the predicted (A) Extracellular and (B) Outer membrane proteins of Bm IND1 using PSORTb and CELLO.

Collaborative Research Projects

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

Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad

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Others members	Amit Kiri	JRF
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Collaborators	Dr. Brahmanandam M	HCU, Hyderabad
	Dr. Paresh Sharma	NIAB, Hyderabad

Objectives

India is the largest producer and consumer of milk and dairy products in the world. This depicts both the nutritional and economical importance of milk in the Indian prospect. Milk biosynthesis is a complex process that involves interplay of various hormonal and related factors. Of all the constituents, milk protein (casein) and milk sugar (lactose) biosyntheses are majorly under hormonal control. Prolactin (PRL) is the predominant lactogenic hormone while growth hormone (GH) and insulin-like growth factor (IGF) are also involved in the regulation. As the process of lactation is shown to be highly conserved throughout the mammalian species, HC11 cells derived from pregnant mice mammary epithelial cells were selected as a model system. To comprehensively profile the genes and their pathways involved in milk biosynthesis, we majorly focus on gene expression profiling in mouse mammary epithelial cell line HC11 in response to PRL signaling by performing genome-wide expression profiles from normal, PRL-stimulated and Stat 5α depleted HC11 cells. Then we shall determine physical gene interaction mapping in mammary epithelial cells in response to PRL treatment which reveals the clustering pattern of differentially expressing co-functional and co-regulated genes to the sites of active transcriptional centers. Then we shall determine the global reorganization of chromatin during PRL stimulus by performing HiC procedure. We shall validate gene products and pathways involved in milk biosynthesis that can help us in constructive manipulation of pathways thereby can increase both the quality and quantity of milk production. Finally by combining the results obtained from the above objectives, we would like to provide the integrated perspective of structural and functional organisation of the genome during lactation and its relation to cell type-specific gene expression programme. The objectives are summarized as follows:

(1) Gene expression profiling in mammary epithelial cells in response to prolactin signaling.
(2) Determination of physical gene interaction mapping in mammary epithelial cells in response to PRL treatment.
(3) Validation of gene products and pathways involved in milk biosynthesis

Summary of work done until the beginning of this reporting year (up to March 31, 2014)

Mouse mammary primary epithelial cell lactogenic differentiation method was further optimized and successfully validated with known differentiation marker genes such as Casein and Wap. Microarray analysis of gene expression profiles from normal and PRL induced HC11 cells were conducted. Analysis has revealed selective induction of various biological pathways upon PRL induced lactogenic differentiation. More importantly, sorting of genes based on RMA expression values between normal and PRL induced HC11 cells and its relationships with biological pathway analysis has uncovered strong correlation between specific biological pathway genes to have highly similar gene expression levels reinforcing the fact that genes related to particular biological pathway to have similar stoichiometric levels of transcripts reinforce the idea of clustering of co-regulated and co-functional genes within the nucleus for their coordinated transcriptional control. We further show by taking example of ribosomal protein genes to have highly similar expression levels between normal and PRL induced HC11 cells. This forms the basis for our hypothesis that genes cluster within the nucleus in order to have similar stoichiometric levels of transcriptional output. To test this hypothesis, it is important to study 3-dimensional organization of genome in normal and PRL induced HC11 cells. To this end, we have also generated HIC libraries from both normal and PRL induced HC11 cells, which were sent for high throughput paired end sequencing service. We further improvised 4C and HIC data analysis pipeline and this will be used for the analyzing HIC data sets. We also optimized the methods required for isolation of mouse mammary epithelial cells for virgin, pregnant and lactating mouse mammary tissues.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Gene expression dynamics during lactogenic differentiation:

We profiled global gene expression in normal, glucocorticoid-induced and PRL-induced mouse mammary epithelial cells by using Agilent 8X60K array representing approx. 30,000 mRNAs and 4600 LincRNAs (Fig. 1a). Glucocorticoids were known to play an important role in setting up cell-type specific gene expression program upon binding to glucocorticoid receptor (GR) directly or indirectly by binding to chromatin regions or by protein-protein interactions respectively. Prolactin was known to play an important role in setting up cell-type specific gene expression program by facilitating dimerization of Stat5α and Stat5β and its translocation into the nucleus and regulation of multiple genes upon binding to chromatin regions directly. We observed differential expression of various genes and pathways (Fig 1b, 2a, b, c, d). From these, we could be able to deduce the gene regulatory networks that orchestrate the milk biosynthetic pathways in mammary epithelial cells. Further co-expression analysis of LincRNAs and mRNAs suggest a significant correlation between many LincRNAs for the presence or absence of specific mRNAs.

Integrative analysis of LincRNA and mRNA dynamics during lactogenic differentiation:

Dynamic expression profiling of mRNAs and LincRNAs in Normal, glucocorticoid-induced and PRL-induced epithelial cells gives us an opportunity to understand the relationship between dynamic expression of mRNA and LincRNAs. Based on the similarities in either up regulation or down regulation and fold enrichment of similar genes one would predict the co-regulation or functionally related mRNA and LincRNAs. To this end, we used coexpression clustering algorithm to computationally predict inter related mRNAs and LincRNAs. Our analysis has predicted strong positive or negative correlation between occurrences of a particular LincRNAs with multiple mRNAs which are involved in milk biosynthetic pathways (Fig. 3).

Summary of the work done during the academic year 2014-15

Lactogenic hormone signaling promote sequential up and down regulation of multiple mRNAs as well as LincRNAs in mouse mammary epithelial stem cells. Glucocorticoid signaling shutdown expression of proteins that promote cell division and DNA replication suggesting requrirement of cell cycle arrest during initial stages of cellular differentiation. Functional and pathway analysis of top up-regulation of genes during lactogenic differentiation suggest lysosome function, melanogenesis, focal adhesion, tight junctions, sulfur metabolism and endocytosis. Lactogenic hormone signaling enhances the expression of genes that encode for carbohydrate, lipid and protein breakdown in to respective monomers such as glucose, galactose, fatty acids and amino acids that become part of milk constituents. Protein interaction network analysis suggest selective up or down regulation co-functional genes upon lactogenic hormonal signaling. Co-expression clustering analysis predicts physically or functionally inter-related mRNAs and LincRNAs. However, these prediction require experimental validation. Genome wide correlation analysis would potentially identify putative mRNA targets for LincRNAs. Currently we are investigating functional significance of some of the LincRNAs during lactogenic differentiation.

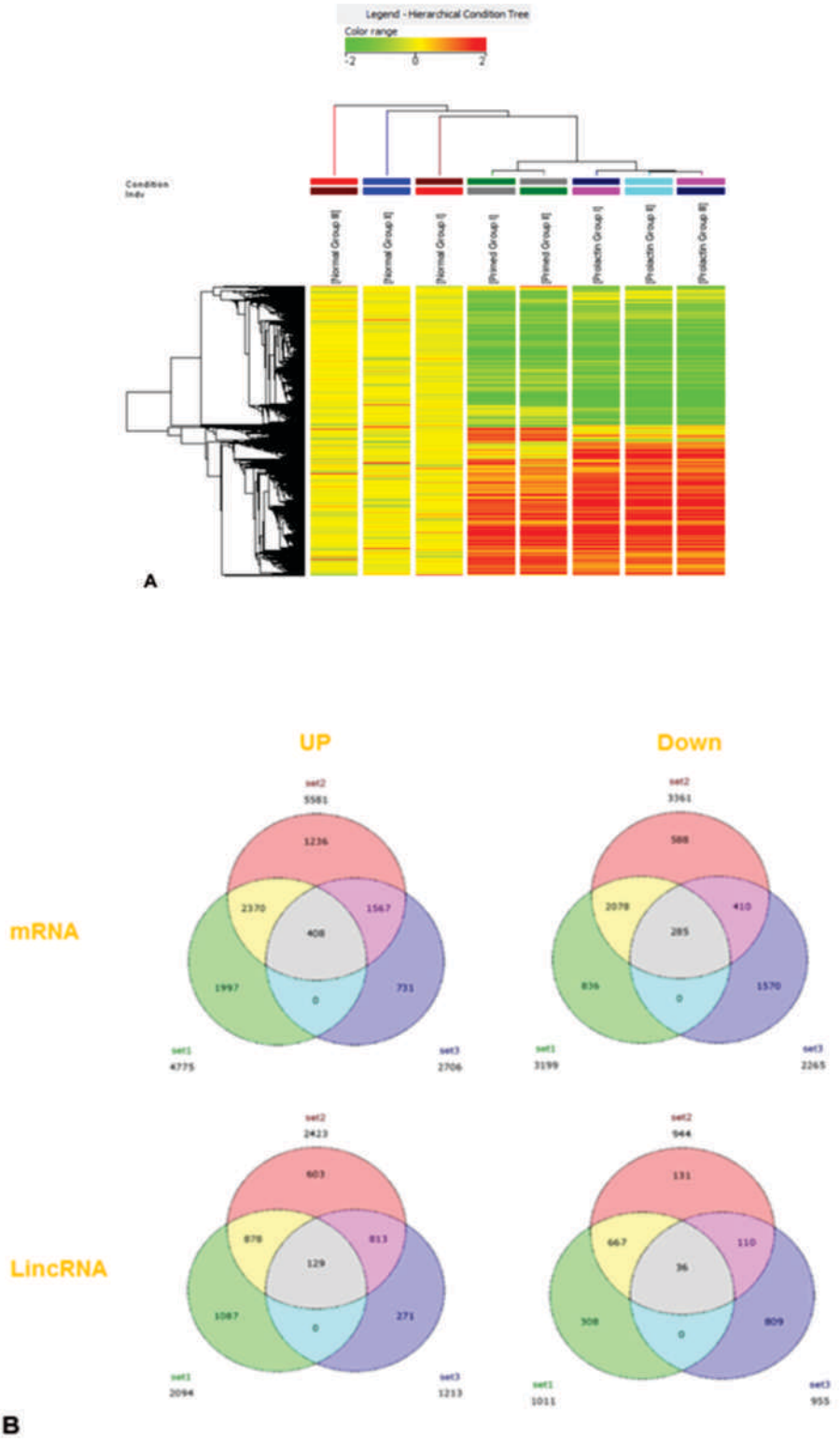

Fig. 1. Gene expression profiling in normal, glucocorticoid-induced and PRL induced mouse mammary epithelial stem cells: 1a. Hierarchical clustering analysis of differentially expressed mRNA, LincRNAs in normal, glucocorticoid-inducecd and PRL-induced mouse mammary epithelial cell. 1b. Pie chart showing number of differentially expressed genes between normal vs. glucocorticoid-induced (set 1), normal vs. PRL-induced (set 2) and glucocorticoid vs. PRL-induced (set 3).

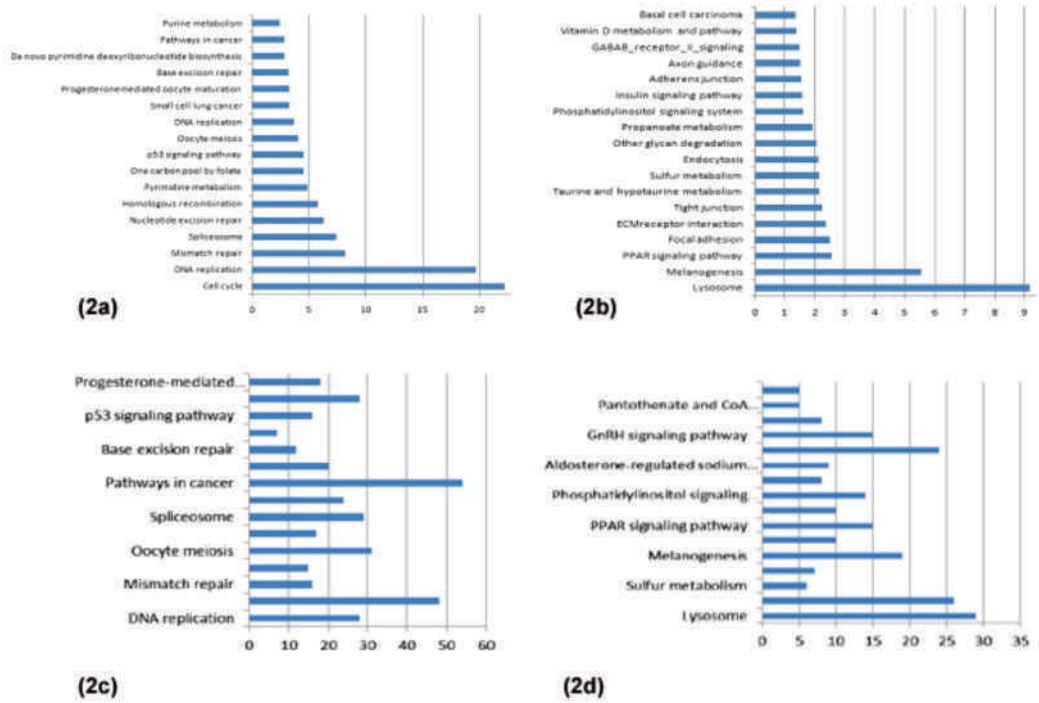

Fig. 2. Functional and pathway analysis of top up- or down-regulated genes upon glucocorticoid and PRL signaling in mouse mammary epithelial stem cells by using DAVID data base: (a) for top down-regulated genes and (b) top up-regulated genes upon glucocorticoid signaling; (c) for top down-regulated genes and (d) top up-regulated genes upon PRL signaling.

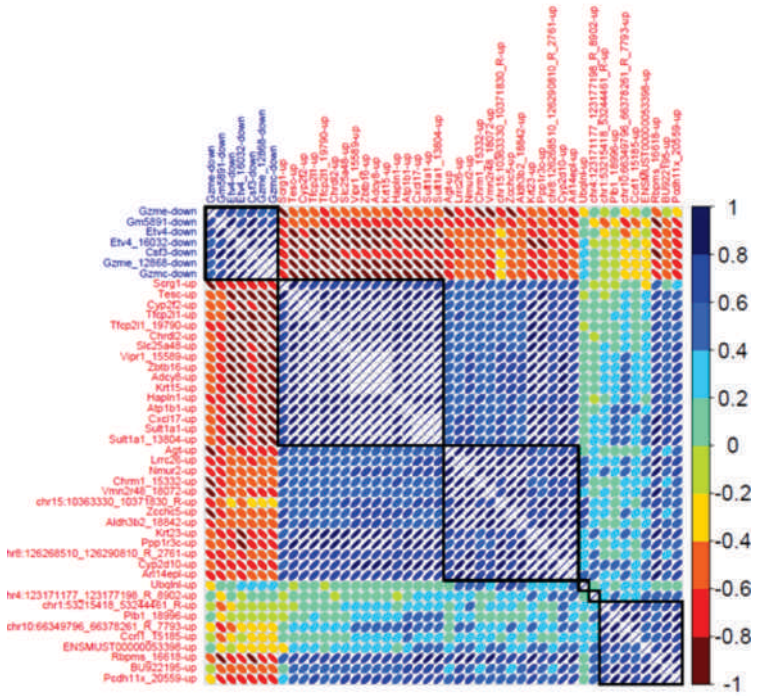

Fig. 3. Integrated co-expression clustering analysis of LincRNAs and mRNAs during lactogenic differentiation identifies putative inter related mRNA and LincRNAs: Heat map of correlation coefficients for top up- and down-regulated genes (fold change > ~ -6.2 or ~ +6.2 before and after GR signaling. Distortion in the ellipse is directly proportion to significance of the correlation. Blue square boxes denotes the highly co-expressed clustered. If LincRNAis co-clustered with other genes in the squared boxes, it is most likely to positively regulate them.

Antibiotic Resistance of Bacterial Pathogens and Cytokines Mediated Mammary Tissue Damage in Bubaline Mastitis : Role of Polyphenols and NSAIDs in Control

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Objectives

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 mastitis in buffaloes and cows could be controlled. This project aims to address these issues and the objectives of this project are as follows:

1) To evaluate the antibacterial activity of polyphenolic compounds (individually or in cocktail or in combination with reduced doses of antibiotics) on mastitis pathogens *in vitro.*

2) To study the sensitivity of mastitis pathogens to antibiotics in combination with NSAIDs *in vitro.*

3) To analyze the levels of expression of different inflammatory and immunomodulatory cytokines in bubaline mammary epithelial cells during *in vitro* infection with *S. aureus* and *E. coli.*

4) To test the action of polyphenolic compounds in regulating the levels of inflammatory and immunomodulatory cytokines in bubaline mammary epithelial cells.

Summary of work done until the beginning of this reporting year (upto March 2014):

At SVVU, Gannavaram:

E. coli was isolated from certain mastitic milk samples of buffaloes. The *E. coli* isolates were provisionally confirmed by conventional cultural and biochemical tests, later further confirmed in PCR by using *E. coli* specific oligonucleotide primers. Antibiotic resistance pattern in *E. coli* isolates revealed varied degree of sensitivity to the antibiotic enrofloxacin in antibiotic sensitivity test (ABST). Whereas these *E. coli* isolates are found to be resistant to amoxicillin and ceftriaxone in in vitro Minimum Inhibitory Concentration (MIC) assays, the polyphenol cinnamic acid exhibited antibacterial activity at 1.25mg/ml concentration for half of the isolates, and for the remaining isolates its antibacterial activity was at 2.5mg/ml. For majority (75%) of *E. coli* isolates, quercitin exhibited antibacterial activity at 2.5mg/ml. and gallic acid at 5mg/ml. Cinnamic acid has to be further studied for antibacterial activity in combination with other polyphenols and conventional antibiotics.

Staphylococcus species other than *S. aureus* were isolated from majority of mastitis milk samples. It is interesting to note that the five isolates GDV 5, GV 7, KSP 14, KSP 15 and KSP 16 are coagulase negative variants of S. aureus 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. The β-lactamase production of *S. aureus* isolates was detected by starch-iodine agar test.

At HCU, Hyderabad:

Initial studies of Inosine-5 -monophosphate dehydrogenase (IMPDH) activity with crude lysate, where the formation of NADH is measured, showed the reduction of NADH formation in the presence of celecoxib but celecoxib did not show any inhibition of IMPDH activity with pure protein at various concentrations. This has clearly shown that celecoxib may be involving in the inhibition of other enzymes/reactions which use NAD as co-factor. As there is no inhibition of IMPDH activity, we did not further proceeded with patent filing.

The β-lactamase was down regulated at gene level and its activity also got suppressed in the combination of celecoxib and ampicillin. But the reason for high activity in celecoxib treated samples was unexplained. MSSA and MRSA growth kinetics were studied in presence/absence of antibiotics alone or in combination, which showed delayed lag phase in the combinatorial treatment.

Twelve isolates of *S. aureus* from mastitis were tested for the combinatorial efficacy using methicillin and celecoxib. These isolates have clearly shown higher inhibition in combination than when they are used alone. Nearly 66 % of the strains are mecC positive which indicated higher prevalence of MRSA in bubaline mastitis.

Details of the progress made in the current reporting year (1st April, 2014 – 31st March 2015).
At SVVU, Gannavaram:

A total of 39 isolates of *S. aureus* were isolated from clinical cases of bubaline mastitis. All these isolates are provisionally confirmed by conventional and biochemical tests, then further confirmed by PCR test. Of these isolates, a total of 24 *S. aureus* isolates were found to be positive for blaZ gene in PCR with specific oligonucleotides, indicating production of β-lactamase that confer resistance to β lactam antibiotics. The β-lactamase production of *S. aureus* isolates was detected by starch-iodine agar test. Seventeen isolates of *S. aureus* were found to be positive for mecA gene in PCR with specific oligonucleotides, indicating their resistance to methicillin (Fig. 1). However, 15 isolates of *S. aureus* were positive for both blaZ and mecA genes. Except two isolates TVCC44 and GV32, all other isolates of *S. aureus* that were positive for mecA gene were also positive for blaZ gene. Ten isolates of *S. aureus* were positive for PanteneValentine Leukocidin (PVL) gene pvl (Figure 1). The detection of pvl gene in *S. aureus* isolates from bubaline mastitis cases assumes significance as the *S. aureus* isolates that produce pvl toxin have significance with regard to community in that habitat.

Biofilm formation in *S. aureus* isolates was assessed by phenotypic studies such as microtitre plate assay and modified Congo red agar. Genetic determinants of biofilm production in *S. aureus* isolates were detected in PCR test by reactivity with oligonucleotide primers specific to icaA and icaD genes. Specific PCR product of 188 bp was observed in some *S. aureus* isolates for icaA gene. Specific PCR product of 198 bp was observed in some *S. aureus* isolates for icaD gene (Fig. 2). It is interesting to note that the *S. aureus* isolates that are positive for pvl gene were isolated from clinical mastitis cases that showed the value of 2 or 3 in California Mastitis Test (CMT). There is also strong correlation between biofilm producing *S. aureus* isolates and antibiotic resistance pattern. MIC assays with conventional antibiotics and polyphenols Quercitin, Cinnamic acid and Gallic acid were formed as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2012. Majority of *S. aureus* isolates were found to be resistant to β-lactam antibiotics. Sensitivity of *S. aureus* isolates to polyphenols Quercitin and Gallic acid showed similar trends as reported with earlier isolates.

Bubaline Mammary Epithelial Cell line (BuMEC) procured from NDRI, Karnal, is propagated. Fresh milk aseptically collected during milking from the mulching buffaloes was also collected and processed for isolation of mammary epithelial cells. The mammary epithelial cells were propagated in RPMI medium. However, the conditions for propagation of cells need certain modifications to yield healthy mammary epithelial cells that can also be used in infection studies. Progress pertaining to infection studies by co-culturing BuMEC with bacterial pathogens is behind the schedule due to certain technical constraints in co-culture studies. Presently study is in progress to assess the levels of expression of different cytokines in BuMEC during infection with *S. aureus* and *E. coli.*

At HCU, Hyderabad:
Ampicillin and Celecoxib co-crystal development:

Various proportions of Ampicillin and celecoxib were used for co-grinding and the co-crystals formed were named as per their proportions. These co-crystals were analysed by powder X-ray diffraction and their stability was tested by Differential Scanning Calorimeter. The results indicate that ampicillin and celecoxib might be interacting with each other by weak electrostatic interactions or van der Waals forces or by hydrogen bonding, which might be enhancing the half-life of ampicillin or because of it the ampicillin might not be cleaved readily by β-lactamase and hence effectively show its action on bacteria (Fig. 3).

Ampicillin-Celecoxib interaction by fluorescence spectroscopy:

Ampicillin-Celecoxib working concentrations were prepared in methanol and their excitation and emission maxima were determined. It was observed that there is no difference in the emission peak of celecoxib (Fig. 4A), but for ampicillin, the peak height was increased in the case of combination (Fig. 4B). This is suggesting that, when celecoxib and ampicillin solutions are mixed, they are showing the weak interactions between them and that electron cloud which is helping in the interactions is raising the fluorescence peak.

MSSA growth monitoring in presence of drugs by CFU counting:

Growth of MSSA was earlier observed by measuring the optical density. Since this method cannot distinguish between live and dead bacteria, we have counted the colony forming units (CFU) of the samples by pour plate method while observing the bacterial growth in presence of drugs. The bacterial growth was inhibited significantly in the presence of combination of drugs.

Effect of combination of ampicillin and celecoxib on S. aureus of mastitis:

Based on the MIC values the *S. aureus* isolates were categorized into three groups, namely, high resistance, intermediate resistance and sensitive. Bacterial ghosts were prepared and the entry of celecoxib and ampicillin was observed after incubation with drug/antibiotic. It was clearly observed that there was increased ampicillin absorption inside the ghost membranes in presence of celecoxib suggesting that celecoxib might be facilitating the entry of ampicillin into the bacteria and thereby increasing the antibiotic efficacy.

Membrane potential (MP) is a mechanism by which bacteria attains resistance. Therefore, to understand the mechanism of action of celecoxib in combination, we have analysed the MP by flow cytometer using Rhodamine 123. MP in MRSA did not change in celecoxib treatment and it was similar to that of untreated control cells. But, it was decreased in ampicillin treatment (Fig. 5B) suggesting that MRSA might be attaining resistance to ampicillin via decrease in MP. However, in combinatorial treatment, membrane potential was regained, close to normal. In the case of MSSA, there was no significant difference in MP in all treatment conditions. MP was also measured with DiOC2.

Summary

A total of 39 isolates of *S. aureus* were isolated from the clinical cases of bubaline mastitis during this report period. They were tested for the genetic determinants of antibiotic resistance (blaZ and mecA genes), biofilm production (icaA and icaD) and also for the antibiotic sensitivity pattern by disc diffusion and MIC assays. There is wide spread resistance in *S. aureus* isolates against β-lactam antibiotics. The polyphenols Quercitin, Cinnamic acid and Gallic acid showed promising antibacterial activity in vitro against *S. aureus* and *E. coli.* The mammary epithelial cells (BuMEC) were propagated for co-culture infection studies with *S. aureus* and *E. coli.* Work is in progress to assess the levels of expression of different cytokines in mammary epithelial cells during infection.

Ampicillin-Celecoxib co-crystals were developed and the study showed that the two drugs have weak interactions between them. Bacterial growth monitoring by CFU method clearly suggested that the combination is effective in inhibiting the bacterial growth than individual drugs. Twenty five isolates of *S. aureus* from mastitis were tested for the combination efficacy. It is clearly observed that the combination of ampicillin and celecoxib is much more effective than the individual drugs and it worked for highly resistant bacteria to sensitive bacteria at much lower MIC values. The increased ampicillin absorption inside the ghost bacterial membranes suggest that Celecoxib might be facilitating ampicillin's entry into the bacteria. The decreased MP of MRSA in presence of ampicillin alone was regained back to normal in the combination of ampicillin and celecoxib. To understand the fate of celecoxib and changes in the metabolites of bacteria to the combinatorial treatment, metabolomics was carried out. However, the data analysis is pending.

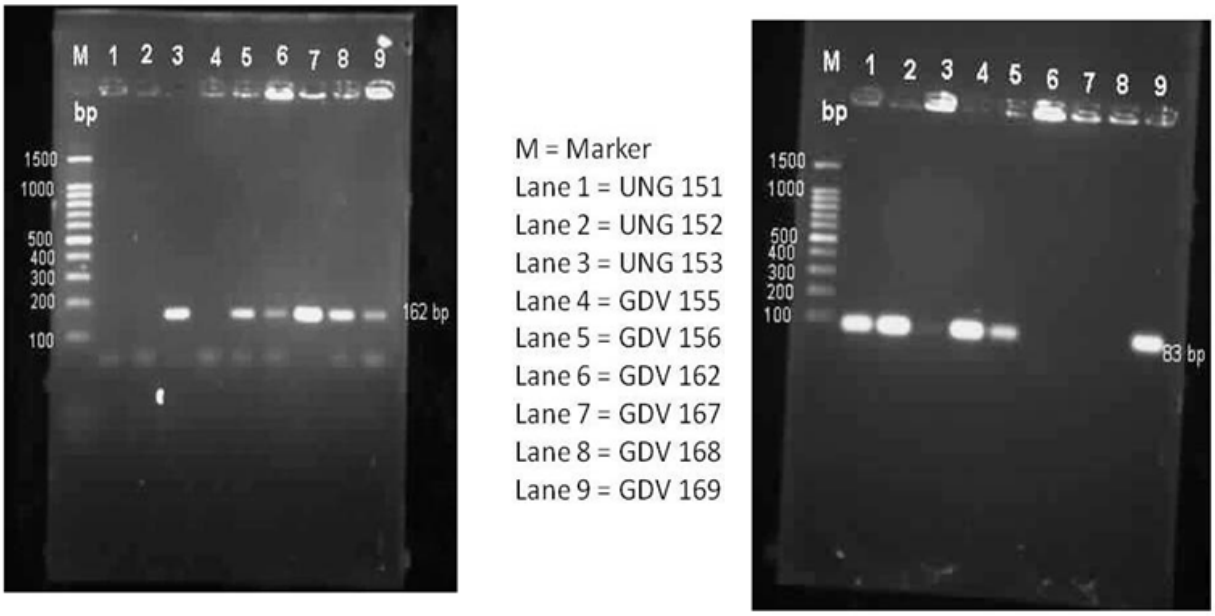

Fig. 1. Detection of mecA and pvlgenes of *S. aureus* isolates of bubaline mastitis.

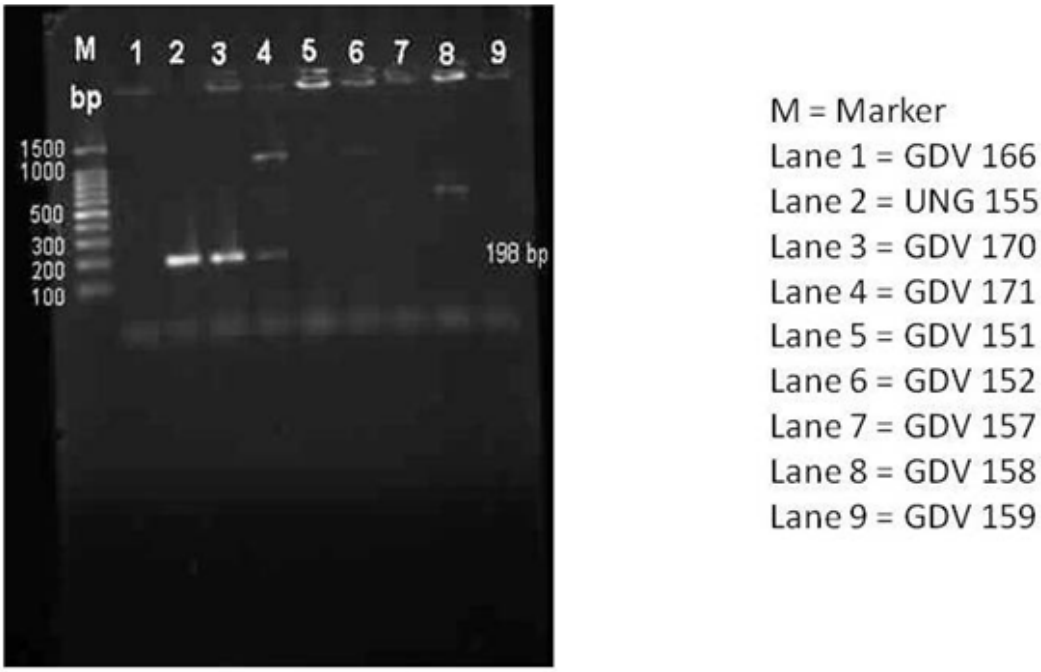

Fig. 2. Detection of icaD gene in *S. aureus* isolates of bubaline mastitis.

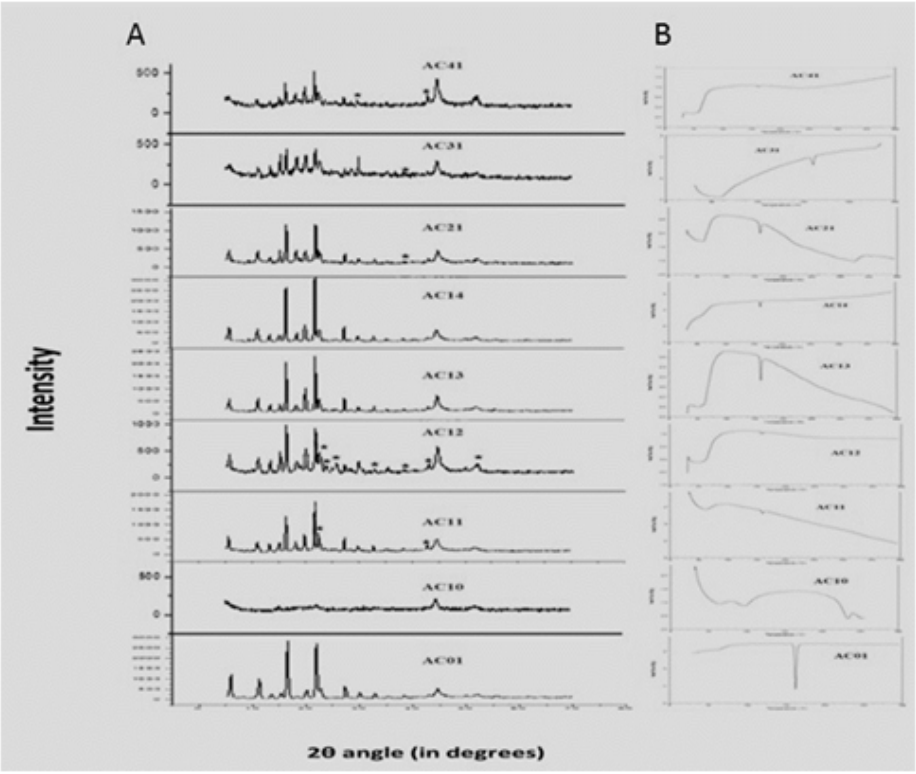

Fig. 3: Figure showing the (A) powder XRD peaks and (B) DSC curves of co-crystals

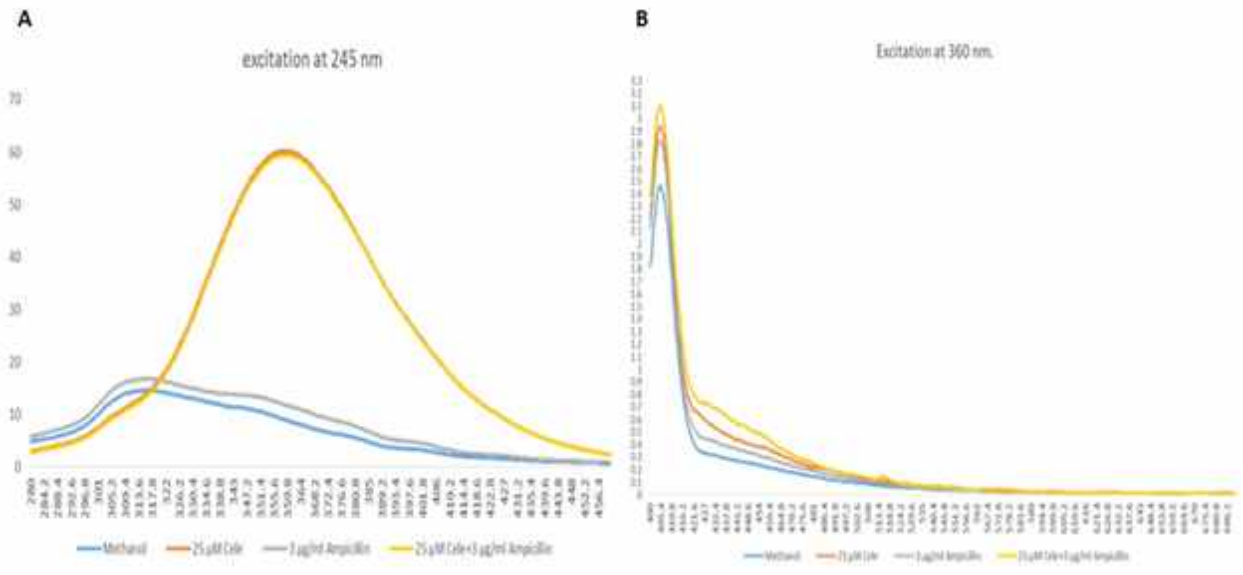

Fig. 4: Figure showing the emission of fluorescence by celecoxib and ampicillin and their combination (A) at 245 nm excitation and (B) at 360 nm excitation.

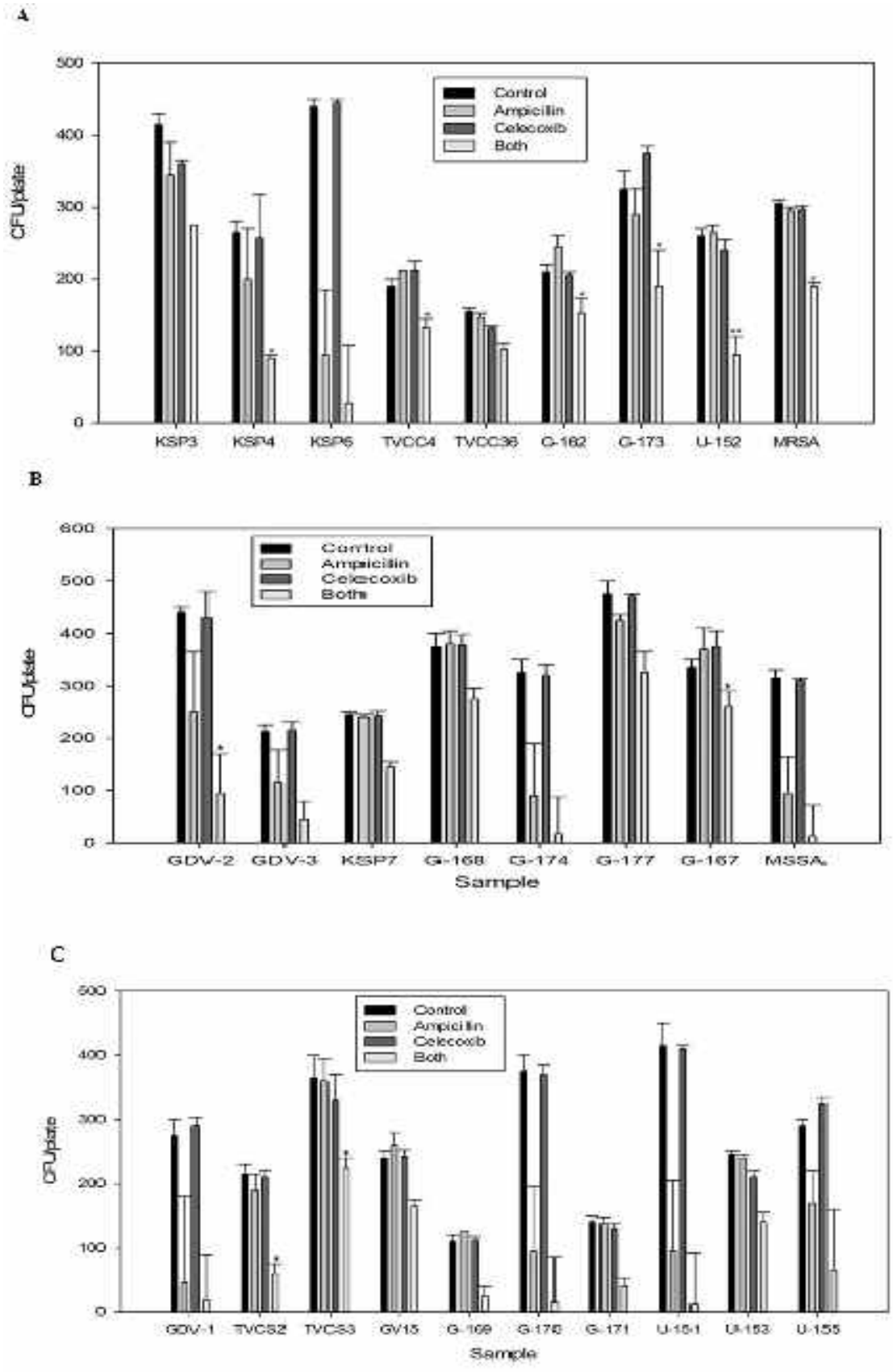

Fig. 5: Figure showing the bar graph drawn between various *S. aureus* isolates and CFU in different drug conditions. Effect of ampicillin + celecoxib combination on (A) highly resistant (B) intermediate resistant and (C) sensitive bacterial strains. *Significant decrease in combination (both) when compared with ampicillin alone.

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

Department of Biochemistry, School of Life Sciences, University of Hyderabad, and Directorate of Poultry Research, Hyderabad

Principal Investigator	G. Ravi Kumar	Assistant Professor
PhD Students	Ravinder Kandi	Senior Research Fellow
	Ram Babu Undi	Senior Research Fellow
	Itishri Sahu	Senior Research Fellow
	Sanjeev Raghuwanshi	Junior Research Fellow
	K. Narasiah	Junior Research Fellow
Other Members	S. P. Venkata Subbiah	Project Assistant
	P. Uma	Project Assistant
	Shilpa Sarvothman	Project Assistant
	P. Satya Ratan	Project Student
	K. Hanuma Kumar	Project Fellow, NIAB (Upto Aug 2014)
Collaborators	Dr. Madhuri Subbiah	NIAB, Hyderabad
	Dr. T. R. Kannaki	DPR, Hyderabad

Objectives

Toll-like receptor (TLR) activation addresses and reinforces both arms of immune response, innate and adaptive, leading to prompt a specific response against pathogens. In this study, non-specific immune mechanisms will be studied because they include the innate or inherent ways in which the chicken resists disease. This protective system is often not considered when designing a poultry health program. Many programs tend to rely primarily on vaccinations and/or antibiotics to maintain flock health. The narrowing base of the genetic stock may lead to a sensitivity of the remaining stock to new diseases that could destroy a genetically uniform population. The importance of non-specific immune mechanisms should be realized. With this understanding, our objective is dissection of the functions of TLR4-MyD88 (Myeloid differentiation primary response gene 88) dependent and independent pathways that poultry innate immune cells activate in distinct signaling pathways.

Summary of work done until the beginning of this reporting year (upto March 31, 2014)

We identified the TLR-4 pathway regulated gene expression in PBMCs of chicken breeds of Broiler (Cobb), Aseel, Dahlem Red and Ghagus after treating with LPS. Different genes from TLR-2 induces MyD88-dependent and MyD88-independent pathways. They are TLR4, MyD88, TNF receptor associated factor 6 (TRAF6), TIR domain containing adapter inducing interferon beta (TRIF), the transcription factors nuclear factor kappa B (NFkB), interferon regulatory factor 7 (IRF7) and interferon beta (IFN).

From our study, we identified that two pathways are crucial for TLR mediated innate immune responses in chicken mononuclear cells. The pathways are MyD88-dependent and TRIF-dependent pathways and we also identified functional TRIF-dependent pathway in Aseel and Ghagus breeds. Based on our results, it is concluded that Aseel breed is comparatively more resistant, Ghagus and broilers are moderately resistant and Dahlem Red is comparatively more susceptible to bacterial infections.

Details of progress made in the current reporting year (April 1, 2014 - March 31, 2015)

Project 1: Investigating the role of TLR-4 signaling mediated bacterial disease resistance in Indian poultry

The TLRs that sense the microbial pathogens are important components of host immune system. TLRs play key roles in the innate defence mechanism against pathogens, in the development of adaptive immunity, and are possibly the major determinants of the susceptibility to infections. To study the resistance pattern in different breeds of chicken, a comprehensive understanding of TLR4 signaling pathways is required. We investigated the TLR-4 pathway regulated gene expressions in PBMCs of chicken breeds of Broiler (Cobb), Aseel, Dahlem Red and Ghagus upon LPS treatment using quantitative RT-PCR approach. Several genes were found to be up regulated in both TLR-induced MyD88-dependent and MyD88-independent pathways. These genes include TLR4, MyD88, TRAF6 and TRIF, and the transcription factors NFkB, IRF7 and IFN β. We have also studied inflammatory cytokines such as IL2, IL6, IL8, IL1 β and TNF α to further understand the downstream signaling of TLR4 pathway. These results showed that higher expression of TLR signaling activation via both MyD88-dependent and TRIF-dependent pathways are

more beneficial to chicken mononuclear cells mediated innate immunity. We observed TRIF dependent pathway in Aseel and Ghagus breeds. Our results are in concurrent with general observation that Aseel breed is comparatively more resistant, Ghagus and broilers are moderately resistant and Dahlem Red is comparatively more susceptible to bacterial infections.

Summary

The inflammatory cytokines such as IL2, IL6, IL8, IL1 beta and TNF α have shown uniform up regulation in Aseel and Ghagus. Broiler also showed up regulation of all cytokines except IFN- β. On the other hand, Dahlem red failed to show up regulation of inflammatory cytokines (Fig.1). To know the morphological changes we observed the cells under scanning Electron microscope. After treating the cells with LPS, there is an effect on cell morphology and on its surface. When compared with control cells, there is an increase in granularity, cell size and identified heavy ridges, rough surface and aggregation of cells in treated samples.

Publications

1. Karnati HK, Pasupuleti SR, Kandi R, Undi RB, Sahu I, Kannaki TR, Subbiah M, Gutti RK (2015). TLR-4 signalling pathway: MyD88 independent pathway up-regulation in chicken breeds upon LPS treatment. Veterinary Research Communications. 39: 73 - 78.

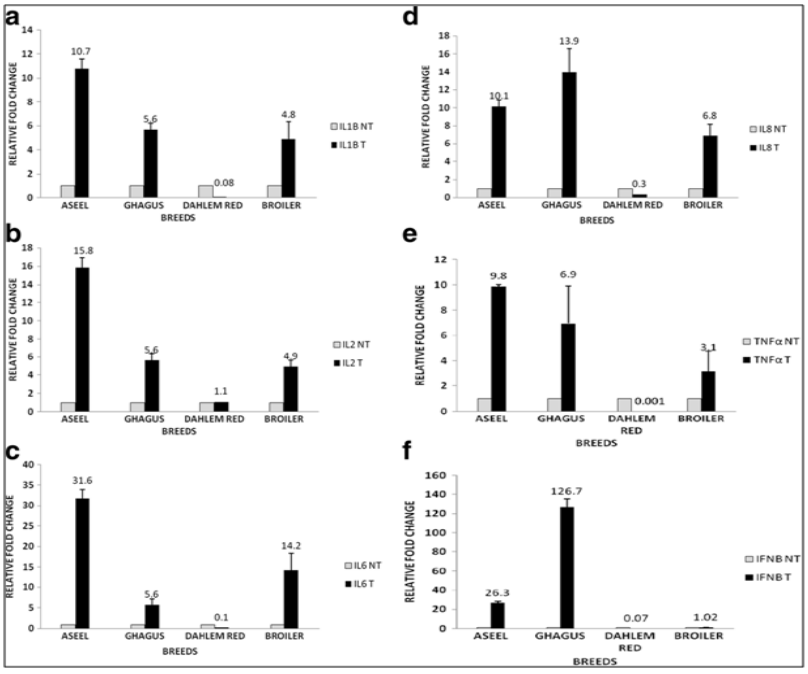

Fig. 1a. Expression of inflammatory cytokines in different poultry breeds. A. IL1β B. IL2 C. IL6 D. IL8 E. TNFα F. IFNβ. Bars represent means±SD of three independent experiments with (*p<0.01). Values were normalized against β-Actin, and LPS treated (T) mRNA values were expressed as relative expression compared to nontreated (NT) sample.

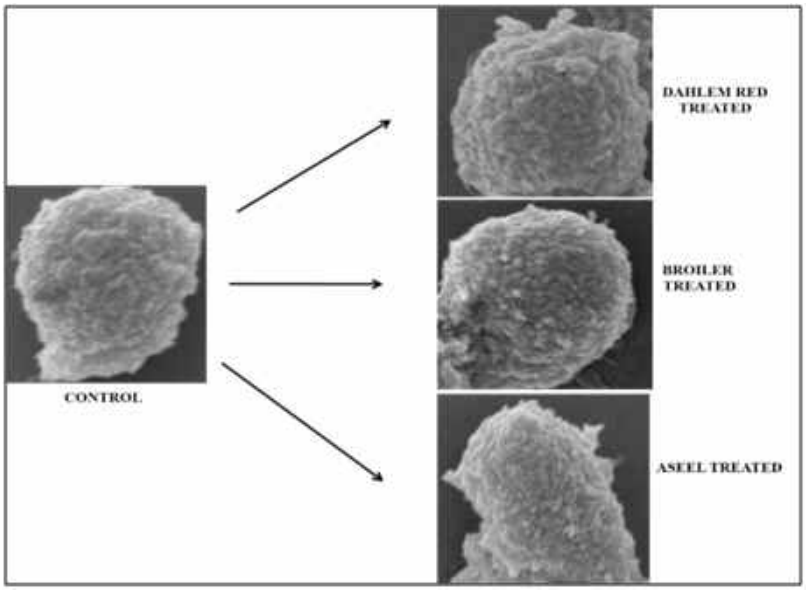

Fig. 1 b. Scanning electron micrographs of PBMCs treated with LPS. PBMCs were isolated from the Dahlem, Broiler and Aseel chicken breeds and treated with LPS (10µg/ml) for 2 hrs at 39°C. These cells were fixed in glutaraldehyde, dehydrated, covered in gold and observed under a scanning electron microscope.

Development and Validation of an Immunoassay for the Screening of Leptospirosis

Department of Animal Biology, University of Hyderabad

Principal Investigator	Manjula Sritharan	Professor, HCU
PhD Students	Reetika Chaurasia	
Collaborators	Dr. Anand Srivastava	NIAB, Hyderabad

Objectives

HbpA is a hemin-binding protein expressed only by pathogenic Leptospira spp. Its usefulness as a candidate antigen in the screening for leptospirosis led us to develop a simple-to-use diagnostic device. The following are the two major objectives:

a) Development of an ELISA-based system for the detection of antibodies against HbpA and sphingomyelinase(s).

b) Development of a lateral flow device for the detection of anti-HbpA antibodies in the serum of suspected cases of leptospirosis.

Summary of the work done till March 2015

Upon analysis of the full length HbpA, a suitable fragment rHbpA-F3 of size 34 kDa was chosen as an antigen based on the highest number of B cell epitopes present in the region. The gene, encoding this protein was cloned into pET28a (+) and extensive optimization for expression and purification of rHbpA-F3 protein was done. ELISA was standardized using this antigen and both bovine and human serum samples from suspected cases of leptospirosis were screened for anti-HbpA antibodies. While the major focus was on HbpA as an antigen for the development of a diagnostic kit, evaluation of rSph3 (sphingomyelinase) has been done. The protein, purified to homogeneity was tested as an antigen in ELISA with both bovine and human samples.

The performance of HbpA-ELISA was highly promising, with high titres seen in suspected cases as compared to the low levels of antibodies in the healthy controls. The results were compared with the microscopic agglutination test and further, comparison with another ELISA-based test using the well-established antigen LipL41 was suggested. In addition, the antigen was tested for its performance in a lateral flow device.

Details of progress made in the current reporting year (April 1, 2014 – March 31, 2015):

Earlier, HbpA-ELISA was done with bovine serum samples. The titres of anti-HbpA antibodies were high in both MAT positive and negative samples. This discrepancy with MAT as the gold standard was due to the fact that only a specific number of serovars were included for the study. In MAT negative samples, it was highly likely that this was so because of the absence of the specific infecting serovar in the assay. Hence, LipL41 ELISA was included for comparing the data obtained in HbpA-ELISA.

Cloning, expression and purification of rLipL41 were completed. ELISA was performed with bovine serum samples (103) and the titres were compared with that achieved with rHbpA as an antigen (Fig. 1).

Human serum samples (131) were screened using HbpA and Sph3 as antigens (Fig. 2). Table 1 shows the statistical analysis of the findings. It can be seen that the performance in human samples was superior to that seen with bovine samples. Fig. 3 shows the lateral flow device that detected antibodies against HbpA in a MAT-positive human serum sample.

Summary

Screening of bovine and human serum samples for anti-HbpA antibodies using ELISA was highly promising. While the sensitivity was good with both bovine and human serum samples, the specificity was low when compared to MAT. However, when compared to LipL41, there was a marked improvement. As also noted by others, MAT though specific does not identify cases when the specific serovar is omitted in the panel of serovars. Hence ELISA-based screening using two antigens can be used. Ongoing work is being done with the human serum samples with LipL41. The results are highly promising with the lateral flow device. Several samples have been screened. Work is ongoing for the refinement of the device.

Table 1: Statistical analysis of HbpA-ELISA, LipL-ELISA and the combined antigens versus MAT

Parameter	rHbpA-F3	rSph3	rHbpA-F3+rSph3
Sensitivity (95% CI)	**91** (82.99 - 96.44)	**92** (85.56 – 97.21)	**96** (89.54 – 99.19)
Specificity (95% CI)	**76** (61.83 - 86.93)	**90** (78.17 – 96.63)	**78** (64.03 – 88.46)
Positive predictive value (95% CI)	**86** (76.89 – 92.57)	**94** (86.00 – 97.92)	**88** (78.96 – 93.66)
Negative predictive value (95% CI)	**84** (70.54 – 93.48)	**88** (76.12 – 95.53)	**93** (80.49 – 98.42)

Degree of agreement among the three antigen-based ELISA tests is evaluated by Kappa statistical analysis using MedCal software (Version 11.5.0.0).

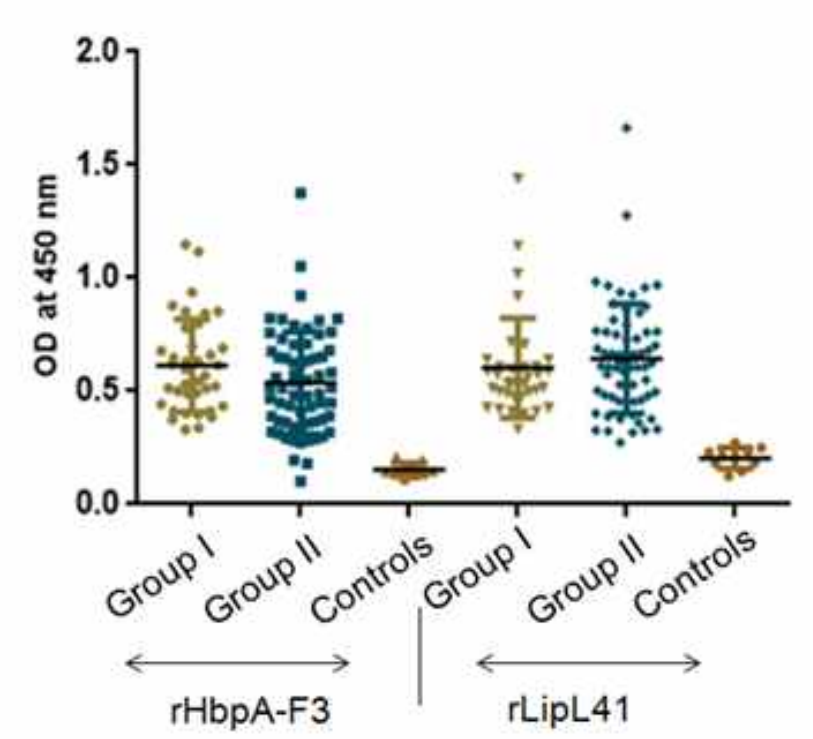

Fig. 1. Performance of HbpA-ELISA vs LipL41-ELISA in the screening of bovine samples. Group I and II represent serum from cattle suspected of leptospirosis that tested MAT positive and negative respectively. The controls represent serum from healthy animals.

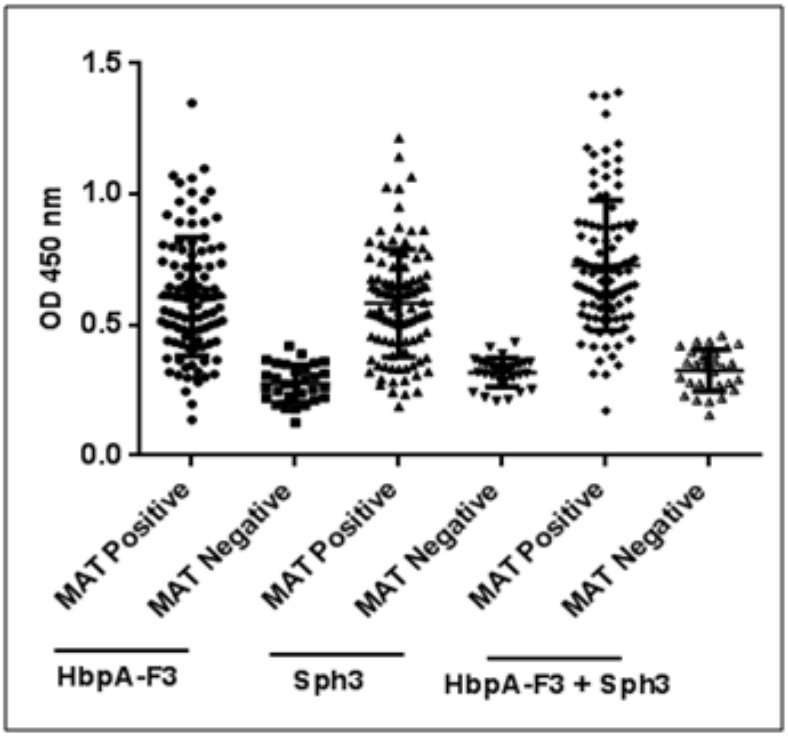

Fig. 2. ELISA-based detection of anti-HbpA and anti-Sph3 antibodies in the serum of patients suspected of leptospirosis. ELISA was performed with three antigens, namely rHbpA-F3, rSph3 and a mixture of both the antigens.

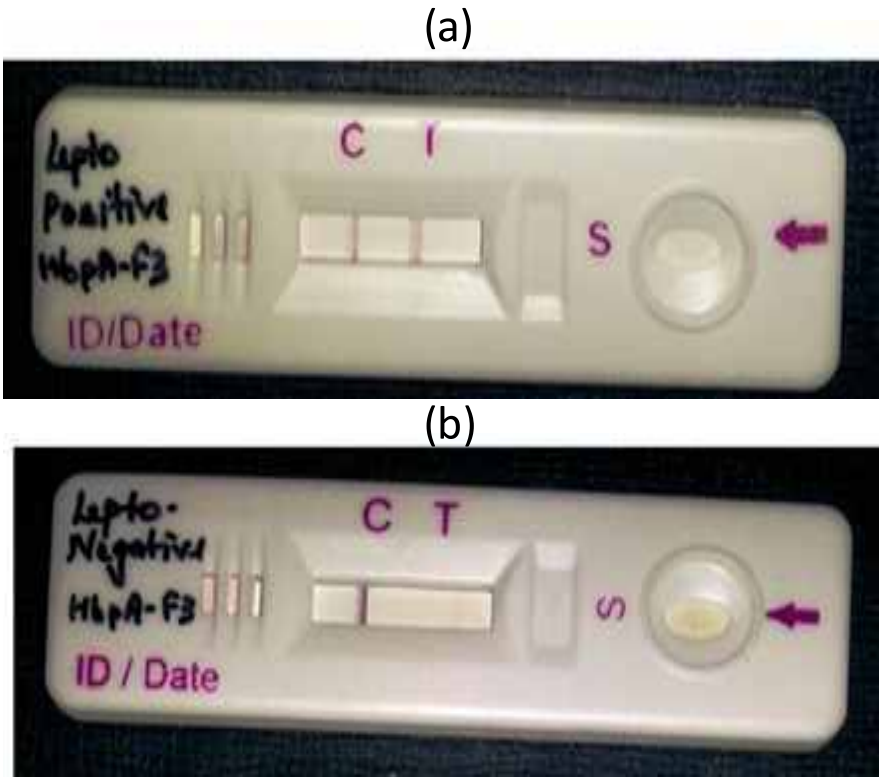

Fig. 3. Lateral flow device for detection of anti-HbpA antibodies. Panel (a) is positive showing both the development of the lines of both control (C) and (T) while Panel (b) represents a MAT negative sample that was also negative for anti-HbpA antibodies.

PUBLICATIONS
(with NIAB affiliation listed)

A. Publications in 2014:

1. Latha TS, Reddy MC, Durbaka PV, Rachamallu A, Reddanna P and Lomada D (2014). γδ T cell-mediated immune responses in disease and therapy. Frontiers in Immunology 5: 571.

2. Muthuchelvan D, De A, Debnath B, Choudhary D, Venkatesan G, Rajak KK, Sudhakar SB, Himadri D, Pandey AB and Parida S (2014). Molecular characterisation of peste-des-petits ruminants virus (PPRV) isolated from an outbreak in the Indo-Bangladesh border of Tripura state of North-East India. Veterinary Microbiology 174: 591 - 595.

3. Kumar KS*, Babu A*, Sundarapandian G, Roy P, Thangavelu A, Kumar KS, Arumugam R, Chandran ND, Muniraju M, Mahapatra M, Banyard AC, Manohar BM and Parida S (2014). Molecular characterisation of Lineage IV Peste des petits ruminants virus using multi gene sequence data. Veterinary Microbiology 174: 39 - 49. (* Equal contribution)

4. Rao SB, Gupta VK, Kumar M, Hegde NR, Splitter GA, Reddanna P and Radhakrishnan GK (2014). Draft genome sequence of the field isolate *Brucella melitensis* strain Bm IND-1 from India. Genome Announcements 2: e00497-14.

B. Publications in 2015:

1. Kallubai M, Rachamallu A, Yeggoni DP and Subramanyam R (2015). Comparative binding mechanism of lupeol compounds with plasma proteins and its pharmacological importance. Molecular BioSystems 11: 1172 - 1183.

2. Karnati HK, Pasupuleti SR, Kandi R, Undi RB, Sahu I, Kannaki TR, Subbiah M, Gutti RK (2015). TLR-4 signalling pathway: MyD88 independent pathway up-regulation in chicken breeds upon LPS treatment. Veterinary Research Communicaitons 39: 73 - 78.

3. Reddy KK, Vidya Rajan VK, Gupta A, Aparoy P and Reddanna P (2015) Exploration of binding site pattern in arachidonic acid metabolizing enzymes, Cyclooxygenases and Lipoxygenases. BMC Research Notes 8: 152.

4. Singh VP, Gurunathan C, Singh S, Singh B, Jyothi Lakshmi B, Mishra AP, Kumar S (2015) Genetic deletion of Wdr13 improves metabolic phenotype of Leprdb/dbmice by modulating AP1 and PPARγ target genes. Diabetologia 58: 384 - 392.

LECTURES, SEMINARS/ PRESENTATIONS AT NIAB

DISTINGUISHED LECTURES

"*Computational Approaches to Biological Systems"*by **Dr. Jan T Kim**, Head of Bioinformatics at the Pirbright Institute, Guildford, United Kingdom on 5th March 2014 in the NIAB Auditorium.

"Genetic determinants of antibody mediated protection in FMD" by **Dr. Mana Mahapatra**, Sr. Scientist , Pirbright Institute, UK on 20th March 2014 in the NIAB Auditorium.

"Recent Concepts of Ovarian Follicle Selection in Monovulatory Species" by **Prof. Mohd Amin Beg**, Professor and Head, Fundamental and Applied Biology Group, King Fahd Medical Research Center, King Abdulaziz University, PO Box 80216, Jeddah 21589, Kingdom of Saudi Arabia on 13th June 2014 in the NIAB Auditorium.

"The role of large animal models in Transplantation/ Translational - Research - Special focus on MHC inbred and gene KO/Tg miniature swine" by **Prof. Kazuhiko Yamada**, Professor and Director, Center for Advanced Biomedical Science, and Swine Research, Kagoshima University, Japan on 4th September 2014 in the NIAB Auditorium

"Quantification of FMDV replicon-derived RNA replication by live-cell fluorescence imaging" by **Dr. UdayPathania**, Wellcome Trust Research Fellow, University of Saint Andrews, UK on 23rd September 2014 in the NIAB Auditorium.

"*Better Vaccines for Brucellosis*" by **Dr. Ramesh Vemulapalli,** Department Head & Professor of Veterinary Immunology/Microbiology, Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, Indiana, USA on 18th November 2014 in the NIAB Auditorium.

"Updates on current research for FMD and PPR: use of reverse genetics techniques to improve existing vaccines" by **Prof. Satya Parida**, Head, Vaccine Differentiation Group, Pirbright Institute, Pirbright, UK, on 11th December 2014 in the NIAB Auditorium.

"Rabies and the lyssaviruses: Ancient enemies, new threats and future perspectives" by **Dr. Ashley C Banyard,** Wildlife Zoonoses and Vector Borne Disease Research Group, Department of Virology, Animal and Plant Health Agency, Woodham Lane, Weybridge, Surrey, UK on 17th December 2014 in the NIAB Auditorium.

"*Oxylipid signaling in host-pathogen interaction*" by **Prof. Hartmut Kuhn**, Professor, Institute of Biochemistry, Charite-University Medicine Berlin, Germany on 27th February 2015 in the NIAB Auditorium.

SEMINAR SERIES

- *"Genetic and epigenetic regulation underlies mammalian development and reproduction"* by **Dr. Ajeet Pratap Singh**, Chromatin and Gene Expression Group, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA on 19th May 2014 in the NIAB Auditorium.
- *"Biosafety and Biorisk Management in Laboratories"* by **Dr. Gopal Pande**, Chief Scientist, CSIR Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007 on 3rd June 2014 in the NIAB Auditorium.
- *"Promoting Innovations in Individuals, Start-ups and MSMEs (PRISM)"* by **Dr. Ramanuj Banerjee**, Scientist D, Department of Science & Industrial Research, Ministry of Science & Technology, Govt. of India on 5th June 2014 in the NIAB Auditorium.
- *"Novel Oocyte Specific Proteins are Associated with Early Embryonic Development"* by **Dr. Monika Sachdev**, Senior Scientist, In-charge Transgenic Facility, Division of Endocrinology, Central Drug Research Institute, Sitapur Road, Lucknow-226 031 on 13th June 2014 in the NIAB Auditorium.
- *"BALT development and augmentation of hyperoxic lung injury in mice deficient in NQO1 and NQO2"* by **Dr. LabanyamoyKole**, Scientist, VINS Bioproducts Ltd., EsshayHouse,Road No.3, Banjara Hills, Hyderabad, Telangana-500034 on 25th July 2014 in the NIAB Auditorium.
- *"cAMP-induced phosphorylation of the 26S proteasome enhances degradation of misfolded proteins"* by **Dr. SudarsanareddyLokireddy**, Department of Cell Biology, Harvard Medical School, Boston, MA 02115 on 11th August 2014 in the NIAB conference room.
- *"Vaccine Development"* by **Dr. S. N. Singh**, Managing Director, Biovet Private Limited, Bangalore on 16th September 2014 in the NIAB Auditorium.
- *"Understanding the Biology of Mycobacterial Molecular Chaperones"* by **Dr. CM Santosh Kumar**, Research Associate, National Centre for Cell Science (NCCS], Pune on 18th September 2014 in the NIAB Auditorium.
- *"Biosafety and Biosecurity for Human and Animal Health"* by **Dr. H. K. Pradhan**, Former Director, High Security Animal Disease Laboratory, Bhopal on 24th September, 2014, in the NIAB Auditorium.
- *"Functions of cell surface receptors in cell migration, cancer and host-pathogen interaction"* by **Dr. Ramprasad O.G**, Senior Research Associate, Indo-American Cancer Hospital and Research Institute, Hyderabad – 500 034 on 21st November, 2014, in the NIAB Auditorium.
- *"Genetic Engineering Strategies to Tailor Milk Composition for Human Needs"* by **Dr. Goetz Laible**, AgResearch, Ruakura Research Centre, New Zealand on 26th February, 2015 in the NIAB Auditorium.

TUESDAY SEMINARS/JOURNAL CLUB

1	Ms. Neena George Project Fellow NIAB, Hyderabad	The structure of *Plasmodium yoelii* merozoite surface protein 19, antibody specificity and implications for Malaria Vaccine design	3rd June 2014
2	Ms. Bindu Bhargavi Project Fellow NIAB, Hyderabad	PPAR -mediated increase in glucose availability sustains chronic *Brucella abortus* infection in alternatively activated macrophages.	17th June 2014
3	Mr. Naveen Gujjar Project Fellow NIAB, Hyderabad	Rational design of thermostable vaccines by engineered peptide-induced virus self - biomineralization under physiological conditions	24th June 2014
4	Mr. Akshay Joshi Project Fellow NIAB, Hyderabad	Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage	1st July 2014
5	Dr. Anil Kumar Kotha Research Associate NIAB, Hyderabad	NCoR repression of LXRs restricts macrophage biosynthesis of insulin- sensitizing omega 3 fatty acids	8th July 2014
6	Ms. Swathi Merugu Project Fellow NIAB, Hyderabad	Evaluation of sperm sex-sorting method using flow cytometry in hanwoo (Korean native cattle)	5th August 2014
7	Ms. S Sriravali Project Fellow NIAB, Hyderabad	Anti-Inflammatory mechanism of poly unsaturated fatty acids in *Helicobacter pylori*-infected gastric epithelial cells	12th August 2014
8	Ms. Rakhi Harne Project Fellow NIAB, Hyderabad	Hemoglobin digestion in blood- feeding ticks: mapping a multipeptidase pathway by functional proteomics	26th August 2014
9	Dr. Himabindu Gali Research Associate NIAB, Hyderabad	Induction of mouse germ-cell fate by transcription factors *in vitro*	2nd September 2014
10	Dr. Padmaja Jakka Project Fellow NIAB, Hyderabad	Inflammatory caspases are innate immune receptors for intracellular LPS	30th September 2014
11	Dr. Abhijit Deshmukh Scientist (Inspire Fellow) NIAB, Hyderabad	Mutations in ORC1, encoding the largest subunit of the origin recognition complex, cause microcephalic primordial dwarfism resembling Meier-Gorlin syndrome	14th October 2014
12	Dr. Aparna Rachamallu Women Scientist NIAB, Hyderbad	RNA viruses promote activation of the NLRP3 inflammasome through a RIP1-RIP3-DRP1 signaling pathway	5th November 2014

13	Ms. Bindu Bhargavi Project Fellow NIAB, Hyderabad	The *Brucella* TIR domain containing proteins BtpA and BtpB have a structural WxxxE motif important for protection against microtubule depolymerisation	18th November 2014
14	Dr. Dileep Kumar Reddy. V Research Associate NIAB, Hyderabad	Nanowire-based single-cell endoscopy	2nd December 2014
15	Mr. Naveen Gujjar Project Fellow NIAB, Hyderabad	Biological characterization and complete genome sequence of "Komarov", a mesogenicvaccinal strain of Newcastle disease virus	16th December 2014
16	Ms. Rakhi Harne Project Fellow NIAB, Hyderabad	Production of Female bovine embroys with Sex-Sorted sperm using intracytoplasmic sperm injection: Efficiency and *in vitro* developmental competence.	3rd February 2015
17	Dr. Vasundhra Bhandari Research Associate, NIAB, Hyderabad	A new antibiotic kills pathogens without detectable resistance	10th March 2015
18	Ms. Hita Project Fellow NIAB, Hyderabad	Covalent bond or noncovalent bond: A supramolecular strategy for the construction of chemically synthesized vaccines	31st March 2015

International Conference on Host-Pathogen Interactions (ICHPI)

12th - 15th July 2014, School of Life Sciences, University of Hyderabad

International Conference on Host Pathogen Interactions (ICHPI) organized by NIAB in association with University of Hyderabad and CR Rao Advanced Institute of Mathematics, Statistics and Computer Science from 12th to 15th July, 2014.

An understanding of the interactions between host and pathogen is very essential for effective prevention and control of infectious diseases. Interdisciplinary approaches are needed for deciphering the interactions between the host and the pathogen. This international conference on host-pathogen interaction (ICHPI) focused on the basic and advance studies of host-pathogen interactions with respect to livestock and poultry including zoonotic infections. ICHPI was conducted mainly to create a platform for scientists, post-docs, and students along with leading industries in veterinary health to gather under one roof and share the cutting edge research findings. The sessions in the conference engaged a variety of disciplines in molecular biology, microbiology, immunology, genetics and genomics, related to host-pathogen interactions. In addition, ICHPI also focused on brainstorming sessions organized on the emerging issues of infectious diseases which are of national and international importance.

Around 400 delegates, speakers, panelists, chair persons and sponsors from different countries attended the conference and provided great intellectual and social interaction for the participants.

The conference included 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)

Alongside hundreds of illuminating presentations on host-pathogen interactions, the FMD brainstorming session was focused on identifying and prioritising research areas, finding collaborative partners and discussing practical difficulties and possible solutions. The fact that FMD gains primacy in discussions at the International Conference on Host-Pathogen interactions attaches a sense of urgency to the need for its absolute control. The recommendations of the session provided key pointers to our policy makers to achieve total control of the disease in the subcontinent.

Based on the panel discussion happened at ICHPI issues related to biosafety and biosecurity procedures faced by various stakeholders was also highlighted. Experts stressed on issues of information and awareness; implementation of regulations and guidelines, education and outreach, capacity building, risk assessment and risk management. In order to increase awareness and training on safety & security in research institutes, academia & industry, it was strongly recommended to the Government of India to have a fresh look at the implementation of the existing laws governing the biosafety and biosecurity to enforce effective bio-risk management in the country and filling in necessary gaps in capacity building. It was further suggested to set up a Bio-risk Management Training Academy (BRMTA), which would provide India an advantage and understanding on bio-risk management in the biotechnology sector. An article emphasizing the need for BRMTA has been published in CBW Magazine by Dr. BM Gandhi with a title as "*Recent Incidences of Global Biosafety and Biosecurity Lapses in Laboratories Need Relook at Implementation of National Policies* by BM Gandhi (July-December, 2014)".

During brainstorming Session on Antimicrobial Resistance significance of rapidly spreading antimicrobial resistance issue in India was widely discussed in various fields such as human health, veterinary science and environment. A dedicated session was organized on 'One Health' concept were eradication of endemic and emerging zoonotic diseases by replacing the pathogen-centric approach with a holistic human-animal-environment approach was thoroughly discussed. Public health experts and scientists present at ICHPI looking at a variety of zoonotic pathogens – bugs which jump from animals to humans – felt that India urgently needs to put in place a 'One Health' programme that focuses on factors beyond pathogens.

Deputations abroad of NIAB personnel

Name and Designation	Period	Country of Visit and purpose
Prof. P. Reddanna Director	08.09.2014 to 16.09.2014	Freie University, Berlin, Germany to attend the on-site evaluation of the Indo-German Research Training Group (IRTG 1673) on "Functional Molecular Infection Epidemiology" at the Veterinary Campus Duppel of Freie University. The Pirbright Institute, UK to attend the Centenary Celebration presentations, to sign MoU between NIAB & Pirbright Institute and to visitThe School ofVeterinary Medicine, University of Nottingham, UK in view of the ongoing collaboration between NIAB & School of Veterinary Medicine as part of BBSRC-India Partnering Award.
Dr. Satish Kumar Scientist-H	25.07.2014 to 03.08.2014	China for attending Business Meeting of ISAG –FAO Advisory Group and to attend ISAG conference
Dr. Syed Faisal Ramalingamswamy Fellow	18.10.2014 to 30.10.2014	USA to participate in the meeting of International Conference and to present paper entitled "Immunostimulatory and Conventional Liposome Adjuvants Induce Distinct Differences in the Magnitude, Qualirt and Kinetics of Innate and Adaptive Immune Responses"

IMPLEMENTATION OF THE RIGHT TO INFORMATION(RTI) ACT, 2005

Appellate Authority : Dr Girish K Radhakrishnan
Central Public Information Officer : Harjit Singh
Details about the RTI applications and appeals received in NIAB

As received under RTI Act 2005	Opening Balance as on 1.4.2014	Received during the year 2014-15			Disposed of during the year 2014-15				Closing Balance as on 31.3.2015
		Received directly	Received as transfer from other Public Authorities [u/s 6(3) of Act]	Total	Decisions where applications accepted / appeals upheld	Decisions where applications /appeals rejected	Transferred to other Public Authorities [u/s 6(3) of Act]	Total	
Applications	0	1	5	6	6	0	0	6	0
Appeals	0	0	Not applicable	0	0	0	Not applicable	0	0

ORGANIZATIONAL STRUCTURE

NIAB SOCIETY

Dr. Jitendra Singh
Hon'ble Minister of S & T & Earth Sciences, GoI.
President (Upto 09th Nov. 2014)

Dr. Harsh Vardhan
Hon'ble Minister of S & T & Earth Sciences, GoI.
President (w.e.f 10th Nov. 2014)

Dr. K. VijayRaghavan
Secretary, DBT, New Delhi

Dr. S. Ayyappan
Secretary, DARE, New Delhi

Ms. Anuradha Mitra
JS & FA, DBT, New Delhi
(Upto 14th Dec 2014)

Shri. J. B. Mohapatra
JS & FA, DBT, New Delhi
(w.e.f 15th Dec 2014)

Prof. Suresh S. Honnappagol
Commissioner, AH, New Delhi

Dr. A. S. Ninawe
Advisor, DBT, New Delhi

Prof. Ramakrishna Ramaswamy
Vice Chancellor, UOH, Hyderabad

Dr. Girish K. Radhakrishnan
Scientist D, NIAB, Hyderabad

Dr. V. A. Srinivasan
Indian Immunologicals, Hyderabad

Dr. M. P. G. Kurup, Bengaluru
(Upto 09th September 2014)

Dr. Vijay Kumar Taneja
Vice Chancellor, GADVASU, Ludhiana
(Upto 09th September 2014)

Dr. Lalji Singh
Vice Chancellor, BHU
(Upto 09th September 2014)

Dr. C S Prasad
Director, NIANP, Bengaluru
(Upto 09th September 2014)

Dr. S N Singh
MD, Biovet Private Ltd., Karnataka
(Upto 09th September 2014)

Dr. Satish Tongaonkar
Consultant Veterinary Biologicals, Pune
(Upto 09th September 2014)

Dr. H. K. Pradhan
WHO, New Delhi
(Upto 09th September 2014)

Dr. Satish Kumar
Scientist-H, NIAB Hyderabad
(upto 20th November 2014)

Dr. Shahid Jameel
Welcome Trust, Hyderabad
(w.e.f 10th September 2014)

Dr. R. N. K. Bamezai
JNU, New Delhi
(w.e.f 10th September 2014)

Dr. A. K. Srivastava
NDRI, Karnal
(w.e.f 10th September 2014)

Dr. K. T. Sampath, Ex-Director
NIANP, Bengaluru
(w.e.f 10th September 2014)

Dr. (Ms). Anuradha Acharya
Oscimum Bio Solutions, Hyderabad
(w.e.f 10th September 2014)

Dr. Suresh Poosala
BMS Preclinical R&D Veterinary
Science and Toxicology, Bengaluru
(w.e.f 10th September 2014)

Dr. S. K. Bandyopadhyay
Member, ASRB, New Delhi
(w.e.f 10th September 2014)

Prof. P. Reddanna
Director, NIAB
(Upto 30th Sep 2014)

Dr. J. Gowrishankar
Director-in-Charge, NIAB
(w.e.f 01st Oct 2014)

NIAB GOVERNING BODY

Dr. K. Vijay Raghavan
Secretary, DBT, New Delhi
Chairman

Dr. S. Ayyappan
Secretary, DARE, New Delhi

Ms. Anuradha Mitra
JS & FA, DBT, New Delhi
(Upto 14th Dec 2014)

Shri. J. B. Mohapatra
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(w.e.f 15th Dec 2014)

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(Upto 09th September 2014)

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(Upto 09th September 2014)

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(Upto 09th September 2014)

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WHO, New Delhi
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(upto 20th November 2014)

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(w.e.f 10th September 2014)

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(w.e.f 10th September 2014)

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(w.e.f 10th September 2014)

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(w.e.f 10th September 2014)

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Science and Toxicology, Bengaluru
(w.e.f 10th September 2014)

Dr. S. K. Bandyopadhyay
Member, ASRB, New Delhi
(w.e.f 10th September 2014)

Prof. P. Reddanna
Director, NIAB
(Upto 30th Sep 2014)

Dr. J. Gowrishankar
Director-in-Charge, NIAB
(w.e.f 01st Oct 2014)

NIAB FINANCE COMMITTEE

Till 10 Sep. 2014

Dr. K. VijayRaghavan
Secretary, DBT, New Delhi
Chairman

Ms. Anuradha Mitra
JS & FA, DBT, New Delhi
(Upto 14 Dec 2014)

Dr. A. S. Ninawe
Advisor, DBT, New Delhi

Dr. J. Gowrishankar
Director, CDFD

Dr. Durgadas P. Kasbekar
CDFD, Hyderabad

Prof. Ramakrishna Ramaswamy
Vice Chancellor, UOH, Hyderabad

Dr. H. K. Pradhan
WHO, New Delhi

Dr. K. T. Sampath, Former Director
NIANP, Bengaluru

Prof. P. Reddanna
Director, NIAB
Member Secretary
(Upto 30th Sep 2014)

Reconstituted w.e.f 11 Sep. 2014

Dr. K. VijayRaghavan
Secretary, DBT, New Delhi
Chairman

Shri. J. B. Mohapatra
JS & FA, DBT, New Delhi
(w.e.f 15 Dec 2014)

Dr. A. S. Ninawe
Advisor, DBT, New Delhi

Dr. J. Gowrishankar
Director, CDFD

Dr. Durgadas P. Kasbekar
CDFD, Hyderabad

Prof. Ramakrishna Ramaswamy
Vice Chancellor, UOH, Hyderabad

Dr. A. K. Srivastava
NDRI, Karnal

Dr. (Ms) Anuradha Acharya
Oscimum Bio Solutions, Hyderabad)

Dr. J. Gowrishankar
Director-in-Charge, NIAB
Member Secretary
(w.e.f 01st Oct 2014)

NIAB SCIENTIFIC ADVISORY COMMITTEE

Till 08th December 2014

Dr. Lalji Singh
Ex-Director, CCMB & Ex- VC, BHU,
Chairman

Dr. A.S. Ninawe
Adviser DBT, Govt of India

Dr. K.M.L Pathak
Deputy Director General (Animal Science),
Division of Animal Science, ICAR, New Delhi

Prof. David Hume
Director
Roslin Institute and Research

Dr. R. K. Singh, Director
National Research Centre on Equines

Dr. N. R. Hegde
Group Leader, Ella foundation

Dr. V. A. Srinivasan
Advisor, NDDB, Hyderabad

Prof. Jagan Pongubala
Dept of Animal Sciences
School of Life Sciences, UoH

Prof. C. Channa Reddy
Distinguished Professor
Dept of Veterinary & Biomedical Sciences,
Penn State University

Prof. P. Reddanna
Director, NIAB
(Upto 30 Sep 2014)
Member Secretary

Reconstituted w.e.f 09th December 2014

Dr. Lalji Singh
Ex-Director, CCMB & Ex- VC, BHU,
Chairman

Dr. A.S. Ninawe
Adviser DBT, Govt of India

Dr. K.M.L Pathak
Deputy Director General (Animal Science),
Division of Animal Science ICAR, New Delhi

Prof. Lothar H. Wieler
Institut fur Mikrobiologie und Tierseuchen, Berlin,
Germany

Prof. S. Ramaswamy
C-CAMP, Bengaluru

Dr. Subeer S. Majumdar
NII, New Delhi

Dr. S. N. Singh
Biovet, Bengaluru

Prof. R. Medhamurthy
IISC, Bengaluru

Mr. Deepak Kapur
Indovax, Gurgaon

Prof. G. Dhinakar Raj
TANUVAS, Chennai

Dr. J. Gowrishankar
Director-in-Charge, NIAB
(w.e.f 01 Oct 2014)
Member Secretary

NIAB BUILDING COMMITTEE

Till 12 February 2015

Dr. J. Gowrishankar
Director, CDFD
Chairman

Dr. A. K. Rawat
Director, DBT

Shri P.C. Singh
Dy Secretary, DBT

Shri B.L.N. Reddy
Superintending Engineer,
HMDA, Hyderabad

Shri Siddartha Reddy
University Engineer
University of Hyderabad

Prof. P. Reddanna
Director, NIAB

Shri V.H. Rao
Senior Consultant
(Upto 31 July 2014)
Member Convener

Reconstituted w.e.f 13 February 2015

Dr. J. Gowrishankar
Director, CDFD
Chairman

Dr. A. K. Rawat
Director, DBT

Shri P.C. Singh
Dy Secretary, DBT

Shri B.L.N. Reddy
Superintending Engineer, HMDA, Hyderabad

Dr. G. Sundararajan
Director, International Advanced Research Centre for Powder Metallurgy and New Materials (ARCI), Hyderabad

Dr. J. Gowrishankar
Director-in-Charge, NIAB

Mr. S. Ayub Basha
Staff Scientist-V (Engineering), CDFD

Shri. Harjit Singh
Sr. Manager, NIAB
Member Convener

Dr. G. Sundararajan, Director, ARCI, will chair the meeting of Building Committee till Dr. J. Gowrishankar is acting as Director- in-Charge of NIAB.

NIAB STAFF

Scientific

1.	Prof. P. Reddanna, PhD	Director (Up to 30th Sep. 2014)
2.	Dr. J. Gowrishankar, PhD	Director-in-Charge (W.e.f. 1st Oct. 2014)
3.	Dr. Satish Kumar, PhD	Scientist- H (Up to 20th Nov. 2014)
4.	Dr. Girish K. Radhakrishnan, PhD	Scientist- D
5.	Dr. Madhuri Subbiah, PhD	Scientist- C
6.	Dr. Anand Srivastava, PhD	Scientist- C
7.	Dr. Paresh Sharma, PhD	Scientist- C
8.	Dr. Sathya Velmurugan, PhD	Scientist -C
9.	Mr. Sarwar Azam	Scientist -B
10.	Prof. Satya Parida, PhD	Visiting Faculty
11.	Dr. Syed Faisal, PhD	Ramalingaswami Fellow
12.	Dr. Abhijit Deshmukh, PhD	Inspire Faculty
13.	Dr. Aparna Rachmallu, PhD	DST Women Scientist (W.e.f. 1st Oct. 2014)

Technical

1.	Mrs. 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 and Support Staff

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 N. Mhadeshwar	Manager (Stores & Purchase)
5.	Mr. V. Ramesh Babu	Service & Maint. Engineer
6.	Ms. K. 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. P. Ramesh	Office Attendant
12.	Mr. Jahid Hussain	Driver

Consultants

1.	Mr. V. H. Rao	Senior Consultant (up to 31st July 2014)
2.	Mr. V. Lachaiah	Consultant
3.	Mr. C.S. Murthy	Consultant (Instrumentation)
4.	Dr. George John	Consultant Advisor (up to 10th Sep 2014)

PICTURES GALLERY

Republic Day Celebrations at NIAB Site

Lecture on **Hindi Day** on "*DNA Fingerprinting"* by **Ms. Varsha Srivastava**, Staff Scientist, CDFD, Hyderabad on 24th September 2014 in the NIAB Auditorium.

Launching of "Stores & Purchase Management System (SPMS) Software

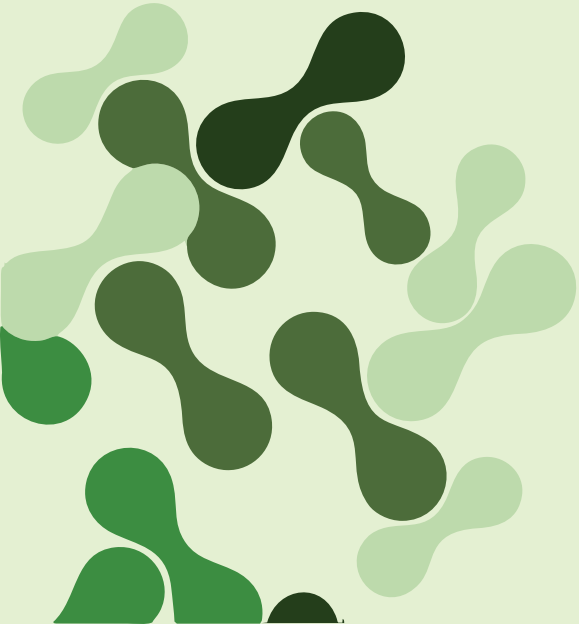
Store Purchase Management System (SPMS) Software was developed by Institute employees viz Shri Santosh Mhadeshwar, Manager (S&P) and Shri A. Harikrishna, Technical Officer. The software was launched by Dr J Gowrishankar, Director-in-Charge NIAB on 20th March 2015. Certificate of Appreciation were given to the employees by the Director-in-Charge for in-house development of the software.

"Swachhta Shapath" (Cleanliness Pledge) was administered to all the all the officers/officials on 2 October 2014 followed by cleanliness drive

Visit of Ms Anuradha Mitra, Financial Advisor, Ministry of Science & Technology, to NIAB on 25-08-2014

Visit of Tamil Nadu Agricultural University B. Tech (Biotechnology and Bioinformatics) students visited NIAB as a part of Institutional and Industrial Educational Tour on 19th and 20th September 2014.

AUDITED STATEMENT OF ACCOUNTS
2014-15



AUDITOR'S REPORT

28th April, 2015

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 2015 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 accounts.
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 2015 and
    b) In so far as it relates to the Income & Expenditure account excess of income over expenditure for the year ended on 31st March 2015.

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

Place: Hyderabad
Date: 28th April, 2015

[CH SATYANARAYANA]
Partner M No. 019092

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY,HYDERABAD
BALANCE SHEET AS ON 31st MARCH 2015

(Amount in Rs)

Particulars	Schedule	Current Year	Previous Year
CORPUS/CAPITAL FUND AND LIABILITIES			
Corpus / Capital Fund	1	36,88,87,939.00	29,03,40,484.00
Reserves and Surplus	2	1,85,10,505.52	23,32,293.22
Earmarked / Endowment funds	3	21,28,855.00	20,04,546.00
Secured Loans & Borrowings	4	-	-
Unsecured Loans & Borrowings	5	-	-
Differed Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	16,68,356.00	23,29,514.00
TOTAL		**39,11,95,655.52**	**29,70,06,837.22**
ASSETS			
Fixed Assets	8	36,04,34,175.00	27,80,78,152.00
Investments- From Earmarked / Endowment Funds	9	-	-
Investments - Others	10	-	-
Current Assets, Loans, Advances etc.	11	3,07,61,480.52	1,89,28,685.22
Miscellaneous Expenditure		-	-
TOTAL		**39,11,95,655.52**	**29,70,06,837.22**
Significant Accounting Policies	24		
Contingent Liabilities and Notes on Accounts	25		

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,HYDERABAD
Income And Expenditure Statement as on 31st MARCH 2015

(Amount in Rs)

Particulars	Schedule	Current Year		Previous Year	
INCOME					
Income from Sales/Services	12		-		-
Grants/Subsides	13		9,50,00,000.00		6,00,00,000.00
Fees/Subscriptions	14		-		-
Income from Investments	15		44,82,171.00		23,30,055.00
Income from Royalty, Publications etc.	16		-		-
Interest Earned	17		-		18,32,327.00
Other Income	18		5,19,610.00		4,38,584.00
Increase/(decrease) in stock of Finished goods and works-in-progress	19		-		-
TOTAL (A)			**10,00 ,01,781.00**		**6,46,00,966.00**
EXPENDITURE					
Establishment Expenses	20		2,19,03,120.00		1,72,98,727.00
Administrative Expenses etc.	21		6,18,92,358.70		4,88,08,751.00
Expenditure on Grants, Subsides etc.	22		-		-
Interest	23		-		-
Depreciation (Net Total at the year-end - corresponding to Schedule 8)		2,14,68,449.00		1,07,92,541.00	
Less: Transferred to Grants-in-Aid		2,14,68,449.00	-	1,07,92,541.00	
Provision For Salaries and other Expenses			28,090.00		5,56,759.00
TOTAL (B)			**8,38,23,568.70**		**6,66,64,237.00**
Balance being excess of Income over Expenditure (A-B)			1,61,78,212.30		- 20,63,271.00
Transfer to Special Reserve (Specify each)					
Transfer to/from General Reserve					
BALANCE BEING SURPLUS/(DEFLICT) CARRIED TO CORPUS/CAPITAL FUND					
SIGNIFICANT ACOUNTING POLICIES	24				
CONTINGENT LIABILITIES AND NOTES ON ACCOUNTS	25				

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,HYDERABAD

RECEIPTS AND PAYMENTS ACCOUNT FOR THE YEAR ENDED 31st MARCH 2015

(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)	2,19,03,120.00	1,72,98,727.00
b) Bank Balances			b) Administrative Expenses (corresponding to Schedule 21)	6,18,92,358.70	4,88,08,751.00
i) In current accounts	-	-			
ii) In deposit accounts	-	-			
iii) Savings accounts	20,32,060.22	49,53,618.22			
2. Grants Received			2. Payments made against funds for various projects		
a) From Government of India	19,50,00,000.00	18,00,00,000.00	(Name of the fund or project should be shown along		
b) From State government	-	-	with the particulars of payments made for each project)		
c) From other sources (details)	-	-	Projects (Annexure F)	58,09,718.00	5,27,972.00
(Grants for capital & revenue					
exp. To be shown separately)			3. Investments and deposits made		
			a) Out of Earmarked/Endowment funds	-	-
d) Projects (Annexure - C)	59,34,027.00	19,00,000.00	b) Out of Own Funds (Investments-Others)	17,50,00,000.00	19,00,00,000.00
3. Income on Investments from			4. Expenditure on Fixed Assets & Capital Work-in-Progress		
a) Earmarked/Endow. Funds	-	-	a) Purchases of Fixed Assets:		
b) Own Funds (Oth. Investment)	40,36,516.00	16,92,798.00	Books & Journals	84,680.00	4,97,459.00
c) Investments Encashed	17,50,00,000.00	19,00,00,000.00	Equipment -Lab/Office/Furniture	2,37,27,888.00	12,67,49,747.00
			b) Expenditure on Capital Work-in-Progress:	7,99,96,000.00	7,35,35,430.00
4. Interest Received					
a) On Bank deposits	4,45,655.00	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(Specify)					
a) Analysis Charges	-	-	6. Finance Charges (Interest)	-	-
6. Amount Borrowed	-	-			
7. Any Other Receipts(Give Details)			7. Other Payments (Specify)		
I-Remittances (Annexure-A)	40,20,024.00	24,32,522.00	Advances (Annexure-D)	3,32,44,101.00	2,25,53,041.00
			I-Remittances (Annexure-E)	40,20,024.00	24,32,522.00
CPF-SUB, Arrears and adv. Refund/GPF	6,01,000.00	4,74,000.00	CPF A/c / GPF A/c	6,01,000.00	4,74,000.00
Sundry Receipts	85,110.00	1,65,084.00	New Pension Scheme	7,73,558.00	6,02,500.00
Application Fee	1,39,500.00	2,73,500.00			
Provident Fund Salvage	-	-	8. Closing Balances		
Free Gifts - Donations	-	-	a) Cash in hand	-	-
Sale of Tender Forms	2,95,000.00	-	b) Bank Balances	-	-
Leave Salary-Pension Contribution	-	-	i) In current accounts	-	-
License Fee	-	-	ii) In deposit accounts		
Welfare Fund	-	-	iii) Savings accounts	32,27,520.52	20,32,060.22
NPS	7,73,558.00	6,02,500.00			
Advance/Refunds/Recovery/Adj					
(Annexure-B)	2,19,17,518.00	10,05,48,603.00			
TOTAL	**41,02,79,968.22**	**48,55,12,209.22**	**TOTAL**	**41,02,79,968.22**	**48,55,12,209.22**

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 MARCH 2015

(Amount - Rs.)

Particulars	Current Year		Previous Year	
SCHEDULE 1 - CORPUS/CAPITAL FUND				
Balance as at the beginning of the year		29,03,40,484.00		18,11,03,125.00
Add : Contribution towards Corpus/Capital Fund				
NIAB Core - Plan (Non-Recurring)	10,00,00,000.00		12,00,00,000.00	
Capitalised portion of Capital Expenditure of projects	15,903.00		29,900.00	
Others (A.P. Govt. allotted 100 Acres land at free of Cost)	1.00	10,00,15,904.00	-	12,00,29,900.00
Less : Lump Sum Depreciation	-		-	
Less : Depreciation For the Year 2014-2015	2,14,68,449.00	2,14,68,449.00	1,07,92,541.00	1,07,92,541.00
Add : Balance of net income/(Expenditure) transferred from the income and Expenditure Account				
BALANCE AS AT THE YEAR - END		**36,88,87,939.00**		**29,03,40,484.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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	23,32,293.22		43,95,564.22	
Addition during the year	1,61,78,212.30	-	-20,63,271.00	-
Less : Deductions during the year	-	1,85,10,505.52	-	23,32,293.22
TOTAL		**1,85,10,505.52**		**23,32,293.22**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	Current Year		Previous Year	
SCHEDULE 3 - EARMARKED/ENDOWMENT FUNDS :				
(Refer Annexures)				
a) Opening balance of the Funds		20,04,546.00		6,32,518.00
(b) Additions to the Funds :				
i. Donations /grants	59,34,027.00		19,00,000.00	
ii. Income from investments made on account of funds	-		-	
iii. Other additions	-	59,34,027.00	-	19,00,000.00
TOTAL (a+b)		**79,38,573.00**		**25,32,518.00**
(c) Utilisation/Expenditure towards objective of funds				
(i) Capital Expenditure (Refer Annexures I & II)				
- Fixed Assets	15,903.00		29,900.00	
- Others	-		-	
- Total		15,903.00		29,900.00
(ii) Revenue Expenditure (Refer Annexures I & II)				
- Salaries, Wages and allowances etc.	-		-	
- Rent	-		-	
- Other Expenses	57,93,815.00	57,93,815.00	4,98,072.00	4,98,072.00
Total				
TOTAL (C)		**58,09,718.00**		**5,27,972.00**
NET BALANCE AS AT THE YEAR-END [(a + b)-c]		**21,28,855.00**		**20,04,546.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	Current Year		Previous Year	
SCHEDULE 4 - SECURED 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 MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	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	-		-	
EMD	-		6,42,500.00	
Security Deposit	90,808.00	90,808.00	1,37,556.00	7,80,056.00
TOTAL (A)		**90,808.00**		**7,80,056.00**
B.PROVISIONS				
1. For Taxation	-		-	
2. Gratuity	-		-	
3. Superannuation/Pension	-		-	
4. Accumulated Leave Encashment	-		-	
5. Trade Warranties/Claims	-		-	
6. Others (Specify) (Annexure-G)	15,77,548.00	15,77,548.00	15,49,458.00	15,49,458.00
TOTAL (B)		**15,77,548.00**		**15,49,458.00**
TOTAL (A+B)		**16,68,356.00**		**23,29,514.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

SCHEDULE 8 - FIXED ASSTES :	GROSS BLOCK				DEPRECIATION				NET BLOCK	
Particulars	Cost/valuation As at beginning of the year	Addition during the year	Deductions during the year	Cost/valuation at 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 Current year end	As at the Previous year end
A. FIXED ASSETS										
1. LAND										
a) Freehold ***	-	1.00	-	1.00	-	-	-	-	1.00	-
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	12,60,83,059.00	2,25,26,558.00	-	14,86,09,617.00	1,05,18,670.00	1,99,89,552.00	-	3,05,08,222.00	11,81,01,395.00	11,55,64,389.00
4. VEHICLES	22,40,610.00	-	-	22,40,610.00	6,35,523.00	2,40,763.00	-	8,76,286.00	13,64,324.00	16,05,087.00
5. FURNITURE, FIXTURES	2,93,623.00	6,756.00	-	3,00,379.00	43,864.00	25,652.00	-	69,516.00	2,30,863.00	2,49,759.00
6. OFFICE EQUIPMENT	29,58,845.0	2,17,600.00	-	31,76,445.00	3,50,156.00	4,14,224.00	-	7,64,380.00	24,12,065.00	26,08,689.00
7. COMPUTER/PERIPHERALS	4,95,793.00	7,95,909.00	-	12,91,702.00	2,97,476.00	3,72,931.00	-	6,70,407.00	6,21,295.00	1,98,317.00
8. ELECTRIC INSTALLATIONS	-	-	-	-	-	-	-	-	-	-
9. LIBRARY BOOKS	4,97,459.00	84,680.00	-	5,82,139.00	2,50,986.00	2,96,936.00	-	5,47,922.00	34,217.00	2,46,473.00
10. TUBEWELLS & WATER SUPPLY	-	-	-	-	-	-	-	-	-	-
11. OTHER FIXED ASSETS	4,28,605.00	1,96,968.00	-	6,25,573.00	60,223.00	1,28,391.00	-	1,88,614.00	4,36,959.00	3,68,382.00
TOTAL	13,29,97,994.00	2,38,28,472.00	-	15,68,26,466.00	1,21,56,898.00	2,14,68,449.00	-	3,36,25,347.00	12,32,01,119.00	12,08,41,096.00
B. CAPITAL WORK-IN-PROGRESS	**15,72,37,056.00**	**7,99,96,000.00**	**-**	**23,72,33,056.00**	**-**	**-**	**-**	**-**	**23,72,33,056.00**	**15,72,37,056.00**
TOTAL	**29,02,35,050.00**	**10,38,24,472.00**	**-**	**39,40,59,522.00**	**1,21,56,898.00**	**2,14,68,449.00**	**-**	**3,36,25,347.00**	**36,04,34,175.00**	**27,80,78,152.00**

***** LAND OF 100 ACRES ALLOTTED BY GOVT. OF AP. WORTH OF RS. 306.822 CRORES TO NIAB AT FREE OF COST VIDE G.O.MS.NO. 566, DT. 13/09/2012 AT SY NO. 37, GOPANAPALLY VILLAGE, SERILINGAMPALLY VILLAGE, R R DIST. *****

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particilars	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	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particilars	Current Year	Previous Year
SCHEDULE 10 - INVESTMENTS - OTHERS :		
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	-	-
TOTAL	**-**	**-**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	Current Year		Previous Year	
SCHEDULE 11 - INVESTMENTS - OTHERS :				
A. CURRENT ASSETS				
1. Inventory				
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	32,27,520.52	32,27,520.52	20,32,060.22	20,32,060.22
b) With non-Scheduled Banks:				
-On Current Accounts	-		-	
-On Deposit Accounts	-		-	
-On Savings Accounts	-	-	-	-
5. Post Office-Savings Accounts				
TOTAL (A)		**32,27,520.52**		**20,32,060.22**
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)	67,93,456.00		1,05,80,000.00	
b) Prepayments - Deposits (Annexure-I)	2,07,40,504.00		63,16,625.00	
c) Others	-	2,75,33,960.00	-	1,68,96,625.00
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)		**2,75,33,960.00**		**1,68,96,625.00**
TOTAL (A+B)		**3,07,61,480.52**		**1,89,28,685.22**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	Current Year	Previous Year
SCHEDULE 13 - GRANTS/SUBSIDES		
(Irrevocable Grants & Subsides Received)		
1) Central Government (DBT Plan Grant-in-Aid)	9,50,00,000.00	6,00,00,000.00
2) State Government(s)	-	-
3) Government Agencies	-	-
4) Institutions/Welfare Bodies	-	-
5) International Organisations	-	-
6) Others (Specify)	-	-
TOTAL	**9,50,00,000.00**	**6,00,00,000.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	Current Year		Previous Year	
SCHEDULE 15 - INCOME FROM INVESTMENTS				
(Income on Investment 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	44,82,171.00		23,30,055.00	-
TOTAL	**44,82,171.00**	-	**23,30,055.00**	**-**
TRANSFERRED TO EARMARKED/ENDOWMENT FUNDS				

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

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

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	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	73,003.00	26,997.00
5) Other Receipts		
Sundry Receipts	12,107.00	1,38,087.00
Application Fee	1,39,500.00	2,73,500.00
Sales Of Tender Forms	2,95,000.00	-
Licence Fee	-	-
Interest On Computer Advance, Conveyance Advance And HBA	-	-
Leave Salary-Pension Contribution	-	-
Provident Fund Salvage	-	-
Free, Gifts-Donations	-	-
TOTAL	**5,19,610.00**	**4,38,584.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	Current Year	Previous Year
SCHEDULE 20 - ESTABLISHMENT EXPENSES :		
a) Salaries and Wages	1,21,58,217.00	94,77,514.00
b) Allowances and Bonus	87,71,625.00	71,04,689.00
c) Contribution to Provident Fund	-	-
d) Contribution to Other Fund (NPS)	7,73,558.00	6,02,500.00
e) Staff Welfare Expenses - Medical charges	1,99,720.00	1,14,024.00
f) Expenses on Employees Retirement and Terminal Benefits	-	-
g) Others (specify) - Staff leased House	-	-
TOTAL	**2,19,03,120.00**	**1,72,98,727.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	Current Year	Previous Year
SCHEDULE 21 - OTHER ADMINISTRATIVE EXPENSES :		
a) Purchases	1,42,32,828.00	1,64,84,227.00
b) Electricity and power	83,33,182.00	86,173.00
c) Water charges	2,23,429.00	32,669.00
d) Insurance	47,744.00	93,412.00
e) Repairs and maintenance	2,13,154.00	11,17,656.00
f) Rent, Rates and Taxes	2,22,66,461.00	94,27,941.00
g) Vehicles Running and Maintenance	2,64,461.00	2,06,387.00
h) Postage, Telephone and Communication Charges	3,50,801.00	2,92,403.00
i) Printing and Stationary	6,06,742.00	7,61,935.00
j) Travelling and Conveyance Expenses	28,74,025.00	45,90,802.00
k) Expenses on Seminar/Workshops	4,000.00	3,90,186.00
l) Subscription Expenses	4,000.00	17,917.00
m) Expenses on Fees	-	2,500.00
n) Auditors Remuneration	28,090.00	28,090.00
o) Hospitality Expenses	97,335.00	2,21,714.00
p) Professional Charges	-	2,000.00
q) Advertisement and Publicity	8,50,302.00	2,78,057.00
r) Bank Charges	2,896.70	54,841.00
s) Security & Cleaning Contract Charges	52,13,050.00	26,55,657.00
t) Training Course /Symposia	-	-
u) Other Contingencies	5,78,645.00	16,04,137.00
v) Liveries & Blankets	-	-
w) Other Research Expenses	57,01,213.00	1,04,57,028.00
x)Office Books	-	3,019.00
TOTAL	**6,18,92,358.70**	**4,88,08,751.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31st MARCH 2015

(Amount - Rs.)

Particulars	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 MARCH 2015

(Amount - Rs.)

Particulars	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 31/03/2015

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. **Advances:**
   It is observed from the objection book register that advances to suppliers for consumables & Equipments are to be reconciled and adjustment entries are to be passed in the books of accounts.

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

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

Director	Finance Officer	Manager Office (Accounts)	[CH SATYANARAYANA]
NIAB	NIAB	NIAB	Partner M. No. 019092

Place: Hyderabad
Date: 28thApril 2015

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
HYDERABAD

CLARIFICATION ON NOTES ON ACCOUNTS: 2014-15

- **Notes on Accounts 1 to 2 & 4 to 6:** Method of Accounting / Revenue recognition / Fixed Assets/ Inventories / Foreign Currency transactions / Investments:
  These are all only informatory items.

- **Notes on Accounts 3:** Fixed Assets:
  Depreciation has been calculated on Written Down Value method and at the rates prevailing to the concerned Fixed Assets as specified on the Income Tax Act, 1961 and set off against the Grant-in-aid (non-recurring). The details of the Depreciation on Fixed Assets are at Schedule – 8 is an integral part of the financial statements.

- **Notes on Accounts 7:** Advances:
  The observation of the audit has been noted. The action has already been initiated to reconcile the objection book register.

Finance Officer, NIAB

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
Details of Closing balances of various Earmarked / Endowement Funds (Refer Sch-3)
For the Year Ended 31 MARCH 2015

Annexure-I (Amount in Rs.)

Previous year	Proj No	Particulars	Current Year
-	FS001	FELLOWSHIP	1,55,390.00
-	FS002	DBT RA	1,56,719.00
6,29,634.00	SP001	NMMP MODEL NURSERY	- 4,44,030.00
13,74,912.00	SP002	DST INSPIRE FACULTY	24,594.00
-	SP003	RAMALINGASWAMY FELLOW SHIP	83,971.00
-	SP004	NMPB, DEPT. OF AYUSH, MHFW, NEW DELHI	8,42,715.00
-	SP005	DST WOMEN SCIENTIST SCHEME	3,09,496.00
-	SP006	SERB YOUNG SCIENTIST	10,00,000.00
20,04,546.00		**TOTAL**	**21,28,855.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
Details of Fixed Assets Fund (Capitalised Portion of Project Grants)
For the Year Ended 31 MARCH 2015

Annexure-II (Amount in Rs.)

Previous year	Proj No	Particulars	Current Year
29,900.00	SP002	DST INSPIRE FACULTY	-
-	SP003	RAMALINGASWAMY FELLOW SHIP	15,903.00
29,900.00		**TOTAL**	**15,903.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: A Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	I-REMITTANCES	
-	GSLI	1,080.00
9,48,420.00	Income Tax	11,99,196.00
720.00	LIC	-
35,800.00	Professional Tax	44,800.00
2,50,859.00	Service Tax	4,38,390.00
11,69,885.00	TDS	23,36,558.00
26,838.00	Works Tax	-
24,32,522.00	**TOTAL**	**40,20,024.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: B Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	ADVANCE REFUNDS/RECOVERY/ADJUSTMENTS	
91,265.00	LTC [Advance]	1,37,353.00
1,34,266.00	TA India & Abroad [Advance]	1,61,399.00
-	Telephone [Advance]	5,000.00
-	Transport maintenance [Advance]	20,000.00
-	Advertisement & Publications [Advance]	26,365.00
52,563.00	Insurance [Advance]	47,744.00
-	Others [Contingencies Advance]	1,00,000.00
-	Others [Maintenance Advance]	10,000.00
-	Chemicals [Advance]	56,275.00
-	Consumables, glassware and Spares [Advance]	16,573.00
-	Others [Including Animal House Advance]	14,607.00
-	Other Research Expenses [Advance]	1,11,107.00
9,38,92,447.00	Equipment [Advance]	1,31,23,734.00
9,83,003.00	Vehicles [Advance]	-
59,140.00	Office Equipment [Advance]	-
17,79,423.00	General Deposits And Advances	69,87,361.00
14,47,500.00	EMD	-
90,808.00	Security Deposit	-
5,00,000.00	Workshop & Conference [Advance]	-
15,18,188.00	GDA [Others]	11,00,000.00
10,05,48,603.00	**TOTAL**	**2,19,17,518.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: C Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	PROJECTS-RECEIPTS	
-	FS001	3,87,665.00
-	FS002	3,93,200.00
-	GAP001	1,24,162.00
-	SP001	-
19,00,000.00	SP002	-
-	SP003	21,09,000.00
-	SP004	11,00,000.00
-	SP005	8,20,000.00
-	SP006	10,00,000.00
19,00,000.00	**TOTAL**	**59,34,027.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: D Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	ADVANCES	
1,90,360.00	LTC [Advance]	1,59,617.00
1,37,398.00	TA India & Abroad [Advance]	1,60,967.00
5,000.00	Telephone [Advance]	-
-	Transport maintenance [Advance]	20,000.00
-	Advertisement & Publications [Advance]	26,365.00
-	Printing & Stationery [Advance]	14,894.00
52,563.00	Insurance [Advance]	47,744.00
-	Others [Contingencies Advance]	1,00,000.00
-	Others [Maintenance Advance]	10,000.00
-	Chemicals [Advance]	69,39,106.00
-	Consumables, glassware and Spares [Advance]	48,23,844.00
-	Computer maintenance [Advance]	15,000.00
-	Others [Including Animal House Advance]	14,607.00
-	Other Research Expenses [Advance]	1,16,107.00
1,16,54,814.00	Equipment [Advance]	85,74,232.00
-	Major Software [Advance]	7,62,958.00
2,26,904.00	Vehicles [Advance]	-
59,140.00	Office Equipment [Advance]	-
19,41,505.00	General Deposits And Advances	79,94,058.00
8,10,000.00	EMD	6,42,500.00
-	Security Deposit	46,748.00
74,75,357.00	GDA [Others]	11,00,000.00
-	Prepaid Expenses	16,75,354.00
2,25,53,041.00	**TOTAL**	**3,32,44,101.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: E Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	I-REMITTANCES	
-	GSLI	1,080.00
9,48,420.00	Income Tax	11,99,196.00
720.00	LIC	-
35,800.00	Professional Tax	44,800.00
2,50,859.00	Service Tax	4,38,390.00
11,69,885.00	TDS	23,36,558.00
26,838.00	Works Tax	-
24,32,522.00	**TOTAL**	**40,20,024.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: F Forming part of Receipts and Payment a/c

(Amount in Rs.)

Previous Year	Particulars	Current Year
	PROJECTS - EXPENDITURE	
-	FS001	2,32,275.00
-	FS002	2,36,481.00
-	GAP001	1,24,162.00
2,884.00	SP001	10,73,664.00
5,25,088.00	SP002	13,50,318.00
-	SP003	20,25,029.00
-	SP004	2,57,285.00
-	SP005	5,10,504.00
5,27,972.00	**TOTAL**	**58,09,718.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: G Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
15,49,458.00	March Salaries	15,49,458.00
-	Audit Fee	28,090.00
15,49,458.00	**TOTAL**	**15,77,548.00**

NATIONAL INSTITUTE OF ANIMAL BIOTECHNOLOGY
For the Year Ended 31st MARCH 2015
Annexure: H Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	LOANS & ADVANCES	
1,05,80,000.00	Equipment [Advance]	60,30,498.00
-	Major Software [Advance]	7,62,958.00
1,05,80,000.00	**TOTAL**	**67,93,456.00**

NATIONAL INSTITURE OF ANIMAL BIOTECHNOLOGY
For the Yar Ended 31st MARCH 2015
Annexure: I Forming part of Balance sheet

(Amount in Rs.)

Previous Year	Particulars	Current Year
	PREPAYMENTS / DEPOSITS	
99,095.00	LTC [Advance]	1,21,359.00
3,132.00	TA India & Abroad [Advance]	2,700.00
5,000.00	Telephone [Advance]	-
-	Printing & Stationery [Advance]	14,894.00
-	Chemicals [Advance]	68,82,831.00
-	Consumables, glassware and Spares [Advance]	48,07,271.00
-	Computer maintenance [Advance]	15,000.00
-	Other Research Expenses [Advance]	5,000.00
2,52,229.00	General Deposits And Advances	12,58,926.00
59,57,169.00	GDA [Advance]	59,57,169.00
-	Prepaid Expenses	16,75,354.00
63,16,625.00	**TOTAL**	**2,07,40,504.00**

NIAB
Hyderabad
SP001: NMMP MODEL NURSERY
P.I: Prof. P Reddanna
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
6,32,518.00	Opening Balance	6,29,634.00			
-	Grant In Aid	-	1,500.00	Salaries - Manpower	3,000.00
-		-	-	Consumables	0.00
-		-	1,384.00	Contingencies	10,70,664.00
-		-	-	Travel	-
-		-	-	Overheads	-
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
6,32,518.00		**6,29,634.00**	**2,884.00**		**10,73,664.00**
-	Excess of Expenditure over Income	4,44,030.00	6,29,634.00	Closing Balance	-
6,32,518.00		**10,73,664.00**	**6,32,518.00**		**10,73,664.00**

NIAB
Hyderabad
SP002: DST INSPIRE FACULTY
P.I: DR. ABHIJIT S DESHMUKH
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	13,74,912.00			
19,00,000.00	Grant In Aid	-	3,84,516.00	Salaries - Manpower	9,72,689.00
-		-	71,388.00	Consumables	3,67,701.00
-		-	6,251.00	Contingencies	420.00
-		-	6,036.00	Travel	1,505.00
-		-	26,997.00	Overheads	8,003.00
-		-	29,900.00	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
19,00,000.00		**13,74,912.00**	**5,25,088.00**		**13,50,318.00**
-	Excess of Expenditure over Income	-	13,74,912.00	Closing Balance	24,594.00
19,00,000.00		**13,74,912.00**	**19,00,000.00**		**13,74,912.00**

NIAB
Hyderabad
SP003: RAMALINGASWAMY FELLOW SHIP
P.I: DR. SYED FAISAL
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	21,09,000.00	-	Salaries - Manpower	11,22,500.00
-		-	-	Consumables	6,65,532.00
-		-	-	Contingencies	50,817.00
-		-	-	Travel	1,70,277.00
-		-	-	Overheads	-
-		-	-	Equipment	15,903.00
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**21,09,000.00**	**-**		**20,25,029.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	83,971.00
-		**21,09,000.00**	**-**		**21,09,000.00**

NIAB
Hyderabad
SP004: NMPB, DEPT. OF AYUSH, MHFW, NEW DELHI
P.I: PROF P REDDANNA / DR PARESH SHARMA
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	11,00,000.00	-	Salaries - Manpower	1,11,174.00
-		-	-	Consumables	1,04,145.00
-		-	-	Contingencies	1,966.00
-		-	-	Travel	-
-		-	-	Overheads	40,000.00
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**11,00,000.00**	**-**		**2,57,285.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	8,42,715.00
-		**11,00,000.00**	**-**		**11,00,000.00**

NIAB
Hyderabad
SP005 DST WOMEN SCIENTIST SCHEME
P.I: DR. APARNA RACHAMALLU
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	8,20,000.00	-	Salaries - Manpower	1,75,000.00
-		-	-	Consumables	2,97,254.00
-		-	-	Contingencies	-
-		-	-	Travel	13,250.00
-		-	-	Overheads	25,000.00
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**8,20,000.00**	**-**		**5,10,504.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	3,09,496.00
-		**8,20,000.00**	**-**		**8,20,000.00**

NIAB
Hyderabad
SP006 (VB): SERB YOUNG SCIENTIST
P.I: DR. VASUNDARA BHANDARI
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	10,00,000.00	-	Salaries - Manpower	-
-		-	-	Consumables	-
-		-	-	Contingencies	-
-		-	-	Travel	-
-		-	-	Overheads	-
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**10,00,000.00**	**-**		**-**
-	Excess of Expenditure over Income	-	-	Closing Balance	10,00,000.00
-		**10,00,000.00**	**-**		**10,00,000.00**

NIAB
Hyderabad
FS001: FELLOWSHIP
P.I: DR. HIMABINDHU GALI
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	3,87,665.00	-	Salaries - Manpower	2,32,275.00
-		-	-	Consumables	-
-		-	-	Contingencies	-
-		-	-	Travel	-
-		-	-	Overheads	-
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**3,87,665.00**	**-**		**2,32,275.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	1,55,390.00
-		**3,87,665.00**	**-**		**3,87,665.00**

NIAB
Hyderabad
FS002: DBT RA
P.I: DR DILEEP KUMAR
Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	3,93,200.00	-	Salaries - Manpower	2,28,800.00
-		-	-	Consumables	7,681.00
-		-	-	Contingencies	-
-		-	-	Travel	-
-		-	-	Overheads	-
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**3,93,200.00**	**-**		**2,36,481.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	1,56,719.00
-		**3,93,200.00**	**-**		**3,93,200.00**

NIAB

Hyderabad

GAP001: TRANSCRIPTOME ANALYSIS IN BUFFALO SPECIES AND THE GENETICS OF INNATE IMMUNITY ON FARMED ANIMAL DISEASE AND HEALTH

P.I: DR SATISH KUMAR

Receipts and Payments Account from 01/04/2014 to 31/03/2015

(Amount in Rs.)

Previous Year Amount Rs.	Receipts	Current Year Amount Rs.	Previous Year Amount Rs.	Payments	Current Year Amount Rs.
-	Opening Balance	-			
-	Grant In Aid	1,24,162.00	-	Salaries - Manpower	71,125.00
-		-	-	Consumables	-
-		-	-	Contingencies	47,557.00
-		-	-	Travel	5,480.00
-		-	-	Overheads	-
-		-	-	Equipment	-
-		-	-	Books	-
-		-	-	AMC	-
-		-	-	Others	-
-		-	-	Transfer of Funds	-
-		**1,24,162.00**	**-**		**1,24,162.00**
-	Excess of Expenditure over Income	-	-	Closing Balance	-
-		**1,24,162.00**	**-**		**1,24,162.00**

मानव कल्याण के लिए पशु स्वास्थ्य

Animal Health for Human Welfare

NIAB

National Institute of Animal Biotechnology

4th and 5th Floors, Axis Clinical Building, Opp. Talkie Town Theatre
Miyapur, Hyderabad - 500049