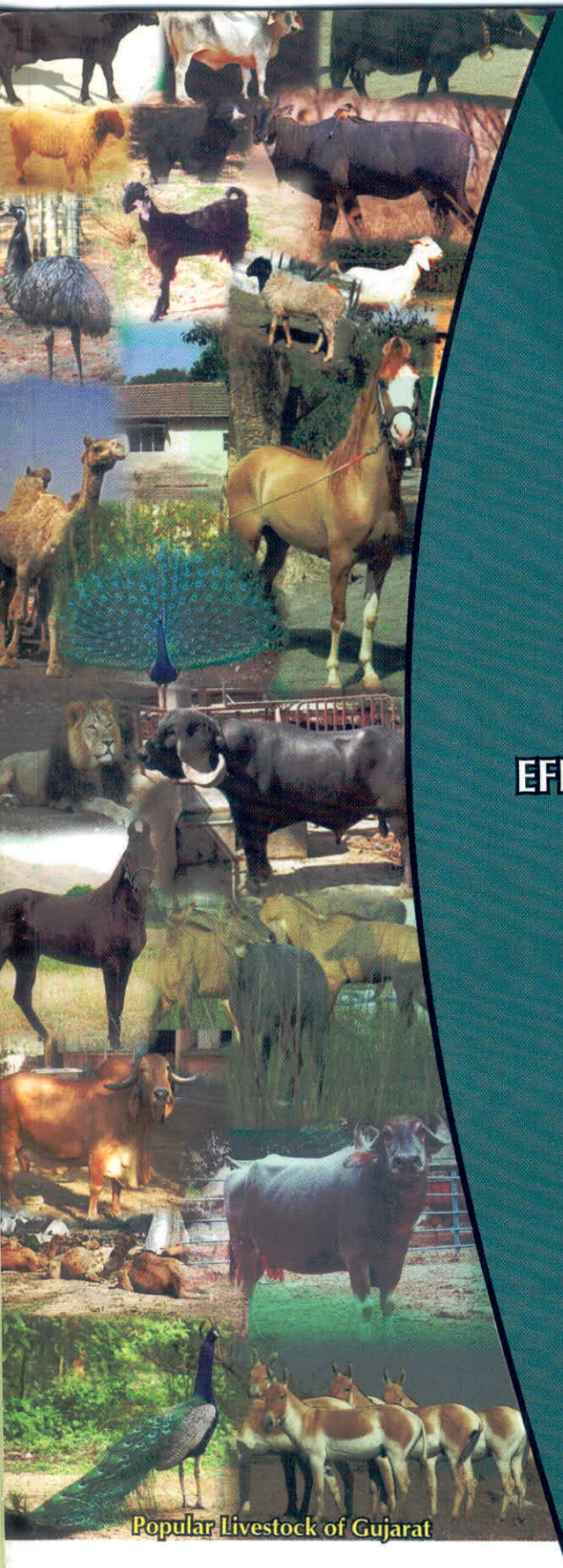

Popular Livestock of Gujarat

Compendium

XVIII Annual Convention of ISVIB

&

NATIONAL SYMPOSIUM

ON

EFFECTIVE UTILIZATION OF TRANSLATIONAL RESEARCH PLATFORMS FOR ANIMAL BIOTECHNOLGY

Organized by

Department of Animal Biotechnology,
College of Veterinary Science and Animal Husbandry,
Sardarkrushinagar Dantiwada Agricultural University,
Sardarkrushinagar-385 506 (B.K.) Gujarat, India

in collaboration with

Indian Society for Veterinary Immunology & Biotechnology (ISVIB)
&
Kamdhenu University, Gujarat, India

12 - 14 December, 2011

XVIII Annual Convention

of

Indian Society for Veterinary Immunology & Biotechnology (ISVIB)

&

National Symposium

On

EFFECTIVE UTILIZATION OF TRANSLATIONAL RESEARCH PLATFORMS FOR ANIMAL BIOTECHNOLOGY

December 12-14, 2011

Department of Animal Biotechnology
College of Veterinary Science & Animal Husbandry
Sardarkrushinagar Dantiwada Agricultural University,
Sardarkrushinagar-385 506 (B.K.) Gujarat-India

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NARENDRA MODI
Chief Minister,
Gujarat State

सत्यमेव जयते

CHIEF MINISTER
GUJARAT

MESSAGE

It gives me immense pleasure to note that Sardarkrushinagar Dantiwada Agricutural University is going to host a National Symposium on "Effective Utilization of Translational Research Platforms for Animal Biotechnology" and is also organizing the XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) in collaboration with Kamdhenu University, Gujarat from December 12-14, 2011.

Biotechnology has every potential for sustainable economic growth of the country in terms of ensuring food security and thereby alleviation of poverty. Therefore, technological development through biotechnological interventions particularly in Agriculture and Allied sector is my vision. This is evident from the term I have evolved from biotechnology (BT) that it is "Bharat tomorrow".

I hope this symposium will bring about a logical convergence of aspirations, experiences and ideas to mould into a vibrant activity in Agricultural Education, Research and Entrepreneurship.

I extend my best wishes for the success of the symposium as well as for the release of the compendium on this occasion.

(Narendra Modi)

Shri Dileepbhai Sanghani

सत्यमेव जयते

Minister
Agriculture & Rural Development
Govt. of Gujrat
1/7, Sardar Bhavan,Sachivalaya,
Gandhinagar-382010

MESSAGE

I am extremely happy to know that the Sardarkrushinagar Dantiwada Agricultural University, College of Veterinary science and Animal Husbandry is organising a three day symposium on ***"Effective Utilization of Translational Research Platforms for Animal Biotechnology"*** in collaboration with Indian Society for Veterinary Immunology & Biotechnology (ISVIB) & Kamdhenu University, Gujarat from December 12-14, 2011.

I hope that the symposium will provide much needful database along with meaningful recommendations for their implementation by the policy makers in the state. I further hope that the deliberation in the symposium will explore the role of biotechnology in enriching the popular breeds of livestock of the state and their productivity.

I convey my best wishes for the success of the symposium.

Dileep Sanghani

(Dileepbhai Sanghani)

Dr. D.H. Brahmbhatt, IAS

सत्यमेव जयते

Secretary
Animal Husbandry,Cow
Breeding, Cooperation &
Fisheries Department &
Chairman
Gujarat Livestock Development Board
Krushibhavan,Sect. 10-A, Gandhinagar.

MESSAGE

It is a matter of great pride and privilege for the College of Veterinary science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar that the Department of Animal Biotechnology is going to organise a three day symposium on "Effective Utilization of Translational Research Platforms for Animal Biotechnology" in collaboration with the Indian Society for Veterinary Immunology & Biotechnology (ISVIB) & Kamdhenu University, Gujarat from December 12-14, 2011.

I firmly believe that the outcome of the proceedings of the symposium would be of immense value not only to researchers, teachers and students of Animal Biotechnology, but the allied disciplines also.
I wish the National symposium a grand success.

(D.H. Brahmbhatt)

Prof. K. M. Pathak
Deputy Director General
(Animal Science)

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
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MESSAGE

It gives me immense pleasure to know that College of Veterinary and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Gujarat is organizing National Symposium on ***"Effective Utilization of Translational Research Platforms for Animal Biotechnology"*** and is also organizing the XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and Kamdhenu University,Gujarat during 12-14 December, 2011.

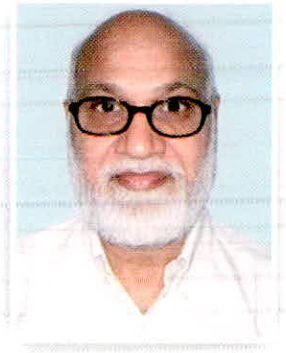
Agriculture is the major contributor to the economy of the developing countries like India and generates more than half of the annual gross domestic products (GDP). In recent years, biotechnology has greatly altered the production in Agricultural and Allied sectors. Among these, livestock sector is growing faster than any other sector and by 2020 livestock is predicted to become the most important agricultural sector in terms of added value.

I am confident that this symposium will provide an excellent platform for fruitful interactions between the biotechnology professionals to improve agricultural productivity as well as excellence in agricultural Education, Research and Development to meet the challenges in future.

I wish the Symposium a grand success.

K M Pathak

(K.M.Pathak)



Dr.Gaya Prasad
Assistant Director General
(Animal Health)

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MESSAGE

I am glad to learn that College of Veterinary and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Gujarat is organizing National Symposium and the XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and Kamdhenu University,Gujarat during 12-14 December, 2011

I appreciate the efforts of the organizers of the symposium to provide an excellent opportunity for an effective interactions between the technologists and hope their active participation and feedback information will contribute significantly to the theme of the symposium "***Effective Utilization of Translational Research Platforms for Animal Biotechnology".***

I wish the Symposium all success.

G. Prasad

(Gaya Prasad)

सत्यमेव जयते

Mukeshbhai B. Gadhvi
Member of Parliament
(Loksabha)
34, Meena Bagh,
Opp. Vigyan Bhavan,
Maulana Azad Road,
New Delhi 110011
Tel. : +91-9013180153

MESSAGE

I am pleased that College of Veterinary and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Gujarat is organizing National Symposium and the XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and Kamdhenu University,Gujarat during 12-14 December, 2011

I hope that the deliberations by the eminent scientists will be of immense value for future research programmes and developing strategies for the advancement of Veterinary Sciences. So, as to exchange the ideas and stimulate discussion on current problems and development activities in the field of Veterinary and Animal Sciences education.

I wish the symposium a grand success.

(Mukeshbhai B. Gadhvi)
Member of Parliament
Banaskantha (Gujarat)

Chairman
GCMMF, Anand and
Banas Dairy, Palanpur

MESSAGE

I am glad to know that College of Veterinary and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Gujarat is organizing National Symposium and the XVIII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and Kamdhenu University, Gujarat during 12-14 December, 2011

Animal Husbandry is the life line of North Gujarat contributing a lion's share to the state as well as national GDP. To encourage Animal Husbandry practices by way of augmenting animal production in terms of milk and milk products through biotechnological intervention in the need of the hour.

I hope and pray almighty for grand success of symposium.

(Parthibhai Bhatol)

SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
SARDARKRUSHINAGAR 385 506

Vice-chancellor
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MESSAGE

It gives me immense pleasure to know that the Department of Animal Biotechnology, College of Veterinary science and A. H., SDAU, Sardarkrushinagar is organising a national symposium on "EFFECTIVE UTILIZATION OF TRANSLATIONAL RESEARCH PLATFORMS FOR ANIMAL BIOTECHNOLOGY" and XVIII Annual convention in collaboration with Indian Society of Veterinary Immunology & Biotechnology and Kamdhenu University, Gandhinagar on 12-14 December, 2011.

I hope that the galaxy of scientists attending the symposium and convention would deliberate on Utilization of Translational Research in Animal Biotechnology. Their fruitful discussion will surely conclude with recommendation that ultimately will help the animal owners to boost their economy.

I wish the symposium a grand success.

(K. Sreedharan)

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SARDARKRUSHINAGAR 385 506

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MESSAGE

It is a matter of great pride & privilege for college of Veterinary science and A. H., Sardarkrushinagar, Dantiwada to organize and host XVIII Annual convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) and National symposium on "EFFECTIVE UTILIZATION OF TRANSLATIONAL RESEARCH PLATFORMS FOR ANIMAL BIOTECHNOLOGY" during 12th - 14th December, 2011.

Translational Research is a new & rapidly evolving domain. The primary goal of Translational Research is to integrate advancements in molecular biology with taking research from the "Lab to Land."

I hope that the present symposium would provide common platform to the researcher's scientists & development planners to deliberate on the emerging issues, exchange ideas and come out with specific recommendations that could be useful in EFFECTIVE UTILIZATION OF TRANLATIONAL RESEARCH IN ANIMAL BIOTECHNOLOGY.

I heartly congratulate the organising secretary and his team for sincere efforts to make the event successful.

(K. R. Tajane)

SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
SARDARKRUSHINAGAR 385 506

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MESSAGE

Organising a National Symposium on "Effective Utilization of Translational Research Platforms for Animal Biotechnology" is truly commendable step of Department of Animal biotechnology, College of Veterinary Science & Animal Husbandry, SDAU, Sardarkrushinagar within 4 years of its birth. Biotechnology is the focal issue to be discussed in the Symposium by a galaxy of eminent Scientists, Researchers and planners coming from across the country.

We have a large variety of Livestock and poultry besides cultivated and wild flora & fauna which serve as backbone of rural economy particularly for developing Nations. Animal Biotechnological interventions can open up new dimension to resolve the problems faced by rural people.

I take this opportunity to compliment the organisers of the Symposium for choosing this important and relevant topic. It would be right approach to harness this potential in ensuring food security for millions of countrymen through livestock development.

I wish the symposium a grand success.

(H. N. Kher)

Indian Society for Veterinary
Immunology and Biotechnology
Madras Veterinary College
Chennai- 600 007

MESSAGE

I am pleased that the staff, Department Biotechnology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar Gujarat, has accepted to hold the XVIII Annual convention of Indian Society for Veterinary Immunology and Biotechnology (ISVIB) during December 2011.

I am indeed happy that this Dept, relatively young Department has immense desire to update its scientific thirst by arranging a concurrent symposium on Effective Utilization of translational research platforms for animal biotechnology with its limited resources.

I am quite confident that the scientists in the related fields at this blossoming institution will join together as a team, understand the national need in the fast changing global scenario in animal biotechnology and immunology and overcome the challenges ahead.

The college of Veterinary Science and Animal Husbandry under the aegis of SDAU will be a source of wisdom, inspiration, realization and guiding light for the present and future students of veterinary science.

My best wishes to it for reaching newer heights in education and research.

On this auspicious occasion I convey my greetings to the alumni, staff and students for successful conduct of this convention.

(Prof. K.S.Palaniswami)

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Dr. Gami Bhavesh
Dr. Uttam. V. Gelot

CONTENTS

UNDERSTANDING RUMEN ECOSYSTEM USING BIOTECHNOLOGY

K.M. Singh[1] and C.G.Joshi[2]

1. ARIBAS, New Vidhya Nagar, 2. Department of Animal Biotechnology,
Anand Agricultural University, Anand

Applied animal biotechnology used to characterise complex microbial communities without incubation are now being employed regularly in ruminant nutrition studies. Conventional culture-based methods for enumerating rumen microorganisms have been superseded and are now used mainly to obtain pure isolates of novel organisms. The foundation of the molecular ecology techniques is ribosomal gene sequence analysis which has provided a phylogeny based classification scheme for enumeration and identification of microbial community members. The use of this marker gene in assays involving the use of single nucleic acid probes or primer sets is rapidly evolving to high throughput approaches such as microarray analysis and new generation sequencing technologies. While these analyses are very informative for determining the composition of the microbial community and monitoring changes in population size, they can only infer function based on these observations. The focus of nucleic acid research is now shifting to the functional analysis of the ecosystem which involves the measurement of functional genes and their expression in the predominant or specific members of the rumen microbial community. Functional gene studies are less developed than 16S rDNA-based analysis of community structure. Also for gene expression studies there are inherent problems involved in extracting high quality RNA from digesta, and priming cDNA synthesis from bacterial mRNA.

Role of high throughput sequencing to explore rumen microbes

Recently, metagenomes of extreme environments have also been used as sources of novel biocatalysts. The employment of next-generation sequencing techniques for metagenomics resulted in the generation of large sequence data sets derived from various environments, such as rumen, soil, human gut, and ocean water. Analyses of these data sets opened a window into the enormous taxonomic and functional diversity of microbial communities. To assess the functional dynamics of microbial communities, metatranscriptomics and metaproteomics have been developed. The combination of DNA-based, mRNA-based, and protein-based analyses of microbial communities present in different ecosystems is a way to elucidate the compositions, functions, and interactions of microbial communities and to link these to physiological processes. The diversity of microbial metabolisms represented in the rumen microbial community has a significant influence on nutrient utilization in ruminant animals.

For microbial diversity study, 454 sequencing systems have become the standard for ribosomal RNA/DNA identification and whole genome surveys having enabled an unprecedented view of microbial diversity in such environments as the human gut and mouth, rumen, soil, coral reefs, deep sea thermal vents, drinking water, and much more. The clonal reads provided by 454 sequencing systems enable population characterization without laborious cloning protocols.

Advantages of next generation sequencing technologies

- Analyze the relative abundance of microbial species under varying environmental conditions
- Discover new genes and functional predictions in uncultivable species
- Perform gene expression analysis and functional annotation within microbial communities
- Identify novel pathogens in viral outbreaks by sequencing fragments of amplified RNA from infected individuals

16S and 18S rDNA and rRNA gene amplicon sequencing

rDNA or rRNA gene PCR products are designed to cover predetermined variable regions of the 16S and/or of the 18S ribosomal gene. Ribosomal DNA amplicons will provide diversity of total bacterial/fungal community with only disadvantage that organism may be functionally active or dormant and live or dead will be detected. This limitation can be overcome by using ribosomal RNA amplicons where live and functionally active organisms will be recorded. Organism-specific differences in the sequence of variable regions allow identification of the source

organism for individual reads using a public database (BLAST) search or other mapping strategies. The merits of sequencing the different variable regions of rRNA genes are discussed in a variety of publications. Wu et al. (2010) discussed the performance of different 16S amplicons in the publication "Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags". 454 sequencing can instantly generate hundreds of thousands of long clonal sequence reads that accurately enable the sensitive detection of rare mutations. Ultra deep sequencing provides an essential tool for research on virome and their treatments. The ability to use 454 sequencing to detect rare bacterial species is a crucial research tool to better understand the mechanism of drug resistance.

Shotgun Metagenomics

Shotgun metagenomics is a random fragment sequencing application that is used on a sample derived from a pool of microorganisms. High throughput (GS-FLX) sequencing reads provide a significant advantage in shotgun metagenomics experiment as they are better able to distinguish between genes from related organisms, thereby providing a more accurate picture of diversity than shorter reads. Long reads enable de novo assembly within metagenomics samples which provides possibility of reconstruction of genomes from these reads. In our study (Singh et al., 2011) in rumen of Surti buffalo ecosystem, genetic profile characteristic of fermentation of carbohydrates in high roughage diet was observed and could help to determine the role of rumen microbes and their enzymes in plant polysaccharide breakdown, which is fundamental to understanding digestion and maximising productivity in ruminant animals.

cDNA Metagenomics – Metatranscriptomics

A metaproteomics approach reveals proteins with functions related to translation, carbohydrate metabolism, and energy production. Analysis of a cDNA library is an approach at the transcriptome level that can be used to investigate the gene expression of mixtures of microorganisms. The application of this approach in rumen microbial communities already showed that several functional genes of environmentally important processes could be detected. Although that study provided useful information about the activity of the ecosystem under study, application to the far more complex bacterial ecosystems such as in the livestock rumen with a large number of different bacterial genomes present will be far more complicated.

Uncultivable microorganisms

Technological advances in second-generation sequencing methods are fueling a rapid increase in the number and scope of metagenome projects. While metagenomics provides information on the gene content, metatranscriptomics aims at understanding gene expression patterns in microbial communities. Metatranscriptomics analysis of the sample reveals subject/substrate-specific expression profiles and also revealed key metabolic pathways and indicates their intermediate metabolites. Based on the information generated by metatranscriptomics analysis, we will be able to formulate culture media composition for uncultivable microorganism.

Data Analysis tools

There is a wide variety of publicly available tools for the analyses of metagenomic data.

- **http://www-ab.informatik.uni**-tuebingen.de/software/meganMEGAN, a Metagenome Analyzer, allows a single scientist to analyze large data sets and group sequencing reads into taxonomic units.
- **http://metagenomics.anl.gov/- MG-RAST** is a fully-automated service for annotating metagenome samples.
- **http://img.jgi.doe.gov/cgi-bin/m/main.cgi**- IMG/M provides tools for analyzing the functional capability of microbial communities based on their metagenome sequence, in the context of reference isolate genomes, using a variety of public functional and pathway resources.
- **http://camera.calit2.net/-** CAMERA is a user-driven site dedicated to providing the scientific community with metagenomics data and analysis tools.
- **http://webcarma.cebitec.uni-bielefeld.de** -CARMA is a software pipeline for the characterization of species composition and the genetic potential of microbial samples using unassembled reads.
- **http://galaxyproject.org** - Galaxy includes higher eukaryotes, such as insects in the analysis pipeline.

- **http://greengenes.lbl.gov/cgi-bin/nph-index.cgi**-Greengenes provides access to comprehensive 16S rRNA gene sequence alignment
- **http://qiime.sourceforge.net/** - QIIME provides a full workflow for processing 454 16S metagenomics experiments.
- **http://rdp.cme.msu.edu/**-The ribosomal database project has a pyrosequencing specific pipeline.

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GENOMIC APPROACHES FOR IMPROVING DISEASE RESISTANCE AND PRODUCTION IN LIVESTOCK

V.K. Saxena,

CARI, Izatnagar

In animal production system, health and production are the two important facets. Good health of flocks is the key for achieving optimum production level which is the basis for sustained viability of animal sector. Augmenting immunocompetence without compromising production is a difficult task since in most cases; production and immunocompetence traits are inversely correlated. The feed consumed by the individual is ultimately partitioned into production and immunity & anti stress responses. Therefore, for optimization of feed utilization, host needs to possess such genes that are involved in growth and immunity pathways. However, in all livestock species including poultry, mostly the breeding programs have been focused exclusively on exploitation of economic traits (e.g. milk, meat, egg and fiber) with drugs, vaccines and chemicals as virtually the only disease management strategy. Thus the long-term genetic selection only for production traits has operated against immune response capabilities/ genetic resistance of individuals as a result, the genes/alleles conferring resistance or involved in higher immunity levels have come down to lower frequencies in the high yielding populations. Supported with modern vaccination and drugs, such unfit genotypes survive in the population which has resulted into a serious reduction in the genetic resistance potential of our livestock populations leading to increasing the virulence of the pathogens and problem of drug residues in animal products. At this stage, a more balanced strategy with reduced intervention of medicaments which would be coupled with development and incorporation of strains/ genotype having increased genetic resistance to endemic diseases may be advocated. Enhanced disease resistance will also lead to reduced need of inputs for drugs thus will help in resolving the problem of drug residues in animal products and would be especially beneficial in low-input agricultural systems. The genetics of an individual or flock has a profound impact on its ability to resist disease, because genetics define the maximum achievable performance level. Disease therefore, occurs when environmental insult meets genetic predisposition (Warner *et al.*, 1987). The resolution of genes controlling disease resistance and production in livestock and poultry is a somewhat daunting but rewarding task and the advance genomic approaches may be of wide applications.The technological advancements have made it possible to explore the genome of an individual for assessing genetic variability and to associate it with phenotype or expression. The power of top-down genome wide scans can be combined with bottom-up DNA microarray to identify candidate genes based in map positions. The new alleles identified by genomic/bioinformatic tools may be subjected to their functional validation using functional genomics. The available genome sequence draft and genome maps for some of the livestock species have further increased the number of polymorphisms.

Genomic approaches

DNA markers are the basic tools for genetic studies. The use of DNA markers to define genotype and predict performance of an animal is a powerful aid to animal breeding. The ultimate use of DNA markers is to identify QTLs for MAS. DNA marker may be classified into two type i.e. Type I (markers derived from known genes of known functions) and Type II (markers derived from anonymous DNA segments) (OBrien, 1991; Emara and Kim, 2003). There are two approaches for genetic dissections of complex and quantitative traits, i.e., genome-wide scanning and candidate gene approach. Genome wide scan does not need any priori information about functional features of traits but it is expensive and resource intensive. Alternatively, using few selected markers (STR or RAPD or AFLP markers) the QTLs have identified instead of going for a genome wide scan.

Genome analysis for QTL identification

There are a large number of reports on detection of QTL for livestock (Bidanel and Rothschild, 2002; Bovenhuis and Schrooten, 2002; Hocking, 2003), most of these were identified in experimental populations using crosses between breeds or lines (Andersson, 2001) but these results cannot be used directly for selection within breeds. However, they can provide an important stepping stone for identification of LD (Linkage disequilibrium) markers for QTL that segregate within breeds using positional candidate gene approaches (Rothschild and Soller, 1997).

An alternative is to follow a breed-cross QTL analysis with an LE (Linkage-equilibrium) QTL analysis within commercial lines in identified regions. Various aspects of commercial application of marker- and gene-assisted selection in livestock have been reviewed by Dekkers (2004). Cattle QTLs for milk production (344) and mastitis related traits (71); loci with sequence variations that show specific allele-phenotype interactions associated with milk production (24) or mastitis (10) in cattle; genes with expression profiles associated with milk production (207) or mastitis (107) in cattle or mouse; cattle milk protein genes that exist in different genetic variants (9) have been listed (Ogorevc *et al.*, 2009). Major genes and QTLs affecting wool production and quality have been reviewed by Purvis and Franklin (2005). Bidinost et al. (2008) identified wool QTLs in Merino sheep by analyzing specific regions of chromosomes 3, 4, 11 and 25. Siwek *et al.* (2003) identified three and five QTLs for SRBC primary and secondary responses, respectively in chicken. Zhu *et al.* (2003) mapped QTLs associated with resistance to coccidiosis and growth in two commercial broiler lines. So far, genome wide scan have revealed 14 QTL for resistance to MD (Yonash *et al.*, 1999) one for salmonella resistance (Marriani *et al.*, 2001) and one for resistance to coccidian (Zhu *et al.*, 2003). Three QTL were found associated with general immune response to ND Virus and E.coli (Yonash *et al.*, 2001). QTLs conferring resistance to Marek's disease (MD) in commercial layer chickens were identified; seventeen markers were associated with MD survival (McElroy, *et al.* 2005). The field data on milk recording was used in a genome search for quantitative trait loci (QTL) in Norway. QTLs significantly affecting clinical mastitis were verified on Chromosome (Chr) 6. Additional putative QTL for clinical mastitis were localized to Chrs 3, 4, 14, and 27 (Klungland *et al.*, 2010).

Candidate gene analysis

Major Histocompatibility Complex genes

To start with analysis of disease resistance related candidate genes, the MHC being the key locus containing numerous genes playing critical role in immune response regulation (Dausset, 1981; Lamont, 1998 b) may be chosen first. Besides disease resistance, many traits of economic importance are also affected by MHC (Dietert *et al.* 1991; Sander, 1993). In different livestock species the association of MHC genes with different disease has been well established. Caprine DRB alleles (18 of the 22 alleles) were distinguished in MHC class II DRB gene by PCR-RFLP. Close associations have been found between RFLPs and amino acid substitutions at positions at expected antigen-recognition site (ARS) of the DR molecule suggesting the role of RFLPs in disease resistance (Amills *et al.* 1996). Two microsatellite loci DRBP1 (MHCII) and BOBT24 (IL4) were found positively associated with Cowdriosis susceptibility; significant association of the microsatellite locus SPS113 with Trichostrongylus *colubriformis* resistance was detected; however, MHC class II locus DYA (P19) was found weakly associated with susceptibility in both diseases (Obexer *et al.*, 2003). Ruff and Lazary (1988) reported the association of MHC gene in cprine arthritis encephalitis (CAE). Association of OLA haplotypes with resistance to Trichostrongylus colubiformis has been reported (Outteridge *et al.*, 1988 and Schwaiger etl., 1994). In poultry, association of Class II and Class-IV sub region of MHC with parasitic and viral diseases has been reported (Lillehoj *et al.*, 1989; Caron *et al.*, 1997; Uni *et al.* 1995; Laxmanan *et al.* 1997). SNPs in Class I and II genes have been found associated with antibody response kinetics to bacterial antigens and sheep RBCs (Zhou and Lamont 2003). SNPs have been reported been reported in *Tapasin gene* sequences from exon 5 to 6 among three avian species viz., chicken, turkey and pheasant (Sironi *et al.*, 2006).

Immunity and production traits related genes

The non-MHC gene candidates related to immunity, group genes involved in immune pathways and apoptosis (e.g cytokines, chemokines, TLRs, iNOS, caspases, bcl-2, bcl-xL etc.). The growth related candidate genes come from major growth axis (*GH-GHR-IGF-1*) and growth factors like *MRFs, MYFs, GDFs, TGFs, FGFs* etc. Calpains and myostatin genes are the preferred candidates for meat quality. Similarly, for milk production the genes like *DGAT1, ABCG2, GHR, LGB, LEP, LTF, PRL STAT5A, CSN1S1, CSN3* etc are candidates. Recently, a new method of candidate gene approach i.e. digital candidate gene approach (DigiCGA) has emerged and been primarily applied to identify potential candidate genes in some studies (Zhu and Zhao, 2007). A total of four genes viz. AHCY, PRKDC, HNRPU, OSTF1 were suggested as potentially involved in mastitis defense (Schwerin *et al.*, 2003). A candidate gene, Daxx, was identified, which is thought to be strongly associated with resistance to trypanosomiasis infection (Fisher *et al.*, 2007). A previously undescribed polymorphism in ARHGAP15 in the Bta2 trypanotolerance QTL was identified by transcriome analysis and sequencing of ESTs of N Dama

(Trypanotolerant) and Boran (susceptible) cattle post Trypanosoma congolense infection. Genetic analyses showed that selective sweeps had occurred at TICAM1 and ARHGAP15 loci in African taurine cattle, making them strong candidates for the genes (Noyes *et al.*, 2010). Association of three genes (IL8RA, TLR4 and BoLA-DRB3) with mastitis resistance or susceptibility has been reported (Ogorevc *et al.*, 2009). SNPs were identified in IGF-1 and MYF5genes using SSCP and the genotypes were found association with growth traits (weight at 3, 12 months and ADG) in Korean cattle (Chung and Kim, 2005).In poultry, the SNPs in cytokine genes, their receptors/promoter and signal transduction factors have been reported to be associated with immune response/ disease resistance (Zhou *et al.* 2001; Kramer *et al.*, 2003; Zhou and Lamont, 2003b; Ahmed, *et al.*, 2007b). iNOS polymorphism has been found to be associated with bacterial resistance (Kramer *et al.*, 2003; Malek and Lamont 2003). SNPs associated with resistance to bacterial and viral pathogens have been detected in TLRs (Beaumont *et al.*, 2003; Bochud *et al.* 2008). IGF-1 SNP has been found associated with various growth traits in broilers (Amillis *et al.*, 2003; Zhou *et al.*, 2005, Pandey, 2009 and Pandey *et al.*, 2011).

Allele Mining

Identifying naturally occurring genetic variants that regulate gene expression is an important route for connecting genotype to phenotype based on changes in gene expression. The identification of polymorphisms that influence heritable variation in gene expression is an important but challenging task limited by difficulties in predicting the nucleotide changes which are responsible for changes in gene expression particularly in eukaryotic genomes (Levine and Tjian, 2003). Allele mining for discovering new and unknown valuable alleles may also be carried in the genebank collection. In first step suitable candidate genes are chosen through functional genomics or gene discovery. The collection is further short listed by choosing a subset ('core collection') of highly distinctive accessions. For each new allele discovered at a candidate gene the functional significances are to be determined. Recently, a web-based contig and DNA sequencing trace browser 'MAVIANT' (Multipurpose Alignment Viewing and Annotation Tool) has been developed for predicting SNPs that could be visually evaluated based on the underlying sequence chromatograms (Panitz *et al.*, 2007). Using MAVIANT a subset of candidate SNPs was selected in pigs for experimental validation by re-sequencing and genotyping. Gene Ontology (GO) based schema and queries have been presented for livestock positional candidate gene discovery associated with QTLs (Harhay and Keele, 2003) within minutes.

Genomic Selection

Development of "next-generation" sequencing technologies and high-throughput genotyping platforms has led to creation of high-density SNP array as a state-of-the-art tool for genetics and genomics analyses of domestic animals. The most promising applications of these arrays in agriculture could be genomic selection for the improvement of economically important traits. Genomic selection is an advanced form of marker assisted selection (MAS) which concentrates on all markers across the whole genome (Meuwissen *et al.*, 2001; Calus, 2010). Meuwissen *et al.* (2001) proposed the original concept of genomic selection, *i.e.*, predicting breeding values of animals using information offered by thousands of SNPs across the genome (genomic estimated breeding value, GEBV), by assuming the availability of abundant SNPs scattered throughout the genome and LD relationships between SNPs and QTL.The large number of SNPs essentially required for the design and construction of arrays can be obtained through different methods and resources e.g. predicted SNPs generated from genome sequencing and HapMap studies, completing reduced representation library (RRL) sequencing (Matukumalli *et al.*, 2009, Amaral *et al.*, 2009; Ramos *et al.*, 2009) downloading SNP information from dbSNP of NCBI (*http://www.ncbi.nlm.nih.gov/projects/SNP/*) etc.. The candidate SNPs for array design should be validated and have high minor allele frequency (MAF) in the testing populations. Illumina's BeadArray based on single-base extension or allele-specific primer extension (*http://www.illumina.com*) and Affymetrix's GeneChip based on molecular inversion probe hybridization (*www.affymetrix.com*) are the two biggest and most competitive SNP chip genotyping platforms. Currently, the majority of the commercially released SNP arrays for domestic animals (dog, cattle, horse, pig, sheep) are constructed using the BeadArray platform with Illumina's iSelect Infinium technology (Fan *et al.,* 2010). Chicken 60 K SNP array (Groenen *et al.*, 2011) is not available commercially at present, however, may be available for research in public domain in India.

Functional Genomics (Trancriptomics)

The regulation of gene expression is the key process for adaptation to changes in environmental conditions and thus for survival. The subset of genes transcribed in a given organism is called the transcriptome. Transcriptomics

describes this process in a genome-wide range. The extremely powerful techniques used in transcriptomics are microarrays, which allow determining the mRNA expression level of practically every gene of an organism. However, at smaller level, the expression profiles of candidate genes involved in pathways of immune response or production traits have been analysed in livestock species including and poultry (Lee *et al.* 2003; Pfaffl *et al.* 2003; Swanson *et al.* 2004; Djeraba *et al*, 2002; Sundersan *et al.*, 2005; Sundersan, 2007; Ron *et al.*, 2007; Korteweg and Gu, 2008; Suba, 2008, Pandey, 2009).

Microarray

Microarrays are the combination of robotics, chemistry, computer science and biology for studying whole genome at a time. Arrays offer the systematic means to survey the variation at DNA or RNA level in entire genome. Microarray has great promises for understanding the molecular pathways of various diseases and biological processes. The differentiation between diseased and healthy cells may also be carried out. Microarrays also have potential application for identification of pathogenic strains, drug discovery, and diagnosis of diseases.

DNA/cDNA Microarray

Based on techniques used the microarray are of two types i.e. Spotted DNA microarrays (cDNA and Oligo arrays) and in-situ synthesized oligo arrays (commercial companies are Affymatrix- using photolithography (masks), NimbleGen- using mirror photolithography (mask less) and Agilent-using inkjet synthesis). DNA Microarary is a small chip of finger nail size containing thousands of spots. In each of these spots are thousands of DNA probes. Using these probes and set of targets thousand of genes may be scanned at a time by scanning these chips for fluorescent signals with computer and analyzed using software developed for microarray data analysis. A 14K Chicken Integrated Systems Microarray has been developed (Cogburn et al., 2004). Using this microarray a number of polymorphic functional genes in key metabolic pathways that could control important phenotypes in chickens have been identified. More recently, the oligonucleotide arrays have been developed. The GeneChip uses oligonucleotide sequences as its probe. These oligonucleotides are about 25 bp in length. The GeneChip (Affymatrix) for about 32,773 transcripts of poultry genes are now available. Arizona Gallus gallus 20.7K Long Oligo Array (oligos length 65-75nt), FHCRC Chicken 13K Array with multi-tissue cDNA microarray of 13,007 features and ARK Genomics using 1153 clone chicken embryo array, a 5,000 cDNA chicken immune array, and a 4,800 clone chicken neuroendocrine array, are so far on record. The Chicken Genome Array also contains 689 probe sets for detecting 684 transcripts from 17 avian viruses. In cattle also cDNA libraries in of various tissues e.g. liver, intestine, skeletal muscle, endomertium, ovary, embryos etc have been constructed. A bovine cDNA array (CattleArray 7600 from Pyxis Genomics; Hocquette *et al.*, 2007) became available commercially in 2003. Since this array has been made with cDNAs derived from spleen and placenta, it may not be suitable to explore muscle biology and hence beef quality. A number of high-density cDNA arrays constructed for assessing genome-wide gene expression changes in cattle now include genes from muscle-specific libraries. The NBFGC in the USA has selected 18 263 genes from the pooled tissues MARC 1–4 libraries for printing on a high-density microarray. Another array with a set of selected 10 608 EST from MARC 1–4 libraries which include all the known genes and unknown, putative or hypothetical genes expressed in muscle, liver or adipose tissue had been developed. A new molecular array been called the BoviAnalyser has been developed which is suitable to analyse cell proliferation and differentiation in response to disease resistance or the ability of the animals to produce marbled beef. Roslin Institute's ARK-Genomics Centre, UK have constructed high-density microarrays based mainly on its collection of immune-specific EST and AgResearch has been using a 10 204 bovine cDNA microarray with broad genome coverage in bovine and ovine transcriptome studies. French scientists have also prepared cDNA libraries from bovine mammary gland. In addition, a selection of 13 168 clones from the MARC 1–4 libraries (75%) and from INRA clones (25%) has been made to prepare an array suitable for many applications in cattle (http://sigenae.jouy.inra.fr/). Many bovine specific oligo arrays have also been developed for transcriptomic studies in cattle (GeneChip- Affymatrix-23000 gene probes, Bovine whole genome long oligonucleotide expression array, Bos taurus eArray-Agilent- 21,475 unique probes) (Hocquette *et al.*, 2007).

Conclusions

Because of complex genetic mechanisms and inverse relationship between production and immunological traits, the suitable methods for optimizing immunocompetence and production in commercial breeding are yet to be finalized. At the same time, ignorance of genetic resistance in selection programs may further worsen the

disease situation. Enhancement in genetic resistance to diseases may therefore be given due consideration in the selection programme so that a balance may be maintained between production and disease resistance status. The individuals selected for high functionalities of immune system may have better capabilities to resist the infection or the effect of infection along with better antibody response to ongoing vaccination programs. The recent information generated on disease pathogenesis / immuneresponsiveness and production traits by advanced genomic approaches will help tremendously in generating better tools for improving production as well as genetic resistance to the myriad of pathogens that face livestock and poultry. The recent high density array based genomic selection for predicting genomic breeding values estimates using information from thousands of SNPs across the genome is expected to have high promise in improving production traits and disease resistance in livestock and poultry.

Reference on request

VETERINARY BIOTECHNOLOGY : TIME FOR A PARADIGM SHIFT

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The recent and continuing advances in the life sciences are making a reality of the prediction that this will be the century of biotechnology. Capturing the economic, environmental, health and social benefits of biotechnology will challenge government policy, public information, law, education and the scientific and technological infrastructure, and will affect our societies and many aspects of our life as profoundly as information technologies have already done. For a country like India, biotechnology is a powerful enabling tool that can revolutionize animal husbandry and agriculture, healthcare, industrial processing and environmental sustainability. The Indian Biotechnology sector is gaining global visibility and is being tracked for emerging investment opportunities.

Biotechnology has made the world a different place. Biotechnology has made it possible to identify the genetic causes behind many different inherited diseases. Biotechnology has made it possible for people to survive to a much higher population density by providing more food per acre. The advent of modern molecular biology and genetics has advanced our understanding of the genomes of a wide range of organisms from viruses and bacteria to trees and humans. The application of this knowledge has revolutionized the sciences, changing them from a descriptive nature to a variety of disciplines that provide new products such as drugs, vaccines, and foods.

Livestock sector in India and biotechnology

In a developing country like ours, biotechnology solutions relating to livestock problems need to be tailor-made for such animal owners, who are resource-poor and small-scale operators, who own little or no land and few animals, and who are responsible for more than 70 % of animal holdings in the nation. Livestock is becoming increasingly important to national growth in the face of agricultural saturation, and the application of biotechnology is largely coming to be dictated by commercial considerations and socio-economic goals. Using technology to support livestock production is an integral part of viable agriculture in multi-enterprise systems. Molecular methods are increasingly finding place in the identification and selection of particular genes that lead to these desirable traits and it is now possible to select superior germplasm and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies (ARTs). These technologies have been used in the genetic improvement of livestock, particularly in cattle and buffaloes, and the economic returns have been rewarding.

Globally, livestock production is growing faster than any other sector; in 2005, livestock production accounted for about 43% of the gross value of agricultural production worldwide and by 2020 livestock is predicted to become the most important agricultural sector in terms of added value. In developed countries livestock accounts for more than half of agricultural production, while in developing countries the share is about one-third. This latter share, however, is rising quickly due, largely, to the ongoing increases in livestock production resulting from population growth, urbanization, changes in lifestyles and dietary habits. In India, the contributions made by livestock to both agriculture and gross domestic product (GDP) have risen at a time when the contribution of agriculture to GDP has fallen. Socio-economic studies reveal a dismal fact that the biotechnological interventions under commercialized agro-livestock practices have diminished the nutritional security of the poor because small farmers face greater difficulty than larger farmers in adopting the new technologies, and are left behind. The demand for livestock products is a function of income, and the poor are likely to spend a relatively higher proportion of any incremental income on food, so increases in livestock production achieved through the use of biotechnology can have major nutritional implications, particularly if the technology is aimed at the poorest producers. The modern veterinary biotechnologist must, therefore, aim to catalyze a distinct shift in the economic returns from livestock whereby the actual stakeholders should benefit, and livestock enterprise is encouraged amongst the masses.

Veterinary biotechnology in India

The use of biotechnology in animal production has advanced more quickly than its applications in plant production. Worldwide, more than one-half of all biotechnology research and development expenditures are in the field of human health. At the experimental stage, a large number of drugs, diagnostic probes, vaccines etc. are frequently applied in livestock production prior to becoming available for use by humans. Developments in the pharmaceutical industry, therefore, have had considerable outcomes for animal production since many innovations in this area are also applicable to animals.

A little over two decades ago, applications of biotechnology to animal production in India was envisaged covering four broader fields:

- Animal health;
- Feeding and nutrition;
- Growth and production; and
- Reproduction, selection and breeding.

The short story is—'Much has been tried with little turn around'. In fact in veterinary biotechnology efforts have been there at few better developed places as IVRI, NDRI, NRCC, NRCE, PDCm, SAG, few universities viz. TANUVAS, GBPUAT etc. All these have contributed and advancements have been made in areas like - Elite animal production through embryo biotechnology, ova pickup technology, semen biology and cryopreservation, molecular and immunological approaches to animal disease diagnosis, novel vaccines against bacterial and viral diseases, innovative drug design and delivery, herbal based immunomodulation, augmentation of growth and productivity, breed characterization and molecular genetics, epidemiology and pathobiology of animal diseases, and rumen biotechnology etc.

In spite of all such efforts it's surprising and sad to note that not much has reached the poor farmer or the animal owner. The major limitations/ bottlenecks are namely i) Cumbersome and de-motivating bureaucracy and imperialism; ii) Lack of need based/ problem based research projects iii) Manpower-shortness and lack of relevantly trained manpower iv) Lack of private-funding/ clientele based funding.
As a developing country, India too has other constraints. The major constraints on applying biotechnologies have been enumerated by Madan and include:

a) the absence of an accurate and complete database on livestock and animal owners so that programmes can be implemented
b) the biodiversity present within species and breeds in agro-ecological systems
c) the fact that models of biotechnological intervention differ distinctly between developed and developing economies
d) the fact that many animal species and breeds are unique to the developing world; each has its own distinct developmental, production, disease resistance and nutrient utilization characteristics
e) the lack of trained scientists, technicians and fieldworkers to develop and apply the technologies, both in the government and in the private sectors
f) the absence of an interface between industry, universities and institutions, which is necessary to translate technologies into products
g) the inability to access technologies from the developed world at an affordable price in order to make a rightful, positive and sustainable contribution to livestock production and the economic welfare of farmers
h) the high cost of technological inputs such as materials, biologicals and equipment
i) the failure to address issues of biosafety and to conduct risk analyses of new biologicals, gene products, transgenics and modified food items, and, above all
j) the negligible investment in animal biotechnology.

Veterinary biotechnology in India: What needs to be done?

In the light of above facts, need and constraints following approaches regarding already mentioned broader veterinary fields would be worth adaptation.

Animal health can be improved with new biotechnology methods of diagnosis, prevention and control of animal diseases. Diagnostic tests based on the use of antibodies and new vaccines against viral and bacterial diseases are also particularly relevant for developing countries and have a wide application for prevention of cattle epidemic and diseases. Nanofabricated systems should be used to make smarter yet cheaper diagnostic devices with improved farmer acceptability and accessibility. There is also a dire need to transcend the concept of conventional vaccines. Our animals today are being vaccinated against a-never-before number of diseases, and probably, still as sick as they were some centuries ago. One apparent reason here is that we are still depending on the production techniques borrowed or copied from developed nations. We are still to develop our own indigenous protocols using local pathogen strains and variants.

The large number of immunogens and stressors that our animals gets exposed to has overwhelmed their immune systems, which are often found to exist in a state of compromised anergy. We need to focus on our indigenous vaccine technology with better efficacy. Sincere efforts are needed to reduce the number of vaccines that go into our livestock and modern biotechnology has much to offer. The arrival of multi sub-unit vaccines on the horizon promises protection against several different diseases with a single shot. It is also not always necessary to burden the immune system with a large number of immunogens. There are many diseases whose probability of occurrence is remote but which can result in severe losses, and the farmer vaccinates his animals against the disease for the fear of the losses lest it rarely appears in his herd/ flock. Pulsed antibody presenting cells and memory cells against such rare diseases can be used to bolster the cellular immunity discarding unnecessary vaccinations and weighing down of the immune system. In the event of an exposure to the pathogen, the pre-primed cells can quickly mount a sufficient immune response. Improved bio-design towards the development of edible vaccines and DIVA vaccines is a bright avenue under the existing scenario.

Animal feeds and fodder improvement is an area where we have lots of potential as we have a vast access to non conventional feed resources and we at the same time have a good amount of traditional wisdom. Biotechnology research in the field of animal nutrition concentrates on improvements in the enzymatic treatment of feed and decreasing the anti-nutritional factors in certain plants which are used as feed. In developing countries like ours, such techniques might eventually increase the potential range of crops used to feed larger herds of livestock. Here it is important to note that India is blessed with a long coastline. Possibilities for the development of halophyte based nutritional regimes for livestock are immense. If we could succeed in optimizing sea derived foliages that can support livestock well, the issues pertaining to feed and fodder scarcity in livestock nutrition may be addressed for good. Manipulation of resident gut microbiota for improved assimilation of nutrients and reduced methanogenesis shall not only improve feed efficiency but, also address the long-impending concern over greenhouse emissions from livestock.

Experiments with hormones to improve production are the subject of much debate because of their possible negative effects on animals and consumers. In developing countries like ours, however, specific needs for increases in productivity are a pressing consideration which may lead to earlier large-scale adoption of this technology. This field is another area of biotechnology application where research could prove beneficial.

In the field of reproduction, new bio-techniques such as embryo transfers, in vitro fertilization, cloning and sex determination of embryos have been developed for different types of livestock; for example, cattle. However, all these technologies need to be made available for as many of the livestock species as possible. Throughout history, animal husbandry has made significant contributions to human health and well-being. The convergence of recent advances in reproductive technologies with the tools of molecular biology (gene targeting and array analysis of gene expression) adds a new dimension to animal breeding.

For a country like India, major prerequisites for success and safety will be the continuous refinement of reproductive biotechnologies according to our domestic conditions and indigenous resources available and a rapid completion of genomic sequencing projects in our indigenous livestock. At global level it is anticipated that within the next five to seven years genetically modified animals will play a significant role in the biomedical arena, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts.

Transgenesis and genetic engineering are probably the most powerful tools available to us today, offering solutions in all fields of animal biotechnology, and thus, the wielding of these technologies also warrants proper caution. Genetic modifications in animal production, in their early stages of R&D, include improvement of disease resistance, increased birth rates in sheep, altered sex ratio in poultry, increased egg production in poultry by creating two active ovaries, and improved feed conversion in the 'enviropig' (environment friendly pigs that excrete less phosphorus). It is noteworthy, that most of these interventions are one-time investments; a breed with an improved digestive enzyme profile or a chicken breed with two ovaries, once developed, can be perpetuated without any further, extra costs.

Until recently, pronuclear microinjection of deoxyribonucleic acid (DNA) was the standard method for producing transgenic animals. This technique is now being replaced by more efficient protocols based on somatic nuclear transfer that also permit targeted genetic modifications. Lentiviral vectors and small interfering ribonucleic acid technology are also becoming important tools for transgenesis. Transgenic farm animals are important in human medicine as sources of biologically active proteins, as donors in xenotransplantation, and for research in cell and gene therapy.

Biotechnology has opened doors to making proteins with new functions, and even new biochemical pathways with altered products. With new proteins and new biochemical pathways, it seems only logical to find ways to incorporate the new functions into crops, into animals, and, it is hoped, into people with genetically based illnesses. Only a short time ago, agriculturists largely relied on green fingers to get good yields; today they use green fluorescent protein to assess gene expression in transgenic crops. The ability to make such direct changes will result in major changes for the future.

Gene 'pharming' entails the production of recombinant biologically active human proteins in the mammary glands of transgenic animals. This technology overcomes the limitations of conventional and recombinant production systems for pharmaceutical proteins and has advanced to the stage of commercial application. The mammary gland is the preferred production site, mainly because of the quantities of protein that can be produced in this organ using mammary gland-specific promoter elements and established methods for extraction and purification of that protein.

Nano-biotechnology and veterinary applications

One field of research and development which needs special mention is nanotechnology. India has proven its mettle in IT and engineering and therefore holds promise in this area. Nanotechnology, as a new enabling technology, has the potential to revolutionize agriculture and food systems throughout the world. Nanotechnology can provide new tools for molecular and cellular biology and new materials for pathogen detection, so there are several areas in which nanotechnology could be applied to the science and engineering of agriculture and food systems, e.g. agricultural and food systems security, disease treatment delivery systems, and the protection of the environment.

Animal health is an increasingly important issue, both for animal agriculture and pet owners. Feneque, a veterinarian, writes that nanotechnology has the potential to significantly affect the way veterinarians practice veterinary medicine. Food security and safety and an increasingly aged pet population, along with heightened costs for medication and veterinary care create a need for new solutions. Nanotechnology has the potential to provide these solutions, since the possible applications of the technology in medical and veterinary applications are almost mind-boggling. Although much research and major company developments are necessary before nanotechnology is common place in veterinary medicine, there are numerous glimpses of the future in applications for drug delivery, disease diagnosis and treatment, breeding and identity preservation.

Nanotechnology, although still in the early stages of its development, is beginning to equip scientists, engineers and biologists to work at the cellular and molecular levels for significant benefits in healthcare and animal medicine. It is reasonable to presume over the next couple of decades that nano-biotechnology industries and unique developments will be revolutionizing animal health and medicine. It is therefore seriously required to train ourselves not only as a receiving end for the western world technologies, but as world leaders.

Need for developing technical manpower

It's the same old diction—"It's the Man behind the Machine". One can never—ever underestimate the necessity of developing relevantly trained manpower.

Biotechnology in its essence is an applied science. The ultimate success of biotechnology is dependent upon advances in and support for the fundamental sciences which underpin it. Short cuts, empiricism and superficial attention to basic scientific principles are likely to lead at best to poor process performance and at worst to expensive failures. Therefore it's quite worthwhile to mention the need to develop veterinary biotechnology research and academic areas.

There is a serious need to develop veterinary biotechnology programme in the form or opening dedicated departments/ divisions at various veterinary colleges and institutes; develop need based short term projects, both of research and applied-training levels; undertake need-based/ client-based/ local resourse-based research projects; develop value-addition biotechnologies for animal produce; revisit ancient/ traditional biotechnologies for generating green-biotechnologies etc.

Epilogue

Seemingly, the prospects are very promising, the potential is very strong, but at the same time the challenges and constraints too are big. If India has to be a world leader in any way, we must strive to be the future food-bowl for the world and veterinary sector can play a crucial role here. Its time for a paradigm shift for veterinary biotechnology in India.

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SIGNALING PATHWAYS AND THE FUNCTIONAL MOLECULES REGULATING PLURIPOTENCY OF EMBRYONIC STEM CELLS

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Assisted reproductive technologies have made significant advances during last fifty years through the development of newer and innovative techniques for the improvement of farm livestock production and human health, majority of these advances are flanked by commercial potential and opportunities. Developmental biology has been revolutionized through the embryonic stem cells (ESCs), these cells are a potential tool for understanding the molecular mechanisms involved in the process of differentiation from the embryonic to the adult stage. The pluripotent ESCs are harvested from the inner cell masses (ICM) of the blastocyst that can self-renew and generate all cell types of the three embryonic germ layers, i.e., ectoderm, mesoderm and endoderm, but are not able to generate the extra embryonic trophoblast lineage. Application of ESC based cell-replacement therapy (CRT) is of great advantage it provides an unlimited, renewable source of cells capable of replacing or repairing tissues that have been damaged in virtually any degenerative diseases. Therapeutic and commercial possibilities are enormous in livestock and pets, once ESC lines are developed, established and validated from these species, it will be above the ethical constraints applicable to the ESCs derived from human embryos and shall expand very rapidly in Veterinary Sciences for the regenerative purposes. Identification of different auto- and cross-regulatory signaling network pathways mediated through various transcription factors expressed in pluripotent stem cells of livestock have made an advancement to understand of the molecular mechanisms which govern ES cell pluripotency. It helps providing an insight related to the crucial role of the complex network of transcription factors such as Oct4, Sox, Nanog, cMyc, Klf4, FoxD3 etc. which are utilized extensively to characterize embryonic stem cells in livestock and maintains the pluripotency through an intricate interplay. Each of these factors are required for pluripotency both in vivo and in vitro. In vivo, the pluripotency is maintained for brief transient period through the coordination of transcription factors and intracellular signaling molecules. Whereas, in vitro, these pluripotent stem cells require different extrinsic factors in addition to the feeder layers and various mitogens. Requirement of such cytokines/growth factors are species specific, their removal may lead to spontaneous differentiation and loss of pluripotency. To maintain pluripotency and inhibit differentiation during in vitro culture, ES cells require a fine balance of the transcriptional factors and signaling molecules of different pathways. Different signaling pathways are reported in human, mouse and livestock controlling ES cell pluripotency either directly or through specific ligand interactions, including several growth factors and cytokines which are responsible for triggering these pathways at single or multiple dots to regulate transcription factor expression, hence an enhanced understanding of the molecular mechanisms that regulate ESCs propagation and pluripotency will allow better utilization for therapeutics.

Network of ESC Transcription Factors

During the early pre-implantation stages of embryonic development, Oct4 the transcription factor of the POU family, encoded by *pou5f1* plays an essential role in the establishment of pluripotency. Oct4 is an important marker of ES cell pluripotency, expressed in tropectoderm, primitive endoderm and undifferentiated cells, subsequently it is downregulated upon cell differentiation into somatic lineages. It is also expressed in unfertilized oocyte, ICM and epiblasts of pre-gastrulation embryo as well as in primordial germ cells during embryonic development. Nanog, originally named as early embryo specific natural killer was first identified as a homeobox-containing gene with homology to members of the NK (natural killer) gene family and is mainly expressed in the inner cell mass of blastocyst, epiblasts and ectoderm of the primitive streak. It maintains ES cell pluripotency independent of LIF/STAT3 pathway. Down-regulation of this gene occurs during formation of the mesoderm and endoderm. Inner cell mass which are, nanog-null fails to develop an epiblast and differentiates into the extraembryonic endodermal lineage. Sox genes, SRY-related belong to the family of high mobility group (HMG) - box encodes certain transcription factors which are involved in the regulation of early embryonic development, germ layer formation and maintenance of pluripotency in ES cells. It regulates the pluripotency by controlling

expression of FGF4 in ES cells and forms a complex with Oct-4 to regulate its own expression. Recent reports suggest some other transcriptional factors like cMyc, FoxD3 and Klf4 are also actively involved in maintenance of the ES cell pluripotency. Based on the crucial role of these transcription factors in pluripotency of ES cells it could be proposed that Oct4, Sox2, Nanog, cMyc, Klf4, FoxD3 collaborate to form regulatory circuitry consisting of auto-regulatory and feed-forward loops that contribute to pluripotency and self-renewal of ES cells.

Signal pathways of ESCs

Major signaling pathways involved in self renewal of embryonic stem cells are JAK/STAT pathway, BMP/SMAD signaling pathway, MAP- Kinase/ERK pathway, PI3K/AKT pathway, TGF-â/activin pathway, FGF pathways and Wnt pathway.

JAK/STAT/LIF Pathway

Leukaemia inhibitory factor (LIF) belongs to the interleukin-6 cytokine family. LIF binds to a heterodimeric receptor consisting of the low-affinity LIF receptor and gp130, with downstream signals being transmitted through gp130. The JAK-STAT (Janus Kinases- Signal Transducers and Activators of Transcription), one of the important pathways involved in ES cells self-renewal is activated by LIF required to allow embryonic cells to remain pluripotent when cultured in the presence of serum, binds to the LIF receptor/gp130 heterodimeric receptor complex at the cell surface, upon binding, receptor-associated JAK phosphorylates the tyrosine residues of the ligand-bound receptors to interact with the signal transducer STAT3 and then activates. Upon activation, STATs with phosphorylated tyrosine residues are subjected to dimerization to form homodimers, before being translocated to the nucleus where it binds to DNA for regulation of gene expression and acts as a transcription factor. STATs are also activated in response to growth promoting factors such as Epidermal Growth Factor (EGF) or Platelet-Derived Growth Factor (PDGF). Thus, the JAK/STAT pathway plays an important role in maintaining pluripotency of ES cells in response to growth promoting factors and cytokines.

Wnt Pathway

Wnt signaling pathway is proposed to be an important member of the ESC core regulatory network and is amongst the most evolutionary conserved pathways, implicated in a variety of cellular, embryological and physiological activity. The Wnt pathway consists of more than 30 extracellular Wnt-ligands, which interact with receptors of the frizzled family and this signaling pathway results in an elevated transcript level of pluripotent genes such as *Oct4*, *Sox2* and *Nanog*. Wnt pathway is activated on binding of the Wnt protein to the Frizzled receptor on the cell membrane and activates a protein called Dishelved (Dsh). Dsh inhibits the Glycogen-Activated Kinase-3 (GSK-3) which phosphorylates and targets the â-catenin-Adenomatous Polyposis Coli (APC) complex for ubiquitination and proteolytic degradation. Upon Wnt signaling, â-catenin is stabilized, accumulates in the cytoplasm and translocates to the nucleus, where it interacts with DNA-binding proteins of the T-cell Factor / Lymphocyte Enhancer binding Factor (Tcf/Lef) family to maintain self renewal of ES cells. In the presence of â-catenin, Tcf/Lef act as transcriptional activators of proliferation stimulating genes such as c-myc and cyclin D1.

FGF signal pathway

Fibroblast growth factor (FGF) is another important factor since it maintains the undifferentiated state of ES cells. FGF acts via fibroblast growth factor receptors (FGFRs) and its expression is regulated by Oct3/4 for self renewal. Withdrawal of bFGF results in ESC differentiation, and loss of TRA-1-60 and Oct3/4, hence making it and its receptors an essential component which maintains the pluripotency of ES cells by increasing the expression of ECM through activating Akt/PKB signal pathway. Removal of FGF or inhibition of PI3K/Akt/PKB cause the rapid down-regulation of ECM and result in the differentiation of human ES cells.Receptor binding of FGFs is modulated by extracellular matrix (ECM) molecules and O-linked carbohydrates on ECM for maintaining the pluripotency of ES cells. In addition, FGF promotes the self-renewal in ES cells by antagonizing the BMP pathway and MEK/ERK signaling.

TGF-â/activin/nodal pathways

The Transforming Growth Factor-â (TGF-â), a prototypic member of a large superfamily of related growth and differentiation factors play a key role in maintaining pluripotency of ES cells. The family has more than 40 members, including TGFb, Activin, Nodal, and bone morphogenetic proteins (BMPs). Like Wnt pathway, the

TGF-â pathway has three major divergences: the SMAD1/5/8, the SMAD2/3 and the TAB/TAK. The activation of the pathway is mediated by TGF-â ligands binding to the extracellular domain of Type I and Type II TGF-â receptors. By direct serine phosphorylation the signal is transmitted to latent cytoplasmic transcription factors, the SMADs. The TGF-â signaling pathway has two divisions: a) Bone Morphogenic Protein (BMP) and Growth Differentiating Factor (GDF) ligands bind Type I receptors ALK1, ALK2, ALK 3 and ALK6, leading to phosphorylation and activation of SMAD1 and 5. Activin and Nodal ligands bind ALK4, ALK5 and ALK7 receptors and trigger phosphorylation and activation of SMAD2 and SMAD3. Phosphorylated SMADs form a higher-order complex with SMAD4, translocate to the nucleus and regulate transcription of a broad range of genes and transcription factors which regulates the ES cell pluripotency.

BMP-SMAD signal pathway

Bone morphogenetic proteins (BMPs), members of the TGFâ superfamily bind to serine/threonine heterodimer kinase receptors. The downstream effectors of BMP4 are the well-characterized SMAD proteins (SMAD1, 5, 8). The function of BMP4 has been usually described more as controlling cell differentiation rather than promoting self-renewal. BMP-SMAD pathway is stimulated by BMP4 which binds its receptor to transduce signals from plasma membrane to the nuclear targets involved in embryonic development via SMAD proteins. The BMP-SMAD pathway is regulated by both intra and extra-cellular proteins that interact with BMPs or components of the pathway. BMP binds to four different receptors: BMPIá (ALK2, ALK3), BMPIâ (ALK6), BMPR1a and BMPRII to form heterodimers. BMPR1a and BMPRII heterodimer activates Smad as result of which members of the inhibitor of differentiation (Id) family genes are expressed. However, in the presence of LIF, it inhibits neural differentiation by allowing the expression of ID (for inhibition of differentiation) genes. Thus BMPs regulate the ES cell pluripotency via activation of Id family genes. In contrast with rodents, BMP signaling does not contribute to self-renewal of human ES cells. It causes the down-regulation of Nanog and Oct3/4, which subsequently induces trophoblast differentiation of human ES cells.

The Map Kinase/Erk Pathway

The RAS-Mitogen Activated Protein Kinase (MAPK) pathway plays an important role in maintaining pluripotency by transducing signals from cytokines and growth factors through binding of Tyrosine Kinases Receptor (TKR) and promotes cell adhesion, proliferation, migration and survival. This signaling cascade, a small membrane-bound GTPase shuttles works between two conformational states: active GTP-bound and inactive GDP-bound. RAS is activated by Son of Seven less (SOS), a guanine exchange factor usually found in the cytoplasm of the cell. Upon receptor signaling, SOS shuttles catalyze the nucleotide exchange reaction of RAS and activated GTP bound RAS-RAF complex. This complex further activates the mitogen-activated protein kinases (MAPK) also known as extracellular signal-regulated kinase (ERK). ERK1/2 translocate to the nucleus where they activate the Jun/Fos transcription factors to maintain the pluripotency of ES cells. Numerous factors inhibit the activity of this pathway like RKIP, RAF kinase inhibitor, protein obstructs of MAPK, RAS and RAB Interactor 1 (RIN1) which competes with RAF for binding of GTP-bound RAS. MAPK pathway also be activated by extracellular matrix molecules and changes in focal adhesion, however, most of these signals are relayed through Focal Adhesion Kinase (FAK) and Phosphatidylinositol 3-Kinase (PI3K) for activating the transcriptional factors.

PI3K/AKT Pathway

Phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway likes to the MAPK/ERK pathway responds to a variety of extra- and intracellular signals. The PI3 kinase pathway is activated by exogenous factors such as insulin, but also activated endogenously by Eras (ES cell-expressed Ras), a novel small GTP binding protein specifically expressed in mouse ES cells. It works through binding of cytokines to hormonal receptors and phoshorylates the transmembrane tyrosine kinase-linked receptors (RTK) which activates transcriptional factors and regulates cellular proliferation, cell death and cytoskeletal rearrangements. Activation of class I PI3K occurs through interaction of the p85 subunit with various activating proteins such as protein kinase C, RHO, RAC, mutated RAS, SRC and leads to activation of the p110 catalytic subunit. Upon activation, PI3K phosphorylates phosphatidylinositol-4, 5-biphosphate (PIP2) converting it to phosphatidylinositol-3, 4, 5-biphosphate (PIP3). PI3K/ AKT signaling is counteracted by the Phosphates and TENsin homologue (PTEN) (and SHIP1, SHIP2), which dephosphorylates PIP3–PIP2. Activated AKT is localized at the cell membrane where it interacts with and is phosphorylated by Phophoinositide Kinase 1 (PDK1) and maintains the cell proliferation and maintain the pluripotency.

Conclusion

Many signaling pathways along with different transcription factors regulate pluripotency and self-renewal of ES cells and determine the fate of ES cells, generating more knowledge related to the pluripotency signaling pathways will help to meet these challenges and provide the necessary tools for the manipulation of ES cells for basic science and research application, therapeutic purposes and for the reproductive cloning. Mouse, rabbit, goat, bovine, buffalo and human ES cells show similar markers of pluripotency such as Oct4, Nanog, and high levels of telomerase activity. But, there are notable differences between ES cells amongst these species and these differences suggest that although the same pluripotency genes are expressed in ES cells however, their function and downstream signaling pathways may differ between species. Although our understanding for the maintenance of stemness is increasing fast yet there are many questions related to the molecular mechanism of pluripotency which need to be answered, such as cross talk between the signaling pathways and functional molecules, whether there are other existing pathway in livestock then that of the established pathways of human and mouse. To address these fundamental queries related to the stemness and its basic molecular nature is necessary before the ESCs are used for clinical application.

GENETIC ENGINEERING AND RELATED ETHICAL ISSUES

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Just the 20th century was golden age of computing and green revolution. 21st century is the age of DNA technology. We are on verge of being able to transform, manipulate and create any number of organisms for productive purposes. From medicine to agriculture to construction , Industry and even computing , we are within the reach of an age when manipulating the genetic codes of various organisms promises to alter the way we relate to natural world. The genetic engineering has potential to improve our health and well being dramatically, helps to conserve limited resources and produce new wealth. Provided it is well regulated, being in mind its ethical concerns relating to dignity, harmful consequences and justice, its potential benefits outweigh its harm. Biotechnology should be understood as an extension of already accepted and well established techniques, such as directed breeding, combined with sophisticated understanding of evolution and genetic techniques. As with any revolutionary technology, anxieties, fear and moral objections are going to be there. Some of these objections are well grounded and suggest cautions, while others are products of misinformation's and religious prejudice.

History

Humans have altered genomes of species for thousands of years through artificial selection and more recently through mutations The direct DNA manipulations has only existed since 1970s. In 1972, Paul Berg created the first recombinant DNA molecule combining DNA from monkey virus with that of Lambda virus. In 1973, Herber Boyer and Stenley Cohen created the first transgenic organism by inserting antibiotic resistant gene in the plasmid of an E. coli bacterium. One year later, Rudolf Jaenisch created the transgenic mouse by introducing foreign DNA in to its embryo making the first world's transgenic animal. In 1976 the first human protein (Somatostatin) was synthesized in *E.coli.* In 1978, Herbert Boyer and Robert Swanson engineered human insulin which was patented and approved by U.S. Drug Administration in 1982.First field trial of genetically engineered plants occurred in France and U.S.A. in 1986. Tobacco plants were engineered to be resistant to herbicides. Republic of China introduced Virus resistant tobacco in 1992. In 1994, tomato having long self life was introduced. In 1995 Bt potato was approved safe by Environment Protection Agency. In 2009, 11 transgenic crops were grown in 25 countries including India. In 2010, the scientists at J. Craig Institute claimed to have synthesized complete bacterial genome, when this genome was introduced in DNA free bacterium the synthesis of new species of bacteria resulted. The resulting bacteria named SYNTHIN was world's first synthetic life form.

Technique

Genetic engineering also called genetic modification (GM) is the direct human manipulation of an organism's genome using modern DNA technology.It involves introduction of foreign DNA or synthetic gene into organism of interest. The organism is considered genetically modified organism. The most common form of genetic engineering involves the insertion of new genetic material at an unspecified location in host genome using gene gun in embryonic cells. This is accomplished by isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence containing the required genetic elements for expression and then inserting this construct in to the host organism. The other form of genetic engineering includes genetic targeting and knocking out specific gene via engineered nucleases and endonucleases. .e.g. Knock out mouse or Onco-mouse.The genetic engineering alters the genetic make up of the organism after the introduction of heritable material prepared out side. The cell fusion technique, (Hybridoma) is also used in genetic engineering. The genetic material is either introduced indirectly through microinjection, macro injection , micro encapsulation or cell fusion technique. The cloning and stem cell technologies are not considered a part of genetic engineering but new genes can be introduced in clones and stem cells. The animal where the new genetic material from other species has been introduced is known Transgenic animal. The animal where new genetic material has been introduced from same species is known as Cisgenic animal. The gene can be introduced in somatic as well as germ cells. The genetic engineering using somatic cells provide effects only for that generation, while gene introduced in germ (Germ line) cells will provide effects in many generations to come and it is long lasting.

Application of genetic engineering

The genetic engineering has wide spread application right from human, animal, plants, lower forms of life, microbes, viruses, fish, pollution control and to industry

Human : The human recombinant DNA when introduced into microbe plasmids the microbes in culture synthesize hormones such as insulin, Growth Hormone, Follicle Simulating Hormone (FSH), steroids and various pharmacologically active substances. The human blood protein fractions such as clotting factors, human albumin and enzymes such as lactase and amylase etc have been synthesized by introducing human genes in goats, cattle and rice. The monoclonal antibodies get synthesized through hybridoma technique . the human edible vaccines (Hepatitis-B) are synthesized by introducing specific genes into food grains and fruits. The defective genes in the human causing chronic ailments such as Cancers. Metabolic diseases, Hereditary disorders such as cystic fibrosis, alzheimer's disease, sickle cell anemia and wound repair etc. can be knocked out and healthy gene therapy can be used for cure. The human stem cell treatment and organ transplantation as xenotransplants have been nearly successful in developing new tissues and organs .The attempts are going on in suppressing the genes controlling ageing process towards extending life span to 100 to 150 years. The genetic defects now can be corrected right from embryonic stage itself so the child born is without such defects (e.g. mental retard ness, Cleft palate etc.) In case the human cloning is made legal, the attempts will be towards developing Super Human and Sub Human as per government and country needs.

ANIMALS : Many species of higher mammals and invertebrates have been exploited towards aims of increasing production (Milk, Meat, Eggs, wool, leather) or producing DNA recombinant human proteins or the products suitable to man. The breeding bulls have been produced through cloning so that the growth, meat and milk production and quality can improve fast. The birds with low fat meat have been produced with application of genetic engineering. The trials are on way to produce disease resistant farm animals and birds. The Australians (CSIRO) have introduced human gene in dairy cows for the purpose of producing humanized milk having human milk proteins and low fat .The goats have been used for synthesizing human blood clotting factors in their milk towards the treatment of clotting disorders of man. The spider silk protein gene is introduced in goat embryos for the synthesis of strong steel for industry and bullet proof jackets cars. Histocompatible genes producing pigs are developed for transplantation of pig organs in to man. The knockout mice have been developed by knocking out disease causing and cancer causing genes towards the research and treatment of human cancer and other genetic disorders. The development of clones of farm animals such as cows, goats, sheep, horses and pet animals with high quality genetic make up have been successful. The trials are on way towards preservation of endangered species such as Tasmanian tigers and Panda have been successful. The development of Malaria and Dengue resistant mosquito for controlling the spread of malaria and dengue in man has been achieved. The wound repair through fruit fly gene is successful, this repair do not produce scar. The Salmon fish has been developed for more meat production. The recombinant DNA for the synthesis of bovine Somatotropin , FSH and other animal protein hormones have been synthesized in microbes. The rumen bacteria have been improved for the better digestibility of crude fiber.

AGRICULTURE : The major objectives of using genetic engineering in agriculture is to protect the crops from pests, improving the production and quality of food grains , fruits and fodder. Since the land of cultivation on the earth is limited and already the green revolution using chemical fertilizers, pesticides and insecticides is no more further increase food production the newer approach is to introduce quality genes so that food production can keep pace with ever increasing human and animal population. The consumption of animal protein has been increased three times in last 10 years. The crops which have been genetically engineered are as under :

Food grains: Bt Maize, Golden rice (with Vitamin A Gene), Soybean, Bt corn and oil seeds with low cholesterol and more fat content. Fruits such as tomato, potato, sugar beets, banana, papaya and sweet potato, fodder like alfa alfa and super weeds. Fibers like *Bt* cotton,biodegradable plastic. TNT and RDX clearing weeds.

MICROBES AND VIRUSES : The viruses are the first vectors used in genetic engineering. The microbes are easy to handle in genetic engineering work. The advantage with microbes is that they multiply fast. In 1970s the microbes were the only tools for genetic engineering research. The synthetic live forms of microbes have been

developed in 2011.Thr role played by microbes in genetic engineering is as under:Synthesis of pharmaceutical products such as insulin, human growth hormone, FSH and steroids. Synthesis of edible and non edible vaccines, Cleaning oil spills and carbon waste in industry, Cleaning sludge in sewage purification , Absorption of radioativity and methane from environment, Transfer of waste products in to ethanol. Synthesis of new strains of bacteria and viruses. Production of improved varieties of cheese, Improving digestibility in rumen, warfare in battles.

INDUSTRY : The biotechnology and genetic engineering have been used in industry for minimizing use of energy and clearing industrial pollution. Development of Lithium batteries, Black and white photography, Sensor as florescent, Gold and copper extraction from mines through digestion of substance in which they are embedded. The biotech microbes clear methane in mines.

Ethical issues related to genetic engineering

The genetic engineering involving the application of microbes, plants, insects, animals , human and fish genes for the food production, health, therapeutics, pharmaceutics and environmental pollution control is creating large number of ethical issues in human and animal world. The scientists have been divided in the for and against groups. In 1976 George Weld, Nobel Prize biologist stated that the recombinant DNA technology (Genetic engineering) faces our society problems unprecedented not only in the history of science , but life on earth. It places in human hands the capacity to redesign living organisms, the product of some three billion years of evolution. It presents probably the largest ethical problem the science has ever had to face. Potentially, genetic engineering could breed new animal and plant diseases, new sources of cancer and novel epidemics. These statements by great scientist clearly show that we can not necessarily depend on the biotechnologists to make our ethical decisions for us. Too much is at stake. However James Watson, Nobel laureate is in the favor of genetic engineering. Stephen Hawking (physicist) suggests that once the intelligent life form reaches the stage we are at now, it proceeds to destroy itself. The genetic engineering should attempt to change the aggressive nature of man . The ethical issues facing genetic engineering are (1) Safety of Genetically modified (GM) products for man, animals and plant world. (2) Religious and Secular concerns, (3) Dignity of man, (4) Animal welfare, (5) Economic and social concerns, (6) Justice and Equality, (7) Ecological and environmental risks, (8) Public perception, (9)Allergy, (10)Biowarfare, and (11) Altering humans.

SAFETY OF GM PRODUCTS :

The genetically modified foods, vaccines, pharmaceutics and gene therapy as a products are how much safe is matter of dispute. The advantages and disadvantages have been discussed by large many scientists. Therefore, legality of GM products needs to be discussed. Some advanced country like U.S.A. (Food and Drug Administration, FDA) has already passed the regulations about commercial sale in US markets and many GM food products are sold since past 15 years. The UK government has not certified all the GM foods and pharmaceutics safe for human animal consumption. WHO and FAO has approved all the GM foods for human consumptions since the animal trials have not shown untoward reactions and the chemical and nutritional compositions do not differ from natural foods. However, a group of people insists that the GM products should be specifically labeled before sold in market. The people's choice is an important factor for GM foods. The safety trials of short term duration have not shown any untoward reactions except for GM Soybean. Several scientists insist upon long term trials conducted by government agencies rather than corporate sectors (Manufacturers).The organizations such as Greenpeace and World Wild Life Fund insist that the long term health risks associated with GM foods have not yet been adequately investigated. The scientists should see to the injuries caused to GM animals. The Royal Society of Medicine (UK) and National Academy of Science have reported that after consumption of GM foods for last 15 years, no adverse effects on human health has been documented. A review published in 2009 by Dona and Arvanitoyannis concluded that " results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal and reproductive damage and may alter haematological, biochemical and immunological parameters. Introduction of Bt Brinjal in India is still a disputed matter. The Environmental Protection Group protested with naturally equivalent finding by corporate of GM foods with the arguments that scientists are not allowed to publish the true findings by corporate sectors as a result the research access to common man is what corporate feeds them .

Religious and secular concerns

The arguments based upon life sacredness suggest that altering life forms violates the will of Creator. The genetic engineering constitutes a misuse of our free will. All scientists do not believe in God or crater theory. Defying God's will mean defying religion's interpretation. The speed of predictability of genetic engineering is faster than natural selection. The religions have accepted clothing, agriculture and weaponary invented by man but contraceptives and antibiotics have interfered with natural order of evolution of man and animals. The Christians and Muslims believe that every embryo has the right to develop into an adult man and animal. We destroy large number of embryos in genetic engineering, cloning and stem cell technology. The later act also hurt the feelings of Budhists and Jain feelings of Ahimsa. The Hindus have nothing to tell about GM animals and Man because most of gods of Hinduism are genetic engineered. The Budhists and Jains believe on principle of Karma that the quality of an individual is as per the karmas of last life. The Jewish generally oppose GM foods since the religion has specified the types of foods and feeding habits. Even mixed cultured crops are not allowed in Jewish religion. The introduction of animal or human genes in food grain and fruit crops will affect the feelings if vegetarians.

Dignity of man

The introduction of animal or plant or bacterial gene into is reverting the process of evolution. This will affect the dignity of mankind and legal problems of inheritance will arise. A fearexpressed by scientists and common man is that the genetic engineering may introduce animal instincts and behavior in man. The society may not accept genetically modified man.

Animal welfare

The proponents of animal welfare believe that the genetic engineering, embryo transfer and other embryo technology is causing cruelty on animals. Large number of embryos are killed in these processes. The genetically modifies animals are not normal animals they are stresses not only for the faster growth and production but they suffer from large number of anatomical physiological abnormalities. They live short life with joint problems and circulatory problems. This is very much true for caged poultry pigs and calves.

Economic and social concerns

Since the genetic engineering research and patent rights are held by corporate research institutes situated in developed countries the GM food and other items will remain as monopoly of developed nations. There are chances that the under developed nations will be financially exploited by corporate sectors of developed countries and money will flow there. This will cause under developed countries over dependence on developed countries. In India the farmers are dependent on Monsanto produced Bt cotton seeds and the local varieties are becoming extinct. There is also the problem of how genetic engineering affect distribution of social goods as well as political rights .There is a concern that genetic interventions, especially genetic enhancements or reverse may enhance already existing inequalities as well creating new ones. The genetic engineering can increase the skills, body characters and qualities of children but this will not only cause social disharmony but only rich people can afford the technology.. We will have to redefine species and genus with the introduction of newer organisms.

Ecological and environmental issues

The large scale growth of GM foods and animals may have both positive and negative effects on ecology and environment. These may be both direct effects on the organism that feed on or interact with the crops or wider effects on the food chain. As an example ,the insect resistant BT crops will reduce the number of pest insects feeding on plants, but as there are fewer pests, the farmer will not use the pesticide. Which in turn tends to increase the non-pest insects in these fields. This will affect ecological balance .The Monarch Butterfly population increased due to decreased pesticide use. Other possible effect might come from the spread of genes (GM pollan) from genetically modified to unmodified relatives which might produce sterile seeds and weeds known as super weeds. Increasing or decreasing farm weeds have impact on wild life population. Growing herbicide resistant varieties , the production of weeds will be negligible and the wild life is adversely affected.. The GM animals such as pigs and poultry are so much deformed because of body weight gain that they are not acceptable to other animals to same species. These animals have to be reared in special environments. The genetically modified organisms are living organisms that have potential to disperse to new habitates, colonise there and multiply. This also will disturb the ecological balance. Once GM organisms get established they can not be called back.

Public perception

American consumers do not support banning uses of gene technology, but rather seek an active role of regulators to ensure that the product is safe. Only two percent of Britons were said to be happy with GM foods. More than half Britons are against eating GM foods .However, opposition to GM food is decreasing in Britain. In Australia, multiple surveys shown that while 45 % of the public accept GM foods, some 90 % demand genetically engineered foods to be labeled before sale. According to inter national survey in 2010, approximately 15 million farmers grew biotech crops in 29 countries. 6.3 million farmers each in India and China are growing Biotech crops. Introduction of Bt Brinjal in India has created discussion in parliament about its safety for human consumption. The survey on public perception about GM food has not been made in India.

Allergy

Some environmental organizations such as European Green Party and Greenpeace have suggested that GM foods might trigger food allergies, although other environmentalists have implicated that these allergies are due to green house effects of increasing pollen level, greater exposure to synthetic chemicals and molds in the houses. The well known case of a GM plant that did not reach the market due to its allergic reaction was a new form of Soybean intended for animal feed. A gene transferred from Brazil nut in Soybean was caused this allergic reaction. The GM Soybean was rich in protein and methionin but was caused immune reactions in poultry. The pest resistant GM pea crop developed allergy in mice. These cases of products that failed to safety testing can either be viewed as evidence that genetic modification can produce unexpected and dangerous changes in foods. A hypoallergic strain of Soybean has been produced by genetic engineering by knocking out genes for allergy, antitrypsine and antiamylase activities. Similarly grasses with low pollen allergens have been produced so that the incidence of Hay Fever is much more minimized. The reports on incidence of allergy to gene therapy, hormones , stem cells and pharmaceutics are not available at present.

Biowarfare

The biowarfare is a genetic engineering for the production of highly virulent microbes that can cause deadly disease in enemy country. These microbes are resistant to most of antibiotics. A secret mission employing 35000 scientists was set up in U.S.S.R. during cold war period. The project was closed after disintegration of U.S.S.R. Similar attempt was made by terrorist groups after 9/11 incidence USA. The Anthrax organism spores were dispatched to many Americans

Altering humans

Several geneticists and biotechnologists have called for ban species altering technology and have asked their governments to decide the level of introduction of human genes in animals, plants, and microbes. Similarly the human cloning has been objected with an argument that some day genetic engineers may alter humans into slaves, designer children and athletes. In 1998, scientist Jeremy Rifkin and Stuart Newman applied for " Humanzee" part human and part Chimpanzee to fuel debate on misuse of genetic engineering but they were denied the patent by US Government. This interspecies was to be used as slave. The US constitution do not grant permission for developing slaves.Shall we give the legal rights to such inter species?

Conclusions

The genetic engineering for food crops and human health has good motives and the technology can provide predictable results and production. However, the technology being new it is facing ethical problems. Consider the series micro-organisms –plants - animal – human. Should we draw a line limiting genetic manipulation at some point ? if so, where and what grounds ? Which potential benefits, if any (Therapeutic medicine), might be thought to justify animal genetic manipulations, which would not, which criteria we should apply ? Should we eat food stuffs which have been genetically manipulated using human genes ? How does this affect our religious feelings and dietary aws ? Is profit motive too dominant a driving force in biotechnology ? Are we reducing animals and nature in general in to a commodity ? How great are the potential risks involved in releasing genetically modified organisms in to the biosphere without knowing all the possible consequences ?
Are we opening a Pandora's Box ?

BIOTECHNOLOGICAL APPROACH TO IMPROVE THE NUTRITIVE VALUE OF LOW GRADE ROUGHAGES THROUGH RUMEN FUNGAL MANIPULATION

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Introduction

Ruminants are one of the most widely distributed groups of mammals on earth, having adapted to arctic, temperate and tropical environments. This global distribution is possible because of the unique ability of ruminants to digest a wide variety of vegetation. In tropical countries like India, the majority of livestock subsists on low grade roughages such as wheat straw, paddy straw, stovers, sugarcane bagasse and tree foliages. These straws and stovers are bulky and poorly digested in the rumen, due to their low protein and high lignin contents. Sixty percent of such feeds are unable to be digested by the animals. However, these feeds are the potential source of energy due to the presence of hetro and homo polysaccharides, such as hemicelluloses and cellulose. High lignin contents of these roughages reduce their digestible energy as lignin encrusts the cell wall contents and develops ester covalent bonds with hemicelluloses, which limits the plant cell wall degradation by rumen microorganisms. So, upgrading of low grade roughages is still a central issue for improving ruminant's productivity in tropics. Research workers have given technologies to enhance the digestibility of poor quality lignocellulosics feeds by the addition of various physical, chemical or microbiological treatments or feed additives. Chemical feed additives like antibiotic, ionophores and hormones increased the productive performance of animals but have the problem of antibiotic resistance and residual effects in the meat, milk and their products. Hormones alter the normal physiological functions, increased the unnecessary stress to the animals. Among microbial additives, there are evidences of definite positive relationships between elite rumen anaerobic fungi in the rumen and the increased digestibility of nutrients of fibrous feeds, growth and milk production (Phillips & Gordon, 1988; Dey et al., 2004, Sehgal et al. 2008, Saxena et al. 2010). An understanding of relationship between plant cell wall constituents and their degradation is important to the economics of animal production. The unusual nature of rumen anaerobic fungi and their potential importance of fiber-degradation in herbivores nutrition have made these a subject of interest during recent past. Rumen Anaerobic fungi is an emerging group of animal probiotic, account for up to 8-12% of the microbial biomass in the rumen and actively colonize plant cell-walls (Rezaeian et al., 2004, Sehgal et al., 2008) to improve digestibility of nutrients..

Degradation of low quality roughages

The B*utyrivibrio fibrisolvens*, R*uminococcus albus*, R.*flavefaciens, and* F*ibrobacter succinogenes* are regarded as the primary fiber degrading bacteria in the rumen. The genetic diversity and phenotype of Ruminococcus have received little attention and very few investigations have been carried out in a systematic manner, but for other fibrolytic bacteria, a number of studies are available. These bacteria act on peripheral portions of the substrate only. The 16S rDNA analysis has confirmed the DNA–DNA hybridization results of B.*fibrisolvens* and showed that the bacterium is polyphyletic. A study to assess the genotype and extent of fiber digestion of a medium quality grass varied from 0-16%. The genotype was based on ribosomal DNA sequence analysis. It is believed that, if this phenotypic diversity is represented within an individual rumen, than increasing the number of highly fibrolytic Ruminococci, it could potentially improve the rates of cell wall digestion in the rumen. Krause et al., (2001) also collected the highly fibrolytic Ruminococcus strain and evaluated them for their ability to colonize the rumen and to enhance fiber digestion. Tracking systems based on strain specific 16S rDNA sequences indicate that inoculated Ruminococcus strain did not persist for more than 3 weeks before reaching undetectable levels. Hence, the biggest problem is the ability to introduce bacterial strain and maintain the same in the mixed rumen population, and its survivability is not well understood.The role of rumen fungi in the degradation of plant fibre has been examined extensively (Samanta et al. 2001; Paul et al. 2004; Dey et al. 2004; Lee et al. 2004; Thareja et al. 2006; Dayanand et al. 2007; Tripathi et al. 2007a, b; Sehgal et al. 2008, saxena et al 2010, Jha , 2009). The

rhizoids or bulbous holdfasts of vegetative thalli are better for penetrating into the scleryncyma portion of plant tissue than bacteria and protozoa, thus they gain access to the plant material not accessible to other rumen microorganisms (Orpin and Joblin, 1988). It was suggested that such penetrations lead to a faster and complete degradation of forage entering the rumen. Degradation of lignified plant cell walls is an important characteristic of rumen fungi. Zoospores of many fungal species colonize the lignified tissues preferentially and establish colonies localized on sclerenchyma and xylem cells. Anaerobic fungi penetrate the cuticle, the rigid structural barrier on the outside of the plant epidermis. Rumen fungi also show protease activity that may have role in protein degradation, because the plant structural proteins increase the integrity of plant cell wall. Species of Piromyces, Neocallimastix, Orpinomyces and Anaeromyces degrade fibre to a substantial degree. Caecomyces species degrade fibres but lesser than other genera perhaps because of the lack of an extensive rhizoidal system. These findings suggest that the ability to degrade fibre varies among fungal genera, and that plants differ in their support for fungal growth. The greater ability of rumen fungi, compared to rumen bacteria, to weaken forage fibres may be vital to enhancing its utilization by the host animal (Borneman and Akin, 1990).Further rumen microbes have the ability to synthesize S- amino acids viz. methionine, cysteine and cystine from degradable inorganic sulphur. Utilization of nitrogen, assimilation, and metabolism of number of microelements in body depends upon sulphur. The activity of superior anaerobic fungi, which play a significant role in the structural degradation of fiber in the rumen, requires and necessitates the dietary sulphur and contributes in the synthesis of S- amino acids. As urea is a constituent of the concentrate mixture in practical ration, the proliferation of rumen microbes, especially, growth of rumen anaerobic fungi necessitates the dietary sulphur in the ration. Addition of sulphur enhanced fermentative activities, with the increased number of rumen anaerobic fungi proliferated in the rumen through complete feed blocks incorporated with rumen fungal zoospores, and enhanced the digestibility of nutrients and nutritive value in terms of total digestible nutrients in buffalo calves. In this essay, progress and problems related to manipulation of rumen ecosystem through inoculation of natural rumen anaerobic fungi will be discussed.

Taxonomy and classification of anaerobic rumen fungi

The ruminal anaerobic fungi, isolated as early as 1910, were thought to be flagellate protozoa (Liebetanz, 1910; Braune, 1913) and were placed in the genera Callimastix, Sphaeromonas and Oikomonas. These flagellates were recognized as fungi for the first time in the 1970s (Orpin, 1975) with the first named species N*eocallimastix frontalis*. The flagellate zoospores encyst and germinate on ingested forage with radiating rhizoids that produce a single zoosporangium. In terms of lifecycle and morphology, N. *frontalis* is similar to members of Chytridiomycota and its fungal affinities are confirmed by chitin in the cell wall (Orpin, 1994); though uniquely among the fungi it is an obligate anaerobe. There are 18 different species of anaerobic rumen fungi reported in various ruminant and hindgut-fermenting mammals (Table 1).

Table 1: Classification of genera and species of anaerobic rumen fungi.

Genus/species	Source(s)	Reference(s)
Caecomyces: C. *communis;* C. *equi*	Sheep; Horse, Elephant	Gold et al, 1998Nagpal et al, 2010
Piromyces: P.*communis;* P.*mae;* P.*dumbonica;* P. *rhizinflata;* P.*minutus;* P.*spiralis;* P.*citronii*	Sheep; cow; Horse; Elephant; Deer; Goat; Donkey, Blue bull	Julliand et al, 1998; Li et al, 1990; Breton et al, 1991; Ho et al, 1993a, b; Gaillard et al, 1995. Tripathi et al, 2007a.
Neocallimastix: N. *frontalis*; N.*patriciarum*; N. *hrleyensis*; N. *variabilis.*	Sheep; Cow, Goat	Webb and Theodorou, 1991; Ho et al, 1993c, Thareja et al, 2006. Sehgal et al, 2008

Anaeromyces: A.*elegans*; A.*mucronatus*	Cow; Sheep	Ho et al, 1993d; Breton et al, 1990, Sehgal et al, 2002.
Orpinomyces: O.*joyonii*; O.*intercalaris* Cyllamyces:	Sheep; Cow, Buffalo	Ho et al, 1994, Sehgal et al, 2002.
C. *aberensis*	Cow	Ozkose et al, 2001

Distribution of anaerobic rumen fungi

Anaerobic fungi play a key role in plant fiber degradation in the rumen, by releasing various enzymes such as cellulases, hemicellulases, proteases, and esterases, justifying their use as animal feed additives for improved ruminant nutrition (Lee et al., 2004 and Nagpal et al., 2009). It is now well established that these ruminal fungi efficiently take part in fiber digestibility in ruminants, leading to a more rapid degradation of forage entering the rumen (Lee et al. 2004). These fungi have been found in all of the geographic regions of the world, being ubiquitous in foregut fermenters and ruminants such as cattle, buffalo, sheep and goats (Ho et al. 1993a, b; Sehgal et al. 2002 and Thareja et al. 2006) and the wild bluebull (Boselaphus tragocamelus) (Paul et al. 2004a and Tripathi et al. 2007a). These fungi have also been isolated from feacal samples of horses, zebras, donkeys, rhinoceroses, and Indian elephants (Li et al. 1990 and Nagpal et al. 2009), all of which are hindgut fermenters. Rumen anaerobic fungi actively colonize plant cell walls and account for up to 8-12% of the microbial biomass in rumen (Rezaeian et al. 2004). Prior to their discovery, it was assumed that only rumen anaerobic bacteria and protozoa were involved in hydrolysis of plant biomass. But now, it is well established that these ruminal fungi effectively take part in fibre digestion in ruminants (Dey et al. 2004, Lee et al. 2004). The rhizoids of their vegetative thalli penetrate deep into plant tissues better than bacteria and protozoa, and thus achieve access to plant materials otherwise unavailable to other rumen microorganisms. This infiltration leads to a more rapid degradation of forage entering the rumen (Nagpal et al. 2007b). These fungi secrete high levels of very active fibre-degrading enzymes (cellulases, hemicellulases, xylanases, avicelases, glycosidases, p-coumaroyl esterase and feruloyl esterase etc.) found to be associated with rhizomycelia (Williams et al. 1994; Lee et al. 2001 and Kumar et al., 2011).

***In vitro* studies**

Anaerobic ruminal fungi play an active role in fibre degradation as evidenced by the production of different fibrolytic enzymes in culture filtrate. Thareja et al., 2006 isolated 16 anaerobic fungal strains from ruminal and faecal samples of sheep and goats. Based on their morphological characteristics, they were identified as *Anaeromyces, Orpinomyces, Piromyces* and *Neocallimastix*. Data in table 2 shows that Isolates of Neocallimastix sp. from goat rumen showed a maximum activity of CMCase (47.9 mIU ml^{-1}) and filter paper cellulose (48.3 mIU ml^{-1}), while *Anaeromyces spp.* from sheep rumen showed a maximum xylanolytic activity (48.3 mIU ml^{-1}). The cellobiase activity for all the isolates ranged between 178.0 – 182.7 mIU ml^{-1}. Based on the enzymatic activities, isolates of *Anaeromyces spp.* from sheep rumen and *Neocallimastix spp.* from goat rumen were selected for their potential of in vitro fiber degradation.

Table 2 : Hydrolytic activities (mIU/ml) of anaerobic fungal isolates using different substrates

Isolate	Genus	CMCase	Filter paperCellulase	Cellobiase	Xylanase
SRj*	*Anaeromyces* sp.	41.2	_	178.9	28.3
SR_2	*Piromyces* sp.	42.6	-	178.7	24.7
SR_3	*Neocattimastix* sp.	42.4	37.4	179.2	41.7
SR*	^mwromyrts sp.	43.9	40.3	178.7	48.3
SR_5	OrpHwwycw sp.	41.3	40.2	178.6	22.7
SF,"	*Piromyces* sp.	42.5	44.1	182.7	22.5
SF_2	*Piromyces* sp.	47.4	-	182.5	31.4
SF_3	*Orpinomyces* sp.	42.0	37.4	182.4	42.9
SF_4	^noerowj'ws sp.	43.3	-	182.2	22.3
SF_5	*Orpinomyces* sp.	41.4	39.2	182.5	26.0
GR/	*Neocattimastix* sp.	47.9	48.3	182.4	39.5
GR_2	*Orpinomyces* sp.	40.3	-	178.4	23.7

GR_3	*Piromyces* sp-	40.0	37.4	178.8	23.6
GF_1	*Piromyces* sp.	38.7	43.5	178.4	22.3
GF_2	*Anaeromyces* sp.	37.3	-	178.9	26.1
GF_3	*Orpinomyces* sp.	42.9	40.8	178.0	23.9

•**SR,** Isolated from rum **goat;** *GF, Isolated fronr of sheep; #SH, Isolated from fae " sheep; +GR, Isolated fro

Shelke et al. (2009) studied the Hydrolytic activity (mIU/ml) of fungal cultures in sugarcane bagasse and TMR assayed after 48h incubation at 39 °C. They observed that inoculation with *Neocallimastix spp.,* GR1 and *Piromyces spp.* WNG12 significantly increased (P<0.05) CMCase, FPCase and xylanase of cultures compared to control (Table 3). The results were in agreement to that of earlier reports (Paul et al. 2004; Thareja et al. 2006 and Tripathi et al. 2007a). Since *Neocallimastix spp.* GR1 and *Piromyces spp.* WNG-12, increased IVDMD and TVFA of sugarcane bagasse and TMR signifi-cantly, hence, these were found to be promis-ing direct fed microbial for ruminants to improve the nutritive value of fibrous feeds, ultimately leading to increased ruminant productivity in domestic animals.

Table 3 : Hydrolytic activity (mIU/ml) of fungal cultures in sugarcane bagasse and TMR assayed after 48h incubation at 39 °C

Treatments	CMCase		FPCase		Xylanase	
	Neocallimastix spp. GR1	*Piromyces* spp. WNG-12	*Neocallimastix* spp. GR1	*Piromyces* spp. WNG-12	*Neocallimastix* spp. GR1	*Piromyces* spp. WNG-12
Control T1(C)	13.71 ± 0.41	15.70 ± 0.95	9.09 ± 0.21	7.09 ± 0.36	23.37 ± 0.62	21.85 ± 0.42
Treatment T1(A) & (B)	17.95 ± 0.61*	17.61 ± 0.41*	12.58 ± 0.75*	10.91 ± 0.24*	29.28 ± 0.94*	27.85 ± 0.29**
Control T2(C)	15.71 ± 0.84	18.36 ± 0.08	10.73 ± 0.71	8.46 ± 0.90	25.45 ± 0.32	24.84 ± 0.95
Treatment T2(A) & (B)	23.15 ± 0.38*	21.01 ± 0.12**	16.85 ± 0.91*	13.45 ± 0.80*	32.29 ± 1.08*	30.80 ± 0.35*

Values are mean ± SD of three replicates.
Treatments significance of difference for control is indicated by * significant (P < 0.05) and ** significant (P < 0.01).

In another experiment Mamen et al 2010 studied the effect of inoculation of two different fungal organisms isolated from sheep *Anaeromyces spp.* (CTS-67) and goat *Orpinomyces spp.* (CTS-91) in to mixed rumen micro flora of buffalo on levels of certain major extra cellular fibrolytic enzymes (Table 4-5). They concluded that between two fungal isolates investigated *Anaeromyces spp.* isolated from sheep had highest stimulating effect on true, apparent and neutral detergent digestibility of lignocellulosic feed after 24 and 48 hours of incubation.

Table 4 : Effect of inoculation of two different anaerobic fungi isolated from sheep (CTS-67) and goat (CTS-91) into mixed rumen micro flora of buffalo

Enzyme activity	**Control**	**CTS-67**	**CTS-91**
CMCase (mIU/ml)			
24h	10.41^{b}±0.06	14.71^{a}±0.10	10.69^{a}±0.20
48h	11.15^{c}±0.22	15.71^{a}±0.25	13.17^{b}±0.17
Xylanase (mIU/ml)			
24h	23.26^{c}±0.08	31.25^{a}±0.05	25.38^{b}±0.22
48h	24.34^{b}±0.25	29.46^{a}±0.15	24.55^{b}±0.14
Â-Glucosidase (mIU/ml)			
24h	310.76^{c}±0.20	380.12^{a}±0.12	330.92^{b}±0.15
48h	288.22^{c}±0.02	369.08^{a}±0.13	308.64^{b}±0.04

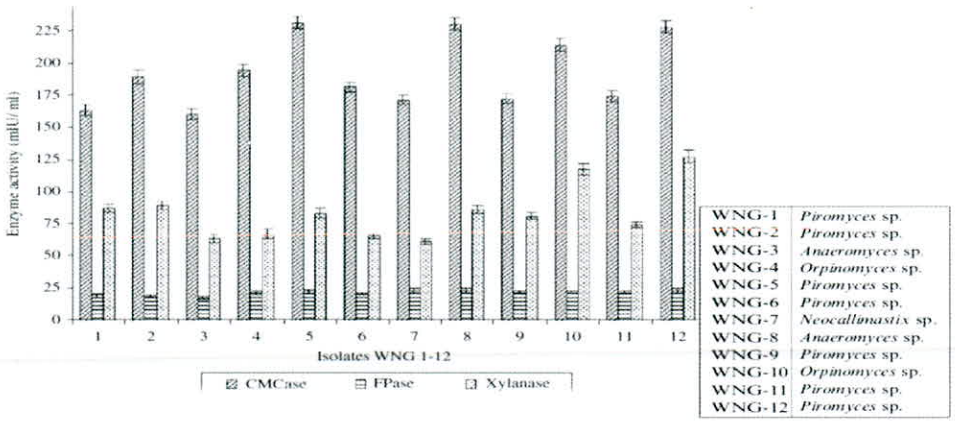
^{abc} Values in the same row are significantly different (P<0.05)

Table 5 : Effect of inoculation of two different anaerobic fungal organisms isolated from sheep (CST-67) and goat (CST-91) into mixed rumen micro flora of buffalo on *in vitro* feed digestibility

Digestibility (%)	Control	CTS-67	CTS-91
True digestibility			
24h	28.52c	35.28a	30.14b
48h	34.05c	43.31a	38.72b
Apparent digestibility			
24h	36.18c	44.50a	39.12b
48h	42.63c	52.74a	47.25b
NDF digestibility			
24h	18.32b	30.11a	15.62c
48h	29.81c	39.43a	34.45b

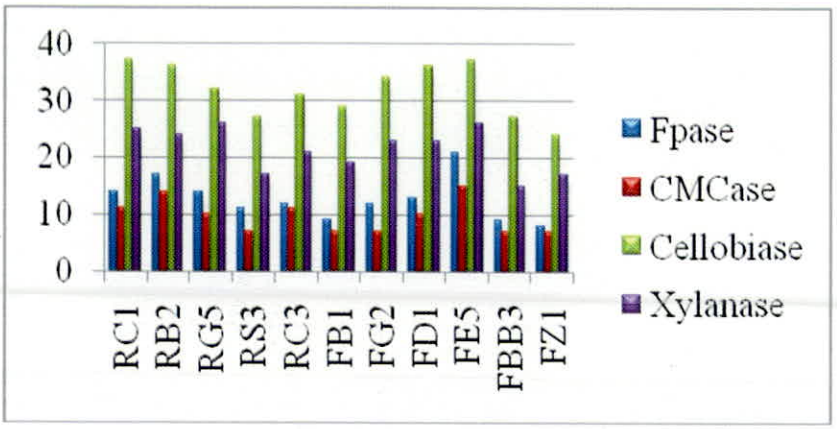
Tripathi et al., 2007 studied the hydrolytic activities of different anaerobic fungi from wild blue bull (Fig 1). Twelve anaerobic fungal strains were isolated from the faecal samples of wild blue bull, and identified as species of *Piromyces, Anaeromyces, Orpinomyces* and *Neocallimastix* based on their morphological characteristics. Isolate WNG-12 (*Piromyces spp.*), showed maximum filter paper cellulase (23mIU ml-1) and xylanase (127mIU ml^{-1}) activity, while WNG-5 (*Piromyces spp.*) showed maximum carboxymethyl cellulase activity (231mIUml^{-1}). Based on the results obtained, they stated that *Piromyces spp.* WNG-12 was a promising isolate in utilizing fibre rich diets in the rumen as evidenced by the production of hydrolytic enzymes *in vitro*.

Figure 1: Hydrolytic activities of different anaerobic fungi from wild blue bull



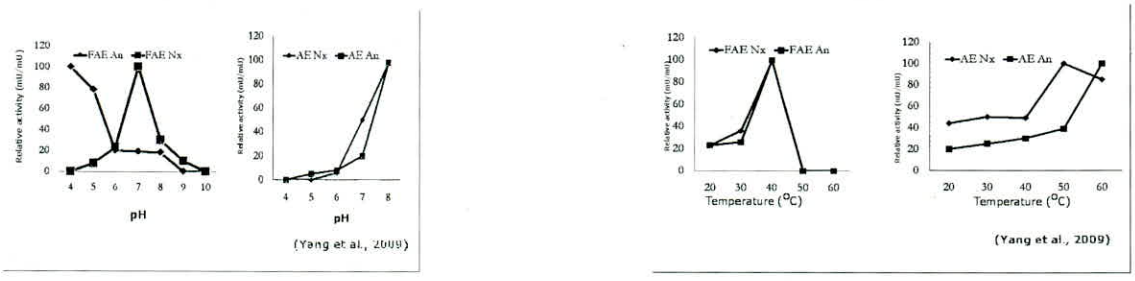
Nagpal et al., 2010 isolated different anaerobic fungi from different ruminants and non-ruminants; i.e., cattle, buffalo, sheep, goats, wild bluebulls, elephants, deer, and zebras.These were identified as *Anaeromyces, Orpinomyces,Caecomyces, Piromyces*, and *Neocallimastix spp.*, based on their morphological characteristics. These isolates possessed significantly higher *in vitro* hydrolytic enzyme activities(Fig 2); however, an isolate of *Caecomyces spp.* from elephant was found to exhibit maximum activity, i.e., filter paper cellulose (Fpase; 21.4 mIU/ml), carboxymethyl cellulose (CMCase; 15.1 mIU/ml), cellobiase (37.4 mIU/ml), and xylanase 26.0 mIU/ml).

Fig 2: Hydrolytic enzyme activity of different isolates using different substrates (mIU/ml/h)



The anaerobic rumen fungi secrete two enzymes to breakdown ester linkages or side chain linkages between hemicelluloses and lignin i.e. p-coumaroyl and p-ferulyl esterase. Yang et al (2009) studied the effect of pH and temperature on the relative enzyme activity of these two enzymes produced by Neocallimastix sp YQ1 and Anaeromyces sp YQ3. They concluded that p-ferulyl esterase produced by Neocallimastix sp had optimal enzyme activity at pH 7 while as same enzyme produced by Anaeromyces sp show optimal enzyme activity at pH 4(Figure 3). Acetyl esterase enzyme produced by both species showed optimal activity at pH 8. The effect of temperature on the enzyme activity was also studied. They concluded that optimal activity of p-ferulyl esterase produced by Neocallimastix sp YQ1 and Anaeromyces sp YQ3 was recorded at a temperature of 40 ^{0}C while that of acetyl esterase produced by Neocallimastix sp YQ1 and Anaeromyces sp YQ3 was at a temperature of 50^{0}C and 60 ^{0}C respectively. This means that ruminants were unable to degrade lignocellulosic roughages completely because the enzymes required for the degradation of fibrous feeds are unable to show their cent percent activity under rumen physiological conditions.

Figure 3 : Effect of pH and temperature on ferulic acid esterase and acetyl esterase activity of Neocallimastix sp YQ1 and Anaeromyces sp YQ3



Enzyme p-coumaroyl and p-ferulyl esterase activity of Orpinomyces joyonii

Kumar et al., (2011) studied the p-coumaroyl and p-ferulyl esterase activity of zoospores of Orpinomyces joyonii. They were grown for 6 days on paddy straw, wheat straw and TMR medium, containing 0.5% paddy straw, 0.5% wheat straw and 0.5% TMR respectively as the sole carbon source and strained rumen liquor to provide unidentified growth factors. Enzyme activity of p-coumaryl esterase and p-ferulyl esterase of Orpinomyces joyonii grown on paddy straw, wheat straw and TMR separately are shown in Table 6. One unit of activity was defined as the amount of enzyme that catalyses the release of ìmol product (ferulic or p-coumaric acid)/hour. p-coumaroyl esterase and p-feruloyl esterase enzymatic activity of Orpinomyces joyonii when grown on paddy straw was 39.98±1.93 and 178.93±4.91 (micro mol/ml/hour) respectively. The similar enzymatic activity of Orpinomyces

joyonii when grown on wheat straw alone was 89.14±5.61 and 205.09±10.23 (micromol/ml/hour) respectively (Table 6)

Table 6 : Enzyme p-coumaryl and p-ferulyl esterase activity (micro mol/ ml/ hour) of Orpinomyces joyonii grown on paddy straw, wheat straw and TMR. (Mean± S.E).

Treatment•	p-coumaryl esterase	p-ferulyl esterase
PS+Buffer+SRL without FZ	24.09±2.73	78.93±3.92
PS+Buffer+ SRL+FZ	39.98±1.93**	178.93±4.91**
WS+Buffer+SRL without FZ	29.94±4.72	84.92±5.11
WS+Buffer+ SRL +FZ	89.14±5.61**	205.09±10.23**
TMR+Buffer+SRL without FZ	45.82±3.71	99.34±9.01
TMR+Buffer+ SRL +FZ	109.13±2.99**	295.83±5.88**

•samples were analyzed in triplicates, **Significant (P<0.01)

p-coumaroyl and p-feruloyl esterase enzymatic activity of Orpinomyces joyonii grown on TMR was 109.13±2.99 and 295.83±5.88 (micromol /ml/hour) respectively. p-coumaroyl esterase and p-feruloyl esterase enzymatic activity of Orpinomyces joyonii when grown on paddy straw, wheat straw and TMR increased significantly (Pd"0.01) as compared to their respective control group. Little information regarding the activity of enzyme p-coumaroyl esterase and p-feruloyl esterase on paddy straw, wheat straw and TMR could be found in the literature. Thus it can be seen that anaerobic rumen fungi Orpinomyces joyonii is able to produce higher p-coumaroyl esterase and p-feruloyl esterase enzymatic activities and it has a potential to release more energy from lignified roughages as these two enzymes can break down the lignin to free cellulose and hemicelluloses to provide more digestible energy. Hence in order to increase the release of nutrients from highly lignified feedstuffs, elite Orpinomyces joyonii should be exploited under field conditions as direct fed microbial.

***In vivo* studies**

Lee et al.,(2004) studied the *in vivo* digestibility (%) of dietary nutrients by sheep receiving daily intra ruminal doses of 200 ml of fungal medium (FM), fungal enzyme (FE) or whole fungal culture (FC) . They found that direct administration of cultures of a polycentric fungal strain, Orpinomyces strain KNGF-2 isolated from a Korean native goat, to the rumen of sheep (FC) increased nutrient digestibility of DM, CP and cell wall components (table 7) and results were in agreement with results of Van Horn et al. (1994) who also observed increased DM digestibility with the addition of A. oryzae fermentation extracts. The improvement of digestion coefficients of nutrients by the administration of FC would be expected from improved ruminal fermentation parameters, and increased microbial numbers and enzyme activities in the rumen content of sheep.

Table 7 : *In vivo* digestibility (%) of dietary nutrients by sheep receiving daily intra ruminal doses of 200 ml of fungal medium (FM), fungal enzyme (FE) or whole fungal culture (FC) .

Item	FM	FE	FC
Dry matter	71.5±0.6b	70.8±0.9b	75.2±0.6a
Crude protein	68.6±0.6b	69.1±1.1b	71.9±0.7a
Ether extract	69.2±0.9	68.8±1.0	70.5±0.9
NDF	65.1±1.0b	62.8±1.0b	68.9±0.7a
ADF	57.3±0.9b	57.0±1.2b	62.9±0.8a
Hemicellulose	75.1±1.0a	71.2±0.7b	77.1±0.5a
Cellulose	68.4±0.6b	70.9±0.4b	79.0±0.4a
Cell contents	72.3±0.5	73.5±1.0	74.4±0.6

a Mean ± S:E: Means in the same row and item with different letters differ significantly (P < 0.01).

Saxena et al. (2010) studied the productive performance & feed efficiency of milk production in lactating buffaloes fed on wheat straw based total mixed ration(roughage : concentrate ratio 50:50 along with 5 kg green per animal per day) with and without fungal culture. For this study they selected fifteen lactating buffaloes (Murrah) in their second to fourth lactation (30 to 60 days after calving and yielding 7-10 kg milk/d) from the NDRI herd. The control group was also dosed with broth media without any fungal culture. Group II and III were offered the same diet as the control group along with *Orpinomyces spp.* C-14 or Piromyces sp. WNG-12 broth cultures, respectively. They observed that, the DMI/animal/day remained statistically same in all groups, but the average milk yield was higher (Pd"0.10) in group III (8.48 kg/d), followed by group II (8.42 kg/d) and the control (8.03 kg/d) as shown in Table 8.

Table 8 : Productive performance& feed efficiency of milk production in lactating buffaloes fed on wheat straw based total mixed ration with and without fungal culture

Parameters	Control	Orpinomyces	Piromyces	CD value (Pd"0.10)
Avg IMY (kg/d)	9.33[a]	9.34[a]	9.24[a]	NS
Avg MY (kg/d)	8.03[a]	8.42[b]	8.48[b]	0.25
Avg 6% FCMY (kg/d)	9.58[a]	10.28[b]	10.48[b]	0.31
MILK COMPOSITION (%)				
Fat	7.68[a]	7.90[b]	8.02[b]	0.21
SNF	9.51[a]	9.06[a]	9.15[a]	NS
Protein	4.24[a]	4.27[a]	4.50[a]	NS
DMI and Feed efficiency				
Avg. DMI(kg/d)	11.79[a]	11.50[a]	10.62[a]	NS
FE(kg milk / 100 kgDMI)	67.14[a]	72.99[a]	81.13[b]	6.41

Fat corrected milk (6%) showed an increase of 700 and 910 g/animal/day in group II and III, respectively compared to the control. The increase in milk production clearly indicated a better use of the wheat straw based diet in buffaloes dosed with rumen fungi, evidenced by an increase in the number of zoospores in the fungal culture administered group, which in turn might have increased the use of rumen ammonia nitrogen for the proliferation of bacteria. These fungi increased fibre utilization, as their enzymes break the bonds between lignin and hemicelluloses, and can also solubilise the lignin (Borneman et al., 1990; Phillips and Gordon, 1995) leading to the increased availability of digestible energy for higher milk production (Pd"0.05). Inspite of the fact that the DMI of the experimental animals was virtually the same in all groups, due to variation in their milk production, the feed efficiency was found to be higher in group III and II compared to the control. The present study was in consistent with that of Dey et al. (2004), Tripathi et al. (2007b) and Sehgal et al. (2008). The changes in milk composition (solid non-fat and protein) were statistically non-significant, except for increased milk fat in group II and III, compared to the control. This might be because high fibre degradation increased the acetate production in total volatile fatty acids that consequently act as a precursor for milk fat synthesis. Sehgal et al, (2008) studied the influence of anaerobic fungal administration on Animal performance, total feed intake, feed efficiency and nutrient utilization in female buffalo calves. Daily gains were higher in fungal culture administered group compared with control, and the differences were statistically significant ($P<0.05$) with nearly similar feed intake (Table 9). There was an improvement in body weight gains of calves' fed with TMR administered with fungal culture over control group of animals. As the growth in the present study pertains only to the accelerating phase of growth, the differences in growth performance in two groups could be attributed to the better utilization of TMR by the animals of fungal administered group, because of the availability of more digestible energy from the breakdown of ligno-cellulose and ligno-hemicel-lulose bonds of the wheat straw by Neocallimastix sp. GR1. Lee et al. (2000) used a similar technique to enhance the fiber digestion and fermentation in the rumen by increasing the activity of the anaerobic rumen fungi to breakdown the ligno-cellulolytic bonds of the straw in sheep, reported enhanced production efficiency.

Table 9 : Influence of anaerobic fungal administration on Animal performance, total feed intake, feed efficiency and nutrient utilization in female buffalo calves

Item	Control	Treatment	t-value
Initial body weight, kg	121.5 ± 4.5	122.6 ± 6.4	0.2^{NS}
Final body weight, kg	168.3 ± 6.6	182.0 ± 6.0	2.7*
Total body weight gain, kg	46.8 ± 2.9	59.3± 1.9	3.8*
Gain/day, g	520.1 ± 33.0	659.8 ± 21.6	3.8*
Total DMI[1], kg	372.9 ± 15.1	370.4 ± 17.5	0.2^{NS}
DMI/day, kg	4.1 ± 0.1	4.1 ± 0.2	0.2^{NS}
Feed efficiency#, %	12.5 ± 0.7	16.1 ± 0.8	3.8*
Total DCP[2] intake, kg	24.7 ± 0.8	26.5 ± 1.3	2.0^{NS}
DCP intake/day, g	275.1 ± 9.6	295.4 ± 14.8	2.0^{NS}
DCP intake/kg gain, g	538.9 ± 36.9	490.8 ± 10.8	1.6^{NS}
Total TDN[3] intake, kg	196.6 ± 8.2	221.1 ± 11.6	2.9*
TDN intake/day, kg	2.1± 0.1	2.4 ± 0.1	2.9*
TDN intake/kg gain	4.2 ± 0.2	4.0 ± 0.1	1.3^{NS}

* - significant (Pd"0.05); NS - non significant; # - kg gain/100 kg DMI; 1 DMI - dry matter intake; 2 DCP - digestible crude protein; 3 TDN - total digestible nutrients.

Dey et al. (2004) observed an improvement of 15.4% in body weight gains of crossbred cow fed on TMR and administered with fungal culture from cows at a weekly interval. Tripathi et al. (2007b) reported that there was a significant improvement in body weight gains of male calves fed TMR and administered with *Piromyces* sp. or *Orpinomyces* sp. Even though the dry matter intake of the calves was similar in two groups, due to variation in body weight gains, the feed conversion ratio was found to be lower in fungal administered group and the differences were statisti-cally significant. Dey et al. (2004) also reported that the feed conversion ratio was lower and the feed efficiency was higher in fungal culture administered group than control in crossbred male calves. Tripathi et al. (2007b) also reported the feed conversion ratio to be lower in *Piromyces* sp. administered and *Orpinomyces* sp. administered group of animals than control.

Effect of feeding lignified roughage based complete feed blocks fortified with anaerobic fungi, as probiotic, on nutrient digestibility.

Gordon and Phillips (2000) reported that the *in vivo* digestibility of DM was increased by 3-8% points in the presence of rumen fungi. It was also reported that the *Neocallimastix spp.* and *Piromyces spp.* efficiently degraded plant tissues.Sanjay et al (2009) incorporated anaerobic rumen fungi, *Neocallimastix spp.* GR1 in the wheat straw based complete feed blocks fed to buffalo calves and reported an increase in the digestibility of various nutrients as shown in table 10. Anaerobic rumen fungi has ability to produce various enzymes that break down not only the main chain of lignocelluloses/ lingo-hemicelluloses but also break the side chain of high lignified wheat straw based complete feed block (Yang et al., 2009).

Table 10 : Digestibility coefficient (%) of various nutrients of wheat straw based complete feed block without or with fungal (*Neocallimastix spp.*, GR1) Zoospores in growing male Murrah buffalo calves

Particulars	Groups		't' values
	Control	Treatment	
Dry matter	53.0 ± 0.4	61.7 ± 1.2	4.7*
Organic matter	53.3± 0.5	62.4 ± 1.0	5.1*
Crude protein	57.5 ± 1.0	65.1 ± 0.4	6.1**
Ether extract	76.0 ± 1.0	78.8 ± 1.2	1.3NS

Crude fibre	48.8 ± 0.9	58.9 ± 1.1	6.6**
Nitrogen free extractives	53.8± 0.6	62.2 ± 1.6	3.6*
Neutral detergent fibre	47.5 ± 1.3	57.2 ± 0.6	5.5**
Acid detergent fibre	44.2 ± 0.6	54.2 ± 0.7	7.2**
Cellulose	57.3 ± 0.6	68.1 ± 0.8	7.1**
Lignin (ADL)	52.6±9.1	65.2±10.1	11.2**

NS = Non-significant., ** Significant ($p \leq 0.01$), * Significant ($p \leq 0.05$).

Effect of feeding lignified roughage based complete feed blocks fortified with anaerobic fungal zoospores, as probiotic on growth

Due to unique potential of anaerobic rumen fungi to produce variety of hydrolytic enzymes, they are able to penetrate the lignified roughage feed particles entering the rumen and are said to be primary colonizers on feed particles in rumen. These fungi are able to break down the ligno-cellulose and ligno-hemicel-lulose bonds and expose the structural carbohydrate units to other microbes (bacteria and protozoa) which otherwise did not have access in rumen. Sehgal et al. 2008 reported higher growth rate in buffalo calves fed straw based diet fortified with fungal culture of *Neocallimastix* sp. Sanjay et al. (2009) has observed higher body weight gains in treatment group (515.52 g/day) compared to control group (436.74 g/day) with almost at similar level of DM intake was due to the incorporation of elite fungal zoospores of Neocallimastix sp., GR1, as probiotic, in low grade roughage based complete feed block in buffaloes as shown in table11.

Table11 : Animal performance, total feed intake and percent feed efficiency in male Murrah buffalo calves fed wheat straw based complete feed blocks without or with fungal Zoospores of (*Neocallimastix spp.*, GR1)

Particulars	Groups		't' values
	Control	Treatment	
No. of animals	6	6	
Av. initial body wt. (kg)	81.2 ± 7.3	82.3 ± 6.6	0.2NS
Av. final body wt.a (kg)	156.3 ± 13.1	171.0 ± 14.3	8.3 *
Total body wt. gain (kg)	75.1± 4.3	88.4 ± 3.3	4.8*
Gain day-1 (g)	436.7 ± 35.1	515.5 ± 20.1	3.8*
Dry matter intake (DMI) (kg)			
a) From CFB	525.9 ± 30.5	512.4 ± 16.7	0.2NS
b) From green Maize	72.7 ± 0.3	72.7 ± 0.4	0.2NS
Total DMI (kg)	598.5 ± 30.5	584.7 ± 16.7	0.2NS
Av. DMI day-1 (kg)	3.4± 0.2	3.3 ± 0.0	0.2NS
Feed conversion ratio (kg DM/kg gain)	8.0 ± 0.1	6.5 ± 0.1	3.8**
% Feed efficiency (kg gain/ 100 kg DMI)	12.4 ± 0.1	16.1 ± 0.1	3.9*

NS = Non-significant., ** Significant ($p \leq 01$), * Significant ($p \leq 05$).

Dey et al. (2004) also observed an improvement in daily gain after administration of fungi Orpinomyces sp. C-14 to calves. Compared to the control group, they observed a 15.4% higher body weight gain fed wheat straw based complete feed mixture when supplemented with Orpinomyces sp. C-14. Tripathi et al. (2007) reported that body weight gain of buffalo calves was improved by 20.6% and 29.7% after feeding diets supplemented with *Orpinomyces spp.* C-14 or *Piromyces spp.* WNG-12, respectively. Gordon et al. (2000) showed that sheep supplemented with a non-indigenous rumen anaerobic fungus, having highest cellulolytic activities, increased voluntary DMI by 12%. Thus the administration of *Neocallimastix spp.* GR-1, as probiotic, has improved the nutrient utilization from low grade roughage based complete feed blocks and increased the growth rate of ruminants.

Effect of feeding lignified roughage based complete feed blocks fortified with anaerobic fungi, as probiotic, on Rumen fermentation parameters.

pH of rumen is important for the normal functioning of rumen microbes. Incorporation of fungal zoospore in wheat straw based complete feed block in treatment group showed significant (P<0.01) decrease in rumen pH. Reduction in pH might be due to increase production of total volatile fatty acids in fungal zoospore incorporated group of buffalo calves as shown in table 12 (Sanjay et al. 2009). The TCA-ppt Nitrogen is also increased significantly in treatment compared to control group. TCA-ppt nitrogen represents the microbial protein so incorporation of fungal zoospores in wheat straw based complete feed block not only increases the TVFA production but also increased the microbial protein synthesis. High quality microbial proteins at intestinal level are digested, absorbed and was responsible for the increased growth rate in male buffalo calves fed low grade roughage based complete feed blocks.

Table 12 : Effect on rumen fermentation parameters and number of rumen microbes.

Particulars	Groups		't' values
	Control	Treatment	
pH 7.1 ± 0.0	6.9 ± 0.0	4.3**	
Total VFA (mM/100 ml)	10.2 ± 0.1	13.4 ± 0.1	5.6**
Tota lnitrogen (mg/100 ml)	78.2± 0.6	106.5 ± 0.5	7.5**
Ammonia nitrogen (mg/100 ml)	13.5 ± 0.2	9.1 ± 0.1	19.6**
TCA-ppt nitrogen (mg/100 ml)	52.5 ± 0.7	71.5 ± 0.5	20.2**
Avg no. of fungal zoospores/ml	1.6 x 105 ± 0.6 x104	4.3 x 105 ± 1.2 x104	10.3**
Average number of bacteria/ml	1.9 x 1010 ± 2.2 x108	2.7 x 1010 ± 3.2 x108	6.5**
Average number of protozoa/ml	2.1 x 106 ± 2.4 x104	1.5 x 106 ± 3.1 x104	11.4**

** Significant (p≤0.01)

Effect of zoospores of *Neocallimastix spp.* Gr-1 on methane production in buffalo calves fed wheat straw based complete feed blocks

Sanjay et al. (2009) as shown in table 13 reported 33% reduction in methane production in male buffalo calves fed wheat straw based complete feed block incorporated with fungal zoospores of Neocallimastix sp GR-I compared to control indicating that even with the increased digestibility of straw based diet with fungal zoospores, methane production reduced significantly may be due to the change in pathways for the production of high energy supplying products. Co-cultures of the fungal zoospores with methanogens resulted in an enhanced cellulose fermentation rate and less methane production with fungal zoospores due to hydrogen sink which is utilized by the anaerobic fungus.

Table 13 : Effect of anaerobic fungal zoospores on methane production (Liter/24 hrs) in growing male buffalo calves

Particulars	Groups		't' values
	Control	Treatment	
1st trial(3 buffalo calves)	45.2±12.2	32.4±10.2	5.3**
2nd trial (3 buffalo calves)	53.2±15.2	33.4±9.6	6.5**
Total methane production (Lit/24 hrs)	98.4±9.6	65.6 ±9.8	10.1**

** Significant (p≤0.01)

Effect of incorporation of fungal zoospores and sulphur in wheat straw based complete feed blocks fed to calves

The size of the anaerobic fungal populations in the rumen increased after either an application of a sulphur fertilizer to the pasture used to make the hay or a sulphur-containing dietary supplement to the low sulphur hay. Probably the form and distribution of sulphur in herbage of low sulphur content was as important as the total sulphur content in determining the size of the fungal population in the rumen during these studies. Phillips and Gordon (1991) indicated the need for reduction of supplementary sulphate in rumen before it would be available for anaerobic fungi. Addition of 1% sodium sulphate (0.22 % S) in wheat straw based complete feed blocks incorporated with fungal zoospores of *Neocallimastix spp.* GR-1 enhanced the growth rate, percent feed efficiency and fungal zoospores count compared to complete feed blocks without fungal zoospores and with fungal zoospores alone (Jha et al., 2011).

Factors affecting fungal population

There are so many factors, such as composition of diet, feed pretreatment, feeding frequency, defaunation, inophores, fungistats, probiotics and dietary phenolic compound, and interaction with rumen microbes that affect fungal population (Sehgal et al., 2011).

Diet composition

It has been observed in general that if fibre content is more in feed, fungal population increases. Few anaerobic fungi were seen in the rumen of animals that were fed lush pasture (either legume or grass when green and leafy) compared with the same pasture when it was mature. Thus, fibre or lignocellulose content of the diet was a critical factor for the growth of fungal population.The influence of diet composition on rumen fungal population by offering successively to a rumen fistulated cow 11 different diets rich in fibre, starch or soluble carbohydrates, was studied. At the same time, the colonization of four different plant substrates introduced into the rumen in nylon bags was investigated. They found that the population of rumen anaerobic fungi particularly was abundant with lignocellulose rich diets, decreased with starch or soluble-carbohydrate and the fungi selectively colonized the plant tissue with thick or lignified cell wall. If free lipid content of feed increased, fungal population decreased. Feeding of free lipids to ruminants could have a detrimental effect on rumen fermentation, retarding fibre digestion. Perhaps coating effect of fats on fungal cells could explain this. The addition of grass lipids (extracted from fresh alfalfa grass) to the medium, significantly decreased fungal growth and cellulase activity. However, low chain fatty acids (palmitic, stearic and oleic acids) did not have any significant inhibitory effects on fungal growth and enzyme activities. Unsaturated fatty acids (oleic, linoleic acids) addition increased fungal growth and activities perhaps due to utilization as alternative hydrogen sink. But, linolenic acid showed an inhibitory effect. The size of anaerobic fungal population in the rumen increased dramatically either after an application of S to the pasture used to make the hay, or due to a S- containing dietary supplement to the low S-hay. It has been reported that microbial growth enhanced with the supplementation of S in the diet of ruminants and addition of the sulfur supplementation resulted in increased feed intake and increased flow of microbial protein to the small intestines when the animals were fed a poor quality roughage low in S (0.1%) and N. Both supplements improved rumen function by stimulating the microbial population to incorporate more ammonia N for protein synthesis. The study also demonstrated that the animals would benefit from additional supplemental urea for complete utilization of the S based supplements.

Feed pretreatment

Alkali treatment (ammonium hydroxide) increased the in vitro digestibility of wheat straw by an efficient fibre degrading anaerobic fungus. Earlier it was reported that ammoniation of wheat straw increased the dry matter digestibility of the feed by 8-10% and normally faunated sheep consuming it had higher fungal counts in the rumen (1.6-3.0 folds). did microscopic studies of structure and rumen fungal colonization in sheep fed different alkali treated wheat straw and showed the effects of chemically treated wheat straw with calcium oxide (CaO), sodium hydroxide (NaOH), and alkaline hydrogen peroxide (AHP) on its subsequent colonization and degradation in-sacco by rumen fungi in sheep. AHP caused the greatest fragility to straw particles followed respectively, by NaOH and CaO treatments. Untreated straw showed relatively less fungal colonization compared with treated straws being heavily colonized by rhizoids and thalli. The thalli of this study resembled mono- and poly-centric genera of anaerobic Chytridiomycete fungi. The fibrolytic activities of rumen fungi in terms of dry matter loss,

plant cell wall degradation, and enzyme (cellulase and xylanase) activities, when grown in vitro, on either untreated or sodium hydroxide treated stems of barley straw over a 12 day period. Showed that in vitro degradation of cellulose and hemicellulose was higher in the treated than that of untreated cultures. Although, comparatively, xylanase activity was higher than that of cellulase, the cellulose fraction of the straw was degraded more than hemicellulose in both treated and untreated straws. Piromyces spp. WNG-12 from Nilgai had shown better digestibility of urea-NH_3 treated wheat straw than Orpinomyces C-14 from cattle in vitro.

Defaunation

Ionic detergents caused increased populations of rumen fungi and it was found that with the increase in rumen anaerobic fungal count there was a decrease in protozoal count, but the bacterial count was more in the rumen of buffalo calves fed wheat straw based ration incorporated with superior fungal culture.

Ionophores

Monensin, tetronasin, salinomysin, polyoxins were found to decrease fungal count. The effect of the antibiotic lasalocid on the rumen anaerobic fungus Neocallimastix frontalis RK 21 was examined as it was thought that lasalocid increased the efficiency of feed utilization by cattle by altering the rumen fermentation. The effect of steroidal saponins from Yucca schidigera extracted from rumen microbes concluded that steroidal saponins from Y. schidigera inhibited cellulolytic rumen bacteria and fungi, but their effects on amylolytic bacteria were species dependent and similar to the effects of ionophores.

Fungistats

Sodium propionate had no effect on the number of fungi in sheep at a rumen dose rate of 20-40 g/ day, only Neocallimastix become dominant over Piromyces.

Other fungal probiotics

Feeding of live *S. cerevisiae* to sheep had no effect on the numbers of fungal zoospores in the rumen. The effects of a live yeast strain of *Saccharomyces cerevisiae* on zoospore germination, metabolism, and cellulolytic activity of the anaerobic rumen fungus Neocallimastix frontalis was observed and results showed that yeasts could enhance plant cell wall colonization by *N. frontalis*. The yeasts could therefore also be a good tool to optimize the microbial degradation of lignocellulosic materials on certain diets.The effects of *Aspergillus oryzae* fermentation extract, Amaferm, on the rumen fungus *Neocallimastix frontalis* showed that the secretion of cellulase was increased by 67% and rhizoid development was increased by 3.8-fold in the presence of this extract. These results supported the idea that by accelerating fungal growth and metabolism, Amaferm increased the rate of fibre degradation caused by rumen fungi and that this in turn, may contribute to enhanced animal performance.

Conclusion

Rumen anaerobic fungi produce wide variety of enzymes with broad substrate specificity including the ability to degrade microcrystalline cellulose.Rumen Fungi plays a leading role in degradation of lignified roughages, as it increases the availability of nutrients to other rumen microbes.Administration of Rumen anaerobic fungal cultures improve the productive performance of animals fed low quality straw based ration.Rumen fungi can be exploited as Direct Fed Microbial to enhance the digestibility of nutrients and rumen fermentation of wheat straw based ration.Optimum pH and temperature plays an important role for the maximum activity of enzymes.Incorporation of fungal culture utilizes complex polysaccharides of sugarcane bagasses that can be used by animals for increasing the productive performances

Avenues for future research

Milk and meat products from ruminants are important source of energy rich nutrients and bioactive compounds. Future research on biohydrogenation potential of rumen fungi is needed. Genetic modification of fungi to synthesize enzymes that show optimal activity under normal rumen environmental Conditions (pH, temperature etc). Genetically-modified ruminal bacteria have an important role to play in altering rumen function, but the challenge is not simply the molecular manipulation of a strain as ecological factors ultimately have an overriding influence on the outcome of the process.There is an urgent need to develop the zoospores of the superior fungi in larger quantity and then to make their dried powder or paste which can be incorporated into diet of the ruminants for proliferation of these fungi in rumen.

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BIOTECHNOLOGY AND MODIFICATIONS OF RUMEN MICROBIAL ECOSYSTEM[1]

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Role of ruminants in the developing countries

Ruminant animals are one of the mans' most valuable and renewable resources providing livelihood for many people in both developed and developing countries. Ruminants have the ability to convert low quality feeds into high quality protein and to utilize feeds from land not suitable to grow crops for human consumption.Preliminary estimates indicate that of the 1.3 billion poor worldwide, (around 42%) depends on livestock as part of their livelihood (Pica-Ciamarra, 2005). It is also seen as having a key role in the reduction of poverty. Livestock production is a complex function of climatic and environmental conditions that prevail in the particular area, availability of feed quantity and quality, genotype which fits it to a particular production system,control of various diseases, health and reproduction.

According to FAO food balance sheets, in the developing world meat demand will grow to 327 millions tons by 2020 and milk consumption to 648 millions tons. India has been uniquely successful in converting crop residues into milk-expanding production from 20 million tons in 1961 to 80 million tons in 2001, and without feeding grain. Recent knowledge and research in biotechnology can help to increase livestock production to meet the demand of ever increasing population world wide.

The Rumen Ecosystem

The rumen ecosystem is very complex. The rumen which hold up to 95 litres of food & fluid is having unique microbial digestive mechanism that work within physiological range of rumen temperature fairly constant 37°C, pH (5.5 to 7.0) and humidity (Johnson ad Johnson, 1995).However, this ideal niche for development is controlled by feeding (type and level), mixing of rumen contents, salivation and mastication, diffusion and secretion into the rumen and absorption from the rumen and passage of material down the digestive tract. The rumen having diverse and complex microbial ecosystem also have genotypic and phenotypic diversity in different ruminant. Rumen microbial eco-system consist of population of bacteria (10^{10}–10^{11} cells/ml, more than 50 genera) (Prescott et al., 2005), anaerobic fungi (10^{3}–10^{5} spores/ml, representing five genera), ciliate protozoa (10^{4}–10^{6}/ml, from 25 genera), and bacteriophages (10^{8}–10^{9}/ml). Less than 10-20% of the rumen microbial population is culturable (McSweeney et al., 1999) thus large population of rumen microorganism is non cultural but is active in rumen fermentation. In the rumen, there is presence of cellulolytic, hemicellulolytic, amylolytic, proteolytic, and acid-utilizing microbes,etc. Rumen metagenomics coupled with biotechnology has the potential to contribute to all these pressing needs. These technologies have the potential to revolutionize the understanding of rumen function and will overcome the limitations of classical based techniques, including isolation and taxonomic identification of strains. important to efficient rumen function and better understanding of the roles of microorganisms in relation to achieving high productivity and decreasing environmental pollutants (Shakira and Atiya, 2009)

Contribution of Rumen Microbes

(A) Rumen Bacteria

The ruminants are born with sterile gastro-intestinal tract where bacterial colonization occurs rapidly. Rate and extent to which fiber is degraded is determined to a considerable degree by factors such as microbial accessibility to substrate, physical and chemical nature of the forage and kinetics of ruminal digestion. Major bacterial species of the rumen and their biochemical activities, are given in Table 1 (RobertB. Hespelln. 1987). Ruminococci are to the extent of 50–94% in Cattle &Buffalo (Jalaludin et al., 1992). *Streptococcus bovis*, one of the cellulolyitc bacterial species found to be tolerant to O_2 and depressed rumen pH (Bowmen and Sowell, 2003).

(B) Rumen Fungi

Fungi excrete wide variety of enzymes required for lignocellulose degradation and may also play an important

synergistic role in the ruminal digestion of forages by physically disrupting the lignified stem tissue so that ruminal microbes can have greater access to the plant stem and the digestible portions of the plant. (Akin and Ringsby, 1987) because of the presence of different enzymes like proteases and esterases in addition to cellulases and hemicellulases (Fonty and Joblin, 1990,Tenkanen et al., 1991).Five genera of anaerobic chhytridomycetous fungi have been isolated from the rumen and involved in fibre degradation are: *Anaeromyces, Caecomyces, Neocallomastix,Orpinomyces* and *Piromyces* (Ho and Barr,1995). High xylanases have been isolated from *Neocallomastix patriciarum* and *Orpinomyces jayoni* (Gilbert et al. , 1992, Li et al. , 1996).

(C) Rumen Archeaea

The methanogens by the way of capturing hydrogens for the benefit of fermentation leads to a significant loss of gross energy consumed by the animals. The relative contribution (%) of greenhouse gases to atmospheric warming is Carbon dioxide—49%, Methane –18%,CFCs—14%, N oxide—6%,others—13%. Relative contribution of biological resources to the global production of CH_4 in the atmosphere is Ruminants-18%, Paddy fields-31%,Marsh-13%,other biological 6% and non-biological 32% (Bolle *et al.,* 1986, Leng, 1990). Seven different species of methanogens have been reported from the rumen of different animals are *Methanobacterium formicicum, Methanobacterium bryanti, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanomicrobium mobile, Methano-sarcina barkeri* and *Methanoculleus olentangyi* (Joblinet et al.1990; Jarvis et al. ,2000).

(D) Rumen Protozoa

The total number of ciliate protozoa is lower in buffalo than that in Cattle, The enzymatic profile of holotrich protozoa indicates that they have amylase, invertase, pectin esterase and polygalacturonase in sufficiently large quantities (Bailey and Howard,1963; Williams,1979).Ciliate protozoa present in cattle and buffalo include *Entodinium, Diplodinium, Eremoplas-tron, Eudiplodinium, Elytroplastron, Metadinium, Ostra-codinium, Epidinium, Dasytricha* and *Isotricha* (Shimizu et al.,1983). Defaunation has resulted in 30–60% increases in growth rate of sheep, cattle and buffalo (Bird *et al.* 1990) and a preliminary study indicates a promise of up to 2 litres/day more milk in Friesians (Moate, 1989).

(E) Rumen Bacteriophages

The specificity of the bacteriophages for lysing a particular rumen bacterium may be exploited for selectively killing rumen methanogens or removal of unwanted rumen bacteria from the ecosystem. (Klieve et al.,1999; Bach et al., 2002, Ed Charmley, 2009).

Limitations of Rumen Microbes in feed utility

In many cases greater than 50% of the dietary fibre remains as an undegraded form (Cherney et al.; 1991). Cattle lose 6% of ingestion energy as methane (Johnson and Johnson, 1995). Overall only 10–35% of energy intake is captured as net energy. Limitations to plant cell wall digestion in the rumen could result from following major factors that regulate ruminant fiber digestion are: (1) Plant structure and composition, which regulate bacterial access to nutrients(2) Nature of the population densities of the predominant fiber-digesting microorganisms; insufficient quantities or types of enzyme production by the ruminal microbes It can also be due to inability of degradative enzyme(s) to interact with the target substrates (3) Animal factors that increase the availability of nutrients through mastication, salivation and digesta kinetics. Conditions in the rumen not being optimal for activity (low ruminal pH).(4) Feed associated constrains include anti-nutritive factors (protective for plants) present in plant tissues like tannins, lignins, saponins, phytohaemagglutinins, mimosine, protease inhibitors, and cyanogens in legumes, and glucosinolates, etc. They may act as inhibitors for some of the rumen microbes.

Biotechnology and Manipulation of Rumen Microbial Ecosystem to increase digestibility

The shortage of feed in most developing countries and the increasing cost of feed ingredients, emphasizes a need to improve feed utilization. Different methods of optimizing feed conversion into nutrients in the rumen are now available to maintain the P/E ratio (ration of protein digested and absorbed from the intestines to the VFA produced in and absorbed from the rumen). The rumen microbial ecosystem is not as efficient for 100% digestion of ingested feed as evident from the sizable portion of undigested feeds in the faeces and production of large amount of methane gas in the rumen which could be otherwise utilized as source of energy by the animals. Biotechnology and Manipulation of Rumen Microbial Ecosystem are Potential Targets for Improving Fibre Digestion.

Biotechnology in animal production has grown faster than its applications in crop production. The applications of biotechnology to animal production broadly covers different areas viz. Reproduction and breeding, Animal health, the growth and production, and food and nutrition. Given our present inability to culture the vast majority of microbes from this ecosystem the Rumen metagenomic approaches offer the only methodology currently available to access these unique and useful bioresources.(Shakira and Atiya, 2009) **Biotechnology** can be broadly said to be :

1. Traditional biotechnology includes the bakes bread, brew alcoholic beverages, and breed food crops or domestic animals and 2. Modern biotechnology (genetic engineering) includes the integration of microbiology, biochemistry and chemical and process engineering. Animal genetic modification and cloning technologies considered as a part of modern biotechnology. Genetic engineering is the process of transferring individual genes between organisms or of removing, modifying or adding genes to a DNA molecule for a desired trait or characteristic. Metagenomics study aims at uncultured organisms to understand the true diversity of microbes, their functions, cooperation and evolution, in environments such as soil, water, ancient remains of animals, or the digestive system of animals and humans (Huson *et al.*, 2009).Some of the major objectives of rumen manipulation are (Santra and Karim,2003) (a) Enhance fibrolytic activity, enhanced cellulolysis, reduced methanogenesis anddecreased proteolysis/deamination (b) Increase microbial protein synthesis (c) Reduction in proteolysis or the degradation of protein and deamination of aminoacids in the rumen should be discouraged (d). The provision of alternate hydrogen sinks in case of reduction in methanogenesis in the rumen may help in increasing the availability digestible energy(DE) for production. Methane generation in the rumen is a wasteful process as 5-10% of GE intake of ruminants is converted in to methane. e. Prevention of acidosis in high grain fed animals, the level of lactic acid can be controlled to avoid acidosis and inhibition of feed utilization due to lowered pH of the rumen liquor,f. Shifting acetate to propionate production in fattening beef/lambs. g. Metabolism of plant toxins during rumen fermentation can be manipulated for efficient utilization of feeds. The plants contain anti nutritional factors viz. tannin, saponin, mimosine etc,The rumen must be considered as an integrated system and this makes it difficult to rationalize manipulation. The observed result of any treatment is a combination of several interactive reactions. Any change to one component of the system has several uncontrolled effects on other components.

The methods of rumen manipulation

Modification of rumen microbial composition and their activity has been attempted by several methods. It can be classified in two i.e., non genetic manipulation and genetic manipulation.Non genetic manipulation of the rumen can be done by (a) Chemical additives/treatments of forages (b) Microbial/probiotics treatments of forages and (c) Manipulation of dietary inputs and feeding management. In genetic manipulation, attempts were made to develop genetically engineered rumen microbes by gene transfer/manipulation. Introduction of naturally occurring or genetically modified foreign microbes into the rumen and genetically manipulation of existing microbes in the rumen ecosystem. Further, Interspecies trans-inoculation of rumen microbes was also successfully used for annulment of dietary toxic factor. To overcome the difficulties and limitations associated with cultivation techniques, several DNA-based molecular methods have been developed. In general, methods based on 16S rRNA gene analysis provide extensive information about the taxa and species present in an environment. However, these data usually provide only little if any information about the functional role of the different microbes within the community and the genetic information they contain of microbial niches (Streit and Schmitz, 2004). Accordingly, rumen protozoa were eliminated by defaunation for reducing ruminal methane production and increasing protein outflow in the intestine.

A. Non genetic manipulation

A.1 Chemical treatment and enzymes

A1.1 Inorganic Chemicals

The addition of buffers and alkalinizing products (sodium bicarbonate, magnesium oxide, sodium bentonite) to the diet of lactating dairy cows can improve fibre digestion by adjusting pH of rumen. Chemical treatments such as sodium hydroxide, potassium hydroxide and ammonia will partially solubilize hemicellulose and lignin, as well as hydrolyze acetic, phenolic and uronic acid esters. Sulphuric acid and hydrochloric acid bring about reduction in the pH and resultant degradation of lignin and extensive solubilization of structural carbohydrate (Fahey et al. 1993).

A.1.2 Organic Chemicals

Supplementation with organic acids could improve rumen efficiency by maintaining higher pH, optimum ammonia-nitrogen (NH 3-N), thus reducing methane (CH4) and increasing microbial protein synthesis and essential volatile fatty acid (VFAs), for enhancing ruminant productivity. Silage feeding quality can be improved by use of additives tried like chloroform, toluene and cresol (to inhibit bacterial growth) (Fahey et al. 1993). Formaldehyde and other aldehydes, and formic acid are widely studied.

A.1.3 Ionophores and Antibiotics

The ionophore (monensin, lasalocid, salinomycin, lysocellin, narasin and tetronasin) can improve cellulose digestion of diets high in readily available carbohydrate by inhibiting the growth of lactate-producing bacteria, thereby decreasing lactate concentrations and increasing ruminal pH. (Wallace 1994). Monensin can bring about 10% reductions in methane formation (Ed Charmley, 2009). Ionophores inhibit Gram-positive bacteria more than Gram-negative bacteria. Protozoa and fungi are also sensitive to ionophores to different extents.

A.1.4 Enzymes

Within the rumen, exogenous enzymes could act directly on the feed or could stimulate synergistic effect between ruminal microorganisms and exogenous fibrolytic enzymes to increase the hydrolytic potential within the rumen environment (Morgavi et al.(2000).Exogenous enzymes may remain active in the lower digestive tract, contributing to the post-ruminal digestion of fibre or could indirectly improve nutrient absorption in the lower tract by reducing viscosity of intestinal digesta. Most of the fibrolytic enzymes used as feed additives in ruminant diets were originally developed as silage additives (Feng et al.,1996) and are of bacterial and fungal (Trichoderm longibrachiatum, Aspergillus niger, Aspergillus oryzae) origin. Addition of exogenous dose of fibrolyitc enzymes in cows, increased dramatically the total digestibility of nutrients (Rode et al. 1999), and that applying the fibrolytic enzymes onto feed prior to feeding was more effective than dosing directly in to rumen (Wallace,et al.,2001). Enzyme products marketed for livestock are derived primarily from only four bacterial (Bacillus subtilis, Lactobacillus acidophilus, L. plantarum, and Streptococcus faecium, spp.) and three fungal (Aspergillus oryzae, Trichoderma reesei, and Saccharomyces cerevisiae) species (Muirhead, 1996). Commercial hemicellulase and cellulase enzyme cocktails are now available and improve the fermentation process considerably. Considerable emphasis has been placed on the improvement of fibrous crops by the strategy of growing soft-rot fungus (*Phanerochaete chrysosporium*) which produces lignase enzyme which causes a high degree of depolymerisation of lignin.The lignin gene has to date been cloned and sequenced from *P. chrysosporium* (Rege, 2000).

A.1.5 Defaunating agents

The process of making the rumen of animals free of rumen protozoa is called defaunation. Defaunation is resulting in improvement in growth and feed conversion efficiency of the animals. The different means by which defaunationn can be brought about by chemical treatment(copper sulphate,manoxol,sodium lauryl sulphate), dietary manipulation(pH below 5.0 ciliate protozoa are killed), isolation of new born animals(Santra and Karim,2003).

A.2 Microbial /probiotics (live microbial cultures) feed additives

Additionally, probiotics of bacterial and yeast origin have been used in animal feeding to stabilize rumen fermentation, reduced incidence of diarrhoea and thus improving growth and feed conversion efficiency of young stalk.High-protein yeast (Saccharomyces cerevisciae , 50g/d),stabilizes the pH, stimulates certain bacteria, create a healthy balance of bacteria in the digestive tract. and can increase gain, feed, and efficiency. Yeast enhances the breakdown of fiber in the rumen, resulting in increased microbial protein production. Fungi (Aspergillus orizae), is also used as used as probiotics. Directly fed microbials, or probiotics, as additives remove oxygen from the ruminal environment, thereby increasing bacteria viability, and result in pH stability and increased rate (but not extent) of cellulolysis (Wallace 1994). The primary micro-organisms currently used in animal feeding are: (BACTERIAL ORIGIN)B*acillus licheniformis,* B*acillus subtilis,* B*ifadobacterium adolescentis,* B*ifadobacterium animalis,* B*ifidobacterium bifidus,* L*actobacillus casei,* L*actobacillus delbrueckii,* L*actobacillus fermentum,* L*actobacillus cellobiosus* L*actobacillus lactis,*

Lactobacillus plantarum, Bifadobacterium infantis, Bifadibacterium longum, Bifidobacterium peudolongum, Lactobacillus salivarius, Lactobacillus sporogens, Bifidobacterium suis, Lactobacillus reuteri, Bifadobacterium hermophilum, Lactobacillus acidophilus, Streptococcus intermdius, Streptococcus thermophilus Lactobacillus bulgaricus, Lactobacillus brevis(YEAST ORIGIN) Aspergilus oryzae, Saccharomyces cerevisiae.

A. 3 Manipulation of dietary inputs and feeding management

Feeding of urea-treated (5%) straw increases rumen fermentation.Feed processing —development of bypass protein supplements for ruminants.Methane gas formation can be reduced by feeding— fats and oils (cotton seed oil—5 to 10% reduction), canola oil and seedssoyabeen oil and seeds, sunflower oil and seeds, fish oil, faxed seed oil, Muristic acid, coconut oil, copra meal. legumes (10% to 30% reduction), feeding Halogens (bromochorometahne—80% reduction), feeding organic acid—Produce propionate and mop up hydrogen (30% reduction). Molasses supplementation reduces methane in L/Kg digested organic matter intake—At zero proportion of molasses methane liberated was 60L and at 0.4 proportion of molasses it was 34L methane. (Ed Charmley, 2009).Herbs have been evaluated for their ability to alter ruminal fermentation and improve nutrient utilization in ruminants (Wang et al., 2000; Wallace et al., 2002Greathead, 2003). Animal farming operations involving high degrees management input have an increased probability for controlling the rumen ecosystem and are better suited for the introduction of genetically engineered micro-organisms, as well as for the maintenance over time of the introduced species. In contrast, animal farming operations involving low management input or use of grazing animals would seem to be less amenable to the use of genetically engineered rumen micro-organisms.

(B) Genetic manipulation

Gene-based technologies are being increasingly used to improve animal nutrition, either through modifying the feeds to make them more digestible or through modifying the digestive and metabolic systems of the animals to enable them to make better use of the available feeds (Bedford, 2000). (i) The genetic manipulation in forages includes manipulation of plant genomes to increase the efficiency of nutrient availability to animals and to decrease emissions into the environment or incorporate vaccines or antibodies into feeds that may protect the animals against diseases. (ii) Genetic engineering of ruminal microbes to improve ruminant animal production is one of the most useful and less controversial fields. If genetically engineered ruminal microorganisms are to be used for nutritional purposes, following three scientific objectives must be met (Wallace, 1993) (1)To insert new genetic material into ruminal species and ensure that it is expressed, (2) To select a gene product(S)s that will benefit the nutrition of the host animal, (3) To establish a means by which the new organism can survive. Aside from these aspects are ethical considerations and regulatory constraints, which are complex and changing. The foundation of the molecular ecology techniques is 16S/18S rDNA sequence analysis which has provided a phylogenetically based classification scheme for enumeration and identification of microbial community members. (Briesacher, 1992). Specific DNA probes can also be used to enumerate a single bacterial strain in rumen (Attwood,1988). The focus of nucleic acid research is now shifting to the functional analysis of the ecosystem which involves the measurement of functional genes and their expression in the predominant or specific members of the rumen microbial community.

The general methods in Modifying Rumen Bacteria

Isolation /selection of desired genes for Enzymes— cellulose degradation is to be improved, the bacteria which are deficient in cellulose degradation should be selected. *Fibrobacter succinogens* is one such example. These bacteria can be modified to contain a large number of genes for cellulose degradation so that cellulose degradation in the rumen become increased.Preparation of Anaerobic Bacterial Plasmids Suitable for Gene Insertion—for carrying the gene to the recipient cell is required. The recombined plasmid is then transferred back into rumen bacteria to facilitate the insertion of desired genetic property. Strategies proposed (Wallace 1994) for recombinant DNA technology to develop new strains of bacteria for improved fiber digestibility have included the following: 1) Inserting the cellulase gene into numerically predominant species (B. ruminicola); 2) Increasing the competitiveness of cellulolytic organisms (F. succinogenes,

Ruminococcus) by conferring the ability to utilize xylose and pectins, thereby allowing earlier colonization of particulate matter;3) Increasing the competitiveness of cellulolytic species present in the rumen in low numbers by according the ability to adhere to feed particles; 4) Inserting an acid-tolerant cellulase gene into acid-tolerant bacteria (Lactobacillus) to allow fiber fermentation at a ruminal pH less than 6; 5) Developing a cutinase activity in predominant bacteria; and 6) Allowing predominant species to degrade arabinose side chains, thereby overcoming the rate-limiting effect of lignin. Other points to be attended through biotechnology are as under:The problem of excessive proteolysis might be approached by linking the protease gene(s) of one or more species to other N metabolism genes whose expression is regulated by ammonia levels. Such a gene would be urease or glutamine synthetase which have been studied in rumen micro-organism species such as *Selenomonas ruminantium* (Smith *et al.* 1981) and *Succinivibrio dextrinosolvens* (Patterson & Hespell, 1985).The identification, selection of strains of anaerobic microbes, particularly with a high fibrolytic enzyme secretion or that secretes fibrolytic enzymes of high specific activity. This is a necessary prerequisite to the successful inoculation of microorganisms into the rumen and which will nutritionally benefit the host Under the assumption that rumen microbes does not produce the correct mixture of enzymes to maximize plant cell degradation. Ruminococcus and fibrobacter which are he fibolytic bacteria of rumen, do not produce exocellulases so that adding this activity would make them more potent. Creation of microbes with greater spectrum of enzyme secretions by recombinant DNAmeans, e.g. create cellulolytic capacities in xylolytic organisms and vice versa.

Polymer of polymeric lignins is not degraded by any known anaerobic organisms. The identification of strains of anaerobic fungi that most rapidly degrade the cell walls of forage plants. The enzymes for solubilisation of lignin, if present in rumen organisms, may be capable of being boosted by genetic means or by selection. The potential for inducing lignin-solubilising enzymes becomes a major possibility.Loss of these hydrogen-gas-utilizing methanogenic organisms would drastically disrupt the entire rumen fermentation system. (Kandler & Konig, 1985). The metabolism of other bacterial species would also have to be genetically engineered to provide a hydrogen sink. One possibility would be to engineer *Eubacterium limosum,* a relatively numerically minor species in the rumen, to preferentially form acetate and butyrate The regulation of fermentation products made by the rumen microbial population would be another target area for use of genetically engineered micro-organisms. Control of the numbers of specific microbial species in the rumen population is equally important.

Developing Detoxification Mechanisms in Rumen Organisms

Interspecies trans-inoculation of rumen microbes was successfully used for annulment of dietary toxic factor.(a) Introduction of the microbe from goats in Hawaii into ruminants in Australia prevented the symptoms of mimosine toxicity in previously susceptible ruminant livestock. Because theses microbes of rumen of the Hawaiian goats could degraded dihydroxy-pyridine, the toxic breakdown product of mimosine (Hegarty, 1982). (b) The successful transformation of rumen bacteria with a single gene for defluorination of fluoroacetate would indicate the possibility of the utilization of a range of other plants, which may be weeds (e.g. lantana), or potential high biomass producers (some legumes and legume trees) and also high protein crops (e.g. canavalia).Development of transgenic bacteria with enhanced cellulotic activity, capability to cleave lignohemicellulose complexes, reduced methane production capability decreased proteolytic and/or deaminase activities, increased capability for nitrogen "fixation" and increased ability for microbial production of specific amino acids. The first successful transfer of foreign genes into rumen bacteria (*Bacteriodes ruminicola*) was reported by Thomson and Flint (1989). In all cases, the prerequisite will be that the organisms are able to grow in and maintain their space in the rumen. The practical applications of genomic studies on rumen microbes could involve industrial production of key enzymes (lygnocellulytic) for pre-treatment of fibrous residues, which could in turn be employed in practical feeding strategies.

Progress made in Genetic engineering of ruminal microbes

Over 100 different genes encoding enzymes for fibre digestion have been cloned from ruminal bacteria (Butyrivibrofibrisolvents, and fibrobacter succinogeneses and *Prevotella ruminalcola*, *Ruminococus albus* and *R. flavefaciens.* In case of ruminal fungi, about 30 genes have been isolated that encode cellulase, xylanases, mannanases and endogenases. About 50% of the fibrinolytic genes have been cloned and have sequenced (Bowman and Sowell, 2003) have powerful fibolyitc activity.Cellulose and xylanase genes from ruminal protozoa

have been cloned and most of the fibrolytic genes cloned have also been sequenced. It has resulted in enzymes to make 10 times higher specific activity, changed pH and temperature optima and increased substrate binding activity than the native enzyme (Sellinger et al.,1996)After xylanase gene was introduced successfully from anerobic ruminasl fungus Neocallimastix patriciarum to Butyrivibrio fibrisolven and due to expressed of a foreign xylanase gene (B. fibrisolven NO_4), it secreted xylanase enzyme (Krause et al., 2001).The genetic manipulation/ engineering work has been done in bacteria, easy to handle e.g. *Escherichia*. The genes which have been cloned in *Escherichia coli* are endoglucanase, xylanase, â-glucosidase, amylase, glutamine synthetase from the donor source of *Bacteroides fibrisolvens*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Neocallimastix frontalis*, *Streptococcus bovis* etc.

The problems with the establishment of genetically engineered rumen bacteria:
The problems are too many and very complex.Scientific and technical problems involved in the establishment of these bacteria in the rumen, Technical difficulties like —isolation and taxonomic identification of strains for inoculation and DNA recombination; isolation and characterization of candidate enzymes; level of production, localization and efficiency of secretion of the recombinant enzyme; stability of the introduced gene; fitness, survival and functional contribution of introduced new strains.The existing regulations about the release of genetically engineered microbes in the atmosphere are also a limitation.A more realistic approach will be to study as to whether the introduced genetic product can serve the purpose of improving rumen fermentation (Wallace, 1994).

The stability of the gene product against its degradation in the rumen.Ability of reintroduced bacterium to survive in the rumen:The recombinant did not survive more than 22 days (Wallace, 1994) and it was mainly due to heat sensitive antibacterial factors associating with microbial cells in the rumen (Kobayshi and Yamamoto (2002). According to McSweeney et al,1999, the ability of reintroduced bacterium to survive in the rumen is determined by many factors, which are (i) Competition for substrate used for growth (ii) Growth at a rate faster than the dilution rate of the rumen (iii) Adaptation to and tolerance of the chemical and physical environment in the rumen (iv) Withstanding engulfment by protozoa (v) Resistance to inhibition by baceriocins like activity present in the rumen liquor(Attwood et al., 1988). and infection by bacteriophage.

Conclusions
Many enzyme preparations are currently in use, with no attempt being made to define the types or activities of the enzymes they contain. Enzyme cocktails should be designed specifically to overcome the constraints limiting digestion of a particular type of feed.

Feedstuffs are exceedingly complex structurally, and our lack of knowledge of the factors that limit the rate and extent of feed digestion impedes engineering of designed enzyme preparations. Presently there is a lack in the technology for specific production of many other potentially important enzymes (e.g., cutinase, ferulic acid esterase, acetylxylan esterase, arabinofuranosidase). Further work is needed for successful gene transfer into important cellulytic species Systemic research is needed to determine the conditions that are required to ensure survival of introduced ruminal microorganisms' strains.

The practical applications of genomic studies on rumen microbes could involve industrial production of key lygnocellulytic enzymes for pre-treatment of fibrous residues, which could in turn be employed in practical feeding strategies Without improved nutrition it is unlikely that any modern biotechnology can be successfully applied to ruminants.Almost nothing is known about the biochemical properties of extracellular or cell-surface-bound enzymes (amylases, cellulases, xylanases, pectinases or other enzymes) for degradation of plant cell componentsVery little is known about the effects of growth rate, pH, temperature, induction-repression mechanisms or other physiological factors on the expression of these degradative enzymes. Need to be determined to see whether the genetically modified strain can grow and effectively compete in mixed microbial populations as found in the rumen.For gene expression studies there are inherent problems involved in extracting high quality RNA from digesta, and priming cDNA synthesis from bacterial mRNA.Rumen metagenomics coupled with biotechnology has the potential to contribute to all these pressing needs. These technologies have the potential to revolutionize the understanding of rumen function and will overcome the limitations of classical based techniques, including

isolation and taxonomic identification of strains important to efficient rumen function and better understanding of the roles of microorganisms in relation to achieving high productivity and decreasing environmental pollutants

Table 1. Major bacterial species of the rumen and their biochemical activities (RobertB.Hespelln,1987)

Major bacterial species of the rumen	***their biochemical activities***
Ruminococcus albus	c, x
Ruminococcus flavefaciens	c, x
Butyrivibrio fibrisolvens	C,X,PR,P, A,L, SU,AU
Bacteroides succinogenes	C
Bacteroides amylophilusBacteroides ruminicola	A, PRA, X, P, PR, SU
Selenomonas ruminantium	A, SU, GU, LU, LP
Anaerovibrio lipolytica	L, GU
Lachnospira multiparus	P, A, PR
Succinivibrio dextrinosolvens	D, P
Streptococcus bovis	A, SU, PR (?), LP
Wolinella succinogenes	HU, M
Methanosarcina barkeri	HU, AU, M

*C, cellulolytic; X, xylanolytic; P, pectinolytic; A, amylolytic; D, dextrinolytic; L, lipolytic; PR, proteolytic; SU, soluble-sugar user; GU, glycerol user; LU, lactate user; HU, hydrogen user; AU, acetate user; LP, lactogenic; M, Methanogenic.

Figure 1 : The relative contribution (%) of greenhouse gases to atmospheric warming (World resource Instituste).

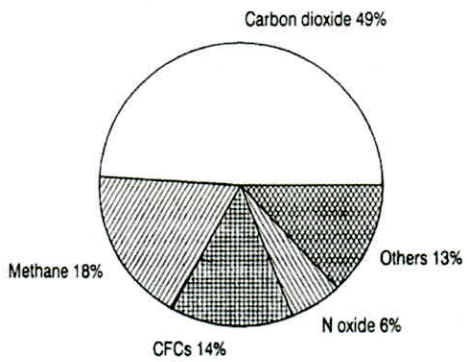
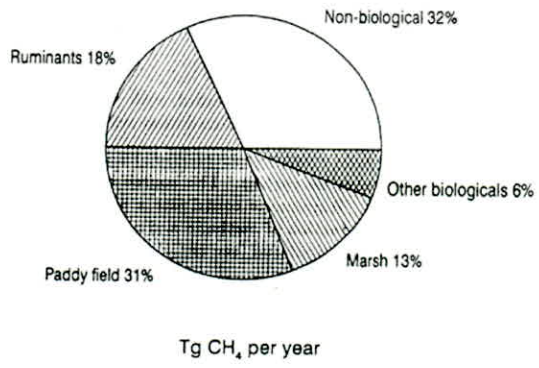


Figure 2 : Relative contribution of biological resources to the global production of CH4 in the atmosphere (Bolle et al., 1986)



Ruminants-18%, Paddy fields-31%,Marsh-13%,other biological 6% and non-biological 32% (Teragram or 10^{12} g)

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MOLECULAR DIAGNOSTICS IN MICROBIOLOGY : CURRENT STATUS AND FUTURE STRATEGY

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Abstract

Systems to detect, identify and characterize infectious agents or to diagnose the infectious diseases should be tightly linked to strategies used to control them. The traditional methods of microbial, detection, identification and characterization rely primarily on the phenotypic characteristics of the organism i.e microbial morphology, growth variables or serology. Bacterial morphology, fermentation, serology, fungal conidiogenesis, parasitic morphology, and viral cytopathic effects (CPE) are a few phenotypic characteristics commonly used across the globe. Some phenotypic characteristics are sensitive enough for strain characterization; these include isoenzyme profiles, antibiotic susceptibility profiles, and chromatographic analysis of cellular fatty acids. However, most phenotypic variables commonly observed in the microbiology laboratory are not sensitive enough for strain differentiation. Hence over the past century microbiologists have been searching for more rapid and efficient means of detection, identification and characterization of microbial pathogens. Advances in molecular biology over the past 20-30 years have opened new avenues for microbial identification and characterization. When methods for microbial genome analysis (genomics) became available, a new frontier in microbial identification and characterization was opened. Earlier DNA hybridization studies were used to demonstrate relatedness amongst bacteria. This understanding of nucleic acid hybridization chemistry made possible nucleic acid probe technology. Advances in plasmid and bacteriophage recovery and analysis have made possible plasmid profiling and bacteriophage typing, respectively. Both have proven to be powerful tools for the epidemiologist investigating the source and mode of transmission of infectious diseases. These technologies, however, like the determinations of phenotypic variables, are limited by microbial recovery and growth. Nucleic acid amplification technology has opened new avenues of microbial detection and characterization, such that growth is no longer required for microbial identification. For example the *Mycobacterium* species, *Dichelobacter nodosus* (causative agent of footrot) etc which are very fastidious bacteria and would take weeks together for isolation and identification. Now these can be detected and diagnosed in hours by using polymerase chain reaction (PCR) without culture. In this respect, molecular methods have surpassed traditional methods of detection for many fastidious organisms like *Mycobacterium tuberculosis* and *Dichelobacter nodosus* (causative agent of footrot). The PCR products, known as amplicons, may be further characterized by various methods, including nucleic acid probe hybridization, analysis of fragments after restriction endonuclease digestion or direct sequence analysis. Similarly, pulsed field gel electrophoresis (PFGE) has emerged as the gold standard molecular approach to the epidemiology analysis of most bacterial pathogens as DNA fragmens larger than 40kbp can be easily resolved now. DNA microarray is another powerful tool for the investigation of various aspects of prokaryotic biology because it allows the simultaneous monitoring of the expression of all genes in any bacterium. Similarly Real-time PCR (RT-PCR) also called quantitative RT-PCR (Q-PCR/ qPCR/qrt-PCR) or kinetic PCR), is used to amplify and simultaneously quantify a targeted DNA molecule in real time. It is anticipated that the optimal selection of appropriate antimicrobials by clinicians, and the ability to monitor the efficacy of prescribed therapies, will be gradually improved with the increasing number of rapid molecular diagnostic tools for the detection, identification and characterization of infectious agents. Thus molecular diagnostics for infectious diseases is an emerging concept in which molecular biology tools are used to provide rapid (less-than-one-hour), accurate and more informative diagnostic microbiology assays, thus enabling better therapeutic interventions. Therefore, it is important that no one working in microbiology and immunology should find themselves distanced from this latest field of genomics for optimum exploration of microbial world, their virulence mechanism and host response and including development of real time diagnostic assays with high sensitivity and fidelity. Thus rapid techniques of nucleic acid amplification and characterization have significantly broadened the microbiologists' diagnostic arsenal.

Introduction

Over the past century microbiologists have been searching for more rapid and efficient means of detection, identification and characterization of microbial pathogens. The traditional methods of microbial identification rely solely on the phenotypic characteristics of the organism. Bacterial fermentation, fungal conidiogenesis, parasitic morphology, and viral cytopathic effects are a few phenotypic characteristics commonly used. Some phenotypic characteristics are sensitive enough for strain characterization; these include isoenzyme profiles, antibiotic susceptibility profiles, and chromatographic analysis of cellular fatty acids. However, most phenotypic variables commonly observed in the microbiology laboratory are not sensitive enough for strain differentiation even at times they become redundant when the organism is non cultivable or takes too long to grow. Advances in molecular biology over the past 20- 30 years have opened new avenues for microbial identification and characterization. When methods for microbial genome analysis became available, a new frontier in microbial identification and characterization was opened. Earlier DNA hybridization studies were used to demonstrate relatedness amongst bacteria. This understanding of nucleic acid hybridization chemistry made possible nucleic acid probe technology. Advances in plasmid and bacteriophage recovery and analysis have made possible plasmid profiling and bacteriophage typing, respectively. Both have proven to be powerful tools for the epidemiologist investigating the source and mode of transmission of infectious diseases. These technologies, however, like the determinations of phenotypic variables, are limited by microbial recovery and growth. Nucleic acid amplification technology has opened new avenues of microbial detection and characterization, such that growth is no longer required for microbial identification. In this respect, molecular methods have surpassed traditional methods of detection for many fastidious organisms. The polymerase chain reaction (PCR) and other recently developed amplification techniques have simplified and accelerated the *invitro* process of nucleic acid amplification. The amplified products, known as amplicons, may be further characterized by various methods, including nucleic acid probe hybridization, analysis of fragments after restriction endonuclease digestion, or direct sequence analysis. Similarly, Pulsed Field Gel Electrophoresis (PFGE), Real time PCR (RT-PCR) and DNA microarray analysis have proved state of art techniques in diagnosis, epidemiological investigations and characterization of the pathogens. Thus rapid techniques of nucleic acid amplification and characterization have significantly broadened the microbiologists' diagnostic arsenal. Hence the immunologist, microbiologists and biotechnologists should not find themselves distanced from the latest fields of genomics and proteomics. Role of molecular methods lies in: (1) the rapid identification of organisms in clinical specimens (2) the identification of organisms isolated in pure culture and (3) the identification of fastidious and non culturable organisms. Presently many molecular methods are available. Insight of some of them is summarized below:

Nucleic Acid Probes

First described by Marmur and Doty (1961), nucleic acid probes are capable of identifying organisms at, above, and below the species level. The quantity of target detectable by the method depends on the size and homology of the probe chosen and the nature of the original specimen; identification of organisms in pure cultures or from isolated colonies is usually easier than detection of organism in a direct specimen. DNA probes facilitate the identification of infectious agents that do not grow rapidly. Additionally, this technique allows for the diagnosis of infections in which the organisms are not easily cultured or cannot be cultured at all. Detection of DNA with direct or culture-amplified gene probe technology has been applied to several organisms *Streptococcus agalactiae*, *Haemophilus influenza*, mycobacteria, Hepatitis delta virus, *Histoplasma capsulatum, Cryptococcus neoformans* etc.,. The technique has been also used to monitor growth as an indicator of drug resistance or to directly detect genes associated with antibiotic resistance.

Plasmid analysis

Plasmid profile analysis was among the earliest nucleic acid-based techniques applied to the diagnosis of infectious diseases and has proven useful in numerous investigations. In epidemiological studies, relatedness of pathogenic bacterial strains can be determined from the number and size of plasmids they carry. This technique is widely used to monitor the spread of resistance-encoding plasmids between organisms and between hospitals, communities, or even countries. The weakness of the analysis is inherent as the plasmids are mobile, extra chromosomal elements, not part of the chromosomal genotype. These can be spontaneously lost from or readily acquired by a host strain thus epidemiologically related isolates can exhibit different plasmid profiles.

Polymerase Chain Reaction (PCR)

For direct application to the diagnosis of infections, nucleic acid analysis without amplification often has the disadvantage of low sensitivity. Nucleic acid amplification techniques increase sensitivity dramatically while still retaining a high specificity. Invented by Cetus scientist Kary Mullis in 1983, PCR is the best-developed and most widely used method of nucleic acid amplification. Commercial systems for PCR detection of DNA targets of *Chlamydia trachomatis* and *Mycobacterium tuberculosis* are manufactured by Roche Molecular Systems. The direct detection method of diagnosing the disease using PCR has eased the way for the organisms that are fastidious and difficult to grow like *Mycobacterium* species, *Dichelobacter nodosus* (*D. nodosus*), Ovine herpes virus 2 (OvHV2) etc. Earlier it used to take 2- 3 weeks for culturing of the *D. nodosus* due to its slow and fastidious nature. Now without culturing, the organism can be identified from clinical specimens collected from footrot lesions through PCR using 16S rRNA specific primers. Similarly, determination of virulence strain of *D. nodosus* can be done by PCR for detection of virulence intergrase (*intA*) gene. Otherwise laborious Gelatin Gel Test was being used for which organism needs to be grown first in pure culture which is very difficult, time consuming and cumbersome affair and also sufficient amount of the culture is required in broth for carrying out the test. Similalry, early detection of Methicillin resistant *Staphylococcus aureus* (MRSA) permits timely implementation of preventive and control strategies and reduces costs. Conventional processing of screening samples takes 2 or 3 days before definitive MRSA identification can be achieved. Duplex PCR for *mecA* (encoding Penicillin binding protein-2A) and *femB* (factors essential for methicillin resistance) provides reliable and unequivocal results for MRSA identification within 18 hours.

Reverse Transcriptase PCR

Reverse transcriptase PCR was developed to amplify RNA targets. In this process, RNA targets are first converted to complementary DNA (cDNA) by reverse transcriptase enzyme and then amplified by PCR. It is used in RNA-containing virus infections, detecting viable *Mycobacteria* species, and monitoring the effectiveness of antimicrobial therapy. This method is commonly used for detection of various RNA viruses like rotavirus, Human immunodeficiency virus etc.

Nested PCR

Nested PCR, designed mainly to increase sensitivity (detect smaller quantities of target), uses two sets of amplification primers. One set of primers is used for the first round of amplification, which consists of 15 to 30 cycles. The amplification products of the first reaction are then subjected to a second round of amplification with another set of primers that are specific for an internal sequence that was amplified by the first primer pair. Nested PCR has extremely high sensitivity because of the dual amplification process. Nested PCR has been developed for *M. tuberculosis*, Adeno virus. For example at present group A rotavirus has 27G and 35P genotypes. Determination of same is crucial in development of vaccine against rotavirus infection. Previously genotyping of rota virus by serological methods was a time consuming and laborious procedure. This methodology requires the pure viral antigen and hyper immune sera against rotavirus. The preparation of antigen involves use of cell culture for virus growth followed by purification of viral antigen using cumbersome technique of ultracentrifugation. For raising anti-rotavirus sera there is need of lab. animals (rabbits/ gunea pigs etc) and consumes at least 4-6 weeks. Thus the process would take weeks together and is laborious. Using nested PCR technique now the G and P genotyping is made so easy that within hours the result can be achieved by using a single forward primer and many reverse primers circumventing all the steps of traditional methodology.

Hemi nested PCR

The only difference with the nested PCR is that here in the second amplification step the forward primer is same as that used in the first reaction while another one is a new primer. Among other application it is used in the diagnosis of a fatal disease of cattle and buffaloes called Sheep associated malignant catarrhal fever (SA-MCF) caused by Ovine herpesvirus 2 (OvHV2) which is yet to be cultivated in the laboratory. The test can be performed in 3-4 hrs after extraction of DNA form the buffy coat of suspected animals. Sequencing of the PCR product gives 100% specificity.

Multiplex PCR

Multiplex PCR is an amplification reaction in which two or more sets of primer pairs specific for different targets are introduced in the same tube. Thus, more than one unique target DNA sequence in a specimen can be

amplified at the same time. For diagnostic uses, multiplex PCR can be set up to detect internal controls or to detect multiple pathogens from a single specimen. Quantitative competitive PCR, a variation of multiplex PCR, can be used to quantify the amount of target DNA or RNA in a specimen.

For example based on pilin antigen *D. nodosus* has ten serogroups designated as A-I and M without any cross protection. Determination of serogrouping is crucial for the development of serogroup specific vaccine against footrot. Previously serogrouping of *D. nodosus* from a footrot lesion required isolation of the organism, purification by subculture followed by antigenic analysis by serological methods, which is a very cumbersome and time consuming procedure. The process would take 3-5 weeks. But now the serogrouping of *D. nodosus* can be achieved in hours through mutiplex PCR. Here serogrouping is based on variation in the sequences of *fimA* gene among the isolates. In this one forward primer and nine reverse primers are used in a single reaction so that single or multiple serogroups (A-I) of the bacteria present in the sample could be detected in a single PCR.

Ribosomal RNA Targets

When identity of a bacterial organism is not known, amplification of DNA encoding ribosomal RNA (rRNA) in conjunction with sequencing of the amplicon has proven to be valuable. The 16S rRNA gene has been most commonly employed for identification purposes, due to it being highly conserved and commonly having several copies in the bacterial ribosome. Primers, which are directed towards highly conserved regions of the 16S rRNA genes, may either be Universal (fD1 and rP2 etc.) which can target any bacterial species in a situation where no preliminary idea or information about the causative agent is available. Or may target specific organisms viz; *D. nodosus*, *Mycobacterium tuberculosis/ bovis/* paratuberculosis, *Salmonella* spp. etc. Sequence-based identification methods including universal/ target specific detection requires the use of appropriate analytical tools like BLAST, FASTA and DNAstar.

Real-Time polymerase chain reaction (RT-PCR)

Real-time, polymerase chain reaction (RT-PCR) has become one of the most widely used methods in the field of molecular diagnostics and research. This approach is a highly sensitive enabling simultaneous amplification and quantification of specific nucleic acid sequences with high speed. In addition to enhanced sensitivity, the benefits of real-time PCR assays over conventional endpoint detection methods include their large dynamic range, a reduced risk of cross contamination, and the potential for accurate target quantification. Real-time PCR is suitable for a wide range of applications, such as i.) Quantitative mRNA expression studies. ii) DNA copy number measurements in genomic or viral DNAs, iii). Allelic discrimination assays or SNP genotyping, iv). Verification of microarray results, v). Drug therapy efficacy and vi). DNA damage measurement. Specific detection of real time PCR is done with some oligonucleotide probes labeled with both a reporter fluorescent dye (fluorophore) and a quencher dye without need to run in the gel. Probes based on different chemistries are available for real time detection, these include: a). Molecular Beacons, b). TaqMan® Probes c). FRET Hybridization Probes and d) Scorpion® Primers. The use of a specific probe facilitates an increase in specificity compared to conventional agarose-gel-based PCR assays. The potential of this format to provide sensitive, specific and swift detection and quantification of viral RNAs has made it an indispensable tool for state-of-the-art diagnostics of important human and animal viral pathogens. Based on these advantageous characteristics, numerous robust RT-PCRs systems have been developed and validated for important epizootic diseases of livestock. RT-PCR assays that have been developed for the detection of five RNA viruses that cause diseases that are notifiable to the World Organisation for Animal Health (OIE), namely: foot-and-mouth disease, classical swine fever, bluetongue disease, avian influenza and Newcastle disease.

Restriction enzyme digestion

Restriction endonucleases recognize specific nucleotide sequences in DNA and produce double-stranded cleavages that break the DNA into small fragments. The number and sizes of the restriction fragments, called restriction fragment length polymorphisms (RFLPs), generated by digesting microbial DNA are influenced by both the recognition sequence of the enzyme and the composition of the DNA. In conventional restriction endonuclease analysis, chromosomal or plasmid DNA is extracted from microbial specimens and then digested with endonucleases into small fragments. These fragments are then separated by size with use of agarose gel electrophoresis or Pulsed field gel electrophoresis (PFGE) depending upon the size of fragments. The nucleic

acid electrophoretic pattern can then be visualized by ethidium bromide staining and examination under UV light. Both the RFLP and PFGE techniques are briefed below.

Restriction Fragment Length Polymorphism (RFLP) Analysis

RFLP is a very useful tool to further characterize PCR products by using the selective DNA cleavage with restriction endonucleases. Restriction endonucleases are enzymes that cleave double stranded DNA at precise sites defined by a sequence of nucleotides. Each enzyme recognizes a specific sequence, usually 4, 5, 6 or 8nucleotide long. RFLP analysis involves the digestion of a PCR product followed by agarose gel electrophoresis to separate DNA fragments. Following electrophoresis, the restriction products are visualized by usual ethidium bromide staining of the gel.

RFLP analysis has numerous applications in the clinical microbiology laboratory. For example serotyping of *D. nodosus* serogroup B can be done on the basis of PCR-RFLP of *fimA* gene which is amplified using *fimA* gene specific primers. The amplified products are digested using 5 different restriction endonucleases (RE) and the banding pattern obtained after digestion of the amplicons forms the basis of the differentiation of six different serotypes from B1 to B6. The serotyping using RFLP-PCR reduces the time and labour. Earlier serotyping of the serogroup B isolates was based on serology using hyperimmune sera raised against each serotype in rabbits, also antigen needs to be cultured in pure form which is difficult and a time consuming affair. Similarly, it can be used to differentiate among herpes simplex virus type 1 (HSV-1) and HSV-2 amplified products. The HSV primers amplify a 476 base pair region of its polymerase gene. The PCR product from HSV 1 contains two *AvaII* restriction sites leading to 3 DNA bands (87, 183 and 296 bp), while the PCR product from HSV 2 contains only one *AvaII* site resulting into two DNA fragments of 87 and 389 bp.

Pulsed Field Gel Electrophoresis (PFGE)

In 1982, Schwartz *et al.* introduced the concept that DNA molecules larger than 50 kb can be separated by using two alternating electric fields (i.e. PFGE). Separation of DNA from a size of few kb to over 10 megabase pairs (Mb) can be achieved. Separation of the resultant fragments is done by passing a current which is reversed regularly in polarity to effect separation of large DNA fragments (up to 10^4 kb) much larger than can be separated by conventional agarose gel electrophoresis (20-30 kb). Enzymes (e.g. *XbaI*) that have relatively few restriction sites on the genomic DNA are selected so that 10 to 20 DNA fragments ranging in size from 10 to 1000 kb are produced.

Representative isolates of *S.* Gallinarum, *S.* Enteritidis and *S.* Typhimurium isolated from different Govt. farms from Kashmir valley were subjected to PFGE for genotyping using *Xba*I as restriction enzyme. The similar PFGE profiles of *S.* Gallinarum isolates from two farms indicated that the same clonal type of *S.* Gallinarum was circulating on these two farms. The *S.* Gallinarum infection of these two farms could be attributed to vertical transmission of the infection from a common source as the chicks for these two farms were supplied from a single farm. The PFGE profile of the one of the isolates of *S.* Gallinarum was quite dissimilar from the profiles of the above mentioned isolates, showing that a different clonal type of *S.* Gallinarum was prevalent in that farm. This may be due to horizontal transmission. This seems to demonstrate that *S.* Gallinarum infection on these farms originated from multiple sources, for example, water, feed or vertical transmission.

Single-strand conformational polymorphisms (SSCP)

SSCP was first described by Orita *et al.* (1989). DNA is subjected to PCR with primers to a region of suspected polymorphism. The PCR products, which usually incorporate a detector marker, are examined after gel electrophoresis. Physical conformational changes in single- stranded DNA are based on the physiochemical properties of the nucleotide sequence. Variations in the physical conformation are reflected in differential gel migration. This technique is sensitive enough to detect single nucleotide substitutions. One area in which SSCP may prove to be of value is in the detection of mutations related to resistance mechanisms. SSCP, and variations on the technique, have been successfully used to examine the genes contributing to the multidrug resistance of *M. tuberculosis*. PCR in conjuction with SSCP has been used to demonstrate strain variation in *Fusobacterium necrophorum*. In this *lktA* gene is targeted and amplicon generated by PCR is put to SSCP. In total four unique patterns of the isolates of the bacteria have been detected so far using this technique.

DNA Microarray

DNA microarrays are a powerful tool for the investigation of various aspects of prokaryotic biology because they allow the simultaneous monitoring of the expression of all genes in any bacterium. They offer a more holistic approach to study cellular physiology and, therefore, complement the traditional "gene-by-gene" approaches. There are several other types of microarrays, like protein microarrays, but the DNA microarray is by far the most widespread and will simply be termed microarray. The essence of microarray technology is the parallel hybridization of a mixture of labelled nucleic acids called target, with thousands of individual nucleic acid species called probes that can be identified by their spatial position in a single experiment. The location of a specific probe on the array is termed spot or feature. Whereas the probes are immobilized on a solid support, the targets are applied as a solution onto the array for hybridization after fluorescent labeling.

Future Applications

Molecular screening for a group of possible pathogens involved in disease is an exciting area of development in molecular microbiology, e.g. Many etiologic agents cause debilitating gastroenteritis in immunosuppressed patient populations, including mycobacteria (i.e., *Mycobacterium avium* complex and *M. genevense*), parasites (i.e., *Cryptosporidum*, *Microsporidum*), viruses (i.e., Rotavirus, Norwalk agent), and typical bacterial pathogens (*E. coli* variants, *Salmonella*, *Shigella*, and *Campylobacter*). So the future of diagnostics lies in targeting all these organism in one test. The techniques being used for molecular screening include the newer nucleic acid "chip" technologies, multiplex PCR, and the use of broad-range PCR primers for simultaneous detection and subsequent nucleic acid sequence analysis. Also rapid detection of microbial resistance and, it is hoped, with the development of more user friendly systems, the expansion of these technologies to smaller institutions and laboratories.

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MOLECULAR DIAGNOSIS AND CONTROL OF ECONOMICALLY IMPORTANT CHRONIC INFECTIONS OF PUBLIC HEALTH SIGNIFICANCE IN DOMESTIC RUMINANTS

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Abstract

Mycobacteria have thwarted detection by scientists for centuries. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is one of the most fastidious of the Mycobacteriaceae, and has been implicated in both animal and human diseases. In domestic livestock, MAP has been cause of Paratuberculosis (PTB) (popularly known as Johne's disease; JD), which given its increasing incidence, is currently a cause for concern, due to the potential for MAP to enter our food chain. Though MAP has many social, health (both human and animal), economic, national, international (trans-boundary) etc concerns, the focus of this paper is on diagnosis and economic concerns of MAP. This review compares the sensitivity and specificity of traditional and modern tools for MAP diagnosis in animals. Recent developments in this field include more rapid methods of MAP culture as well as the development of more accurate and sensitive PCR assays. PTB is a disease which causes considerable economic losses livestock industry and has been recognized as most prevailing and costly infectious diseases of dairy cattle. Present paper focuses on factors associated with economic losses due to PTB. The losses thoroughly described include: loss of milk production and poor body condition followed by death or culling. In contrast, hidden losses arising from a subclinical disease have not been well documented and given less concerns. These hidden include: increased susceptibility to other diseases, poor feed conversion, shortened production age and increased predisposition to other diseases, premature culling of animals and their unrealized future income, expenses for non-active production, herd replacement, diagnostic testing, veterinary care, implication of control programmes. Last but not the least the reputation of farm where mycobacteria infected animals is diagnosed.

Introduction

Disease effects on livestock include direct effects on productivity, disease-control costs and constraints on livestock management including limitations on species and breed choices. Direct effects of animal diseases on livestock productivity include reduced feed intake, changes in digestion and metabolism, increased morbidity and mortality and decreased rates of reproduction, weight gain and milk production. These have aggregate effects that limit economically important herd-management decisions regarding animal selection and optimal longevity. Interactions between disease, nutrition and genetic selection emphasize the need to control the effects of epidemic and endemic diseases before enhanced nutrition and genetic programmes can make an impact. Considerable costs may be incurred in controlling animal diseases, though they may not always be effective. It is particularly true for smallholder farmers, who often lack information and have limited diagnostic data to make disease-control and treatment decisions. Non-compliance to control measures by significant proportion of poor communities, highlight the need for regulatory veterinary services supported by legislation and incentives to comply.

Classification of animal diseases : As per Perry et al. (2001), diseases of small ruminants can be classified in to four groups viz. zoonoses, food-borne, endemic and epidemic. Zoonoses and food-borne diseases have been placed together, since majority of these are food-borne.

Disease Groups

a. **Endemic :** Coccidiosis, Gastro-intestinal (GI) parasites, Ectoparasitism, Goat and Sheep Pox, Contagious Ecthyma, Foot-and-Mouth disease (FMD), Rotavirus infection,CCPP, Colibacillosis, Entero-toxaemia, Haemorrhagic septicaemia, Johne's disease

b. **Zoonoses and food-borne diseases:** Hydatidosis, Cysticercosis, Brucellosis, Tuberculosis (TB), Listeriosis, Leptospirosis, Johne's disease

c. **Epidemic :** Peste des petits ruminants (PPR)

In small ruminant production system focus has been mainly on production and control of diseases has not received similar attention. However, with emergence of 'Commercial Goat Husbandry' as new livestock industry, control of losses due to diseases has become central to the viability of goat farms in the country. In the absence of National surveys, estimation of losses accrued due to diseases (mortality, morbidity, production losses etc.) are non-existant. However, based on our experience in last 27 years in Animal Health Divison of Central Institute or Research on Goats, Johne's disease (JD) and PPR have been major diseases causing mortality, morbidity and production losses in different goatherds (farm and farmer's) across different geo-climatic conditions in the country. Both diseases are endemic in our goatherds and emerged as major cause of losses to goat production system, therefore rightly classified as 'Production diseases'. Control programs for Bovine JD have been in place since 1950s, but in goat husbandry, it has not received major disease, since disease existed only in farm herds, which were fewer in number and limited to government sector. In last decade goat farming has gained momentum and availability of grazing land has been extremely limited. Our experience of last few years show that there is no difference in the incidence of JD in farm or farmer's sector. Disease exists in equal proportion in the two production systems. Since JD is a 'Production disease' needs highest attention and priority for the development and success of 'future, viable and sustainable goat industry' in the country.

Paratuberculosis (PTB) is nowadays viewed as one of the most serious and widespread chronic bacterial diseases of ruminants in agriculturally developed countries (Hruska, 2004). The etiologic agent, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a slowly growing, mycobactin-dependent acid-fast bacilli containing specific insertion sequence IS *900*. It is truly a multispecies infection with widest host range. Infected animals may exhibit clinical signs such as persistent diarrhoea and progressive weight loss; however, asymptomatic animals shed MAP in colostrum and milk, particularly in later stages of disease (Streeter et al., 1995). Most infections are assumed to be acquired during the first 6 months of life (Larsen et al., 1975). Primary source of MAP transmission is faecal oral route from contaminated environment, including contaminated milk and feed, leading to ingestion of MAP by the young ones (Sweeney, 1996). Kids may also get infected in utero from mother (Ayele et al., 2004). Wild animals (Machackova-Kopecna et al., 2005) may also be a potential source of MAP infection. MAP has also been recovered from pasteurized milk and milk products, from primates and human beings from cases of inflammatory bowel disease (Crohn's disease).

Disease has long incubation period and hence animals may show no signs of the infection for many years, and will often test negative on serologic and/or faecal culture tests (Sweeney, 1996). Use of multiple diagnostic tests is essential for diagnosis of JD in a herd. In some herds affected with PTB, economic losses may be so high that farming cannot be profitable any more (Benedictus et al., 1987, Singh et al., 2007). The purpose of the present paper was to review the economic and diagnostic concerns of MAP infection.

1. Economic losses due to paratuberculosis

Economic losses due to PTB may be classified as (i) direct losses and (ii) indirect losses

2.1 Direct losses

a. Mortality of clinically ill animals and decreased slaughter value or complete condemnation of slaughtered animals.
b. Decreased milk production and reduced milk quality, i.e. changes in milk parameters, increased somatic cells counts and increased incidence of mastitis.
c. Decreased pregnancy rate and increased post-partum complications, fertility of animals declines.
d. Feed conversion is poor not only in clinical, but also in subclinical animals.
e. Productive age length is decreased in the infected animals due to early culling.
f. In endemic herds, increased predisposition to other chronic diseases (chronic arthritis, rumenitis, dermatitis, mastitis, etc.) has been recorded.

2.2. Indirect losses:

a. Unrealized future income by breeding animals prematurely culled.

b. Increased expenses for idle production.
c. Increased expenses for herd replacement
d. Expenses for diagnostic testing or expenses for ineffective veterinary care
e. Expenses for a control programme.
f. Lost genetic value of highly valuable animals, which are culled from a herd due to suspected infection.
g. Expenses associated with trade restrictions imposed by the market or by regulation.

2.3 Impact of paratuberculosis on milk production

Decreased milk production has been described in several studies; different authors estimated the consequences from various aspects by different methods. PTB has been documented to reduced milk production in clinical and subclinically infected animals (Ott et al., 1999, Dufour et al., 2004) Degree of these losses depends on the time of initiation of the disease. In cases where clinical signs appear soon after kidding, production is lost for the whole lactation (Dufour et al., 2004). Studies report reduction of 17% and 15% milk production in clinically and subclinically infected animals, respectively (De Lisle and Milestone, 1989, Abbas et al.,1983).

Studies show that daily milk fat and milk protein production are significantly less for the infected animals compared to healthy animals (Sweeney et al., 1994, Collins and Nordlund 1991). Due to this loss of 205 USD per cow per lactation has been estimated (Collins and Nordlund 1991).

It has been reported that PTB has been associated with increased mastitis culling (McNab et al. 1991).

2.4 Poor Feed conversion

Negative energy balance (NEB) is the situation in which intake of feed energy is less than the output of energy from the body (Vandehaar et al., 1995). A higher probability of negative energy balance is assumed in PTB infected cows because of a decreased nutrient absorption in the intestines. In addition to this malabsorption syndrome, protein losing enteropathy occurs during PTB (Kreeger, 1991). The association between the pathological condition and reduction in feed efficiency, milk production, milk fat and protein production, and slaughter weight is clear (Johnson-Ifearulundu and Kaneene, 1997). Vandehaar et al. (1995) found that negative energy balance can reduce the development of corpus luteum with consequent reduction of the serum progesterone level. Despite milk production decline in MAP-infected cattle herds, feed consumption does not change. It is caused by maintained appetite of infected animals, although feed conversion gradually decreases due to chronically affected intestinal mucosa (Ott et al., 1999).

2.5 Diagnostic Expenses

An optimum diagnostic test has to be available for the lowest price and showing the highest specificity. High specificity minimizes the number of false-positive results and consequently the number of unnecessarily culled animals (Collins and Sockett, 1993). It is owner who bears economic consequences resulting from false positive and false-negative diagnostic test results.

2.6 Reduced Fertility

One potential source of economic losses in sub-clinically infected cows is reduced fertility (Johnson-Ifearulundu et al., 1996). Infertility was significantly higher in cows with unapparent MAP-infection than in non-infected cows in the same herd (Merkal et al., 1975). Buergelt and Duncan (1978) have shown that cows with subclinical MAP-infection frequently had infertility problems.

Further studies have found that cows sub-clinically infected with MAP are at a greater risk of being culled for infertility (Merkal et al., 1975; Buergelt and Duncan, 1978). A study also reported that sub-clinically infected cows have a 1.73 month increase in calving interval compared to non-infected cows (Abbas et al., 1983). Johnson-Ifearulundu et al. (1996) focused in their study on the impact of subclinical MAP-infection on the number of days from parturition to conception (days open). This period was statistically significantly ($P < 0.05$) longer (141.5 days in average) in MAP-infected cows than MAP-non-infected cows (104.5 days in average). Their further study (Johnson-Ifearulundu et al., 2000) brought comparable results: ELISA-positive cows had a 28-day increase in days open when compared to ELISA-negative cows ($P = 0.02$).

2.7 Culling of Animals

Buergelt and Duncan (1978) documented in a group of MAP-infected animals with clinical signs that the primary reasons for culling were culture of faeces (50% animals), body wasting (33% animals) and decreased milk production (17% animals). The reasons for culling in the group of MAP-infected animals without clinical signs were: low milk production (46% animals), mastitis (27% animals), infertility (9% animals), positive culture of faeces (9% animals) and positive CFT (5% animals). The primary reason for culling of non-infected cows was low production (47% animals), age of the animals (20% animals), infertility (13% animals), mastitis (7% animals) and body wasting (7% animals).

In the study of Johnson-Ifearulundu et al. (1999) a 3% increase in herd mortality rate associated with PTB was found. This association reflects deaths directly caused by PTB with deaths attributable to an increased risk of secondary disease. Kreeger (1991) reported that annual death losses may range from 3 to 10% in an infected herd.

2.8 Increased Disease Susceptibility

The association between cellular immunity and increased risk of occurrence of secondary diseases has been described by Kreeger et al. (1992). It is supposed that persistence of the disease in an organism may cause an inadequate immune system cell response (Kreeger et al., 1991, 1992). Kreeger et al. (1991) found that infected cattle monocyte response to antigens is reduced. An association between MAP infection and reduced immuno-competence may be the basis for the elevated rate of culling due to mastitis, infertility and other health problems (Johnson Ifearulundu and Kaneene, 1997).

2.9 Premature culling and unrealized future income

In animals in good health and with normal production potential, the average income increases with age (Dijkhuizen et al., 1985). The greatest economic loss was attributed to unrealized future income caused by premature culling of infected cattle (Benedictus et al., 1987). According to the Groenendaal and Galligan (2003) study most of the loss (>70%) attributable to PTB was categorized as a loss of future income. Many animals were slaughtered at a relatively young age and before they reached the peak of their lactation potential (Hutchinson, 1996). The loss in breeding value added another economic component.

2.10 Herd replacement

Expenses for herd replacement result from increased mortality rate and increased culling rate of animals due to paratubercolosis or other reasons associated with PTB (Johnson-Ifearulundu et al., 1999). Animals culled because of PTB are replaced by the purchase of pregnant heifers, and the losses induced by the culling of a sick cow are thus estimated by the price of a pregnant heifer. When an animal is culled there is often a period when a replacement animal is not immediately available (Benedictus et al., 1987). Expenses increase on one hand because the operation costs for the whole herd remain; on the other hand, income decreases because the production of the lost animal has not been replaced yet.

2.11 Export/ Import Restrictions

Herds with positive reactors (in ELISA test; infected or not) would be placed under movement and trade restrictions until the diagnosis could be confirmed or rejected (Paisley, 2001). Animals from farms or areas known to be infected may suffer price penalties or may sell only for slaughter (Kennedy and Benedictus, 2001). Certain trade restrictions lead to losses at National and International level. States suffer losses due to the cost of control programs, loss of revenue from lost income, and trade restrictions between certain states (Whipple, 1991).

2. Diagnosis of Paratuberculosis

Control of JD has been problematic because it has a long incubation period, it is clinically similar to many other common diseases of animals, available diagnostic tests are expensive and relatively low in sensitivity, and there are no accepted standards for diagnosis and control. These problems are compounded by a lack of awareness of the disease and the fact that its slow progression makes financial losses not easily perceptible to the individual producer. The situation got exacerbates with increasing concern over the human health

implications of JD. The possibility that MAP infection could be a cause of some cases of Inflammatory bowel diseases (especially Crohn's disease) in humans, combined with concern that MAP is becoming widespread in the environment and the food chain, could transform JD into a serious public health problem (Shankar et al., 2010). All these concerns have raised questions about our scientific knowledge and nationwide preparedness.

Any conceivable control programme for PTB will be based on reducing within-herd transmission of MAP. Therefore, control measures will be based on combination of management changes reducing the risk of transmission between animals that shed MAP and other non-infected (young) animals, and the removal of infectious animals by early detection (test and cull) or vaccination strategies. Effective control program for JD has been hampered mainly due to lack of rapid and accurate diagnosis. Tests results from sub-clinically infected animals are challenge to interpret, because clinical signs are not present to assist their interpretation.

Diagnostic tests to detect MAP infection can be divided into 2 categories, one that detects the organism (bacteriology and molecular approaches) and other that detects host reactions to the organism (immunological approaches).

2.1 Bacteriology

i **Direct microscopy :** Staining of fecal samples for acid-fast bacilli (AFB) may reveal mycobacterial bacilli, but the sensitivity of this method is low. Moreover, accurately distinguishing MAP from non- pathogenic mycobacteria (saprophytes) in such samples can be difficult even for experienced persons, resulting in low specificity.

ii **Conventional culture :** Isolation of MAP by culture is Gold Standard test (100% specific) (Nielsen et al., 2001). Clinical samples including, feces, tissues (intestine, MLN, SMLN, liver, testes, udder, uterus, etc), blood and milk can be used to isolate MAP from infected animals. Culture medium either HEYM (Herrold's Egg Yolk Media) or modified LJ (Lowenstein-Jensen) supplemented with iron chelator (mycobactin) are preferred by diagnostic laboratories to isolate MAP (Whipple et al., 1991; Singh et al., 1996). MAP colonies are characterized on the basis of slow growth (appearing after 8 weeks), mycobactin J dependency, acid-fastness and IS *900* PCR. Culture can detect infected animals shedding more than 100 CFU/g of feces (Merkal, 1970) and sensitivity of fecal culture is 50% (Shin 1989). Disadvantages of culture methods are- (a) long incubation period (2 months to years); (b) lack of reproducibility; (c) MAP from sheep and human beings frequently fail to grow; and (d) MAP is not distributed homogenously in feces, it is highly likely that fecal samples collected from MAP infected animals may not contain MAP.

iii **Radiometric culture (BACTEC):** *This* method is adapted from the one used to isolate *tuberculosis* in humans. Collins *et al.* (1990) have demonstrated that BACTEC system, if modified, could also be used to diagnose PTB. The main advantage of this method over the standard one is that it can detect low numbers of MAP and can detect the bacterium faster (in 7 weeks). Disadvantages are that the BACTEC method is more expensive, requires specialized instrument and involves handling of radioisotopes.

3.2 Immunological tests

i **Cell mediated immunity (CMI)**

a **Skin testing or delayed type hypersensitivity (DTH) :** This test is performed by intra-dermal inoculation Johnin (extract of MAP). An increase in the thickness of the skin at site of injection >4 mm within 24-72 hr is considered as positive. It is less sensitive (54%) and specific (79%), also has poor correlation with the infectious status of the animal (Hermel, 1998).

b **Interferon-gamma detection (IFN-g) :** IFN-*g* is released by lymphocytes of infected animals after stimulation with antigens. Two assays known as bioassay (Wood et al., 1989) and sandwich enzyme immunoassay (EIA) have been evaluated (Rothel et al., 1990). However, results indicated that the test is non-specific and interpretations are uncertain. Thus, IFN-y detection has limited value in diagnosing PTB (Collins & Zao, 1995; McDonald et al., 1999).

ii **Humoral immune response**

a **AGID :** *The* AGTD test has a high specificity (>90%) in animals with clinical signs, but because of poor sensitivity (30%) this test is not popular (Hermel, 1998).

b **Complement fixation test (CFT) :** *The* specificity of CFT is less than AGTD and ELTSA. Moreover, this test detects antibodies 1 to *5* months later than ELISA (Ridge et al., 1995) and has intermediate sensitivity to AGTD and ELISA. Results yield false positives and false negatives, CFT is not recommended as routine diagnostic test.

c **ELISA :** ELISA is most widely used 'Herd- screening test' for diagnosis of PTB. Sensitivity of ELTSA is high in clinically infected animals and is low at initial and terminal stages of disease (Anergy). In general, ELISA sensitivity for clinical and sub-clinical cases is 85 and 15%, respectively (Hermel, 1998). Absorption of serum with *M phlei* removes nonspecific antibodies (Nielsen et al., 2001). An indigenous ELISA kit utilizing protoplasmic antigen from native MAP 'Bison type' strain has been developed for screening of animals and also human beings (Singh et al., 2007a, 2007b, 2008).

Interpretation of the results of serological tests should be made with caution, since evaluation of performance (sensitivity and specificity) of serological results is done by comparing with culture (Gold standard) and many times poor standardization or over sensitivity of culture may lead to wrong estimates of the sensitivity and specificity of serological test. To avoid errors in the interpretation of ELISA results, S/P ratio (sample to positive ratio) method has been proposed that relates ELISA OD values with the disease status of infected animal (Collins et al., 2005, Singh et al., 2007a).

iii **New antigen discoveries and MAP diagnosis :** Pre-absorption of sera with *M phlel* is a good option for increasing assay specificity, but reduces the sensitivity. Reports indicate that only 1/3rd of animals shedding MAP can be detected by present ELISA tests (Collins et al., 2005). It is essential to characterize promising antigens for sero-diagnosis. The search for MAP specific antigens has led to discovery of many antigens as follows-

a. *34 kDa cell surface protein*- Carboxy terminal end is 100% specific for MAP.

b. *Secreted antigens (9, 14 and 34 kDa)- Al1* are recognized by sera of clinically and sub-clinically infected animals.

c. *Ahp C and Ahp* D- Both have ability to differentiate paratuberculosis and tuberculosis.

d. p43 - Product of 1S900, specific antigen for MAP diagnosis.

e. *14kDa secreted* antigen- Higher levels of antibodies against this antigen were detected in patients with Crohn's disease.

f. *35kDa antigen* - In ELISA, this antigen has highest sensitivity compared to other antigens *(Ag85* A, B, C and SOD).

With complete genome sequencing of international reference isolate MAP K10 post genomic applications will play a pivotal role in identifying MAP specific antigens and some work has already been started in this area.

a. Results of comparative genomics and immunoproteomic analysis of MAP extracts and culture filtrates *25* MAP diagnostic antigens were identified (Leroy et al., 2007).

b. *In silico* comparison of MAP genome with other mycobacterial genomes and cloning and expression of MAP unique protein sequences MAP specific protein antigens were identified that reacted with sera from infected animals (Bannantine & Paustian 2006).

c. Comparative genomics and immunoblots identifiedl4 antigen including Mod D, Pep A, Arg I, - Cob T, Ag85C and 9 hypothetical proteins 50, but the sero-diagnostic evaluation of these antigens reveal that natural forms of these proteins are more useful for sero-diagnosis, since recombinant proteins have lower sensitivity to crude antigens (Cho et al., 2007).

d. Comparative genomic approach identified 21 potential coding sequences specific for MAP and found that *5* of these sequences code for MAP specific antigens (Bannantine et al., 2002, 2004).

3.3 Molecular diagnosis

i **DNA probe (non-culture detection)** : Although highly speciûc for MAP, the DNA probe was unable to detect infected cattle that were shedding low numbers of organisms. However, PCR tests for detection of

MAP in fecal samples have vastly improved in recent years, leading to an increased sensitivity of detection of low shedders, including report of a detection level of 1 colony-forming unit(s) (cfu)/g feces.

ii **PCR :** Various PCR protocols have been designed from different workers world over for the detection of MAP in tissues, milk, feces etc. Most of these methods are based on detection of IS*900* element. However, PCR has been reported to be less sensitive than culture. Low sensitivity may be due to presence of inhibitory ions, inefficient extraction of DNA and low bacilli number in the samples. Various methods have been employed to improve the sensitivity of PCR. Recently, IS*900*-like sequences had been identified in mycobacteria other than MAP which prompted the identification of alternative marker sequences. Recently, a number of markers sequences unique to MAP have been identified for specific diagnosis of MAP.

Genome sequencing of MAP KI 0 had identified 3 more MAP unique IS elements; ISMav2, ISMAPO2 and ISMAPO4 present in 3, 6 and 4 copies, respectively in MAP Kb. Small-scale studies using these newly identified MAP unique IS elements have provided encouraging results. Studies have shown that PCR for ISMav2, ISMAPO2 has comparable sensitivity and specificity to 1S900 PCR for detection of MAP in fecal samples and are 100% specific for MAP (Stabel & Bannantine, 2004; Paustian et al., 2004). Comparative genomics based study (Semret et al., 2005) had identified 17 MAP unique large sequence polymorphisms (LSPs) and PCR analysis for these LSPs across the 383 *M avium* complex isolates (107 MAP +276 non-MAP) reveals that LSP^{P}2, LSP^{P}4, and LSP^{P}I5 are 100% specific for MAP, others are also present in non-MAP mycobacteria. Of these MAP specific LSP^{P}s, LSP^{P}15 consistently give positive results (highly sensitivity) across MAP isolates and are heterogeneously distributed among different MAP isolates.

In summary, accurate diagnosis of infected animals is prerequisite of disease control programs. Efforts are needed on the development of simple, rapid and non-invasive tests based on the newly identified antigens. Range of diagnostic tests is available, but all have inborne limitations. Culture and molecular tests are very specific, but are less sensitive and costly. For molecular tests, it is important to evaluate the newly identified molecular markers on large number of samples since concern of 1S900 specificity for MAP has been raised. But consistent with mobile nature of newly identified MAP specific markers (IS elements and LSPs), they may be variably present among different MAP isolates. Thus, a diagnostic test targeting different markers (LSPs and IS elements) can be designed in multiplex form to test particular clinical sample or MAP colony. Among all tests, ELISA is most affordable and widely used, but it also demands improvements in terms of sensitivity and specificity. Improvement of ELISA requires identification of infection stage and MAP specific antigens to improve the assay sensitivity and specificity. All newly identified antigens may impart major impact on development of diagnostics for paratuberculosis.

4. Control

While options for the control of JD are dictated by ûnancial constraints and farming practices, the overall disease status of the herd, region or country is also an important determinant of what options are applicable, if any. In India, JD has become one of the most prevalent and disease of animals majorly due to lack of awareness, poor hygiene condition of flocks, lack of sensitive indigenous diagnostics, and vaccine and ban on cow culling (even after getting infection), therefore, 'test and cull' method is not feasible to control JD. Moreover, poor or marginal farmers with small flocks will never go for this strategy. Thus, control by vaccination and improved management practices is the only viable option for control of JD in India.

During twentieth century, a number of live attenuated and killed vaccines were developed and vaccination has shown good results in controlling JD in several countries. Existing vaccines against JD are effective in reducing number of fecal shedders and clinically infected animals; however, they are not efficacious in eradicating the disease. Also vaccination against MAP may interfere with diagnosis of tuberculosis. Thus it is important to design a marker vaccine with better efficacy and ability to discriminate vaccinated and infected animals. Also this vaccine should not interfere with tuberculosis diagnosis. Besides vaccination, hygienic practices were another important tool in herd management to prevent transmission of MAP.

Recently in India our group has developed an indigenous inactivated vaccine using highly pathogenic, 'Indian Bison type' strain. Vaccination of goat kids (4-6 months) with this indigenous vaccine followed by double challenge showed that indigenous vaccine is highly efficacious in controlling the disease. Vaccination induced strong humoral and cellular response. Indigenous vaccine successfully reduced fecal shedding and mortality. Histo-pathological examinations of experimental goats showed that even after double challenge, lesions/bacilli were absent in vaccinated animals as compared to control animals, which showed focal, diffused and confluent lesions. Comparatively indigenous vaccine was more successful in reducing fecal shedding and mortality than imported vaccine. Also we have documented first time on comprehensive scale that Indigenous vaccine also had therapeutic effect. Vaccination of ready to cull clinical cases of JD in goats with indigenous vaccine showed marked improvement in animals. Vaccination significantly reduced mortality due to JD and fecal shedding. After 7 months post vaccination all the animals in control group were shedding MAP, however, only 17% animals of vaccinated group were shedding MAP. Externally, there was marked overall improvement in the body conditions of vaccinated animals. Similarly indigenous vaccine was used as control measure in the breeding herd of Jamunapari goats endemic for JD. After 3 months of vaccination, there was increase in body weights, kidding percentage, litter size. After these successful in-house trials, vaccine is now being used in goatherds and sheep flocks and cattle herd located in the different agro-climatic region of the country. In the recent trials of Indigenous vaccine (developed at CIRG, Makhdoom) in endemic cattle herds, marked reduction in the shedding of MAP and severity of clinical sign was observed.

Research on development on subunit vaccine is also running world-wide however, still it will require intense research for recognition of protective antigens. Though currently, vaccination programme using killed vaccine is most popular but, the inability of killed and subunit vaccines to prevent establishment of infection leaves the potential for infected animals to break with disease if protective immunity wanes. This is why now development of genetically attenuated mutants for evaluation as vaccines for JD (conferring long term protection) is becomes major area of JD vaccine research. Conclusively, JD control deserves high priority from national government. Besides Government mandated and directed national JD surveillance and anti-MAP vaccination program we suggest extensive research on development of newer diagnostics and vaccine of better potential along with general awareness to this disease.

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SEGMENT 2 BASED INTRATYPIC VARIATIONS AMONG INDIAN ISOLATES OF SHEEP AND GOAT ORIGIN BELONGING TO BTV1 SEROTYPE

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Bluetongue (BT) is an economically important viral disease for Indian sheep and goat causing morbidity and mortality. In present study, a total of 12 BHK-21 cell culture adapted Indian isolates of BTV-1 were taken. Of these 12 isolates, eight belonged to goat from Utter Pradesh (U.P.). The rest of 4 isolates were of infected sheep from Andhra Pradesh (A.P.). All the samples were confirmed as bluetongue virus (BTV) based on 366bp amplicon size (19-384bp) with group specific ns1 gene RT-PCR. All the twelve isolates were confirmed as BTV serotype 1 based on segment 2 (vp2 gene) specific RT-PCR showing specific amplicon size of 605bp (1240-1844bp). Further, these amplicons were targeted for nucleotide sequencing to study the genomic variolations among these isolates. The sequences thus generated were subjected to *in silico* restriction enzyme analysis (REA) with *HindII* enzyme in order to find the diversity. A total of 7 goat sample of UP and 3 sheep sample of AP showed two restriction sites at positions 1250nt and 1531nt. However, the 2 sheep samples from A.P. (NRT35 and NRT39) showed only one restriction site at nucleotide position 1250, where as one isolate each of from Rajasthan state (sheep, AY559058), U.P. (goat, MKD25) and Haryana (sheep, AY559060) also showed one restriction site at position (at nucleotide position 1531) different than that A.P. state samples. The *in silico* REA with *TaqI,* revealed presence of single position at 1513nt in both sheep and goat samples from various states under study. However, one of the sheep sample (BT1) from AP along with one Tamil Nadu isolate (AY559061) in the GenBank showed one extra restriction site at position 1244nt along with at 1513nt. Two RE sites at positions 1448nt and 1646nt were observed in all the sheep and goat isolates of BTV-1 on analysis with enzyme *NdeII*. The variations were observed in one goat sample (MKD22) from UP that showed one extra restriction site at position 1285nt and one sheep sample (NRT35) from AP showed one extra restriction site at position 1685nt. The intratypic variations among Indian isolates of BTV1 of goat and sheep origin from northern and southern states of India have been observed. Haryana and Rajasthan are neighboring states in North, similarly Andhra Pradesh and Tamil Nadu are adjoining states in South and *C. oxystoma* has been reported as common vector species along with other *culicoides spp.* in all of these states. As the disease is vector transmitted and common vector is circulating in all the states reported under study could be the potential source of transmission of BTV-1. Further, the variation has been observed only on the basis of partial sequences, the full gene sequence of vp2 gene is required for better understanding of diversity of BTV-1.

Key Words : Bluetongue virus, BTV serotptype 1, Restriction enzyme analysis, vp2 gene

PARADIGM SHIFT IN ANTIVIRAL DRUG DEVELOPMENT

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Summary

Infection of cells with viruses results in the activation of a variety of intracellular signaling pathways that are in part exploited by the virus to ensure efficient replication. These dependencies may be used to develop novel antiviral drugs that disrupt signal transduction. Receptor tyrosine kinase (RTK), Raf/MEK/ERK and IKK/NF-êB pathway are important signaling pathways that are known to require for efficient influenza virus propagation and have attracted some attention as suitable targets for antiviral interventions. Targeting host cell factor might have an additional advantage in terms of drug resistance because the virus can not easily replace the missing cellular functions by mutations. Using influenza virus as a model, this review will summarize the recent progress in our understanding about the host cell factors that can be exploited for development of antiviral therapeutics.

Introduction

As an obligate intracellular parasite, virus has to rely on host cell machinery for its effective replication. The replication cycle and roles of different viral proteins are well characterized. However, there is a significant gap of knowledge about the host cell factors required by the virus during its replication. Rapidly accumulating evidences suggests the involvement of host cell factors for different steps of virus replication cycle and each essential steps of replication cycle is considered as a potential site for antiviral intervention. Virus encoded protein targets are attractive but selects drugs resistant variants over the time due to mutations; the host cell factors that are required by the virus and at the same time are dispensable for host might be good target in terms of drug resistance as virus can not easily replace missing cellular functions by mutation. The detailed molecular interaction between virus and host is required to define the precise targets. Influenza viruses belong to the family *Orthomyxoviridae* and are divided into the three virus types A, B and C. Influenza A viruses have a broad host range including mammals and birds whereas Influenza B and C viruses cause only mild disease only in mammals. The genome of the Influenza A viruses is having 8 segments (about 13 Kb total) which encodes up to 12 different proteins. The first three segments encode viral RNA dependent RNA polymerase (RDRP; comprises of PB2, PB1 and PA). Although RDRP can initiate RNA synthesis but for full polymerase activity nucleoprotein (NP) protein is also essential which is encoded by segment 4. Other proteins include Haemagglutinin (HA), Neuraminidase (NA), nonstructural proteins (NS1 and NS2) and matrix proteins (M1 and M2). While HA protein is required for receptor binding, NA is required for release of virus from the host cell. Besides suppressing host antiviral response, NS1 has several other functions in transcription and translation. The NS2 (also called nuclear export protein; NEP) together with NP, is associated in export of vRNPs (composed of a segment of RNA with PB2, PB1, PA, NP). The M1 is known to have nuclear localization signals (NLS) potentially involved in import of vRNPs into the nucleus via host cell's classic import pathway (28), M2 is involved in release of nucleocapsid in cytoplasm (58). The second gene products of PB1 i.e. PB1-F2 (PB1 frame-2) and PB1 N40 are newly identified proteins but not all strains encode these proteins (5) (76). Both PB1-F2 and PB1 N40 are nonessential for virus viability but are detrimental to virus replication. PB1-F2 have additionally been shown to localizes to mitochondria and act as an apoptosis promoter in late stage of infection (77).The primary defense against influenza A has been vaccination that is reformulated each year. There are some antiviral medications approved by US Food and Drug Administration (FDA) that are used for both prophylactic and therapeutic treatment during seasonal epidemics of influenza (http://www.fda.gov/drugs/drugsafety). First group comprises ion channel or M2 inhibitors Amantadine (approved in 1966) and Rimentadine (approved in 1993). Second group comprises neuraminidase inhibitors Oseltamivir (Tamiflu) and Zanamivir (Relenza) and both were approved in 1999. The M-2 inhibitors are limited in practice because of lack of their activity against influenza B viruses and rapid emergence of drug resistant variants, which retains their ability to cause disease and transmit disease from person to person (23) (24). The

neuraminidase inhibitors came with great success but by 2009 its resistance has been reported in both seasonal and pandemic H1N1 (43), highlighting the need of an alternative strategy to design suitable antiviral compound that do not have the tendency to easily induce drug resistance due to preexisting selection pressure.

Influenza virus life cycle

Influenza virus is an enveloped virus, it binds to host cell sialic acid via its haemagglutinin (HA) surface protein. The events following binding leads to internalization of virus particles into endosomes, acidification of endosome which trigger conformational change in HA which in turn leads to fusion of viral and endosomal ultimately resulting in release of vRNPs into the cytoplasm (8). In eukaryotic cell, the nucleus is separated from cytoplasm by nuclear membrane which contains nuclear pores for transport of macromolecules across the membrane. While small proteins may passively diffuse through nuclear pores, the transport of RNA and larger proteins/protein complexes (>20 kD) across nuclear membrane is an active energy dependent process and requires specific targeting sequences on substrate viz; nuclear localization signals (NLS) or nuclear export sequences (NES) for nuclear import and export respectively (64). Most nucleocytoplasmic transport processes requires the Ran-GTPase protein which traffic across the nuclear membrane (RanGDP form in cytoplasm and RanGTP in form nucleus). It forms a transport complex composed of RanGTP, cargo molecule and exportins/importins. A gradient of RanGTP/RanGDP across the nuclear membrane which results from the activity of chromatin-associated nucleotide exchange factor regulator of chromosome condensation 1 (RCC1) and the cytoplasmic GTPase-activating protein RanGAP and Ran-binding protein 1 (RanBP1) is the major driving force for nuclear protein transport (30). Influenza virus vRNPs are too large (10-20 nm wide) to diffuse passively through NPC (6). Early studies reported that all of four viral RNA-associated proteins as well as NS1 and NS2 accumulates in nucleus following infection as well following independent over expression via cloned DNA (17) suggesting that they undergoes NLS-mediated vRNPs import (56). A major receptor for export of proteins out of the nucleus is CRM1 (chromosome region maintenance 1) (also referred to as exportin1 or Xpo1). CRM1 is an importin â superfamily of transport receptors and interacts with leucine-rich nuclear export signals (NESs) found in a large variety of proteins, such as transcription factors, certain translation factors and other proteins that have to be excluded from the nucleus (30). The compelling evidences suggests that the Raf/MEK/ERK signaling cascade regulates influenza virus vRNP export blockade of which leads to retention of vRNPs in nucleus (62). The activation of the ERK occurs due to accumulation of HA in cell membrane and its tight association with lipid raft (42). Unlike other RNA viruses, influenza virus replicates in nucleus. Because influenza virus genome is negative stranded, it can not directly serves as mRNA so virus encoded polymerase (PB2, PB1 and PA) first synthesize a complementary copy of vRNA called cRNA which is than used as a template to synthesize vRNA. After entry into the host cell the vRNPs, composed of negative stranded RNA, NP and polymerase subunit PB2, PB1 and PA enters the nucleus where they are replicates and transcribes (25). Newly synthesized M1, NP and polymerase proteins are transported into the nucleus and assembled into vRNPs during genome replication. The vRNPs are then transported back to cytoplasm where together with other viral proteins, assemble into virus particles. Besides the well characterized role of viral proteins (PB2, PB1, PA, NP), rapidly accumulating evidences suggests the role of multiple host cell factors for influenza virus RNA synthesis (48) (47) (51) (53) (73) (69) (12) (75) (37) (36). Translation of influenza virus proteins involves several cis and trans (host) acting factors. Influenza virus NS1 plays an important role in transcription, translation and post-transcriptional processing of RNA (34) viz; upregulation of viral mRNA synthesis (10), inhibition of nuclear export of cellular mRNAs that contain 3' poly (A) ends and suppression of cellular mRNA splicing (18), modulation of host gene expression (39), inhibition of dsRNA-activated protein kinase R (PKR), activation of PI3K signaling (21) and antagonizing IFN-á or IFN-â mediated antiviral response (20). The infection of influenza virus results in selective translation of viral mRNA concomitant with shutoff of the host cell protein synthesis (34). Short 5' untranslated conserved region of influenza viral mRNA increases the translational efficiency of viral transcripts (reviewed in reference (19)). In addition, cellular RNA recognition motif containing RNA-binding protein G-rich sequence factor 1 (GRSF1), specifically interacts with conserved 5' UTR of influenza virus and stimulate selective viral mRNA synthesis (33). The RdRp of influenza virus snatch the 5'cap from cellular mRNA to use it as primer for viral mRNA synthesis. The uncapped cellular mRNA is also prone to degradation contributing in shut off of host cell protein synthesis. Virion assembly and budding are the final part of influenza virus life cycle prior to release of infectious progeny virus particles from infected host. Influenza virus causes acute infection and does not maintain long term relationship to infected host thus the survival of the virus depends on how efficiently it transmits from one host to another which in turn depends on release of progeny virus

from the infected host. The assembly implies the formation of capsid and incorporation of the genome inside it. During assembly, the viral components either singly or in form of complexes are brought to the budding site (plasma membrane) which forms higher order of complexes which may facilitate formation of bud. The budding process involves bud initiation, bud growth and bud scission/release and requires both viral (M1, M2, NA and HA) and host factors. Presence of lipid rafts and assembly of viral components at the budding site causes asymmetry of lipid bilayer and outside pinching of the membrane leading to bud initiation and bud growth. There are several excellent reviews on influenza virus assembly budding and release which covers both virus and host cell factors required for these processes (55) (54) (1) (29). Influenza virus is pleomorphic, the primary clinical isolates predominantly with filamentous morphology while cell culture adapted with spherical. Both viral and cellular proteins has been involved in determine the shape of influenza virions. Matrix protein (M1) is considered as primary viral protein which determines particle shape (15). However the filamentous formation does not occur in absence of intact actin cytoskeleton which suggests that host cell factors are also involved in determining particle shape (63). Unlike many other enveloped viruses including retroviruses (reviewed in ref (3)) influenza virus has not been shown to utilize ESCRET pathway (endosomal sorting complex required for transport) pathway (4). However Rab11 (a small GTP binding protein involved endocytic recycling) and Rab11 family interacting protein 3 (FIP3) which plays a role in membrane trafficking and regulation of actin dynamics are both required to support formation of filamentous virions (3). Additionally Rab11 is involved in final step of budding (release) of spherical particles (3) which all together suggests the role of Rab11-mediated pathway in influenza morphogenesis and budding, a cellular system which normal role, like that of ESCRET machinery is in directing vesicular traffic (pushing force).

The roles of host signaling pathways in influenza virus replication

Living system respond to the environmental stimuli by encoding and transmitting the received information in form of a chain of events called signal transduction that in turn results in change in the behavior of the cell. The signals that are activated upon virus infection to cell might be due to (i) interaction of viral surface proteins with cellular receptors (ii) accumulation viral proteins or RNA inside cell and (iii) overload of host cell protein synthesizing machinery due to viral proteins. In addition, many viral proteins not present in infectious virus but produced during replication in host cell, might also activate signaling pathways. Upon viral infection an array of signal transduction events are initiated by the cells that might be: (i) antiviral (ii) virus supportive (iii) both antiviral and virus supportive and (iv) no role in virus life cycle. The best characterized host cell signaling pathway for influenza virus infection are receptor tyrosine kinase (RTK) (36, 37), Mitogen activated protein kinase (MAPK) (62), Phosphatidylinositol-3-kinase (PI3K) (13) and Nuclear factor-kappaB (NF-êB) (38). Following their activation, the basic outcome of these pathways is the upregulation of genes responsible for cytokine production. However, inhibition of these pathways have been shown to inhibit of virus replication suggesting that the virus misuse the component/s of signaling pathways to support its own replication. The virus supportive activity of such signaling pathways is usually exploited to develop novel antiviral therapeutics.

Receptor tyrosine kinases (RTKs) signaling

Receptor tyrosine kinases are a group of growth factor receptors that, upon ligand binding, undergo autophosphorylation at Tyr residues (65). These phosphorylated tyrosines then recruit Src homology-2 (SH2) and phosphotyrosine- binding (PTB) domain-containing proteins that activate or link to downstream signaling pathways, such as the Ras/ ERK/MAPK, PI3K/Akt, and JAK/STAT pathways (60). Together, the complex signaling network triggered by RTKs leads to regulation of cell growth, migration, metabolism, and differentiation. Due to their critical roles in the development and progression of various cancers, RTKs have recently been studied extensively as targets for anticancer therapeutics. Host signaling through RTKs and other tyrosine kinases has also been shown to play important roles in virus replication. The tyrosine kinase inhibitor genistein was found to block replication of HIV-1, herpes simplex virus type 1 (HSV-1), and arenavirus (71) (72) (68). Src family kinases are known to be important for assembly and maturation of dengue virus and West Nile virus (26). The Raf/MEK/ERK and PI3K/Akt pathways downstream of RTKs play important roles in influenza virus replication (41). Specific RTK inhibitors (RTKIs), known as AG879 and tyrphostin A9 (A9), have strong antiviral activity against influenza A virus, and we have demonstrated that they inhibit the Crm1-dependent nuclear export of the vRNP complex, viral RNA synthesis, and virus release. We showed that diverse interventions targeting TrkA (NGFR) can impede not only influenza A virus replication but also impairs replication of several other viruses, thus validating this specific RTK as a candidate drug target (36, 37).

MAP Kinase pathway

Mitogen activated protein kinase (MAPK) cascades are important signaling pathways that convert extra-cellular signals into cellular responses (reviewed in reference (27). They regulate proliferation, differentiation, cell activation and immune responses. Four different members, organized in separate cascades have been identified so far: (i) ERK (extra-cellular signal regulated kinase), (ii) JNK (Jun-N-terminal kinase), (iii) p38 and (iv) ERK5. For each MAPK different isoforms are known. All these enzymes are activated by phosphorylation mediated by an upstream MAPK kinase (MAPKK, MEKs or MKKs). It is induced by extra-cellular agents, including pathogens such as RNA viruses and DNA viruses (61). ERK activation upon influenza virus infection leads to virus-induced cytokine production and airway inflammation (45), however at the same time appears to support viral replication by facilitating vRNP export (62), (42).

NF-êB pathway

Influenza A virus proteins NP, HA and M activates NF-êB by promoting degradation of IKK-â (16) through ER stress response and release of calcium due to over accumulation of HA or ER independent manner (NP and M) (46). Classic NF-êB comprises a heterodimer of 50-kDa protein named p50/ NF-êB1 and a 65-kDa protein called p56/RelA, this heterodimer is the most common form of NF-êB in most cell types (41). In resting stage NF-êB heterodimer resides in the cytoplasm in a complex with inhibitory kinase proteins (IêB) and can not enter in nucleus. Various NF-êB inducing signals ultimately leads to activation of IêB kinase â (IKK-â) which in turn leads to phosphorylation of IêB resulting in ubiquitination and proteosome-mediated degradation of IêB. Following degradation of IêB, NF-êB enters into the nucleus and activates transcription of several genes (reviewed in reference (41). Nimerjahan et al (57) showed the preliminary role of NF-êB as a host cell factor prerequisite for influenza virus propagation. However the first direct evidence suggesting prerequisite of NF-êB for influenza virus RNA synthesis first came by our work (38); using two known inhibitors of NF-êB viz: Bay11-7082 which inhibit phosphorylation of IkB and Pyrrolidine dithiocarbamate (PDTC) which inhibits with ubiquitin-proteosome-mediated degradation of IkB, we identified that the NF-êB signaling differentially regulated influenza virus RNA synthesis and the NF-êB subunit p65 enhances vRNA synthesis but not cRNA synthesis work (38). Highly pathogenic avian influenza virus of H5N1 subtypes in human and birds leads to bleeding and overproduction of cytokines (cytokine storm) preferentially by attacking endothelial cells. The H5N1 induced overproduction of cytokines depends on functional NF-êB signaling whereas low pathogenic strains are much weaker and less NF-êB dependent (66). Viruses have also evolved strategies to counteract these responses. Recent evidences suggests that influenza A virus also not only suppress IFN-â induction but also suppress type I IFN signaling involving NF-êB dependent induction of Suppressor of Cytokine Signaling-3 (SOCS-3) protein expression which negatively affects JAK/STAT activation ultimately resulting in impaired antiviral response (59).

Phosphatidylinositol-3-kinase (PI3K) pathway

Phosphatidylinositol-3-kinase (PI3-K) has been shown to be activated in response to dsRNA intermediate and mediates activation of the transcription factor interferon regulatory factor-3 (IRF-3) a protein with antiviral functions (13). Inhibition of this PI3-K pathway using chemical inhibitors results in decrease in viral titers due to reduced uptake of virus particles (entry) into host cell (13). Additionally, with mechanism unknown, inhibition of PI3-K pathway has also been reported to inhibit viral RNA synthesis, vRNP export and viral protein synthesis (70). On the one hand influenza virus NS1 inhibit the dsRNA-responsive transcription factors, on another hand in activates PI3-K /Akt pathway to suppress the onset of premature virus-induced caspase activation and apoptosis (14) by negatively regulating JNK (c-jun N terminal kinase) pathway via ASK1 (apoptosis signal-regulating kinase 1) (40).

Genome-wide screens to search host cell factors required for influenza virus replication

Following completion of human genome project in 2003 there is rapid accumulation of the knowledge about host genes involved in influenza virus replication. Several approach have been used to identify host cell factors required for influenza virus replication (7) (52) (49) (50) (69) (48) (31) (44) (11). The RNAi-based genome-wide screen is considered as most powerful approach to study host cell factors involved in virus replication (74). RNAi involves the suppression of a host gene by delivering 20-25 nucleotide long dsRNA homologous to gene concerned. Several independent studies using genome-wide screens have identified several human genes involved in influenza virus replication (22) (2) (35) (32) (67). The data from all these genome-wide screens has been analyzed and reviewed recently (74) which depicts that out of 1449 human genes identified, 128 genes were found in at least

two screens. The authors have analyzed these host genes in several different ways which includes (i) PANTHER classification system which revealed several gene categorized associated with defined molecular functions (nucleic acid binding proteins, kinases, transcription factors, ribosomal proteins, hydrogen transporters and mRNA splicing proteins). (ii) Analysis by reactome which is a curated knowledge of biological pathways revealed several other overrepresented events (translation initiation, Golgi-to-ER transport, regulation of gene expression, processing of mRNA etc). The authors further analyzed it by using GeneGo (GeneGo Inc, MI) and integrated with information on the viral and cellular interaction partners from other sources (35) resulting in a network of host-influenza virus interaction which depicts that each step of influenza virus life cycle is associated with multiple host cell factors. Several of these host cell factors identified using RNAi are previously known to support influenza virus replication but several other need to define their precise role. However the genome-wide screens does not cover all human genes and may represent false positive or false negative results due to knockdown efficiency or cytotoxicity. More detailed functional analyses of these human genes identified in genome-wide screens will allow us to find novel cellular pathways and/or host genes sets important for influenza virus replication.

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APPLICATION OF BIOTECHNOLOGY IN DAIRY AND FOOD INDUSTRY

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Abstract

Biotechnology as technique or as process has numerous application in food processing area to deliver nutritious, wholesome, safe and convenient food products. Biotechnology in the food processing sector targets the selection and improvement of microorganisms with the objectives of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products. In recent years biotechnological interventions in food and dairy industry have numerous success stories including accelerated cheese ripening, designer milk and meat products, recombinant enzymes and many more. Current trends are focused on using LAB as live vaccines delivery vehicles and using them as cell factory for production of nutraceuticals.

Introduction

The term "biotechnology" was coined in 1919 by Kari ErekyanHunagarian Engineer, and in simplistic sense means the exploitation of biological processes for industrial and other purposes. Biotechnology in one form or another has flourished since prehistoric times. When the first human beings realized that they could plant their own crops and breed their own animals, they learned to use biotechnology. The discovery that fruit juices fermented into wine, or that milk could be converted into cheese or yogurt, or that beer could be made by fermenting solutions of malt and hops began the study of biotechnology. When the first bakers found that they could make a soft, spongy bread rather than a firm, thin cracker, they were acting as fledgling biotechnologists. The first animal breeders, realizing that different physical traits could be either magnified or lost by mating appropriate pairs of animals, engaged in the manipulations of biotechnology.New biotechnological techniques have permitted scientists to manipulate desired traits and recombinant rDNA technology has changed the orientation of biotechnological processes for wider spread horizon. Modern biotechnology has its "roofs" in basic sciences and the explosion in this area has resulted in three major branches of biotechnology: genetic engineering, diagnostic techniques and cell/tissue culture techniques.Biotechnological applications in the area of food processing are not new, selective breeding of plants and animals to increase the yield and utilization of microorganisms to manufacture wide array of food products like beer, wine, cheese and broad, are centuries old practices. Food biotechnology integrates biochemistry, chemistry, microbiology, and chemical engineering for the enhanced production of food products. The application of microbiology to food systems encompasses methods involved in the assessment of microbial food safety and the use of microorganisms for the production of foods and beverages, food products, food additives. Microorganisms involved either directly or indirectly with food systems include bacteria, molds, yeasts, and algae. Each of these microbial groups has unique metabolic aspects that are either utilized or circumvented to achieve optimization of various microbial processes.

Biotechnlogical research tools

Advancement in the area of molecular genetics in mid and late 20th century reflected in increased availability of research methods in the field of biotechnology. These tools are highly specific and have potential to ensure not only the food security but many other aspects of human life. Some of these technologies are listed here.

Recombinant DNA Technology

Recombinant DNA (rDNA) molecules are DNA sequences that result from the use of laboratory methods (molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure; they differ only in the sequence of nucleotides within that identical overall structure.

Tissue Culture

This process involves *in vitro* culturing of protoplasts isolated from plant/animal tissues under aseptic conductions and with appropriate amounts of growth substances, hormones. In the growing medium, cells grow and divide to form a mass of undifferentiated tissues called as callus. Callus, in turn incubated under conditions that induce

organ formation or used for initiation of a cell suspension. This technique has been commercialized for the productions of plants, food colours, and many other food ingredients.

Hybridoma technology

Hybridoma technology is a technology of forming hybrid cell lines (called hybridomas) which produce antibodies (monoclonal antibodies). A hybridoma is created by fusing two cells, a secreting cell from the immune system and a long-lived cancerous immune cell, within a single membrane. The resulting hybrid cell can be cloned, producing many identical offspring. Each of these daughter clones will secrete, over a long period of time, the immune cell product. A B-cell hybridoma secretes a single specific antibody.Such monoclonal antibodies, as they are known, have opened remarkable new approaches to preventing, diagnosing, and treating disease.

Biosensors

A biosensor is an analytical device which converts a biological response into an electrical signal. Biosensors are composed of a biological component such as a cell or antibody linked to a tiny transducers. The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest.

Application of biotechnology in food and dairy processing

Biotechnology as technique or as process has numerous application in food processing area to deliver nutritious, wholesome, safe and convenient food products. These applications are summarized in brief here under :

3.1 Improvement of Raw Material Quality

It is established that genetic variation in animal causes the different composition in milk. Transgenic cows with altered genetic make up to produce milk with 2 per cent fat with a greater proportion of unsaturated fatty acids in milk fat, higher levels of milk protein, â- and ê- casein and reduced lactose content in milk have been developed. Such milk is suited ideally for people suffering from lactose intolerance and also to produce improved varieties of specialty cheeses with effective cost price efficiency. Genetically modified bovine somatotropin (BST) also play a role in the regulation of milk yield, growth rate and protein to fat ratio of milk which results in milk composition alteration. Traditional breeding methods were aimed to improve the quality of plant products for their improve the quality of plant products for their efficient processing. The bread making ability of wheat flours have been improved by modifying the gluten content and it is endowed to the transgene responsible for high MW proteins production into wheat varieties. Similar kind of experimentation is in progress to alter the amylose and amylopectin fractions in rice grain to enhance its cooking quality as well as processing characteristics. Barley varieties have been targeted to produce good quality malt for infant foods as well as for production of beer, having novel aroma compounds. Seed proteins confer various functional characteristics like solubility, gelling, foaming, whipping, emulsifications in processed food products. Through protein engineering in conjunction with rDNA technology the attempts are in progress to enrich these seed proteins/protein fraction with desired functionality, like wise production of raw fruits and vegetables with high total soluble solids, higher phytochemical contents, enhanced pigments content, and with desired degree of enzymatic activity is also currently in progress to not only improve pre but post harvesting qualities. High starch content in potatoes produce low fat and crisp products like chips, French fries and through gene alternation high starch containing potato plants have been evolved. Rapeseed has also been modified to produce a high temperature frying oil, low in saturated fat. Improvement in processing characteristics is mainly achieved through employing modern biotechnological tools as they are quick and reproducible.

3.1.1 Designer milk

Milk composition can be dramatically altered using gene transfer. Classical studies have revealed association between major lacto-protein variants and milk production traits. Introduction of DNA technology in the dairy science field has enabled to identify new genetic polymorphism and revealed molecular background of lacto-protein gene expression. Subsequently, several emerging transgenic technologies have focused on the mammary gland.Breast milk is nature's perfect food for human infants providing them all aspects of nutrition and protection against infections. However, a considerable number of infants are fed formulae based on bovine/buffalo milk. The composition of these infant formulations can be improved if the proteins contained

therein more closely resemble those of human milk. It is now possible to add human lactoferrin or lysozyme to bovine milk by genetic engineering to produce new functional foods. The shelf life of such products is also expected to be very high due to ant microbial activity of these proteins. Lactoferrin (LF), the iron-binding protein has antimicrobial properties and may also mediate some effects of inflammation and have a role in regulating various components of the immune system. Its level in human milk is about 1 g/l (in human colostrum about 7 g/l). As the levels of LFin cow's milk are only about one-tenth that in human milk,this has caught the attention of those involved in designinghuman milk replacement formulas. Pharming, NV (Leiden,The Netherlands) developed the first transgenic bull in the late 1980s and a line of transgenic cows to produce several proteins, including human LF (hLF). Human milk contains 0.4 g/l of lysozyme (LZ), an enzyme that provides it with antibacterial activity. Active human lysozyme (hLZ) has been produced in the milk of transgenic mice at concentrations of 0.78 g/l. On the processing front, the expression of LZ in milk results in the reduction of rennet clotting time and greater gel strength in the clot. A double transgenic cow that co-expresses both hLF and hLZ in milk may also reduce the incidence of intra-mammary infection or mastitis. Yet another application of transgenic technology could be to produce the human lipase, which is stimulated by bile salt in the milk of bovines. The lipase thus produced could be used as a constituent of formulas to increase the digestibility of lipids, especially in premature infants who have low â-gal activity. Cow milk allergenicity in children is often caused by the presence of â-lg, which is absent in human milk. Elimination of this protein by knocking out â-lg gene from cow's milk is unlikely to have any detrimental effects on either cow or human formula and might actually overcome many of the major allergy problems associated with cow's milk. Further, as milk protein allergenicity studies demonstrate that all food proteins are potential allergens and that allergenic structures are widely spread throughout the protein molecule, milk is a good model in the search for means of characterizing allergenic structures in food. Therefore, while developing strategies for the identification and evaluation of potential allergenicity in novel foods, many of the technological practices used in the assessment of milk protein allergenicity can be adapted.

3.2 Recombinant Coagulating Enzymes

One of the success stories for the application of genetic engineering is the manufacture of recombinant chymosin and its use as milk coagulating enzyme for commercial cheese production. Concern over the supply of chymosin from traditional source (suckling calves) has led to efforts over the past three decades to develop a recombinant source. Cheese industry has been the major beneficiary of this technological work. The gene coding for the chymosin enzyme has been cloned in the bacteria *Escherichia coli*, the yeast *Kluyveromyces lactis* and the mould*Aspergilusniger*. The enzymatic properties of the recombinant enzymes are indistinguishable from those of calf chymosin. The cheese making properties of recombinant chymosin produces very satisfactory results and its use in commercial plant have been approved by many countries. Maxiren:From*K. marxianus var. lactis* produced by Gist Brocades, NetherlandsChymogen: From *A. niger* produced by Hansen's, Denmark. Chymax:From*E. coli by* Pfizer, USA.All the three chymosin available are identical to calf chymsoin, considered as vegetarian source and accepted by religion group. More knowledge on genetic engineering combined with better understanding of protein structure might one day give us chymosin with higher activities, lower cost and with flavour enhancing properties of cheese during ripening.

3.3 Modified Starter Culture

3.3.1 Genetic modification of LAB

Food-grade genetic modifications are the limited types of modifications that would be considered acceptable for food microorganisms with safety as the chief concern. A food-grade GMO must contain only DNA from the same genus and possibly small stretches of synthetic DNA. A broader definition is gaining acceptance and would allow the use of DNA from other genera of food microorganisms, provided that the donor belongs to the group of organisms referred to as 'generally recognized as safe' (GRAS). By this definition, a *Lactococcus* GMO contains DNA from *Streptococcus thermophilus* or other GRAS bacteria. With either definition, it is acceptable to use DNA from non-GRAS organisms during the construction of the GMO provided that this DNA is removed before the GMO is considered to be food-grade. Sensitive techniques such as DNA–DNA hybridization, Polymerase Chain Reaction and DNA sequencing can be used to confirm that the undesired DNA has been successfully removed. A number of genetic modifications which can be done in a food-grade manner as indicated below. Genes can be deleted from a strain. This is

especially relevant when a gene has an undesired property, for example the production of an undesirable metabolic end product or the destruction of a desirable component of the food. A gene in a strain can be replaced with the homologous gene from another strain. If a strain has a number of useful properties but a desirable enzyme activity is at too low a level, this can be corrected by introducing a more active copy of the gene from another strain. Genes can be inserted into a strain, for example genes that result in increased resistance to bacteriophage. Finally, the expression of a gene can be increased by increasing the number of copies in the cell, using food-grade cloning vectors.The application of gene cloning technology to lactic acid bacteria is the potential process in the generation of enhanced starter cultures for the manufacture of cheese and yoghurt. These starter cultures are mainly made of species of *Lactococcus*, *Lactobacillus* and *Streptococcus*. Modifications of these microorganisms were achieved mainly on three directions for cheese making process. These are:Development of phage resistant cheese culture,Organisms with Probiotic Activity for cheeses,Acceleration of cheese ripening.

3.3.2 Phage Resistant Cheese Culture

The major cause of slow acid production in cheese plants today is bacteriophage (phage). This can significantly upset manufacturing schedules and, in extreme cases, result in complete failure of acid production or "dead vats". Phages are viruses that can multiply only within a bacterial cell. They have a head, which contain the DNA and a tail, which is compared of protein. Morphologically, there are three types of phage for *latococci* as mentioned below: Small isometric headed (spherical headed) phage - most common, Prolate – headed (oblong – headed) phage ,Large isometric – headed phage.Phage multiplication occurs in one or two ways, called the lytic and lysogenic cycle. Multiplication of phage is very fast in their hosts. Microbiologists have enhanced cheese culture performance by genetically engineering bacteria increasing the viability of the culture during cheese making. The new strain of bacteria resist phase contamination and are suitable for prolonged use in milk fermentation. Several phase resistance mechanism, including inhibition of phage adsorption, restriction–modification mechanism and abortive infection mechanism are found in LAB. All of these are commonly encoded on plasmids(Daly, Fitzgerald et al. 1996). A phage resistant starter culture of cheddar cheese, *L. lactis* DPC 5000 have been developed which was shown to embody three effective phage resistant mechanisms. Cheddar cheese manufactured with DPC 5000 compared favorably in term of composition with cheeses manufactured using commercial starter. Two phage resistant thermophilic starter strains DPC 1842 and DPC 5099 have been developed in Cork, Ireland which performed well in commercial plants for Mozzarella cheese preparation. The new cultures provide more predictable performance and reduce the chance of vats failure.

3.3.3 Acceleration of Cheese Ripening

Genetically modified starter cultures with enhanced complements of proteinase and/or peptidase, which could be released early and evenly distributed in the curd would be an ideal method of accelerating cheese ripening. Modified / genetically tailored microorganisms, genetically engineered proteolytic and lipolytic enzymes are now being used for enhancing flavour production in cheese. Enzyme addition is now one of the few preferred methods of accelerated ripening of cheese. Enzyme may be immobilized or encapsulated for long term action on the production for quick action and homogenous distribution in the product. Cheese manufactured with an amino peptidase N-negative clone strain of *Lactococcus* produced bitter off flavour. The gene for the neutral proteinase (neutrase) of *B. subtilis* has been cloned in *Lc. lactis* UC317. Cheddar cheese manufactured with this engineered culture as the sole starter showed very extensive proteolysis, and the texture became very soft within 2 weeks at 8°C. Cheddar cheese made with *Lc. lactis* subsp. *cremoris* Sk11 (cloned with proteinase) revealed that starter proteinases are required for the accumulation of small peptides and free amino acids in cheddar cheese. The strain in which the proteinase remained attached to the cell wall appeared to contribute more to proteolysis than the strain that secreted the enzyme. Cheeses made with proteinase positive starter produce more pronounced flavour than those with proteinase negative strain during ripening. The inactivation of genes in a metabolic pathway can be used to alter end products that accumulate from a given pathway. Application of regulated promoters (in genetic engineering) is the controlled expression of lytic genes resulting in autolysis of the starter culture. This would result in rapid release of enzymes (i.e. peptidase) into the cheese matrix and potentially accelerate cheese flavour development(McKay and Baldwin 1990).

3.3.4 Starters for improved flavor profile

The flavour of fermented food depends on the presence of desirable and the absence of undesirable flavour compounds. Often, the desirable flavour compounds are further degraded by the culture used to manufacture the food. This results in instability in the quality of the food and a shortening of the shelf life. Genetic engineering can be used to increase the production of the desired compounds and increase stability by prevention of their degradation. Increasing the production of a particular compound can be done by increasing the expression of the genes responsible for its synthesis, elimination of genes responsible for its degradation, or elimination of other biosynthetic pathways that consume a rate-limiting biosynthetic intermediate(Hugenholtz and Kleerebezem 1999).A good example of a flavour compound whose content in food can be changed using food-grade Genetically Modified Organisms (GMOs) is diacetyl, the major flavour component of butter and buttermilk. An important intermediate in the production of diacetyl is pyruvate. Inactivating genes coding for enzymes that use pyruvate as a substrate results in a significant increase in the amount of diacetyl produced by a culture. Likewise, increasing the expression of a-acetolactate synthase –an enzyme involved in the conversion of pyruvate to diacetyl-increases diacetyl production. Finally, inactivation of diacetyl reductase, an enzyme that degrades diacetyl to acetoin, results in increased diacetyl levels owing to increased stability. Combining these modifications in one strain has not yet been accomplished but should not be difficult using the food-grade techniques described here.Another example is the flavour peptides formed during the degradation of casein in cheese production. Some of the peptides produced by the action of the proteolytic enzymes present in the cheese process have a bitter taste and can result in an unpleasant-tasting cheese. The formation of these peptides can be blocked by inactivating the aminopeptidase responsible, or by increasing the expression of an aminopeptidase that degrades the bitter peptide. Food-grade GMOs are currently being studied to determine which aminopeptidases are responsible for the bitter peptides and which can eliminate them. This knowledge will allow the construction of improved industrial strains for cheese manufacturing.

3.3.5 Spontaneous genetic deletion for shelf-stable yogurt

Yogurt results from the growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. They grow together in milk where they ferment lactose to lactate and lower the pH from neutral to about 4.5. Upon storage of the product at 4^{0}C for several days (in the supermarkets or at the consumer's home), the pH of the yogurt may drop further to values below 4.0. This post-acidification leads to a gradually increasing acid and bitter taste of the yogurt, thus degrading the initial organoleptic quality of the product. *S. thermophilus* ferments milk into a mild but nonaromatic product. It is *L. bulgaricus* which mostly contributes to the typical yogurt flavour and lowers the pH to values below 4.2. The approach used to limit post-acidification and still produce the yogurt flavour was to regulate the growth and maintenance of *L. bulgaricus* by controlling its energy metabolism. *L. bulgaricus* starter strains were screened for the presence of spontaneous Lac minus mutants, having little or no residual â-galactosidase activity. Mutants were identified which contained deletions within the â-galactosidase gene (lacZ) or beyond the lac region, thereby inactivating another gene vital for growth in milk, encoding the cell wall-bound proteinase. Such Lac minus and Lac-Prt minus mutants were not able to grow in milk as single-strain cultures without the supplementation of glucose and peptones. However, if grown in mixed cultures with a lactose fermenting *S. thermophilus* strain, Lac minus *L. bulgaricus* strains were able to grow despite the absence of glucose. Hence, *S. thermophilus* provides the mutant partner strains with the necessary energy to grow in milk. Once the fermentation process has been terminated, growth and lactose metabolism of *S. thermophilus* and *L. bulgaricus* cease, resulting in a mild, non post-acidifying yogurt product. Such products have been shown to keep their mild taste and organoleptic properties for more than 6 months stored at 4^{0}C(Mollet 1999).

3.3.6 Genetic deletion for an improved clinical probiotic product

Lactobacillus johnsonii La1 is a probiotic lactic acid bacterium available commercially in many countries as part of a mixed culture fermented milk. It has been intensively investigated in clinical nutritional studies for its health beneficial effects, i.e. survival in the gastrointestinal tract and positive immuno-modulating effect on the host. La1 is cultured in milk, where it ferments lactose to a racemic mixture of D- and L-lactate in a 60:40 % ratio. As with standard yogurt products, the presence of D-lactate in the La1 containing milk product and the capacity of the strain to produce D-lactate after ingestion does not pose a problem to the vast majority

of the adult population. Nevertheless, it is documented that D-lactate producing colonizing intestinal lactobacilli are a main factor in the pathogenesis of D-lactic acidosis and encephalopathy in patients suffering from short bowel syndrome and intestinal failures. In view of the probiotic and immuno-defense stimulatory effects of *L. johnsonii* La1, a non-D-lactate- producing variant would help these patients in reconstituting their intestinal micro-flora after e.g. antibiotic treatment. Another application of a non-D-lactate producing La1 strain would be in the nutrition of infants for building-up and regulating their intestinal flora. Lactic acid is formed via reduction of pyruvate by lactate dehydrogenase (LDH) for the regeneration of NAD+. Thereby, the two isomeric forms of lactate, D(-) and (+), are formed by distinct stereospecific NAD- dependent LDHs. The gene encoding the D-lactate dehydrogenase (ldhD) of *L. johnsonii* has been identified and isolated. A small in vitro generated deletion was introduced into the coding ldhD sequence, thus inactivating the functionality of the gene. A copy of this truncated gene was then used to genetically replace the genomically located original ldhD gene of La1. The experiment was designed in such a way that at the end of the procedure no DNA fragments derived from the plasmid vectors or the antibiotic resistance markers used in the constructs remained in La1. Thus, the new construct is equivalent to La1 with the exception of a small DNA fragment missing within the ldhD gene. It was also shown that this new strain effectively re-routes pyruvate to L-lactate, and does not produce D-lactate anymore. This technology to inactivate the ldhD gene rather than a screening for a spontaneous deletion event at this locus was chosen because the presence (and necessity) of a functional L-lactate dehydrogenase gene makes an effective selection procedure very difficult. Equally important is that the use of a targeted gene disruption versus a screening procedure for induced or spontaneous mutations prevents the accumulation of numerous uncharacterized mutations in the bacterial genome. This adds to the safety aspect of the technology and assures the preservation of the desired health beneficial character of the strain(Mollet 1999).

3.3.7 Transfer of genes coding for exocellular polysaccharides for a creamier yogurt

Consumer preferences in the yogurt market have moved towards sweeter, thicker yogurt with a low or no fat content. Increasing thickening properties of the yogurt are required to compensate for the lower fat content. This could be achieved by adding stabilizers, gums, pectin's or starch. However, the use of exocellular polysaccharide (& slime) producing bacteria for the fermentation process better satisfies the market need for natural products and the legal definition of yogurt in some European countries (e.g. Holland, France). The use of exocellular polysaccharides (EPS) producing strains increases the viscosity of yogurt and decreases susceptibility to syneresis. A significant characteristics and problem for the yogurt industry, is the instability of the slimy property. The spontaneous loss of the EPS producing ability in lactic acid bacteria has been related to the involvement and instability of plasmid encoded genes. . However, this does not seem to be true for the thermophilic bacteria like *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which often do not contain such plasmids. The structure of several EPS produced by thermophilic lactic acid bacteria has been determined and revealed their nature as heteropolysaccharides. By taking advantage of an agar plate assay containing ruthenium-red, colonies of EPS and non-EPS producing yogurt bacteria can be differentiated and genetic experimentation has now become practicable. Today, several genetic clusters involved in EPS synthesis and secretion have been identified in ropy starter strains and genetic transfers of the EPS character between different strains has been reported. In this way it now becomes feasible to transfer the good texturing property of a selected EPS producing strain to another, non-EPS producer which is industrially or organoleptically more interesting as a starter strain. Furthermore, knowledge of the functions of different glycosyltransferases encoded by the gene would allow the modification of existing EPS structures and ultimately the design of novel EPS constructs. Such genetic transfers and modifications could be achieved by exchanging DNA between strains of a same species while omitting all vector and marker genes used in the genetic shaping of the strain(Mollet 1999).

3.3.8 Starters for production of L-alanine by Metabolic Engineering

Lactococcus lactis subsp. *lactis,* one of the best studied starter cultures having a single fermentation metabolism converts lactose or glucose to L-lactate as the chief end product with the intervention of L-lactic dehydrogenase which converts the intermediate pyruvate to L-lactate. This step can now be surpassed by rerouting pyruvate to alanine. In this strategy, the L-alanine dehydrogenase gene (alaDH) of a *Bacillus* spp. has been cloned and over-expressed in *Lactococcus lactis* subsp. *lactis* under the control of a nisin

inducible food grade promoter system. The application of such a *Lactococcus lactis* subsp. *lactis* biocatalyst for converting cheap sugars into L-alanine presents interesting possibilities for production of amino-acids in a microorganism for application in foods. It is also now feasible to genetically engineer a yoghurt starter using the aforesaid strategy to produce L-alanine instead of L-lactate during the processing of milk into yoghurt which will have sweeter alanine taste. This is an example of using trans-species genetic engineering in developing novel starter strains for newer product development(Mollet 1999)

3.3.9 Lactic Acid Bacteria as Oral Vaccines

Mucosal routes for vaccine delivery offer several advantages over systemic inoculation from both immunological and practical points of view. The development of efficient mucosal vaccines therefore represents a top priority in modern vaccinology. One way to deliver protective antigens at the mucosal surfaces is to use live bacterial vectors. Until recently most of these were derived from attenuated pathogenic microorganisms. As an alternative to this strategy, nonpathogenic food grade bacteria such as lactic acidbacteria (LAB) are being tested for their efficacy aslive antigen carriers. Unlike the other LAB being developed as vaccine delivery vehicles, *Lactococcus lactis*does not colonise the digestivetract of man or animals. In mice, there is only a passive transit (persistence time <24 h) of *L.lactis* through the digestive tract while in humans, lactococci passthrough the gut within 3 days. As it is expected that *L.lactis* would be likely to have only a limited capacity toproduce and secrete antigens in vivo, attention has been focused on expressing antigens intracellularly, or as fusions to the cell wall anchoring domains of cell surfaceassociated proteins, so that the bacteria are pre-loaded with antigen before they are used for immunization. In order that immunogenic quantities of antigen can be deliveredto the mucosal immune system a high-level inducible expression system which exploits the properties of the E.coli T7 bacteriophage RNA polymerase has first been developed for L. lactis (pLETvectors). Using the lactococcalT7 system, a number of heterologous antigens have been expressed intracellularly at high levels (2-20% total solublecell protein) in *L. lactis* (e.g. tetanus toxin fragment C (TTFC), diphtheria toxin fragment B, the 28 KDaimmunogen of *Shistosomamansoni*, as well as avariety of TTFC fusion proteins including for example TTFCHIV-gp120V3 loop fusion proteins. Additional pLET vectors were further designed to secrete the antigen (up to 3mg/L) or anchor it to the cells surface. Expression vectorswhich incorporate constitutively active promoters of low to medium strength have also been employed for antigen expression. One such vector designated pTREX1 has been used to express TTFC and P28 at levels of 1-3% of total cell protein(Mercenier, Muller-Alouf et al. 2000). LAB has been successfully used for active vaccination of animals like rodents (Table 1.0). Whether LAB will be effective as a mucosal vaccine in humans can only be answered by clinical trials. Furthermore, as the dose of recombinant LAB needed to elicit immune responses in animals is high it is unknown if the necessary dose for use in humans will be feasible and cost effective.

Table 1 : Lactic acid bacteria as oral vaccines [extracted from (Detmer and Glenting 2006)]

Vaccine strain	Foreign insert	Model
Lactococcus lactis	*C. tetani*TTFC	Mouse
	TTFC+IL-2 or IL-6	Mouse
	Human IL-10	Mouse
	*H. pylori ure*B	Mouse
	*H. pylori ure*B	Mouse
	*S. pneumonie*CPS	Mouse
	Rotavirus vp7	Mouse
	B-lactoglobulin	Mouse
	HIV-1 gp120	Mouse
	Malaria MSP-1	Mouse
	SARS Nucleocapsid protein	*In vitro*
	*E. rhusiopathiae*SpaA	Mouse

Lactobacillus plantarum	TTFC	Mouse
	Allergen Der p1	Mouse
	H. pylori (*ureB*)	Mouse
Streptococcus gordonii	Antibody	Rat
	Hornet venom Ag5.2	Mouse
	TTFC	Mouse
Lactobacillus casei	*B. anthracis*(protective Ag)	*In vitro*
	SARS spike protein	Mouse
	Human papillomavirus L1	*In vitro*
	Coronavirus S glycoprotein	Mouse
	*S. pneumonie*PsaAPspA	*In vitro*
Lactobacillus zeae	Antibody	Rat
Lactobacillus johnsonii	TTFC mimotope	Mouse

Conclusion

Biotechnology is bound to play a significant role in reshaping the Dairy Sector. Judicious application of biotechnological approaches is needed for producing high quality food and dairy products, which are not only nutritious, clean and wholesome but also safe for local consumption as well as for export. Biotechnological interventions in food and agriculture is sure to raise numerous ethical and legal questions. Scientists and social activists may be confronting each other in some issues or fighting together to deal some of these issues. However, ultimate acceptability shall depend upon factors of animal welfare, demonstrable safety of the product, enhanced health properties of the product and increased profitability compared with conventional practices.

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DIFFERENTIAL SUSCEPTIBILITY OF RUMINANTS TO *peste des petits ruminants* (PPR) - RECEPTOR EXPRESSION VS INNATE IMMUNE RESPONSES

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Peste des petits ruminants (PPR), also known as 'goat plague', is an acute, highly contagious viral disease of goats and sheep, caused by peste des petits ruminants virus (PPRV) which is a member of the genus *Morbillivirus* under the family Paramyxoviridae. The disease is characterised by high fever, ocular and nasal discharges, pneumonia, necrosis, ulceration of the mucous membrane and inflammation of the gastro-intestinal tract. PPR is clinically common in sheep and goats while cattle and buffalo do not get clinical disease although the replication of the virus and sero conversion has been demonstrated in bovine.Host innate immune system discriminates pathogen associated molecular patterns (PAMP) from self antigens by a family of Pattern Recognition Receptors (PRR) which include Toll-like receptors (TLRs), Nod-like receptors (NLRs) and Rig like receptors. TLRs and NLRs play a crucial role in pathogen recognition. TLRs are type1 transmembrane proteins expressed in almost all cell types that activate the innate immune system upon sensing of PAMPs. In response to ligand binding to the corresponding TLRs MyD88 dependant/ independent pathways are stimulated resulting in the production of inflammatory cytokines. Four main intracellular TLRs sensing viral nucleic acid include, TLR3 (double strand RNA), TLR7 and TLR8 (sing. stranded RNA) and TLR9 (CpG motifs). The location and the basal expression levels of TLR mRNA would indicate the natural PAMP load of the tissue as well its ability to respond to pathogen challenge. Higher expression levels of a particul TLR in a tissue/ or cell may be attributed to greater host resistance. TLR-ligand induced downstream cytoki profiles levels could also play a role in the innate resistance of a species or breed.

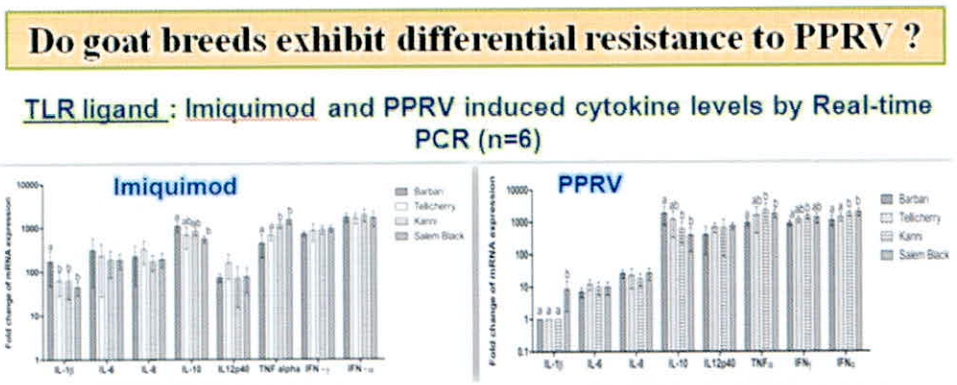
PPR viral receptor – the SLAM

Signalling lymphocyte activation molecule (SLAM) has been shown to be one of the viral receptors The expression of SLAM mRNA in the peripheral blood mononuclear cells (PBMC) of ruminants, quantitative real time PCR (qRT-PCR) was found to be 2.24 folds higher in goats as compared buffaloes (Pawar et al. 2008). Differential expression of SLAM mRNA in the PBMC of four different revealed a 26-fold increase in expression in Barbari goats as compared to the local indigenous br These results indicated that not only the SLAM mRNA levels could be one of the determinants of d susceptibility of ruminant species to PPR but also breed susceptibility variations could also differential expression of the viral receptor. When the SLAM mRNA was inhibited by small interfe the virus was neutralized by anti-SLAM antibodies, the PPRV titre reduced by more than 10 totally abrogated. This suggested that the SLAM could be a co-receptor for PPRV and tha probably also use other receptors, in the absence of SLAM. This emphasised that the differentia of PPR seen in goat and bovine may not all be due to the receptor expression levels alone and pro differential host immune responses also has a 'story to tell' that was explored in further studies.

Innate immune responses among different breeds of goats to PPRV infection

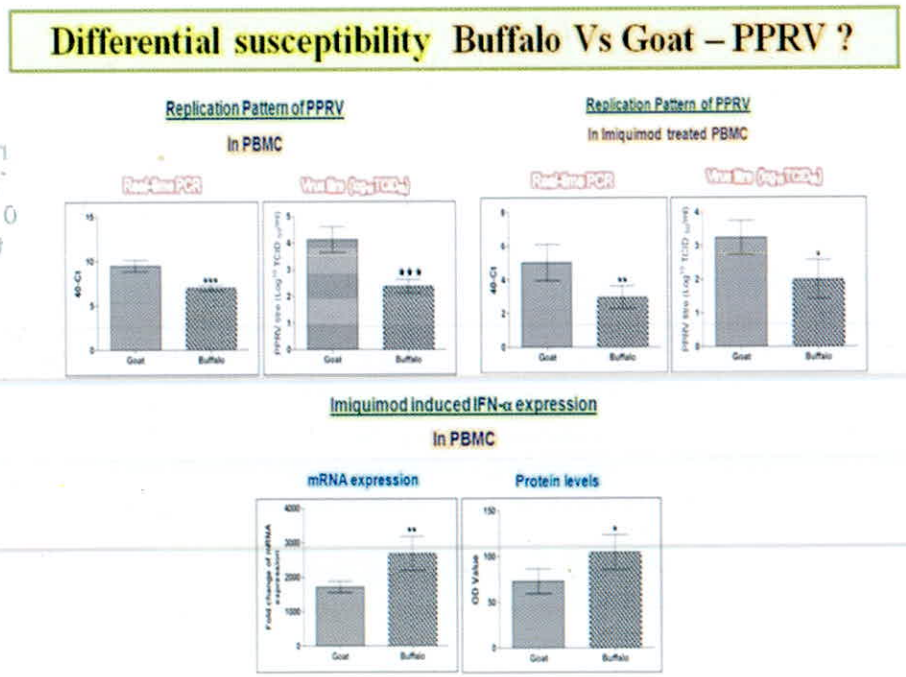
Differential replication patterns of PPRV in different goat breeds such as Barbari, Tellicherry, Kanni and Sale Black was established by qRT-PCR and virus titrations. Kanni and Salem Black breeds had significantly lowe titres and less replication than Barbari and Tellicherry. The expression levels of TLR 7, the ligand for ssRNA we also higher in Kanni and Salem Black breeds than in the Barbari. To further confirm the functional role of TL goat PBMC were stimulated with imiquimod, a synthetic ligand for TLR7 or PPRV and downstream cytok responses were quantified by qRT-PCR and enzyme linked immunosorbent assay (ELISA). Imiquimod stim of PBMCs increased IL1-â and IL-10 mRNA expression in Barbari and TNF-á expression in Kanni and Black. No significant differences in expression levels of IL-6, IL-8, IL12p40, IFN-ã and IFN-á mRNA were ob in all breeds. PPRV stimulation of PBMCs had no significant changes in the mRNA expression of I IL12p40 across the breeds. However, Barbari had significantly higher mRNA expression levels of IL-1(

mRNA expression of TNF-á and IFN-ã. Antiviral cytokine IFN- á expression in Kanni and Salem black breed of goat was significantly higher than Barbari and Tellicherry. Thus the pro inflammatory cytokine, TNF- á and IFN-á was higher in the breeds that supported lower replication of PPRV and may be a likely determinant of host immunity to this virus.



Innate immune responses of goats and buffaloes to PPRV infection

Buffalo PBMC supported PPRV replication less efficiently than goat PBMC. Comparison of TLR7 ligand (Imiquimod and PPRV) induced antiviral cytokine in buffalo and goat PBMC using qRT-PCR and ELISA showed significantly higher expression of IFNá in buffalo PBMC than goat PBMC. The recombinant protein of IFNá could neutralize the PPRV in a dose-dependent manner. Hence it can be postulated that higher expression IFNá, a potent antiviral cytokine in buffalo PBMC limits the PPR virus replication *in vivo* thereby inducing sero conversion but preventing the exhibition of clinical disease in this species.



Sum up...

Thus in the debate on the role of receptor or innate immune responses being attributed to the differential disease susceptibility among species or breeds, it may be summarized from our studies that both may have a role to play. But the role of innate immune responses through over expression of particular cytokines may have a greater part after viral entry into target cells. The role of these cytokines in indigenous breeds is also pivotal since we always anecdotally claim that indigenous breeds are disease resistant. The levels of cytokine expression following viral infections, probably mediated through mutations or single nucleotide polymorphisms (SNPs) in the promoters is a likely 'mechanistic' determinant of disease resistance in these breeds or species.

BIO-SECURITY THREAT PERCEPTION IN RELATION TO FOOD SECURITY

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Introduction

With a global population expected to exceed 9.2 billion by 2050, the security of the world's food supplies is more fraught now than ever. Since the Green Revolution in 1960s followed by White Revolution 1970s, scientists have been developing high yielding varieties in crops and high yielding breeds of food animals to increase the level of food production for its rising demand for growing population. But, the rate at which yields have been improving from year to year has been in decline for the past 25 years. Now, we can't rely on increased yields alone. We need to broaden our approach and implement additional strategies in order to maximize the benefits from improved breeding and ensure that more of what we grow gets to where it is most needed. Biosecurity may be the way. Often overlooked in the continual struggle to produce more, biosecurity aims to protect agriculture from all manner of invasive species, be they vertebrates, weeds, insects or pathogens. Realizing the need to improve biosecurity for agriculture and food, FAO held a *"Consultation on Biosecurity in Food and Agriculture"* at Rome in year 2003. The consultation considered that Biosecurity involves the management of biological risks in a comprehensive manner to achieve food safety, protect animal and plant life and health, protect the environment and contribute to its sustainable use (FAO, 2011).Biosecurity and Food Security are the two terms which have gained much importance during the last decade with growing awareness and concern about the two issues in recent past. The two issues are intricately connected to each other and are dealt together on one or more platforms at national, regional and global level. To understand these issues, it is important to first define each one of them followed by further elaboration and relation between each other. Biosecurity is defined as the application of a combination of measures that are addressed through the coordination of administrative, regulatory and physical security procedures and practices implemented in a working environment for reducing the risks of biological material loss, theft or misuse caused by poor management or poor accountability and protection. Though the term biosecurity encompasses the prevention of the intentional removal (theft) of biological materials from research laboratories, this definition is narrower in scope than the definition used by many experts, including the Food and Agricultural Organization (FAO). Usually biosecurity is referred in context with a laboratory environment, its meaning is frequently extended to encompass practices and procedures applied at livestock farms/ population to prevent or reduce risk of infectious diseases to animals and at agricultural farms to prevent the damage to crops by exotic pathogens and weeds. At national and global level it applies to the sanitary and phytosanitary provisions between two or more countries to prevent the entry of exotic pathogens of livestock and plants into a particular country/ region through transfer/ trade of biological material or products.Another term which is related to Biosecurity is Biosafety which should also be defined here to get a clearer differentiation between the two terms. Biosafety is defined as the application of a combination of laboratory practices and procedures, laboratory facilities, and safety equipment when working with potentially infectious micro-organisms to protect the laboratory staff and, through them, the general public.Food security exists when all people, at all times, have physical, social and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO).

Livestock diseases as threats to food security

Globalization and the increase in the movement of humans, animals and animal products, changes in climate and in natural and cultivated ecosystems, and demographic changes, including accelerated urbanization, changing consumer food habits, and intensified production systems to respond to increases in consumption are contributing to the emergence of new diseases. The emergence of new pathogens has created new threats to the food security of the World. A very recent and definite example of a disease problem which has emerged as a biosecurity threat in relation to food security is the Highly Pathogenic Avian Influenza (HPAI) caused by H5N1 subtype of

avian influenza viruses in India and neighboring countries. In fact, it was after the global emergence of HPAI when the World got alerted to the potential of a virus which can cripple the economy of a country by wiping out its poultry industry creating a food security problem and also pose a major human health challenge. Poultry being a major source of protein in many countries, the diseases of livestock like HPAI can directly pose a threat to the food security and economy of a country. As per the World Animal Health Organization OIE, the best and the safest way to control HPAI in a region is by applying biosecurity measures. The biosecurity practices applied for control of HPAI in India has been elaborately dealt in a review "Avian influenza: a long-known disease and its current threat" by Dubey *et al.,* 2009. The control of HPAI is statutorily governed in India through the Action Plan of Department of Animal Husbandry, Dairying and Fisheries (DADF), MOA, Government of India. The present robust growth of poultry industry of India is maintained due to continued efforts of DADF, MOA, GOI for strict vigilance against HPAI outbreaks with strong diagnostic support from High Security Animal Disease Laboratory which is an OIE reference lab for AI and the National referral facility for AI where diagnosis of AI is carried out as per OIE manual (OIE, 2009). The first incidence of highly pathogenic avian influenza (HPAI), subtype H5N1, in India was reported in states of Maharastra, Gujarat, and Madhya Pradesh in Feb, 2006 (Pattnaik, et al., 2006). The 2006 outbreak led to the loss of thousands of birds and subsequent culling of more than 10.4 lakhs of birds to control the disease. Later on, outbreaks were noticed in 2007 to 2009 involving Manipur, West Bengal, Assam, Tripura states (Tosh et al., 2007; Murugkar et al., 2008; Dubey et al., Nagarajan et al., 2009) and recent cases reported in Nadia and Asansol district of West Bengal. These outbreaks had substantial economic impact on Indian poultry industry. The presence of low pathogenic avian influenza (LPAI) strain, H9N2 has also been reported in some of the states of India (Tosh et al., 2008; Dubey et al., 2009, Nagarajan et al., 2009).With emergence of HPAI and other livestock infections capable of creating major economic impact and threatening food security, a new term Transboundary Animal Diseases (TADs) has been coined by FAO. The TADs are defined as *"Those that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion requires cooperation between several countries"* (OIE, 2004). Some significant TADs which are threats to the food security are the following diseases:

Foot-and-Mouth Disease (FMD),Rinderpest (RP),Contagious bovine pleuropneumonia (CBPP)
Bovine Spongiform Encephalopathy (BSE),Rift Valley Fever (RVF),Peste des petits ruminants (PPR),Classical swine fever (CSF),African swine fever (ASF),Newcastle disease (ND)Avian influenza (AI).

International support to control HPAI and other TADs

In 2008, a new global approach called "One World, One Health" was defined by the four international technical agencies, i.e. FAO, the World Health Organization, OIE and the United Nations Children's Fund, as well as the World Bank and the United Nations System Influenza Coordination. This strategic framework involves multidisciplinary interaction among the public health, animal health, wildlife and national and cultivated ecosystems sectors. The general mobilization carried out since 2004 in response to the H5N1 HPAI outbreak must continue at all levels. Efforts must also be made to anticipate outbreaks of important health crises in order to prevent major health, economic and social impacts. Through Emergency Centre for Transboundary Animal Disease Operations (ECTAD) and Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) initiatives, FAO is working within international frameworks such as the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TAD s) and the newly established One World, One Health (OWOH) initiative to promote a collaborative approach to investigation at the animal–human –ecosystems interface and a coordinated effort for rapid response to emerging infectious diseases. FAO has recognized that protecting livestock against diseases and preventing their spread is one of the keys to fighting hunger, malnutrition and poverty. The EMPRES was established by FAO's Director General in 1994. EMPRES has the mandate to address prevention and early warning across the entire food chain which is done through the following systems: *EMPRES Animal Health:* animal diseases, including aquatic animal diseases.*EMPRES Plant Protection:* plant pests and diseases including desert locust and forest plant pests and diseases and *EMPRES Food Safety.* The mission of the EMPRES is to promote the effective containment and control of the most serious epidemic pests and diseases and food safety threats through international cooperation involving early warning, early reaction, enabling research, and coordination.

Exotic diseases threats to food animals in India

There has been always a risk of introduction of new diseases/pathogenic organisms into a country causing serious animal health problems in terms of mortality and morbidity. Exotic (non native) pathogens, once introduced into a country, can escalate into an epidemic due to the absence of vaccine or effective drugs, lack of resistance in host animals and limited resources to diagnose and restrict the spread of these pathogens ultimately compromising the food supply chain. Based on the currently available literature and reports the following is a compilation of OIE listed livestock diseases exotic to India.

Species	Exotic	Emerging
Bovine-	1. Bovine spongiform encephalopathy.	1. Bovine immunodeficiency virus
	2. CBPP	2. Bovine viral diarrhea
	3. Foot and Mouth disease (SAT 1,2,3)	3. Malignant catarrhal fever
	4. Jembrana disease	
	5. Lumpy skin disease	
	6. Rift valley fever	
	7. Rinderpest	
	8. Ibaraki	
Swine-	1. African swine fever	1. Nipah virus encephalitis
	2. Aujesky's disease	2. Porcine circo virus1 and 2
	3. Porcine reproductive and respiratory syndrome (PRRS)	3. Porcine Parvo virus
	4. Swine vesicular exanthema	
	5. Teschen/ Talfan disease	
	6. Transmissible gastroenteritis	
	7. Swine vesicular disease	
	8. Porcine endogenous retroviral infection	
Sheep/ goats-	1. Akabane disease	1. Bovine viral diarrhoea
	2. Border disease virus infection	2. Caprine arthritis and encephalitis
	3. Louping ill	3. Peste des Petits Ruminants
	4. Nairobi sheep disease	
	5. Rift valley fever	
	6. Scrapie	
	7. Brucells ovis (ovine epididymitis)	
	8. Bunyaviral infections	
	9. Wesselborn disease	
Poultry-	1. Duck viral hepatitis	1. Highly pathogenic avian influenza
	2. Turkey rhinopneumonitis (Avian meta pneumovirus)	2. Turkey Coryza (Bordetellosis)
	3. Turkey haemorrhagic enteritis	3. Avian Myeloid leukosis
		4. VVND

Role of HSADL in preventing ingress of exotic diseases

The preparedness for exotic diseases of animal started at HSADL in 1997. At that time, 39 diseases were considered exotic to India. On the basis of prioritization by scientist at HSADL and as per the need and request from Quarantine Department under DADF, MOA, GOI a few diseases were taken up. These included bovine viral diarrhea (BVD), bovine immunodeficiency virus (BIV), porcine reproductive and respiratory syndrome (PRRS), transmissible gastroenteritis (TGE), avian influenza, caprine arthritis and encephalitis (CAE), rabbit haemorrhagic disease (RHD) and malignant catarrhal fever (MCF). During first ten years HSADL has kept diagnostic preparedness for the above diseases apart from developing a few of them through its own resources and expertise. During the last 5 years since the first outbreak of HPAI in Maharashtra, it has mainly dealt with the diagnosis of HPAI outbreaks apart from taking up new diseases eg. Nipah virus, swine influenza (H1N1), arboviral infections including CCHF etc. Being the first bio-containment lab of the country working for last 10 years, it has gained experience in running and maintenance of bio-containment lab and therefore, it is in a position to be the torch bearer for all those who are now trying to create and run bio-containment labs. Realizing the challenging need of stringent biological safety measures in the era of globalized animal trade and the growing threat of novel pathogens, this lab has to work continuously to develop its R&D and HRD program for extending better services to the nation in terms of food security, employment generation, poverty alleviation and economic prosperity. In the perspective of present day challenges of increasing population, depleting natural resources and global competition, there is need for linking our research programs with the broad aim of 'Food Security'.

Conclusions

Effective measures to guarantee animal health through exclusion and/or containment of emerging as well as trans-boundary or exotic animal diseases is a prerequisite for sustained livestock production and ensuring food security. There is a growing threat of use of biological agents by terrorists to deliberately cause animal diseases in order to destroy animal wealth and to cripple the economy of a country. The challenge of ensuring food security for a world population that will grow to over eight billion people in the next 20 years can be met, in part, by assisting smallholder farmers in developing countries to improve the utilization of locally available land, water, and plant resources to intensify and increase animal production and productivity. This will require not only more sustainable livestock production, but also more efficient approaches, tools, and strategies for preventing, diagnosing and controlling animal diseases. The amount of available animal protein for human consumption is already limited, but the fragile food security situation is further exacerbated by increased movement of animals and animal products due to expanding world trade and the growing effects of climate change that can result in changes in the geographical distribution of pathogens and their vectors. Resource-poor developing countries will become increasingly vulnerable to emergencies caused by the growing prevalence of infectious diseases, especially TADs. In years to come, an important challenge in veterinary public health will be to balance the need for adequate population intake of animal source protein and essential nutrients with the rapid selection, amplification and spread of pathogens in animal production systems. Evidently, addressing disease burdens on host populations must also consider livelihoods, poverty alleviation, food security, and environmental stewardship while constantly reassessing successes, failures, threats and opportunities.

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BIOINFORMATICS DEVELOPMENT AND APPLICATION IN ANIMAL BIOTECHNOLOGY

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It seems part of our nature as a species to manipulate our surroundings. This includes the animals that we use for food and companionship. Genetic manipulation has been going on for centuries as we have bred animals for certain characteristics. Today, we still manipulate animal characteristics by breeding for qualities of interest. The genetic and molecular information detailing has geared up our ability to change. Bioinformatics is conceptualizing biology in terms of molecules an applying information technique to understand and organize the associated with this molecules on a large scale. The Massive data generated on sequential and structural information of biomolecules in last few decades open the new avenue for biologist to think bigger. The development of bioinformatics along with lead to development of new holistic science like Geneomics, proteomics, transcriptomics, Immunoinformatics, system biology etc. This holistic and multidisciplinary new biology approach has also change the Animal biotechnology field also. Genome research in farm animals is largely concerned with mapping genes that influence economically important traits. As currently at different institute large-scale genome sequencing activities are in progress . The Bioinformatics based systems support the better genetic (linkage), quantitative trait locus (QTL), radiation hybrid and physical mapping and allow data sharing between research groups distributed world-wide. There is an increasing awareness of the potential of proteomic technologies to study production animals but the use of proteomic strategies to investigate animal health and disease has been limited by the lack of international coordination and collaboration. The basic problem of animal science is huge biodiversity of animals and the lager versatility in research problem associate to them to deal with such complexity network based approach. Social networking base scientific community project will providing a conduit for the rapid dissemination of knowledge on the techniques and applications of this rapidly advancing area. It will benefit the scientist by providing advanced bioinformatics tools to accelerate the research for enhance animal production, health and welfare, as well as in the assessment of food quality and safety related to the protein in food produced from animal origin. The paper will cover the Genomics and Proteomics development and social networking based research and development opportunities in the area of Animal biotechnology and bioinformatics with special emphasis on Indian conditions.

GENETIC ENGINEERING AND RELATED ETHICAL ISSUES

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The twenty-first century is golden age for Genetic engineering. We are on the verge of being able to transform, manipulate, and create organisms for any number of productive purposes. We can manipulate the genetic codes of various organisms, or engineering entirely new organisms.Genetic engineering has the potential to improve our health and well-being dramatically,revolutionize our manner of living, help us to conserve limited resources, and producenew wealth. Provided that it is appropriately regulated, bearing in mind ethical concernsrelating to dignity, harmful consequences, and justice, its potential benefits outweigh itsharms. There is certainly no reason to reject it outright as "unnatural."[1]Genetic engineering presents intriguing and difficult challenges for 21st century scientists and ethicists .Now we as a society or, perhaps, as a global entity must agree on what beings, human or otherwise, are worthy of moral and legal status and respect, as new intelligent life is created through science and medicine[14].As with any revolutionary technology, anxieties, fears, and moral objections tothe promise of genetic engineering abound. Some are well-grounded and suggest caution, while others arethe product of misinformation, religious prejudice, or hysteria.We should sort out those objections based on sound science and reason from those thatare unfounded.Some religious critics perceive genetic engineering as "playing God" andobject to it on the grounds that life is sacred and ought not to be altered by humanintention. Other objectors argue from secular principles, that it violates the inherent "dignity" of humans and other life-forms to alter their DNA under any circumstances. Religious objections assume theexistence of some creator whose will is defied by genetic engineering, and secularobjections assume that life in its "natural" state, unaltered by human intention, isinviolable because of its inherent dignity.However, it could be argued that "Defying God's will" always means defying some person'sinterpretation of God's will.Even religious sects that reject modern technologies nonetheless embrace some technologies; the essence of technology is to alter the human relationship to nature.Clothing, agriculture, and weaponry have existed since before the dawn of civilizations,and each alters our relationship with nature. These technologies express a rejection of the"natural" order of things, and result from human consciousness and intentionality. For at least 10,000 years—since long before the principles of classical genetics had been scientifically established—human beings have brought about deliberate genetic changes in plants and animals through traditional reproductive methods. Many of the domestic animals, crops, and ornamental plants in existence today are human creations, achieved through selective breeding aimed at enhancing desired characteristics. In a broad sense,such genetic manipulation by breeding for a desired outcome might be considered genetic "engineering."[2]Another argument in favour of genetic engineering is that wholly innocent creatures lead lives of illness or degradation, or die prematurely because of genetic diseases. Nature itself is indifferent to our dignity, and so altering nature cannot violate our dignity. In fact, it dignifies us to use the talents we have to alter our environment and ourbiology to improve our lives and those of the disabled. Technology allows us to overcome natural shortcomings.Although issues in geneticsarouse wide public interest, the initial concern about geneticengineering did not come from the public but from scientists actually involved in theresearch.The fears that exploiting this interchangeability of genetic material couldcause the uncontrollable spread of serious disease or damagethe environment led some of the first scientists working withgene splicing techniques to raise questions about the unpredictable Consequences of their work.It could be argued that "You can stop splitting the atom; you can stop visiting the moon; youcan stop using aerosols.... But you cannot recall a new form of life." If an organism can find a suitable niche it may survive—and even evolve. [3]While religious leaders present theological bases for their concerns, essentially the same concerns have been raised sometimes in slightly different words—by many thoughtful secular observers of contemporary science and technology. They are: Unintended effects,Morality of genetic manipulation in all its forms, and Social and political consequences of new technologies.These points could be further elaborated e.g., crossing species lines that deserve serious consideration. First, gene splicing affords the possibility of creating hybrids that can reproduce themselves (unlike mules, which are sterile). So the possibility of self-perpetuating "mistakes" adds a new dimension of concern, although here again, the point is not that crossing species lines is inherently wrong, but that it may have undesirable consequences and that these

consequences may multiply beyond human control. Second, there is the issue of whether particular crossings of species—especially the mixing of human and non-human genes might not be illicit. The moral revulsion at the creation of human-animal hybrids may be traced in part to the prohibition against sexual relations between human beings and lower animals. Sexual relations with lower animals are thought to degrade human beings and insult their God-given dignity as the highest of God's creatures. But unease at the prospect of human-animal hybrids goes beyond sexual prohibitions.A philosopher concerned with assessingthe risks of genetic engineering has recently noted, the abilityto manipulate genes, both within and across species lines, maybecome a crucial asset for survival.There may...come a time when, because of natural orman-induced climatic change, the capacity to alterquickly the genetic composition of agricultural plantswill be required to forestall catastrophic famine.[4]However, unacceptable uses of gene splicing.described by the World Council of Churches as a "grave hazard," is "the deliberate production of pathogenic micro-organisms for biological warfare or terrorism."[5]It is also necessary that beyond any fear of the malevolent use of gene splicing, attention must be paid to a more basic question about the distribution of power: who should decide which lines of genetic engineering research ought to be pursued and which applications of the technology ought to be promoted? Genetic engineering research on animals needs to address specific concerns relating to transgenic animals and xenotransplants. Some of the concerns raised by scientists, religious bodies, animal welfare societies, and the public at large,[6,7,8,]are as under:Transgenic animals raise several particular moral issues (quite apart from any damage they might do to the environment) Are animals that combine species an unethical alteration of the natural order of the universe ? Is it unethical to modify an animal's genetic make-up for a specific purpose, without knowing in advance if there will be any side-effects that will cause suffering to the animal ? Does 'creating' animals by genetic engineering amount to treat the animals entirely as commodities ? Is it unethical to create 'diseased' animals that are very likely to suffer ? Suffering may last for a long time in these animals as researchers want to conduct long-term investigations into the development of diseases.

Religious views on transgenic animals

Against transgenic animals

God laid down the structure of creation and any tampering with it is sinful.Manipulating DNA is manipulating 'life itself' - and this is tampering with something that God did not intend humanity to meddle with.

Transgenic animals and religious food laws

Transgenic animals pose problems for religions that restrict the foods that their believers can eat, since they may produce animals that appear to be one species, but contain some elements of a forbidden species.

In favour of transgenic animals

As human beings have been given 'dominion' over the animals, they are entitled to tamper with them.Palaeontology shows that the structure of creation has changed over time as some species became extinct and new ones came into being. They say that this shows that there is nothing fixed about the structure of creation.

Public concerns regarding animal welfare in India

In India, respect for animal welfare is rooted in religious beliefs. Animals and birds are thought not only as *Vahanas* or Vehicles on which God rides, but much moreuseful as well. Over the centuries this has brought about a very healthy respect in the Indian mindfor all forms of life. The cow is sacred not because it is a divine vehicle alone, but because it has anoverall utility value. Buddhism and Jainism carry this attitude further, leading to vegetarianism andrespects for all living beings. To the Sufis, steeped in equally considerate attitudes the prevalentIndian mind set was extremely acceptable. Thus, in the East, regardless of specific sects orreligions, the attitude to other life forms was not exploitative, but appreciative. Even pigs, boars,buffaloes and monkeys are referred in holy books and the Indian mind set can become easilysensitive when it comes to these animals. These religious sentiments could be one major reason whythe animal activism in this country has found firm roots, while in the West it may be because of the writings of some secular philosophers.[7,8,13]

Concerns about safety

A utilitarian justification for producing and using genetically modified animals must take into account potential risks to humans and other animals, as well as to the wider environment. While this is a major concern of regulatory

bodies, these vary in scope and efficacy between countries, and much more research on safety aspects is needed. Several related categories of concern about risks to safety need to be considered [9] :Concern that modified animals might 'escape' and breed with other domestic or wild animals, so transferring the new gene(s) to these other populations.Concern about risks from the use of retroviruses as DNA vectors during production of genetically modified animals: e.g. risks that genes might inadvertently be transferred to other individuals or species, or that retroviruses might infect other organisms.Concern about possible risks to human and animal health from consumption of genetically modified animals and their products.Concern about risks that drug resistance gene markers used in some genetic engineering procedures might inadvertently be transferred and expressed.Ecological concerns, e.g. about the wider effects of producing disease-resistant animals.In xenotransplantation, concern about risks that human recipients of animal organs might become infected with animal viral diseases, which might then infect the wider population.These ethical issues relating to safety, animal welfare, religious views, country specific concerns, etc. are discussed by the national and international bodies such as ICMR,[7] UNESCO[10], WHO[11,12], and recommendations framed accordingly, for the law maker's considerations at appropriate forums in the respective countries.

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CANCERS- A ZOONOTIC PERSPECTIVE

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The World Health Organization (WHO) defines zoonotic diseases as "...disease(s) or infection(s) that (are) naturally transmissible from vertebrate animals to humans." In a study of 1415 pathogens known to affect humans, 61% have been reported to be zoonotic diseases Typically, the zoonotic diseases include diseases caused by bacteria, viruses, parasites, fungii, pria; but in recent times, cancers/ neoplastic diseases are also being encompassed within the domain of zoonotic diseases.

Although, etiological agents for various neoplastic diseases include: 1) Physical agents, 2) Chemical agents and 3) Biological agents, current estimates report that 21% of all human cancers are linked to infections, and some experts believe this figure is even too low. Available data support the concept that primary element in human cancer might be a virus and that all other agents might play only a secondary role.

Cancer is a leading cause of death in companion animals. An estimated 1,100 out of every 100,000 dogs and slightly fewer cats develop cancer each year, roughly the same rate as humans.

In Veterinary Medicine, use of conventional turkey herpes virus (HVT) vaccine, a kind of anti-cancer vaccine, had been ever used, since late 20th Century, for providing protection against the Mareks' disease in chickens. However, in Human Medicine, hepatitis B virus and high-risk human papillomavirus vaccines, which have offered the first-ever means for preventing specific widespread cancers by vaccination has been a recent endeavour.

Hence, now the attention is focused in search for newer virus-etiology in rest of the human cancers, because the viral etiology is already implicated in 64% of the known infection-related cancer burden, with bacteria and parasites accounting for the remainder. For example, men with prostate cancers (40%) had a virus similar to murine leukemia virus (MLV).

Dr. Harald zur Hausen, winner of the 2008 Nobel Prize in Medicine for his work on human papillomaviruses as the major cause of cervical cancer, sees a number of other malignancies as being prime candidates for potential linkage to infections, including childhood lymphoblastic leukemias, basal cell carcinomas, Epstein-Barr virus–negative Hodgkin's lymphomas, colorectal and breast cancers, and lung cancers in nonsmokers. Torque teno virus (TTV) family, an extremely heterogeneous family of single-strand DNA viruses, shows particular promise in this regard.

The TTVs, first described by Japanese investigators in 1997 (Rev. Med. Virol. 2007;17:45-57), are an extremely heterogeneous family of single-strand DNA viruses. There are well over 100 genotypes. TTVs are widespread in all human populations, and have been found in umbilical cord blood and are vertically transmitted from mother to child, even prenatally. They frequently rearrange their genomes; and transmissibility and replication have been demonstrated to occur even for small portions of the TTV genome.

In fact, even if a small portion of these speculations turn out to be true, the implications for cancer prevention could be huge. It is estimated that if both the hepatitis B vaccine and the HPV vaccine were to be applied globally, overall cancer burden in women could be reduced by 12%-14% and in men by 4%- 5%.

Further, multiple lines of circumstantial evidence also indicated that plethora of transmissible oncogenic agents *viz.*, bovine leukemia virus (BLV), papilloma virus, and polyoma virus in cattle; mouse mammary tumor virus (MMTV) and feline leukemia virus (FeLV) in cats; Jaagsiekte sheep retrovirus (JSRV) in sheep (Buehring *et al.*, 2003; Wootten *et al.*, 2005), pig endogenous retrovirus in pigs (PERV) (Heneine *et al.*, 2001); papilloma virus, polyoma virus and simian sarcoma virus (SIS) in monkeys and; avian leukosis/ sarcoma virus (AL/SV) in chickens

(Fadly and Payne, 2003) might play possible important roles in some human cancers. Role of animals in transmitting infections of oncogenic importance could be summarized, as under :

Animal food as possible source of various human cancers

Animal food can be possible source of various human cancers (Willett, 2005; Chao *et al.*, 2005), because it acts as a carrier for various biological agents transmitted from food animals to humans. Cattle, sheep, pigs and poultry provide main source of food for vast majority of mankind. It is interesting that the possible link between occurrence of cancers in general population and transmissible agents originating from animal food would be consistent with the report that dietary sources are the single most important cause of cancer occurrence in humans, possibly accounting for as many as 35 % of all cancers in the US (Doll & Peto, 1981).

Dr. zur Hausen even hypothesized that some as-yet unidentified bovine virus, which is non- oncogenic in its normal host, can become carcinogenic when transmitted to humans. For example, basal cell carcinomas are known to have a predisposition to arise in smallpox vaccination scars. Smallpox vaccines were prepared by inoculating vaccinia virus into the skin of calves and then harvesting the crusted skin, which could in theory contain contaminating bovine viruses. Also, colorectal cancer, and to a lesser extent breast cancer and lung cancer in nonsmokers, have repeatedly been associated with beef consumption in epidemiologic studies.

The observed link between a red meat–rich diet and increased rates of colorectal and other cancers is often attributed to the formation of aromatic hydrocarbons and other known carcinogens during cooking or meat curing. But, Dr. zur Hausen believes this interpretation might be inadequate. Temperature achieved in the center of a piece of beef is only 40 - 50° C, yet TTVs, papillomaviruses, and polyomaviruses are able to survive in a protein environment at temperatures of 80° C for 30 minutes or more. Thus, confirmatory data are yet to be established, yet for now, it can be speculated that beef consumption may play a significant role in the development of colorectal cancer.

Similar reports also exist for AL/SV entities found in chickens as well. AL/SV exposure is widespread and virtually universal and occurs on consistent basis in all human populations that consume chickens, eggs and their products. The viruses have been shown to be present in commercial eggs in supermarkets at a prevalence rate of atleast 14 % (Pham *et al.*, 1999), and also in apparently healthy chickens and turkeys and their products destined for human consumption. In one study (Spencer *et al.*, 1977), infectious AL/SV were isolated from 45 % commercial eggs that had been previously stored at 8 ^{0}C for 0-6 days, and from 21 % of eggs stored for 7- 34 days.

Contaminated live virus vaccines of humans and animals as possible source of various human cancers

Human vaccines, derived from chicken embryo cultures, are plagued with some very serious viral contamination problems. Evidences clearly suggest presence of particularly avian leukosis virus (ALV) genomes, reverse transcriptase activity, endogenous retroviruses (ev and EAV elements) as adventitious agents in human vaccines that are prepared in embryonated eggs or in cultures of chicken embryo fibroblasts (CEF), *viz.,* influenza, yellow fever and measles, mumps and rubella (MMR) vaccines (McRearden, 2003). The fibroblasts contain and

express endogenous retroviral genomes. In any vaccine, adventitious agents in the cellular substrate may contaminate the biological product. In live, attenuated vaccines, such contaminants are not inactivated, and endogenous retroviruses by their very nature as Mendelian transmitted genomes are particularly difficult to eliminate. Endogenous retrovirus release also has ramifications for pharmaceutical proteins made in cell substrates (e.g., monoclonal antibodies) and for xenotransplantation.

First evidence of contamination of vaccines for human use with AL/SV or its segments came to light in 1960s, when yellow fever vaccine was found to contain ALV (Harris *et al.*, 1966). Since then, it is common knowledge in vaccine industry that these viruses (or its components) still exist in human and animal vaccines (Payne *et al.*, 1966). Virtually, all yellow fever vaccines used during World War II were also contaminated with AL/SV (Waters *et al.,* 1972). In fact, Field's Virology text (2001 Edn.) states, "at the present time, vaccines produced by some of the world's 12 manufacturing institutes are contaminated with ALVs" (Knipe *et al.*, 2001). A study by the US Centers of Disease Control (US CDS) report that virtually all stocks of measles and mumps vaccine currently in use in United States are contaminated with endogenous form of AL/SV (Tsang *et al.*, 1999).

However, reports are contradictory concerning effects of AL/SV or its components on humans in terms of transmission, infection and possible subsequent disease. A study of the US CDC reported no avian viral presence in frozen serum samples from children that had received MMR vaccinations (Hussain *et al.*, 2001); however subsequent reports on exposed poultry workers (Johnson and Griswold, 1996) and workers with no occupational exposure to these viruses (Johnson *et al.*, 1995; Choudat *et al.*, 1996) were reported to have antibodies in their sera specifically directed against AL/SV. Reports also indicated that given the known behavior of these viruses in mammalian cell culture, a blood serum test would not always provide correct evidence of viral presence in human body. In other words, viruses (or viral antibodies) need not be actively present in blood stream at the time of blood-drawl? It may also be possible that the viral particles may have retreated into other tissues?

Exposure of recipients of contaminated vaccines has been associated with effects ranging from benign to demonstrable transmission of infection with or without subsequent diseases. Considering that ALV can easily capture human erbB oncogene; (erbB and myc oncogenes are strongly associated with common form of human breast cancers), it seems that the issue of ALV vaccine contamination would deserve a high level of attention. As is common with other viruses, strains of ALV will show particular affinities for certain type of tissues or growth conditions (Arshad *et al.,* 1997). Further investigations are required whether the virus has been integrated into the human genome to assess public health significance of these results (Johnson *et al.*, 1995). Thus, an accurate assessment of viral presence or long term effects from numerous ALV associated "offspring" virus have yet to be established.

In addition, AL/SV is also reported to be potential contaminant of live virus vaccines of poultry. Samples of commercially available Marek's disease (MD) vaccines were found to be positive for both endogenous subgroup E ALV and an exogenous subgroup A ALV, which can cause neoplastic disease and other production problems in susceptible chickens (Silva *et al.,* 2007).

Emergence of new viruses from "parent" Retroviruses as possible threat for various human and animal cancers

Retroviruses can evolve unexpectedly and pose serious new threats for the human health and animal health including poultry. For instance, AL/SV can be considered a "parent" virus, as it easily transforms into a dizzying array of related viruses by transducing one of numerous cancer- related gene segments from its host (including human), and inserting it into its own genome (Felder *et al.*, 1994; Johnson, 1994). It has additional capability of inserting itself into the host (including human) genome as latent virus, and causing cancerous cell transformation from that location. Given right growth conditions, ALV can easily transform into closely related viruses, thus, continuing viral mutations will result in appearance of AL/SV with new disease producing properties.

Viruses that originate from the "parent" ALV include potent Rous sarcoma virus (RSV), Rous-associated viruses (RAV), avian myeloblastosis virus (AMV), avian myelocytomatosis virus (AMC), avian erythroblastosis virus (AEV), Fujinami sarcoma virus (FSV) *etc.*, (Nevins, 2001). Serial passaging of a retrovirus (that does not carry an oncogene) on such cultures may lead, with a high frequency, to the emergence of new viruses that have transduced oncogenes. For example, ALV- J strain is thought to be a new strain of virus due to genetic recombinations, possibly among exogenous AL/SV with that of endogenous viruses or even non-retroviral host genes, such as cellular oncogenes.

Besides, mutations also occur most frequently in *env* gene resulting in change in antigenicity and host range manifesting unusual tumors in field. For example, ALV- J strain is a new variant strain of virus causing an unusual tumor, the myeloid leukosis. ALV-J *env* gene might have arisen by multiple recombination event between one or major EAV family of endogenous virus and exogenous virus. A widespread variant virus of undefined subgroup found in Israel had tropism for endothelial cells and it caused hemangiosarcomas (Burstein *et al.*, 1984).

Role of Pet Animals

Cats are also reported to be transmitting certain important cancer biological entities. A subset of cats infected with a close homologue of MMTV from mice, may transmit the virus to humans, possibly after selection for variants with an expanded host range. Detection of MMTV-related sequences have been evidenced in DNA isolated from human breast cancers (BC), however, the reports are conflicting, as others failed to detect the se-

quences. Moreover, *Betaretroviruses* are present in much wider range of species than previously known, including rodents, felines, and primates.

FeLV causes leukemia, lymphoma, anemia and immunosuppression in cats, and most cats die due to immunosuppression. According to the Vet Record in 1989, in the UK, about 18% of sick cats and 5% of healthy cats are FeLV positive. The FeLV can infect and multiply in cells from other species in laboratory conditions, and FeLV-B and FeLV-C can be grown in human bone marrow cells in the laboratory. Further, there have been reported clusters of cancers or leukemia in human patients and simultaneously in their pets. Although there is no evidence that FeLV can infect or cause disease in humans, from a public health perspective it is often recommended that immunosuppressed people and possibly also children should have limited contact with infected cats.

Cat-human link also exists for *Helicobacter pylori,* the bacteria for which the 2005 Nobel Prize in Medicine was awarded. Reports indicated that *H. pylori* was *first* transmitted to humans via sheep's milk. Roughly half of the world's population may now be infected with *H. pylori*, responsible for the peptic ulcer and stomach cancer-associated infection, perhaps the most common chronic infection afflicting humanity (Suerbaum and Michetti, 2002).

Dogs also appear to share a possible link (?). Each year, about 6 million of 65 million pet dogs in the USA will be diagnosed with spontaneous, naturally occurring malignancies that share many features with human cancers such as osteosarcomas, prostate and breast cancers, non-Hodgkin's lymphoma, melanoma, soft tissue sarcoma, head and neck carcinoma and virally induced lymphomas. Currently, dog cancers are taken as model for various human cancers. However, 2 ancient transmissible cancers in dogs, *viz.*, canine transmissible venereal tumor (CTVT), and Tasmanian devil facial tumor (TDFT) have transmissible cancer cells, which have transplanted between dogs, a mechanism responsible for global spread of these 2 canine cancers. Alarmingly, TDFT is feared now as the cause even for the extinction of Tasmanian devils. Importantly, newer evidences are mounting that cancer cells can evolve to become infectious agents and be transmitted between individuals.

Role of Wildlife (?)

Eventually, cancers are killing wildlife at higher rates, as that in humans. Wildlife Conservation Society's (WCS) Global Health program in its Press-release, identified California sea lions, dolphins, porpoises, and green sea turtles as creatures with cancer rates that were higher than expected. Most of the described cancers likely have a viral source, but it is possible that human-caused pollution—including loose, carcinogenic chemical waste—could exacerbate the maladies.

Apparently, frustrated, they reported that there appeared no scientific basis for the Darwinian philosophy that living forms find a way to adapt and improve, ever-increasing their repertoires of functional biological mechanisms. Rather, in the bigger picture, fossils show clearly that nature has "deselected" the vast majority of life forms in the past through widespread catastrophes. And now nature continues to "deselect" more through disease. Rather, extinctions are the rule. In almost every animal and plant phylum, there has been a decline in variety, with most varieties having been catastrophically buried and fossilized. The grim reality is that over the long haul, if a created kind can avoid natural disasters, it will eventually succumb to cancer from mutations.

Factors Contributing to the Emergence of Zoonotic Cancers

Many elements can contribute to the emergence of a new zoonotic disease: microbial/ virologic determinants, such as mutation, natural selection, and evolutionary progression; individual host determinants, such as acquired immunity and physiologic factors; host population determinants, such as host behavioral characteristics and societal, transport, commercial, and iatrogenic factors; and environmental determinants, such as ecologic and climatologic influences. Emergence of new zoonotic pathogens seems to be accelerating for several reasons: global human and livestock animal populations have continued to grow, bringing increasingly larger numbers of people and animals into close contact; transportation has advanced, making it possible to circumnavigate the globe in less than the incubation period of most infectious agents; ecologic and environmental changes brought about by human activity are massive; and bioterroristic activities, supported by rogue governments as well as organized amateurs, are increasing, and in most instances the infectious agents of choice seem to be zoonotic.

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Oral Presentation

In vitro LYTIC ACTIVITY OF COLOSTRUM AGAINST *Staphylococcus aureus* AND *E. coli*

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Colostrum is a rich source of immune factors that provides passive immunity to newborn. The initial secretion present in the mammary gland at or near parturition is termed colostrum and unlike milk it is rich in immunoglobulin, growth factors and tissue repair factors. In addition to immunoglobulins colostrum contain viable cells including macrophages and neutrophills, which secrete a range of immune related components. A total of 25 colostrum samples were collected from different dairy farms and *in vitro* lytic activity of colostrum samples were studied to augment best calf management. *In vitro* lytic activity of bovine colostrum was assessed against fresh cultures of pathogenic *Staphylococcus aureus* and *E. coli* bacteria by Modified Pour Plate method on 20 whole colostrum samples. Results indicated that visible growth of *Staphylococcus aureus* was observed on each plate of colostrum agar out of which 10% with poor growth, 50% with moderate growth and 40% with rich growth. In case of *E. coli,* inhibition of growth in presence of colostrum was more pronounced as indicated by complete absence of growth in 30% plates, 30% plates showing poor growth, moderate growth in 40% plates and none were showing rich growth on colostrum agar. Bovine colostrum has a potent antimicrobial action as evidenced by its capability to partially or completely inhibit the growth of *Staphylococcus aureus* and /or *E coli in vitro.*

IgG CONCENTRATION (mg/ml) IN WHOLE AND FAT FREE COLOSTRUM OF DAY 1 BY SRID

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Colostrum provides passive immunity to newborn as it is a rich source of immunoglobulins, viable cells including macrophages and neutrophills, which secrete a range of immune related components into the colostrums and tissue repair factors. In bovine, colostrum is not only major source to provide effective passive immunity but also give adequate time to prime and boost active immune response. An estimation of colostral immunoglobulin (Ig) is required to establish the quantity and quality of colostrum prior to feeding, and to avoid an incipient failure in passive transfer of immunity from inferior colostrum. Secondly, pooled colostrum may be extended over a greater number of neonates by adjusting the amount fed to a minimum volume that delivers a fixed mass of Ig to the neonate. The immunoglobulins have been subdivided into IgG, IgM, IgA, IgD and IgE according to their structure. The predominant immunoglobulin in the colostrum of most of the major domestic animals is IgG, which may account for 65% to 90% of its total antibody content; IgA and the other immunoglobulins are usually minor but significant components. A total of 25 colostrum samples were collected from different dairy farms. Immunoglobulin (IgG) concentration (mg/ml) in Whole and Fat Free Colostrum of Day 1 by Single Radial Immunodiffusion (SRID) were studied to augment best calf management. Mean specific gravity of excellent, moderate and poor categories colostrum were 1.048, 1.041 and 1.031 and their corresponding mean globulin concentration were 54.18 mg/ml, 38.86 mg/ml and 13.38 mg/ml respectively. In all the three categories SRID was performed on whole colostrum which revealed mean IgG concentration of 38.61 mg/ml, 23.40 mg/ml and 8.00 mg/ml in excellent, moderate and poor quality colostrum, respectively. Slightly higher values were observed in SRID performed on fat free colostrum. In view of this it is concluded that possible variation in determination of globulin concentration between the technique of SRID and the specific gravity can be corrected by optimizing salt concentration extent of cellular components, bacterial load *etc.* in the colostrum at day 1.

IDENTIFICATION OF PUTATIVE DIFFERENTIAL METHYLATED REGION (DMR) WITHIN XIST GENE IN GOAT (*Capra hircus*)

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XIST (X chromosome inactive specific transcript) gene which codes an untranslated RNA and central to X-chromosome inactivation (XCI) and thus plays a pivotal role in dosage compensation during embryonic development. Aberrant patterns of X chromosome inactivation may affect the SCNT (Somatic Cell Nuclear Transfer) efficiency and development patterns of SCNT derived clones. Over recent years, studies of X inactivation provided crucial insights into fundamental epigenetic mechanisms of gene silencing and regulation patterns of gene expression through development. DNA cytosine methylation is an important epigenetic modification associated with gene silencing, developmental processes such as XCI and genomic imprinting. The incomplete reestablishment of DNA methylation at CpG island (Differential methylated region, usually present at promoter region of genes) after nuclear transfer is one of the possible reasons of the low success rate of cloning. In the present investigation, we studied methylation profile of all CpG sites within DMR of XIST gene in goat. Methylation status of all the CpG sites was analysed using "Bisulfite sequencing PCR" method. After bisulfite conversion of extracted DNA, all the unmethylated cytosine of CpG dinucleotide converted to uracil (U) and all the methylated cytosines (C) within CpG sites remain unchanged. The uracil nucleotides get replaced with thymine in subsequent amplifications. We designed normal as well as bisulfite sequencing primers (BSP) using flanking sequences of XIST DMR (exon 1) of bovine. Bisulfite primers don't contain any CG dinucleotide and all 'C' nucleotides being replaced with 'T'. We conducted our study in fibroblast and cumulus cells for being more preferred donor cells for SCNT. We prepared fibroblast cell lines from ear pieces of female goat and cumulus cell lines from ovaries. Genomic DNA was isolated using standard protocol and the desired region was amplified, cloned and the PCR product was sequenced. A sequence of 221 bp, predicted to represent DMR of XIST in goat was obtained and was found to contain 10 CpG sites. Again, the extracted DNA was subjected to bisulfite conversion using EpiTect bisulfite kit (Qaigen). With the help of BSP primers, the converted DNA was amplified and sequenced and was compared with previous one. It was found that all the CpG sites are methylated. The methylation study of CpG sites at putative DMR, also studied in reprogrammed fibroblast as well as cumulus cells. The traditional "serum starvation method of cell synchronisation" was used in reprogramming. The methylation profile of all these CpG sites remained same (*i.e.* exhibit complete methylation at all CpG sites). Thus, from the above study it was found that the methylation status of this region is well maintained.

RECOVERY OF *Escherichia coli* SEROTYPES FROM DIFFERENT PATHOLOGICAL CONDITIONS OF POULTRY

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The present study was undertaken with a view to isolate *Escherichia coli* (*E.coli*) from different pathological conditions (colisepticaemia, yolk sac infection and egg peritonitis) of poultry from 69 poultry farm (broiler and layer) in and around Anand, Gujarat State. A total of 597 samples from different sources of 253 necropsied birds with above stated conditions were collected and recovered 506 (84.76 per cent) *E.coli* isolates. On serotyping of selected and tested 150 *E. coli*, (serotyping was done by National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, H.P.) 10 (6.67 per cent) isolates were found untypable, 15 (10.00 per cent) were found rough and the rest typable 125 (83.33 per cent) isolates were distributed into as many as 36 different O serotypes viz., O2 (sixteen), O8 (fourteen), O151(nine), O78 and O109 (eight isolates each), O1 and O171 (seven isolates each), O101 (five), O26 (four), O45, O107, O111 and O119 (three isolates each), O9, O20, O23, O38, O40, O50, O58, O83, O91, O131, O135 and O157(two isolates each) and O55, O61, O68, O81, O100, O106, O120, O144, O145, O156 and O161 (one isolate each) while serotypes O151 and O171 were found in all three pathological conditions.

IN SILICO IDENTIFICATION, MOLECULAR CHARACTERIZATION AND EXPRESSION ANALYSIS OF DUCK (*Anas platyrhynchos*) TOLL-LIKE RECEPTORS GENE FAMILY

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Innate immunity, once considered as non-specific immune response of meager role is now considered as a fundamental orchester of overall immune response. Toll-like receptors (TLRs), an important member of innate immune system in vertebrates plays a pivotal role in sensing invading pathogens by virtue of conserved microbial patterns. TLR repertoire of chicken has been well characterized. However TLR family of other avian species is yet to be characterized. The present study identified TLR gene family in duck genome draft by bioinformatic analysis and also quantified the mRNA expression profile of TLRs in a range of duckling's tissues by quantitative real time PCR. We annotated 10 (*TLR1LA*, *1LB, 2A, 2B, 3, 4, 5, 7, 15* and *21*) TLR genes orthologous to chicken TLR gene repertoire from latest assembly of duck genome available at Pre ensemble genome browser using TBLASTN and BLASTP analysis. Analysis of duck TLR sequences revealed an overall similarity of 77-84% with chicken orthologs at amino acid level. Phylogenetic analysis of duck and chicken TLR sequences was done with MEGA program and it revealed that duck sequences clustered with their respective avian orthologs. Similar to that of chicken genome, in duck also *TLR7, 8* and *9* genes were deleted and *TLR1* and *2* genes were duplicated. TLR15, avian specific receptor with no vertebrate ortholog was also found in duck genome and TLR15 of avian species form a distinct clade hitherto close to TLR1 family. Sequence analysis of TLR21 of duck revealed that it might also recognize unmethylated CpG motifs similar to chicken TLR21. TLR genes of avian species are under purifying selection except for the duplicated *TLR2* genes, which are under positive selection. Expression profiling of TLRs in various tissues of day-old ducklings was investigated with the gene specific primers designed from annotated duck TLR sequences. All ten *TLR*s mRNA expressions were significantly higher in bursa than other tissues studied, whereas in muscle overall *TLR*s mRNA expressions were significantly ($P<0.01$) lower except for *TLR15*. *TLR7* gene expression was significantly ($P<0.01$) higher in spleen, bursa and also in lung. In spleen, *TLR5* transcript was least expressed, whereas in bursa *TLR3* was least expressed among all other TLRs investigated.

DETECTION OF SUB-CLINICAL MASTITIS IN CROSSBRED COWS AND ANTIBIO-GRAM OF RECOVERED BACTERIAL ISOLATES

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A total of 500 milk samples comprised of one each from a quarter of 125 milking crossbred cows from Pathri Village of Gandevi taluka, dist. Navsari (Gujarat State) were screened by Modified California Mastitis Test (MCMT), pH of the milk and electrical conductivity test for detection of Sub-Clinical Mastitis (SCM). Samples declared positive (n=41) by these tests were further processed for bacterial isolation through standard culture techniques. Among 37 bacterial isolates recovered, 24 were of *Staphylococcus spp.*, 11 of gram negative rods and two of gram positive bacilli. Antibiogram revealed the highest number of isolates were sensitive to gatifloxacin (81.08%) and enrofloxacin (81.08%) followed by azithromycin (70.27%), kanamycin (70.27%) and erythromycin (59.46%), where as cefixime (16.22%) and oxytetracycline (16.22%) were found to be least effective.

EVALUATION OF Th-2 CELL, CD4 AND CD8 CELLS RESPONSE IN CALVES TO RECOMBINANT- BCG AND CONVENTIONAL BCG VACCINES BY INTERLEUKIN-4 CAPTURE ELISA AND FLOWCYTOMETRY

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In most of developing country, humans and animals share the same microenvironment, especially during droughts and the dry season, thereby potentially promoting the transmission of *M. bovis* from animals to humans. *Mycobacterium tuberculosis and Mycobacterium bovis,* a infectious agent prevalent since ancient times cause a bacterial disease Tuberculosis, one of the most widespread infectious diseases and is the leading cause of death among adults in the world. BCG vaccination in cattle although it appeared to be ineffective in field trials as a vaccine against natural infection but induced some reduction in the severity of disease against experimental challenge. Cell-mediated immunity is essential for the control of mycobacterial infections. BCG vaccines induce strong CD4 and CD8 mediated immune responses in mice, but the two compartments may differ in protection conferred by the vaccine against TB. The comparison of recombinant-BCG and conventional BCG vaccine evaluated in calves by conducting experiment in three groups of 8 calves each, aged approximately 4-6 months were selected from herds with known negative tuberculosis status. They were injected subcutaneously with 10^6 CFU of recombinant-BCG vaccine bacilli expressing Rv3881c protein. A control group received PBS via the same route and third group received 10^6 CFU of BCG bacilli used as vaccine. Primary and Booster vaccinations were administered 21 days apart to 8 calves each, while another 8 were left unvaccinated as Control animals. Humoral (Th-2) immune response was evaluated by Cytokine IL-4 capture ELISA. The immune status scores of individual animals were generally lower in all groups than what was expected. The statistical differences between groups were non-significant and it can be concluded that under the prevailing conditions the recombinant-DNA vaccine was unable to show protective immune TH-2 responses in calves. In the Flowcytometry assay, the difference in the population of CD4 cells produced in rBCG vaccinated calves and BCG injected calves was significant on 30^{th} day ($P < 0.05$), increased to maximum and maintained at high level on 120^{th} day ($P<0.01$) whereas, the difference in population of CD8 cells produced in Control and rBCG vaccinated calves was also significant on day 60, 90, and 120 ($P>0.05$). Under the prevailing conditions the rBCG vaccine were able to show protective responses in calves against the B*ovine* tuberculosis then conventional BCG vaccine.

DETECTION OF *Mycoplasma agalactiae* BY PCR IN EAR SWABS COLLECTED FROM APPARENTLY HEALTHY AND SICK GOATS

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Sixty seven ear swabs were collected from the external auditory canal of the healthy as well as sick goats. The swabs were directly collected in the broth medium and transported on ice. After subsequent transfer to the fresh medium, the samples were incubated for five days. The growth in the broth medium was inoculated on solid media and also subjected to DNA extraction and PCR. PCR was performed using 16S rRNA based *Mycoplasma* genus and *Mycoplasma agalactiae* species-specific primers viz. GPO/MGSO and MagF/MagR. After PCR, specific amplification products of 715bp and 360bp were obtained with primers GPO/MGSO and MagF/MagR, respectively in only three samples. Among these three samples, growth on solid media was obtained in case of only one sample. Therefore, this study shows the importance of PCR in comparison to cumbersome cultural isolation of Mycoplasma in screening the flock for the potential carriers of infection.

MOLECULAR GROUPING OF *Listeria monocytogenes* BY CLONING AND SEQUENCING OF INLJ GENE

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The present study was undertaken to characterize all twenty one *L. monocytogenes* field isolates obtained from different animal species which were maintained at the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand. The PCR amplification carried out for the detection of virulence associated genes *inlJ* (lmo2821) and *inlC* gave a positive result. Among these, one from pathogenic *L. monocytogenes* (LM14) and one from nonpathogenic *L. monocytogenes* (LM08) were processed for cloning of *inlJ* gene using pDrive vector and competent *E. coli* (DH5-α strain) cells. Clones showing the amplification of 750 bp DNA fragment were considered as positive clones carrying desired insert of inlJ gene. Screened products of colony PCR were used for plasmid extraction. The concentration of the plasmid was determined and was subjected to automated DNA sequencing on ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA) using BigDye® Terminator v3.1 Cycle sequencing kit. Sequences with good quality value were selected for further analysis. Both the sequences of good integrity were subjected to *in silico* processing by three ways viz. similarity search using MEGA BLAST at NCBI nucleotide database, ClustalW and Phylogenetic analysis.

IDENTIFICATION AND METHYLATION ANALYSIS OF CpG MOTIFS OF H19 GENE BY PCR BASED METHODS IN GOAT (*Capra hircus*)

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The procedure of somatic cell nuclear transfer (SCNT) is likely to disrupt the epigenetic control of growth related genes especially imprinting genes. Such disruptions can have developmental consequences involving growth disorders such as Beckwith–Wiedemann syndrome (BWS), Wilms tumor and Silver-Russel Syndrome (SRS). The H19 gene has previously been shown to be imprinted and a major cause of these disorders. Since CpG methylation has been implicated in imprinting, the study was conducted with an aim to investigate the dynamics of methylation pattern of CpG motifs of H19 CTCF III binding region before and after reprogramming in cultured somatic cells of goat. Polymerase chain reaction (PCR) was used to amplify the 295 bp fragment of H19 CTCF III binding region from the genomic DNA of cultured cells. The identified nucleotide sequence had 19 CpG motifs and showed 89.6% homology with sheep. Cultured cells before and after reprogramming by serum starvation, were used to isolate genomic DNA samples. Bisulphite conversion of genomic DNA was performed to identify the methylation status of CpG motifs. The amplified products of bisulphite treated and untreated genomic DNA samples revealed different SSCP patterns. DNA sequencing revealed subtle changes in methylation pattern of CpG motifs between reprogrammed and non-reprogrammed cells. Interestingly, reduced level of methylation of CpG motifs was found in reprogrammed cells in comparison to non-reprogrammed cells. The nucleotide sequence of bisulphite converted cultured non-reprogrammed cells revealed methylation of 6 CpG motifs. However, in reprogrammed cells all the CpG motifs were found unmethylated. Finally, it is concluded that serum starvation had an effect on reducing the level of methylation in cultured cells.

IMPLICATIONS OF PROBIOTIC SUPPLEMENTATION ON PLASMA PARAMETERS AND ON PRODUCTIVE PERFORMANCE OF ANESTROUS CROSSBRED COWS

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The study was undertaken to provide information regarding the relationship between circulatory IGF-I, lactoferrin, Haptoglobin (Hp) , Non esterified fatty acids (NEFA) factors and postpartum ovarian activity or conception rate in crossbred Karan Fries (KF) dairy cows with and without ovarian activity postpartum. The cows were classified as anestrous when the ovarian activity was not resumed even after 90d postpartum and confirmed by weekly rectal palpation of the cows. The anestrous cows were supplemented with yeast probiotic *Saccharomyces cerevisiae* (S C, Diamond XP) @ 12g/animal along with concentrate which was a normal constituent of the diet. Blood samples were collected weekly from 4 wk post partum until conception in all animals. The study was carried out for 27 weeks postpartum in supplemented and non-supplemented (NS) anestrous group of animals. Both negative and positive control (NS) groups were maintained. Dry matter intake, monthly body weight and average daily milk yield was also recorded and analyzed. It was observed that anestrous group of animals exhibited greater concentration of plasma lactoferrin ($P<0.01$) and plasma Hp ($P<0.001$) and decrease in conc. of plasma IGF-I ($P<0.01$) with respect to control group. On systematic supplementation of probiotic, the supplemented anestrous group of animals exhibited heat earlier than the non supplemented group and also further were confirmed to be pregnant post AI. A significant difference for DMI between NS and control group($P<0.01$)was observed, but the DMI of supplemented group was significantly more ($P<0.01$) than NS group of animals, exhibited higher plasma IGF I concentration postpartum and also became pregnant to maximum three numbers of services post service. Hp and lactoferrin level were within physiological range (<300 ng/ml) highlighting the important role of IGF I, lactoferrin and Hp as important markers. The average daily milk yield declined by 12 weeks post partum but in the supplemented group, the decline in the milk yield was restricted. No significant difference between body weights and plasma NEFA concentration of NS and supplemented group was observed. Hence it can be stated that S C supplementation proved to be beneficial in improving the productive performance of anestrous KF cows.

DETECTION OF BLUETONGUE VIRUS ANTIGEN FROM LIVESTOCK OF GUJARAT STATE

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Bluetongue (BT) is an infectious, non-contagious disease of domestic and wild ruminants. Bluetongue virus (BTV) causes severe disease in sheep, which is transmitted by insect vector belonging to *Culicoides* spp. It is particularly a viral disease of sheep, occasionally affecting cattle, buffaloes, goats, camels and other wild ruminants. Out of 377 (364-blood, 5-spleen and 8-pooled *Culicoides*) samples 110 (29.18%) and 28 (7.42%) were found positive for BTV antigen by s-ELISA and BT-AGID respectively. Specieswise incidence by s-ELISA recorded was 48.20 per cent in sheep, 57.14 per cent in goats and 2.60 per cent in cattle. However, none of the blood sample found positive from buffalo and camel. Specieswise incidence by BT-AGID recorded was 12.23 per cent in sheep and 15.71 per cent in goats however, none of the blood sample found positive for BTV antigen from cattle, buffalo and camel.

SEROEPIDEMIOLOGY OF *Peste des petits ruminants* IN ORGANIZED LIVESTOCK FARMS OF GUJARAT STATE

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Peste des petits ruminants is an acute, contagious, febrile viral disease of small ruminants especially goats. It is an economically important disease first described in the West Africa in the 1940 (Gargadennec and Lalanne, 1942). In India, first occurrence of PPR was noticed from the Arasur village of Villupurum district in Tamilnadu (Shaila *et al.,* 1989).The present study envisaged appraisal of seroepidemiology of PPR in diverse species of animals by detection of PPRV antibodies in organized livestock farms of Gujarat state. A serological survey of PPRV antibodies was carried out by c-ELISA in different species of Livestock in North Gujarat, Kachchh, Central Gujarat Saurashtra and South Gujarat regions. Out of 756 serum samples, PPRV antibodies could be detected in 218 samples (28.83 %). Specieswise seroprevalence recorded was 41.12 per cent in sheep, 26.95 per cent in goats, 11.25 per cent in cattle, 14.78 per cent in buffaloes and 12.30 per cent in camels. In sheep, regionwise seroprevalence recorded was 56.07 per cent in Kachchh followed by 37.50 per cent in Saurashtra and 33.52 per cent in North Gujarat regions. Districtwise highest seroprevalence was recorded from Kachchh (56.07 %) followed by Rajkot (37.5 %), Patan (35.71 %) and Banaskantha (32.07 %). The rate of seroprevalence was highest in Chokhla breed (64.51 %), followed by Crossbred (Patanwadi x Rambouillet) (54.00 %) ,Marwari (40.74 %), Magara (36.36 %) and Patanwadi (29.33%). Agewise maximum seroprevalence recorded was 53.42 per cent in the age groups of > 3 years of age followed by 36.42% in age group of 2 to 3 years, 27.50 % in 1 to 2 years and 20.68 % in the 1 year of age groups. In goats, districtwise maximum seroprevalence was recorded from Kachchh district (76.47 %). Maximum seroprevalence of 39.13 per cent was found in the age group of > 3 years, followed by 2 to 3 years of age (19.14 %), 1 to 2 years of age (06.66 %) and 10.00 per cent in the 1 year of age groups. In large ruminants out of 260 sera screened from cattle, buffaloes and camels, 34 were found positive yielding over all seroprevalence of 13.07 per cent.

VIRULENCE ASSOCIATED AND TOXIGENIC STUDY OF *Pasteurella multocida* ISOLATE OBTAINED FROM RESPIRATORY TRACT OF APPARENTLY HEALTHY AS WELL AS SICK GOATS BY PCR

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In the present study, single isolate of *P. multocida* was obtained from nasal swab samples collected from respiratory tract of goats. Aseptically collected nasal swab samples were inoculated on suitable artificial media and the growth was observed after incubation at 37°C for 24 hours. The organisms were identified on the basis of colony character, staining, microscopic examination and biochemical tests. Primers used for the virulence associated and toxigenic genes of *P. multocida* isolate were subjected to the detection of *Cap*-D- 657bp (codes for Capsular material-N-acetylheparosan), *Tox*-A- 864bp (Dermonecrotizing toxin), *omp*H- 1000bp (Major outer membrane immunogenic protein), *ptf*A- 435bp (Type-4 fimbriae), *plp*E- 1010bp (*Pasteurella* lipoprotein-E) and *nan*B- 585bp (Enzyme neuraminidase) sizes. The single isolate of *P. multocida* shows the presence of all the virulence and toxigenic associated genes mentioned above. The determination of these virulence and toxigenic nature of the bacterial pathogens proves the contemporary approach for study of pathogenic potential and their disease causing ability in respiratory tract infections of goats.

VIRULENCE ASSOCIATED STUDY OF *Pseudomonas aeruginosa* ISOLATES OBTAINED FROM RESPIRATORY TRACT OF APPARENTLY HEALTHY AS WELL AS SICK GOATS BY PCR

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In the present study, nine isolates of *P. aeruginosa* were obtained from nasal swab and tissue samples collected from respiratory tract of goats. Aseptically collected nasal swab and tissue samples were inoculated on suitable artificial media and the growth was observed after incubation at 37°C for 24 hours. The organisms were identified on the basis of colony character, staining, microscopic examination and biochemical tests. Primers used for the virulence associated genes of *P. aeruginosa* isolates were subjected to the detection of *alg*D (Codes for GDP mannose 6-dehydrogenase)- 1310bp, *las*B (Elastase enzyme)- 300bp and *plc*H (Hemolytic phospolipase-C precursor)- 307bp sizes. The prevalence of *alg*D, *las*B and *plc*H was 33.3%, 44.4% and 44.4%, respectively. The determination of these virulence associated nature of the bacterial pathogens proves the contemporary approach for study of pathogenic potential and their disease causing ability in respiratory tract infections of goats.

MOLECULAR EPIDEMIOLOGICAL INVESTIGATION OF *Chlamydiae* AND OTHER BACTERIAL MICROFLORA ASSOCIATED WITH REPRODUCTIVE DISEASES AMONG RUMINANTS

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Chlamydiosis is considered as one of the major reproductive tract disease manifested in the form of abortions, endometritis, repeat breeding, retained placenta in large and small ruminants in many countries including India. A molecular epidemiological investigation was conducted to detect involvement of different chlamydial species of order *Chlamydiales* in abortions, stillbirths, endometritis and retained placenta cases among bovine, ovine and caprine. Total 205 samples were collected from animals having clinical reproductive tract infections that include cattle (67), sheep (80) and goats (58). DNA was extracted from vaginal swabs (109), uterine discharges (71), aborted feti (2) and placental tissues (23). Samples were screened using *Chlamydiales* order specific primer sets based on 23S rRNA and *Chlamydiacae* family specific primers based on *ompA* gene. The results with order specific and family specific primers showed that 38 per cent cases of endometritis and 23 per cent cases of abortions caes were owing to chlamydial infections. Molecular characterization and genetic variability studies of chlamydial species/strains detected among ruminants by PCR were done either by PCR-RFLP or by study of nucleotide sequence variation of *ompA* gene in VD2 region. For this 37 representative samples processed and result showed that 70.3 per cent and 29.7 per cent of reproductive infections were due to *C. psittaci* and *C. abortus,* respectively. Along with chlamydiae, the presence of various bacterial isolates like *Brucella melitensis* 2 (1.1%), *Staphylococcus* spp. 48 (26.37%), *Streptococcus* spp. 7 (3.84%), *E. coli* 47 (25.82%), *Bacillus* spp. 31 (17.03%), *Klebsiella* spp. 11 (6.04%), *Arcanobacterium* spp. 18 (10%), *Pseudomonas* spp. 13 (7.14%) and other bacterial species. 24 (13.19%) were isolated from different female reproductive disorders of the livestock. Our results point to the involvement of chlamydial species in reproductive tract infections to the significantly higher level that warrant further detailed studies and chlamydia specific preventive and control interventions.

VIRULENCE ASSOCIATED STUDY OF *Klebsiella* spp. ISOLATES OBTAINED FROM RESPIRATORY TRACT OF APPARENTLY HEALTHY AS WELL AS SICK GOATS BY PCR

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In the present study, eight isolates of *Klebsiella* spp. were obtained from nasal swab and tissue samples collected from respiratory tract of goats. Aseptically collected nasal swab and tissue samples were inoculated on suitable artificial media and the growth was observed after incubation at 37°C for 24 hours. The organisms were identified on the basis of colony character, staining, microscopic examination and biochemical tests. Primers used for the virulence associated genes of *Klebsiella* spp. isolates were subjected to the detection of *mag*A (Mucoviscocity associated gene)- 1280bp, *uge* (Uridine diphoshate galacturonate 4-epimerase)- 534bp, and *kfu* (Iron uptake system)- 797bp sizes. The prevalence of *mag*A, *uge* and *kfu* was 12.5%, 62.5% and 25%, respectively. The determination of these virulence associated nature of the bacterial pathogens proves the contemporary approach for study of pathogenic potential and their disease causing ability in respiratory tract infections of goats.

VIRULENCE ASSOCIATED STUDY OF GRAM POSITIVE ISOLATES OBTAINED FROM RESPIRATORY TRACT OF APPARENTLY HEALTHY AS WELL AS SICK GOATS BY PCR

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In the present study, 43 isolates of *Staphylococcus* spp. and 14 isolates of *Streptococcus* spp. were obtained from nasal swab and tissue samples collected from respiratory tract of goats. Aseptically collected nasal swab and tissue samples were inoculated on suitable artificial media and the growth was observed after incubation at 37°C for 24 hours. The organisms were identified on the basis of colony character, staining, microscopic examination and biochemical tests. Two sets of primers used for the virulence associated genes of *Staphylococcus* spp. isolates were subjected to the detection of *coa* (encodes for enzyme coagulase) - polymorphism, *Spa* (IgG binding region of the protein A)- 920bp, and *Clfa* (clumping factor)- 980bp genes, whereas *Streptococcus* isolates were subjected to detection of the *bca* (alpha-C protein)- 535bp, and *rib* (surface Rib protein)- 382bp sizes genes. The prevalence of *coa*, *Spa* and *Clfa*, *bca* and *rib* genes were 11.6%, 32.6%, 25.6%, 14.3% and 0%, respectively. The determination of these virulence associated nature of the gram positive bacterial pathogens proves the contemporary approach for study of pathogenic potential and their disease causing ability in respiratory tract infections of goats.

ASSOCIATION OF *Chlamydophila* species AND *Coxiella burnetii* IN REPRODUCTIVE DISEASE CONDITIONS IN SMALL AND LARGE RUMINANTS

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Infectious reproductive disease conditions presented in the clinical form of abortions, infertility, endometritis or repeat breeding severely affect the productive life of ruminant livestock species. Detection of certain potential infectious agents such as *Chlamydophila* species and *Coxiella burnetii* is difficult in routine laboratory practices. Thus, the present study was planned to appraise the association of various chlamydial species and *Coxiella burnetii* in different reproductive conditions. Total 235 samples from 69 bovine, 86 ovine and 80 caprine, including samples from abortions (109), endometritis/repeat breeders (101), retained placenta (2) and uterine tissues from other reproductive conditions (23). Collected samples include vaginal swabs, aborted fetal tissues and esteral/ uterine discharge. After DNA extractions from samples, screening was done using group/genus specific primer sets. The results with family specific chlamydial primers revealed 19%, 20.79% and 8% involvement of chlamydiae in abortions, endometritis/repeat breeders and other conditions, respectively. Predominatly *Chlamydophila psittaci* followed by *C. abortus* was detected whereas, only 11.70% samples from abortions cases of sheep and goats were found positive for *C. burnetii* by specific PCR test. More incidences of *C. burnetii* associated abortions were found in caprine as compared to ovines. Our findings showed high prevalence of chlamydiae and *C. bunetii* in reproductive track infections thus making it essential to screen samples for presence of such infectious agents and adopting appropriate treatment and control measures.

DETECTION OF BLUETONGUE VIRUS ANTIBODIES IN CATTLE RECOVERED FROM FOOT AND MOUTH DISEASE

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Foot and Mouth disease virus inactivated adjuvanted vaccine is widely used to control FMD in India. The vaccine contains FMDV types O, A and Asia 1 propagated in cell culture, concentrated and inactivated by BEI and adjuvanted. First vaccination is recommended at the age of 3 months, second vaccination is recommended after 4-6 weeks of primary vaccination and thereafter revaccination at 6 monthly intervals is required in enzootic areas.In spite of vaccination campaigns, the occurrence of FMD in cattle is often reported. Dose of antigen used, strain of FMDV used, storage and transport of vaccine, level of challenge, time between vaccination and challenge, correct handling and injection are the factors influencing the vaccination failures. Though clinical bluetongue in cattle is rarely reported, there are reports on the presence of BTV antibodies in the cattle exhibiting FMD like symptoms and lesions. In the present investigation, 28 sera out of 52 sera collected from cattle recovered from FMD showed the presence of BTV antibodies in cELISA. The observation warrants the focus on BTV isolation from the vaccinated cattle exhibiting signs of FMD.

CLONING, EXPRESSION AND PURIFICATION OF IMMUNODOMINANT OUTER MEMBRANE PROTEIN OMP31 FROM *Brucella* SPP

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Brucellosis is a worldwide zoonosis barring a few developed nations where the disease has been eradicated. However, the disease is reported to be re-emerging in several developed countries. In developing countries including India, brucellosis is endemic and heavy economic losses occur due to abortion, reduced milk production, delayed conception as well as due to high cost of treatment. The present study was aimed to produce recombinant outer membrane protein 31 (OMP 31) from *Brucella* spp. in a suitable prokaryotic expression vector so that it can be used as antigen for development of ELISA for sero-diagnosis of the brucellosis in animals or other downstream applications. Primers were designed for OMP31 gene after thorough analysis of the available gene sequences on NCBI database. While designing primers, *Nco*I and *Xba*I restriction sites were included in forward and reverse primers, respectively. A putative signal sequence of 20 aa was removed from the N-terminus of the OMP31 protein (60bp deletion from the 5'end of the OMP31 gene). PCR amplification using designed primers resulted in an amplicon of 726 bp. Prokaryotic expression vector pPRoExHTb (Invitrogen, USA) was used for expression of recombinant protein. After restriction double digestion of the PCR amplicon and prokaryotic expression vector pPRoExHTb by *Nco*I and *Xba*I, ligation was put in 20µl reaction using standard protocol. 10µl of the ligated mixture was transformed in to the freshly prepared DH5α competent cells by heat shock method (42^{0}C) and plating was done on Luria Bertani agar plates containing ampicillin (100µg/ml) as selectable marker. Randomly six colonies were picked and grown in Luria Bertani broth containing ampicillin (100µg/ml). Clones were confirmed by restriction double digestion of the isolated plasmids with *Nco*I and *Xba*I and also by PCR. One of the clones releasing specific size insert and giving specific size amplicon by PCR (~726 bp) was selected for induction of expression. Induction was done by adding 0.6 mM IPTG to the Luria Bertani broth containing selected clone in log phase (0.6 OD). After induction, samples were collected at hourly intervals up to 8 h to study the expression kinetics. Un-induced sample was also collected before adding IPTG for comparison. The recombinant his-tagged OMP31 protein produced was of ~ 31 kDa and expression was optimal at ~6h post induction. Recombinant expressed protein was purified by Ni-NTA affinity chromatography under denaturing conditions using standard protocol (Qiagen, Germany). Affinity purification resulted in the single specific band of expressed protein (~31 kDa) as evidenced by SDS-PAGE. For removing salts (urea), expressed recombinant protein was dialyzed several times against PBS using dialysis tubing of 12 kDa cut off value. For further confirmation, DOT blotting and western were performed by using Ni-HRP as conjugate and 4-chloro 1-naphthol as substrate. DOT blotting resulted in a dark circular brownish spot on nitrocellulose membrane while in western blotting dark brown band at specific location indicating ~31kDa size was obtained. In the present study, recombinant OMP31 was successfully expressed and purified to homogeneity. By using recombinant DNA techniques, large quantity of the highly purified protein can be obtained in a cost effective manner as seen in the present study. Further experiments are underway to evaluate the applicability of recombinant OMP31 as antigen for development of monoclonal antibody as well as ELISA for sero-diagnosis of brucellosis.

CLONING, SEQUENCING AND PHYLOGENETIC ANALYSIS OF HEAT SHOCK PROTEIN 70 (HSP70) GENE FROM RUMINANT SPECIES

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Molecular chaperones, including the heat-shock proteins (HSPs) are a ubiquitous feature of cells in which these proteins cope with stress-induced denaturation of proteins. Heat Shock protein 70 (HSP70) is a predominant member of the HSP family of proteins which play a variety of roles in the cells and are responsible for cyto-protection under stress conditions. The present study was aimed at cloning, sequencing and phylogenetic analysis of *HSP70* gene form ruminant species viz. cattle, buffalo, sheep and goat. In the study, 1926 bp *orf* of *HSP70* was amplified from cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), goat (*Capra hircus*) and sheep (*Ovis aries*) using genomic DNA isolated from lymphocytes. The amplicons were cloned in pGEM-T easy cloning vector using standard procedures and sequenced. On the basis of sequence features, the gene corresponds to the cytoplasmic form of HSP70. Sequence analysis revealed 1926-bp long open reading frame of *HSP70* gene encoding 641 amino acids in ruminant species. The nucleotide sequences of *Bos indicus* showed 99.4% identity with *Bubalus bubalis,* 99.7% with *Capra hircus,* 98.9% with *Ovis aries,* 98.6% with *Canis familiaris,* 98.5% with *Sus scrofa,* 84.4% with *Gallus gallus* and more than 90% identity with *Camelus dromedaries, Felis catus,* and *Homo sapiens HSP70* sequences available of NCBI databse. Based on the nucleic acid sequences of *HSP70* full length *orf*, phylogenetic tree was constructed by Mega 4.1 considering 1,000 bootstrap values. It was found that ruminants and monogastrics are derived from different clusters according to their closer evolutionary relationship. On phylogenetic analysis, sequences in this study clustered away from the avian sequences, closest among bovid group and farthest from primate group. The present study clearly shows the highly conserved nature of *HSP70* gene. *HSP70 orf* was successfully amplified, cloned and sequenced from different ruminant species in the present study which is now available for public domain on NCBI database. Attempts are underway to produce recombinant HSP70 protein for various downstream applications including production of hyperimmune antisera, monoclonal antibodies, immunogenicity in experimental animal models as well as for development of ELISA for detection of stress in animals.

OCCURRENCE OF HIGHLY VIRULENT INFECTIOUS BURSAL DISEASE IN UNIVERSITY RESEARCH FARM

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Infectious Bursal Disease is caused by birna virus and is highly contagious. Prevalence of IBD was widely reported from several states of India both in layer chicks and broilers. In Tamil Nadu, IBD was first reported in 1974 and there after there is regular occurrence with high morbidity (50-100%) and mortality (10-80%). During 1993, very high mortalities in chicken due to IBD occurred in several parts of India. The introduction of IBDV live intermediate and hot strains since 1993 has drastically reduced the incidence of IBD with mortality of <1% in commercial farms. However, there are sporadic reports of occurrence of IBD with higher mortalities in poultry farms. During 2010, high mortality in 8 week old White Leghorn chicken was observed in University Research Farm. There was spiking death curve lasting for 4 days with 80% mortality. The study has revealed that IBDV isolates obtained were identical with standard IBDV serotype 1.

ISOLATION OF BLUETONGUE VIRUS FORM CATTLE, SHEEP AND GOATS

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Bluetongue is an arbovirus belonging to the genus orbivirus of the family Reoviridae. Bluetongue virus infects sheep, cattle, goats, deer and other domestic and wild ruminants. Bluetongue is OIE list A disease and economically important in sheep. Affected sheep show signs of high fever, excessive salivation, swelling of face, inflammation, ulcers and necrosis in and around mouth including gums, cheeks and tongue. Affected sheep also show prominent respiratory symptoms with nasal discharge and difficulty in breathing. Some sheep may show pneumonia and enteritis. Some of the sheep also develop foot lesions especially coronitis leading to lameness. Torsion of the neck is observed in severely affected animals. Some of the sheep may show loss of condition and emaciation. Abortions and congenital malformations can also occur. Mortality may vary from 0% to 30%. Most infections in cattle, goats and other ruminants go undetected. BTV antibodies are often detected in the sera of cattle and goats. Bluetongue in sheep was reported during October 2010 in Tamilnadu. The present study aimed at the isolation of bluetongue virus from sheep, goats and cattle from the outbreak areas. The specimens collected include blood in EDTA from sheep showing raise in temperature whereas spleen, lungs and heart were collected from dead sheep. EDTA blood alone was collected in case of incontact cattle and goats. The specimens were initially passaged in embryonated eggs followed by inoculation onto BHK cells. Bluetongue virus could be isolated from sheep, cattle and goats which was confirmed by FAT and by the migration pattern of different segments of dsRNA. Micro SNT and RTPCR has revealed that the Bluetongue virus isolates from sheep, goats and cattle belong to Bluetongue virus serotypes 16.

OCCURRENCE OF PATHOGENIC *Listeria spp.* IN MILK & MEAT PRODUCTS

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Modern lifestyle markedly changed eating habits worldwide, with an increasing demand for ready-to-eat foods, such as milk products and meat products. Packaging and storage conditions of these products may favor the growth of psychrotrophic bacteria, including pathogenic *Listeria monocytogenes*. In present investigation, milk products (127) and meat products (09) from local market were tested for the presence of *Listeria spp.* by two step enrichment followed by plating on selective media. Tentatively identified isolates of *Listeriae* were tested for the virulence associated genes namely *hly*A , *plc*A, *act*A and *iap* by multiplex and *prf*A by singleplex PCR. A total of 23 (18.11%) *Listeriae* were recovered from milk products and 2(22.22%) from meat products. *Listeriae* were isolated from 18 milk products. Virulence associated genes of listeriae , *hly*A, *act*A, *iap* and *plc*A were detected in two strains, *hly*A, *act*A and *iap* in thirteen strain , *hly*A and *iap* in one strain and only *iap* in three strains. Of the two *Listeriae* recovered from meat products virulence associated genes of listeriae, *hly*A was detected in chicken cutlets and *hly*A and *iap* in chicken chips.Contamination of ready to eat products with virulent *Listeriae* is a cause of concern as there will be increased risk for human health.

L. monocytogenes IN MASTITIC BOVINE MILK SAMPLES

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Quarter milk samples (n= 121) collected from dairy cows were screened for sub clinical mastitis by California mastitis test (CMT). CMT positive samples were examined for the presence of *Listeria monocytogenes*. Biochemically confirmed isolates of *L. monocytogenes* were investigated for virulence marker genes (*hly*A, *plc*A, *act*A, *iap* and *prf*A). A total of 26 (21.48%) strains of *L.monocytogenes* were isolated. Genes , *act*A, hlyA and iap in six strains and *hly*A , *plc*A and *iap* were detected in three isolates . *hly*A and *iap* were in eleven strain and *iap* alone was detected in six strain, respectively. Considering high frequency of *L.monocytogenes* in mastitis milk, consumption of raw milk or product prepared from the milk may contribute to food borne infection in human.

EXPRESSION OF P26 ANTIGEN OF EQUINE INFECTIOUS ANEMIA (EIA) VIRUS AND STANDARDIZATION OF AGAR GEL IMMUNODIFFUSION TEST AND INDIRECT ELISA FOR THE DIAGNOSIS OF EIA

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Equine infectious anemia (EIA) is a retroviral disease Equine infectious anemia virus (EIAV), which was one of the first viruses identified in nature in 1904. EIAV is responsible for causing a chronic, debilitating disease of all equidae, including horses, mules, and donkeys. EIAV infection has been reported worldwide and is recognized as livestock pathogen of significant economic importance to the horse industry. This disease falls under a regulatory control program in many countries including India. Control of EIA is based on identification of inapparent carriers by detection of antibodies to EIA virus (EIAV) in serologic tests. The current internationally accepted standard for diagnosis of EIAV infection is the agar gel immune-diffusion test (AGID), which detects antibodies to the major *gag* gene product, p26. At NRCE, AGID and cELISA test are being performed using reagents imported from approved commercial sources. The objective of this study is to evaluate recombinant protein based immunoassays for EIA diagnosis. The *p26* gene of EIAV was commercially synthesized from GeneScript, USA. Synthetic gene was cloned to pQE-30 expression Vector (Qiagen, Germany) and transformed in JM109 *E. coli* cells. Recombinant clones expressing 26kDa p26 protein were screened and selected by SDS-PAGE after 4h induction with IPTG. Recombinant p26 protein was purified in bulk by Nickel-NTA affinity chromatography (Qiagen, Germany). Identity of p26 protein was validated by western blotting with EIAV infected equine serum. AGID and indirect ELISA were standardized using rp26 protein and assays were compared with commercial kit. Total 1200 serum samples were tested with rp26-AGID assay. The assay showed an excellent diagnostic relative sensitivity (100%) and specificity (100%) when compared to a commercial AGID assay (VMRD, USA). About 3000 serum samples were tested with indirect ELISA using rp26 protein. When ELISA results were compared to standard AGID test, 100% diagnostic sensitivity and 99% diagnostic specificity were observed. The present study demonstrated excellent performance characteristics of the rp26-AGID and ELISA with a high degree of agreement with the commercial AGID test. In conclusion, the rp26-AGID/ELISA could be adopted in India for the diagnosis and control of EIA in this country.

CHARACTERIZATION OF *Leptospira* USING 16S-rRNA PCR

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Leptospirosis is an emerging zoonotic disease with wide spread distribution. Samples collected from rats (299), cattle (26), sheep (42), dogs (13), pigs (15), humans(53) and stagnated water in rice fields (10) were inoculated in EMJH liquid medium with tween 80, antibiotics and 5-flurouracil. A total of 17 isolates were recovered from rats (5), sheep (5), pigs (4), humans (2) and rice field water (1) were purified and maintained in EMJH medium and semisolid medium. Physicochemical characterization, growth at 13°C in the presence of 8-Azaguanine and lipase activity revealed the pathogenicity of the isolates. Molecular characterization of the isolates was studied using 16SrRNA PCR using specific primers. All the isolates yielded 525bp product which is specific to *leptospira*. 16SrRNA PCR sequence analysis revealed the presence of *Leptonema illini, L.hardjo* and *L.inadi* in sheep, *L.naguchi, Leptonema ilini* in rats and *L. pomona* in pigs.

IDENTIFICATION OF NOVEL SPLICE VARIANTS IN HORN CANCER BY RNA-SEQ ANALYSIS IN ZEBU CATTLE

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Alternative splicing (AS) is a fundamental molecular process regulating eukaryotic gene expression and involved in numerous diseases. It is usually postulated as the main mechanism to augment protein diversity from a limited set of protein coding genes. Horn cancer, a type of squamous cell carcinoma, in zebu cattle is an expensive affair in Indian agriculture sector, which accounts for 83.34% of total tumors found. Due to the significance of splicing events in cancer pathway and profitability of the knowledge of these events in the area of cancer research, diagnosis and treatment, it is immensely important to study such processes. Here to obtain cancer specific data of splice variants, for the first time, we employed Roche 454 next generation sequencing platform to sequence *Bos indicus* cancerous and normal horn tissue transcriptome. This resulted into 392,692 and 516,696 high-confidence deep sequencing reads, where upon assembly of these reads using GS De Novo Assembler resulted in 4095 and 5805 contigs for HN (normal horn tissue) and HC (cancerous horn tissue), respectively. All contigs were aligned against *Bos Taurus* chromosome database (Btau_4) using BLAT for finding splice variants. In our study we found 29 alternatively spliced genes in which sixteen splice variations have already been reported. Protein prediction of rest of the thirteen candidates revealed splice variation has caused either premature termination of protein chain, insertion or deletion of stretch of amino acids, formation of unique carboxy terminal domain or frame shift in protein chain formation. The alteration in the protein sequence may thus lead to either gain of function, loss of function or change in the specificity of the protein and its functional diversity. Identification of the unique splice variants by RNA-seq thus demonstrates the potential to identify novel transcripts on high throughput basis.

DIFFERENTIAL TRANSCRIPTOME ANALYSIS OF HUMAN BUCCAL CELL CARCINOMA BY RNA-SEQ

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The differential transcriptome analysis of cancer and normal tissues provides better understanding of molecular pathways leading to cancer which in turn allows designing effective strategies for diagnosis, therapeutic intervention and prediction of therapeutic outcome of cancer. RNA-Seq analysis offers advantages of high throughput transcriptome profiling along with providing relative abundance of each transcripts in a given sample thereby allowing differential gene expression analysis. Here, we aimed to characterize buccal cancer and normal tissue transcriptome by RNA-seq to identify significantly altered transcripts and deregulated biological pathways leading to buccal cancer. RNA-seq analysis of buccal cancer and normal tissue transcriptome identified total of 1797 genes uniquely expressed in normal tissues, 2655 genes uniquely expressed in cancer tissue and 2466 genes expressed in both tissues. Among 2466 genes expressed in both, 1842 and 624 genes were observed to be up regulated and down regulated respectively in cancer tissue. The quantitative real time PCR analysis of selected transcripts revealed consistent expression pattern as observed by RNA-seq analysis. RNA-seq analysis thus demonstrated the feasibility of high throughput and quantitative transcriptome profiling of buccal cancer and surrounding normal tissue with expression pattern reproducible with the real time PCR. The most significantly altered genes in the buccal cancer appeared to follow the hallmarks of cancer based on their known association in the various cancer studies. In our study, we also identified potential transcripts with significantly altered expression in buccal cancer which have not been found to be associated with any cancer so far thus providing the novel targets for developing alternative ways for diagnosis and cancer therapeutics. The KEGG pathway analysis showed enrichment of differentially expressed transcripts to various pathways leading to cancer including p53 signaling pathway. The Gene Ontology analysis by GOMiner showed specific enrichment of down regulated transcripts to actin mediated cell contraction process providing unique insight into role of actin mediated cytoskeleton remodeling in cancer.

ISOLATION AND IDENTIFICATION OF *Pasteurella multocida* FROM NATURALY INFECTED SHEEP AND GOAT

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The present study reports an outbreak of Pasteurellosis in goats maintained at Panjrapole near Vapi, Gujarat. Affected animals had high fever, oedematous swelling on lower neck region, nasal discharge, and dyspnoea. The post mortem examination of fresh carcass of male kids aged 6 months to 2 years revealed bronchopneumonia, consolidation and marbling of lungs, pin point hemorrhage on outer surface of spleen and necrotic foci on liver. A total of ten blood samples, four impression smears and eight nasal swabs were collected for identification and isolation of the organisms. Characteristic bipolar organisms have been observed in all impression smears and blood smears by Geimsa staining. Gram negative coccobacilli were isolated from samples of blood, nasal swabs and tissues on blood agar plate. Further, these organisms were confirmed as *Pasteurella spp.* by PCR. Clinical signs, post mortem examination, direct smear examination, cultural isolation and PCR indicated that mortalities may be due to pasteurellosis.

METAGENOMICS OF VIRULENCE-ASSOCIATED AND ANTIBIOTIC RESISTANCE GENES OF MICROBIAL POPULATIONS IN INDIAN BUFFALO RUMEN ANALYZED USING HIGH THROUGHPUT SEQUENCING

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A major research goal in rumen microbial ecology is to understand the relationship between community composition and community function involved in fermentation process of potential interest. The buffalo rumen microbiota impacts human food safety as well as animal health. Although the bacteria of bovine rumen have been well characterized, techniques have been lacking to correlate total community structure with gene function. We used high throughput sequencing of metagenome DNA extracted from buffalo rumen to characterize general microbial diversity and identify the repertoire of microbial genes present in buffalo rumen, including genes associated with antibiotic resistance and bacterial virulence. Results suggest that over six percent (6.44%) of the sequences from our buffalo rumen pool sample could be categorized as virulence genes and genes associated with resistance to antibiotic and toxic compounds (RATC). This is a higher proportion of virulence genes found in samples of chicken cecum (5.39%), cow rumen (4.43%), camel rumen (5%) and Sargasso sea (2.95%). While not comparable to the proportion found in cow milk (11.33%), cattle faeces (8.4%), Antarctic marine derived lake (8.45%), human fecal (7.7%) and farm soil (7.79%) samples. The dynamic nature of metagenomic data, together with the large number of resistance to antibiotic and toxic compounds (RATC) classes represented in samples from widely different ecology indicates that metagenomic data can be used to track potential targets and relative amounts of antibiotic resistance genes in individual animals. Additionally, these data can be also used to generate sample-specific and temporal antibiotic resistance gene profiles to facilitate an understanding of the ecology of the microbial communities in each habitat as well as the epidemiology of antibiotic resistant gene transport between and among habitats. Keywords: Surti buffalo, metagenome, GS-FLX, Virulence, RATC.

MOLECULAR EPIDEMIOLOGY OF CLASSICAL SWINE FEVER VIRUS INFECTION PREVALENT IN SOUTH INDIA

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Classical swine fever virus (CSFV), a positive stranded RNA virus of 12.3 kb genome is a member of the genus *Pestivirus* of the *Flaviviridae* family, causes Classical swine fever in pigs Although pigs can be infected by Bovine viral diarrhea virus (BVDV), Border disease virus (BDV), Porcine circo virus (Terpstra and Wensvoort, 1988) only CSFV induces severe disease and is often fatal. The disease is characterized by fever, leukopenia and hemorrhage, and can run an acute, chronic, or subclinical course leading to substantial economic losses to the pig industry hence the diseases was reported in Office International des Epizooties (OIE) List A diseases. Six different out break samples, two from Andhra Pradesh and four from Tamil Nadu suspected for CSFV infections between 2008 and 2010, were confirmed by amplifying the NS5B gene and the PCR positive samples were subjected to virus isolation in PK15 cells. Further the isolated viruses were confirmed by indirect FAT done on coverslip cultures using CSFV specific positive reference serum. From the early cell culture passaged isolates, CSFV genes viz. NS5B, 5'UTR and E2 were amplified partially for molecular epidemiological analysis. A phylogenetic tree was constructed separately for each gene and the analysis was made with the available Genbank data. Based on the phyllogenetic analysis it was found that the south Indian isolates were clustered with northeastern isolates and further discussed on the grouping and sub-grouping of the south Indian viruses.

DEVELOPMENT OF MULTIPLEX LATEX AGGLUTINATION ASSAY WITH SEROTYPE SPECIFIC PEPTIDES OF FMD VIRUS FOR DIFFERENTIAL DIAGNOSIS

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In this study 200 serum samples were collected from cattle (141) and buffaloes (59) of different ages and sex from Foot and Mouth Disease (FMD) vaccinated and unvaccinated animals. Serotype specific peptides and non structural peptide (NSP 2B) of FMDV as reported in published literature and confirmed by bioinformatics tool BLAST, were commercially synthesized and conjugated to latex beads of different colors. LAT was employed for all the samples . Out of 200 serum samples, 22 samples were positive for antibodies to FMDV type O, 14 for type A, 14 for type Asia 1, 12 for both type O and A, 26 for type O and Asia 1, 4 for A and Asia 1, 14 were positive for all the three serotypes (O, A and Asia 1) and 94 were negative for antibodies to all the three serotypes. Sixty nine samples showed agglutination of beads conjugated with NSP 2B peptide. On multiplexing, agglutinates of different types were seen only with the aid of microscope. However clumps of type A were not clearly visible. Agglutination was enhanced by using anti bovine immunoglobulin. On comparison of LAT with LPB ELISA it was found that LPB ELISA was more sensitive as it could detect more samples positive for different serotypes. On comparison of commercially available DIVA-ELISA with NSP peptide based agglutination, the latter could detect more positive samples than DIVA-ELISA kit.

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN *Pit-1* GENE SEQUENCES IN INDIAN BUFFALO *(Bubalus bubalis)*

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The pituitary specific transcription factor (*Pit-1*), which is also termed as a Growth hormone factor 1(*GHF1*), is responsible for pituitary development and hormone expression in mammals. This gene is candidate gene for milk production marker because of its role in regulating expression of Growth Hormone and the Prolactin genes. The *Pit-1* gene was explored for searching of single nucleotide polymorphism (SNPs) in Indian buffalo breeds (*Bubalus bubalis*). A total of 40 animals belonging to 10 Indian buffalo breeds were screened for SNPs in Pit1 gene by the polymerase chain reaction using primers covering all six exons and sequencing. DNA sequences of all six exons including partial introns were aligned. The gene sequence comparison revealed 7 sequence variations across Exon1, Intron1, Exon2 and Intron 2 of *Pit -1* gene. Remaining exons did not show any sequence variation. The frequency of minor allele for SNP T264A (exon 1) was 0.05, for SNPs C461T, G472A, G603C (Intron 1) were 0.45, 0.11 and 0.03, respectively whereas frequencies for SNP C720T (exon 2) was 0.14, for SNP T771C and A772A (Intron 2) were 0.42 and 0.39, respectively.

A NOVEL CELL-PENETRATING RATH PEPTIDE FOR EFFICIENT DELIVERY OF OLIGONUCLEOTIDE AND CARGO IN HELA CELLS

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Cell-penetrating peptides (CPPs) have attracted increasing attention in the past decade as a result of their high potential to convey various, otherwise impermeable, bioactive agents across cellular plasma membranes. Different CPPs have proven potent in delivery of different cargoes and there is generally a correlation between high efficacy and cytotoxicity for these peptides. It is, therefore, of great importance to find new, non-toxic CPPs with more widespread delivery properties. In this study we assessed the cargo-delivery properties of peptide, fluoresceinyl-labeled Rath navigator peptide. HeLa cells (200,000 cells/well) were seeded in 12 well plate for 24 hours and were incubated with fluoresceinyl-labeled oligonucleotide with various concentrations of Rath navigator peptide (10µg to 50µg) at 37ºC for two hours for internalization. Cells were observed for fluorescence under the microscope and their percentage was detected by flow cytometry. It was observed that there was a 50% shift in fluorescence in HeLa cells transfected with peptide carrying the oligonucleotide. As an ideal delivery vehicle, Rath navigator peptide has the abilty to protect the cargo molecule in the presence of serum, nucleases and has minimal or no cytotoxicity at higher concentrations studied. Thus the novel Rath peptide can be used as a novel CPP that can be used to translocate different cargoes inside cells efficiently.

ASSESSMENT OF TNF- α LEVEL IN EXPERIMENTAL INFECTION OF MICE AGAINST *Toxoplasma gondi*

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Among the apicomplexan parasites *Toxoplasma gondii* is widely prevalent and reported from all the continents. Felines act as the definitive hosts while all non feline vertebrates including humans serve as intermediate hosts with disseminated tissue infections. Because of its wide prevalence in man and animals, the parasite has significant impact not only on animal production but also on public health throughout the world. In order to assess the cellular response against the parasite, the RH strain of the parasite was taken from the cryopreserved stock maintained in the divisional laboratory. The viability of the tachyzoites was checked under the microscope. Their number was counted under the microscope using Neubaur's chamber. Appropriate dilutions were made using sterile PBS. A total of 102 tachzoites were given to the experimental mice through intra peritoneal route. The sera from the mice was taken daily till the death of the mice. After the death of the mice, all the sera were appropriately diluted with PBS and TNF-α was assessed using sandwitch ELISA based cytokine assay and the OD readings were taken at 450nm and the data was statistically analysed. Marked increase in the levels of all the cytokine was observed prior to death of the mice. In absence of prior immunity, mice were not able to cope up with alarming increase in the level of cytokine and finally scumbaled to infection.

EXPRESSION OF SURFACE ANTIGEN 3 (SAG 3) OF RH STRAIN OF *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite with broad host range. Presently serodiagnosis is mainly based on application of native proteins in the serodiagnostic tools. Surface antigens(SAG) are potent candidates for this purpose. Surface antigen 3 (SAG3) is found both on tachyzoites as well as bradyzoites. It has structural similarity with SAG 1. The primers of SAG 3 were laboratory synthesized and 1158 bp product was amplified and purified. The products were double digested with Eco RI and HindIII restriction enzymes. The expression vector pPROExHT[a] was choosen and doubly digested with the same enzymes. The two were ligated using T_4 DNA Ligase. Competent DH5α *E. coli* cells were transformed and grown in LB at 37°C for 1 hour and later plated on LB agar in presence of X-Gal, IPTG and Ampicillin. Confirmation of recombinant clones was done using colony and plasmid PCR as well as restriction enzyme analysis. The selected positive clones were grown on LB after induction with 1.5mM IPTG and the samples were collected after every hour till 8 hours. The samples collected were run on SDS PAGE along with uninduced control and standard Fermentas Protein Marker and protein profile was analysed. Specific 47kDa band corresponding to SAG 3 were observed in all lanes after 2 hours of induction, maximum being at 6 hours of induction.

MOLECULAR CLONING AND CHARACTERIZATION OF SURFACE ANTIGEN 3 (SAG3) GENE OF CHENNAI ISOLATE OF *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular protozoan parasite with a broad host range. Presently serodiagnosis is mainly based on application of native proteins in the serodiagnostic tools which does not rule out the possibility of a false positive reaction. Surface glycoproteins (SAGs) are considered as promising candidates for unequivocal diagnosis. Surface antigen 3, a 43kDa glycoprotein, is found on the surface of both tachyzoite as well as the bradyzoite stages of the parasite. SAG3 mediates the attachment of *Toxoplasma gondii* to cell surface proteoglycans and has structural similarity with surface antigen 1 (SAG1). The coding sequence of SAG3 of Chennai isolate was cloned in InsTA T/A cloning vector. Initially, total RNA was extracted from the host cell-free tachyzoites. cDNA was synthesized from total RNA using MuMLV reverse transcriptase. Specific primer directed PCR amplification of the full ORF of SAG3 was achieved using *Taq* DNA polymerase. The 1158 bp amplified product was purified and used for T/A cloning. Competent DH5α *E. coli* cells were transformed and were grown at 37°C in LB broth for 1 hr and subsequently plated on LB agar containing ampicillin, X-gal and IPTG, overnight. The recombinant colonies were screened by blue-white selection. The recombinant white colonies were picked up and grown for 16-18 hrs in LB broth containing antibiotic for plasmid isolation. The presence of the insert in the recombinant clones was confirmed by restriction digestion using *Pst*1 and *Eco*R1 as well as by colony PCR. The positive clones were custom sequenced and the sequence was analyzed.

RELATIVE QUANTIFICATION OF *Peste des petits ruminants* VIRUS IN VARIOUS TISSUES USING REAL TIME PCR

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Real time polymerase chain reaction, also called quantitative real time (qrtPCR) PCR or kinetic polymerase chain reaction is a laboratory technique based on polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule (Higuchi *et al.,* 1992). It enables both detection and quantification (as absolute number of copies or relative amount when normalised to DNA input or additional normalising genes) of a specific sequence in a DNA sample. In the present study, real time qrtRT-PCR based relative viral load of PPRV was estimated in selected tissues like lung, spleen and lymphnodes collected from PPRV suspected field cases and animal experimentally infected with PPRV. The choice of samples to study viral load was based on the possible predilection sites of the PPRV. Relative viral load was calculated in lung, spleen and lymphnode samples of experimentally infected animals available at National Morbillivirus Referral Laboratory, Division of Virology, IVRI, Mukteswar along with four samples of present study *viz.,* two lymphnodes, one lung and one spleen collected from field cases. Out of these seven tissue samples, highest (6.44 $TCID_{50}$) and lowest (4.64 $TCID_{50}$) viral load was estimated in lymph node from field sample and lung from experimentally infected animal, respectively. In experimental samples, highest viral load estimated was 5.59 $TCID_{50}$ in spleen followed by 5.06 $TCID_{50}$ in lymphnode and 4.64 $TCID_{50}$ in lung. Among field samples, lymphnode showed highest load of 6.44 $TCID_{50}$ followed by 6.09 $TCID_{50}$ in lung and 5.26 $TCID_{50}$ in spleen.

COMPARISON OF DIFFERENT CULTURE MEDIA ON *In vitro* DEVELOPMENT OF BUFFALO EMBRYOS

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In vitro embryo production (IVP) is currently one of the most important biotechnologies in buffalo breeding and husbandry. However, the efficiency of *in vitro* embryo production is still low with only 15-30 per cent of oocytes developing into blastocysts, probably because, the *in vitro* environment cannot mimic *in vivo* environment and results in embryos that have altered morphology and gene expression. Several factors can influence the IVP efficiency and contribute to the existing differences between *in vivo* and *in vitro* produced embryos. Several commercial media with different composition are available in the market. Selecting the medium through comparison of different culture media on *in vitro* development of buffalo embryos will overcome this problem. The recent development in commercial sequential media increases the embryo production. In this study, we have compared the different *in vitro* culture media such as G-1 TM PLUS, G-2 TM PLUS, complete multiblast medium, TCM-199, 1x ovum culture medium for embryo development. The per cent of morula was significantly higher ($p<0.05$) in G-1 TM PLUS, G-2 TM PLUS (18.38 per cent), complete multiblast medium (17.77 per cent), TCM-199 with BOEC (16.55 per cent), 1x ovum culture medium (12.69 per cent) and TCM alone (10.73 per cent). Exact quantification of these factors remains a distant dream but commercially available media such as G1/G2 medium and complete multiblast medium effectively enhances the per cent of morulae formed in very similar number to that of TCM supplement with BOEC and serum. It was concluded that commercial medium with necessary energy and growth factor could effectively replace the unknown factor supplied by BOEC and serum.

DETECTION OF BLUETONGUE VIRUS IN *Culicoides* MIDGES BY MOLECULAR TOOLS

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Bluetongue (BT), an infectious, haematophagous arthropod (*Culicoides* midges) borne viral disease of domestic and wild ruminants, primarily affects sheep while goat, cattle, buffalo, camel remain sub-clinically infected. The disease is endemic to southern part of the country and has been reported from western and northern states of India. However, no outbreak or typical disease has been reported officially, so far, from eastern and north-eastern part of the country. In this study, the vectors (*Culicoides* midges) were trapped from different districts of West Bengal and identification was carried out up to species level. Predominant species identified were *Culicoides schulzei*, *C. palpifer, C. imicola, C. clavipalpis, C. difinitus* and *C. kamrupi*. From meteorological data it was learnt that the state is having agro-climatic conditions viz. temperature, rainfall, humidity and soil pattern conducive to propagation of the vectors that led us to believe in circulation of bluetongue virus (BTV) amongst vectors and animals of the state. In order to confirm sero-conversion of the viral infection, attempts were made to detect and isolate BTV from *Culicoides* midges, collected from localities having good number of sero-positive animals and finally success was achieved. RNA-PAGE and RT-PCR were carried out to detect BTV, followed by isolation by embryonated chicken egg and BHK_{21} cell lines. The study identified BTV in *Culicoides* midges indicating existence of the virus in this state advocating preparedness to prevent menace of clinical BT in future, if any.

DETECTION OF DUCK ENTERITIS VIRUS IN LIVER OF INFECTED DUCK BY PCR

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Duck virus enteritis (DVE), an acute contagious disease affecting birds of domestic water fowls of the order Anseriforms is caused by Anatid Herpes Virus-1 (AHV-1), a member of the Herpesviridae family. The objective of the study is to detect the duck enteritis (duck plague) virus directly from infected tissue of duck obtained from field outbreak in Khaki Campbell breed in West Bengal. Livers from moribund birds showing typical lesions like oesophageal ulceration, haemorrhages in the visceral organs were collected in PBS for confirmation through molecular technique. Viral DNA was extracted from liver samples using 50 mg of tissue in each case and simultaneously viral DNA also extracted by same method from allantoic fluid (AF) after death of embryonated duck eggs (EDE), inoculated with 100 µl 10% liver tissue suspension through CAM route. Polymerase chain reaction (PCR) was performed in all the samples using 2 sets of primers. First set (DPF1 and DPR1) of primer amplified all viral DNA extracted from liver tissues and AF of dead embryonated duck eggs and the product size was 602 bp. Similarly, the second set of primers (DPF2 and DPR2) also amplified all viral DNA extracted from liver tissues and AF of dead EDE and produced 446 bp amplicon confirming DVE viral genome detection from liver tissue. Based on the observation, it was concluded that PCR could be used conveniently for diagnosis of duck virus enteritis in field directly from necropsy materials, viz. from liver.

PRELIMINARY DETECTION OF GROUP-A ROTAVIRUS FROM DIARRHOEIC FAECAL SAMPLES OF CALF AND PIGLET BY RNA-PAGE

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Rotaviruses are recognized as the major cause of severe gastroenteritis of a variety of mammals including human, specially infants and young children as also avian species. Rotaviruses are members of the *Reoviridae* family within the *Rotavirus* genus, having a segmented genome. The segmented nature of the viral genome allows reassortment in a mixed infection in natural conditions, leading to the emergence of new serotypes of the virus. The present investigation describes the detection of rotaviruses among diarrhoeic calf and piglet. Faecal samples of calf and piglet under six months of age with gastroenteritis were collected from different organized farms of different districts of West Bengal during October 2010 to February 2011 were used in this investigation. All the calf and piglet rotavirus PAGE-positive samples depicted a characteristic group-A rotavirus migration pattern (4:2:3:2) of RNA segments, all the piglet and calf rotavirus positive samples showed a long electropherotype. The electrophoretic migration pattern of a particular rotavirus may be used to characterize the virus strain, which may be used in epidemiological investigation.

METAGENOMIC ANALYSIS OF SUBCLINICAL MASTITIS MILK SAMPLES OF COWS

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The metagenomic analysis of pooled DNA of each breed was carried out after DNA extraction from positive subclinical mastitis milk samples of TP, Kankrej and Gir cows. After DNA extraction, nebulisation of DNA was carried out to generate smaller fragments and then fragment end repair was carried out by applying adaptors on both the ends, followed by emulsion PCR and pyrosequencing using GS-FLX 454 Life Sciences. Data generated after the sequencing were processed using inbuilt software GS Run Browser. Further, the data were analyzed using the SEED subsystem of MG-RAST (Meta Genome Rapid Annotation with Subsystem Technology). Metagenomic analysis of DNA of subclinical mastitis milk sample of TP, Kankrej and Gir cows yielded an out put of 274190 bp, 17,727 bp, 42,548 bp and 1,960, 170, 301 contigs respectively. Average fragment length obtained were 139.89, 104.28 and 141.36 bp for TP, Kankrej and Gir cows respectively. The longest sequence length was 560, 327 and 454 bp, while shortest sequence length was 40, 40, and 41 bp for TP, Kankrej and Gir cows respectively. A total of 54 (2.76%), 39 (22.94%) and 12 (3.99%) sequences for TP, Kankrej and Gir cows respectively could be matched to proteins in SEED subsystems of MG-RAST (using an e-value cut-off of 1e-5). Metagenomic analysis of the three breeds identified bacterial organisms belonging to phyla (5), class (8), Subclass / order (15), Family (19), Genus (23) and species (28) were identified. In pyrosequencing, over all 28 bacterial species were identified from all the three breeds of cows viz. *Leifsonia xyli, Propionibacterium acnes, Streptomyces coelicolor, Chlamydophila abortus, Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus acidophilus, Streptococcus mitis, Burkholderia cenocepacia, Burkholderia cepacia, Ralstonia solanacearum, Nitrosomonas europaea, Pseudoalteromonas atlantica, Salmonella* Dublin, *Serratia marcescens, Azotobacter vinelandii, Pseudomonas aeruginosa, Pseudomonas mendocina, Stenotrophomonas maltophilia, Bacillus subtilis, Lactobacillus delbrueckii, Aster yellows witches'-broom phytoplasma, Parvibaculum lavamentivorans, Thermosipho melanesiensis, Aeromonas hydrophila, Escherichia coli, Shigella boydii* and *Pseudomonas fluorescens.*

CULTURAL AND METAGENOMIC BASED IDENTIFICATION OF SUBCLINICAL MASTITIC PATHOGENS IN COWS

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Pyrosequencing is a relatively new molecular technique with an incredible potential for metagenomic analysis. A total of 77 cows and 301 quarters were screened for subclinical mastitis. A total of 106 isolates belonging to five different microbial genera were recovered from 91 quarters of 41 cows including 15 quarters having mixed bacterial infections by cultural examination. Pyrosequencing reads obtained from breed wise pooled DNA of subclinical mastitis milk samples were analyzed using the SEED sub system database of Meta Genome Rapid Annotation with Subsystem Technology (MG-RAST). Out of five genera Staphylococcus, Streptococcus, Micrococcus, Bacillus and Escherichia detected in the subclinical mastitis milk samples by culture based methods, four genera Staphylococcus, Streptococcus, Bacillus and Escherichia were identified in the corresponding pyrosequencing data, while Micrococcus identified by culture based methods was not found in the pyrosequencing data. In contrast, the pyrosequencing yielded 28 bacterial species, of which only two species S. *aureus* and *E. coli* were identified by the cultural methods. Str. agalactiae, the third species identified by cultural method was not found in the pyrosequencing data.

CELL MEDIATED IMMUNE RESPONSES IN SHEEP TO ANTI-IDIOTYPIC DNA SPECIFIC FOR *Pestes des petits ruminants* VIRUS HN PROTEIN

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Peste des Petits Ruminants is a highly contagious transboundary animal disease of wild and domestic small ruminants. PPR is endemic in equatorial Africa, the Middle East and the Indian subcontinent. PPR is caused by Peste des petits ruminants Virus (PPRV), a negative sense, single stranded RNA virus belonging to the genus *Morbillivirus* of the family *Paramyxoviridae*. In PPR endemic regions of the world, conventional live attenuated vaccines are being used to vaccinate small ruminants. Although live attenuated PPR vaccines have been in use in different countries, they suffer from the same disadvantage of being heat labile. During eradication campaign, it is desirable to develop a thermostable DIVA vaccine. Our previous work has shown that DNA coding for anti-idiotypic antibody specific for HN protein of PPR was capable of eliciting neutralization activity *in vitro* in the complete absence of viral antigen. The present work reports that vaccinated sheep were able to elicit cell mediated immune responses on anti Id DNA immunization as measured by *in vitro* proliferation of immune T cells and IFN gamma secretion. The responses were comparable to the responses seen with live attenuated PPR vaccine.

INCIDENCE OF *Mycobacterium avium* subsp. *paratuberculosis* IN MEHSANA GOATS

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The study of incidence of JD in goat envisaged detection of MAP antibodies, clinicopathological observations in naturally infected goats, detection of MAP in faeces and MAP genome in faeces and blood samples. More than 70 per cent goats showed the clinical signs loss of appetite, dullness, emaciation, loss of hairs (alopecia), pasty or loose faeces suggestive of Johne's disease. Animals were weak emaciated, had rough hair coat and leathery skin. A total of 203 serum samples were screened for detecting presence of MAP antibodies using i-ELISA kit yielding 62.56 per cent seroprevalence. On the basis of the result obtained, the samples *viz.,* fecal and rectal pinch were collected from 15 goats (that were strong ELISA reactors and fecal microscopy positive, respectively) and were screened for MAP bacilli by Z-N staining. Out of these, 14 (28.00 %) showed the presence of MAP bacilli in direct microscopy examination of faeces, whereas, no goats were found to be positive in rectal pinch smear examination. This was further followed by detection of MAP genome in faeces of direct microscopy positive animals and blood from strong positive ELISA reactors by IS*900* PCR. 1 (7.14 %) out of 14 fecal samples and 6 (12.00 %) out of 50 blood samples were positive for fecal PCR and blood PCR, respectively.

MOLECULAR CHARACTERIZATION OF *Listeria monocytogenes* ISOLATES BY PCR AND PCR-RFLP

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Listeria monocytogenes, a facultative intracellular pathogen, is responsible for severe food-borne infections in humans and can also cause invasive disease in many different animal species. Despite being pathogenic at the species level, *L. monocytogenes* is in fact made up of a spectrum of strains or genotypes with varying pathogenic potential. While many *L. monocytogenes* strains are highly pathogenic and sometimes deadly, others are relatively avirulent and cause little harm in the host. The present study was undertaken to characterize 28 *L. monocytogenes* field isolates obtained from different animal species. The template DNA was prepared by boiling method as per standard protocol. The PCR amplification was carried out for the detection of virulence associated genes viz. *inlA* (*lmo0433*), *inlB* (*lmo0434*), *plcB* (*lmo0205*) and *inlJ* (*lmo2821*) gave an expected product in all 28 field isolates of *L. monocytogenes* as well as the reference strain. The desired amplified product of approximately 255 bp, 146 bp, 261 bp and 611 bp was generated from virulence associated genes *inlA, inlB, plcB* and *inlJ* respectively. PCR-RFLP analysis was carried out for ten field isolates and reference strain having intense band. Digestion of 255 bp PCR products of *inlA* gene with *Rsa*I yielded two fragments of approximately 151 bp and 104 bp while *Hind*III resulted in two fragments of 149 bp and 106 bp on 2% agarose gel. Similarly, Digestion of 611 bp PCR products of *inlJ* gene with *Alu*I yielded four fragments of approximately 274 bp, 161 bp, 150 bp and 26 bp on 4% agarose gel. All the 10 field isolates as well as the reference strain yielded similar RFLP profiles with *Rsa*I, *Hind*III and *Alu*I while none of the sample was digested 611 bp PCR products of *inlJ* gene with *Dpn*I.

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *Streptococcus agalactiae* ISOLATES FOR ANTIBIOTIC RESISTANCE

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Twenty seven *Streptococcus agalactiae* isolates obtained after screening 89 cows for subclinical mastitis were studied for antibiotic resistance. Out of 27 isolates screened for the antibiotic resistance by disc diffusion method, the highest resistance was found for streptomycin (85.1%), where as lowest resistance was found for gentamicin (3.7%). The percentage of resistance against tetracycline, erythromycin, co-trimoxazole, ampicillin and enrofloxacin were 55.5%, 33.3%, 11.1%, 11.1%, and 7.4% respectively. On PCR based detection of resistance genes for tetracycline and erythromycin, three isolates were found to contain gene *tet*O associated with the tetracycline resistance, where as seven isolates were carrying the gene *Erm*B associated with erythromycin resistance. No specific pattern of correlation was observed between the antibiotic resistance profiles by phenotypic and genotypic methods for these two antibiotics.

ONCOLYTIC NDV STRAIN'S V AND W PROTEINS EXHIBIT ANTI-APOPTOTIC PROPERTY IN HELA CELLS

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NDV is a poultry virus widely known for its inherent oncolytic activity in human tumor cells. NDV genome consists of 6 structural genes namely nucleoprotein (NP), phosphoprotein (P), matrix protein (M), Fusion protein (F), Haemagglutinin neuraminidase protein (HN) and RNA polymerase protein (L). Additionally, NDV P protein produces two non structural proteins called V and W by RNA editing at a conserved editing site in homologous host. There is no information available whether these two proteins are produced in non-homologous hosts or not. Open reading frames (ORF) of v and w gene of an oncolytic strain of NDV were cloned in pCDNA 3.1+ mammalian expression vector and transfected to HeLa cells. These expressed proteins in HeLa cells had shown anti-apoptotic property as evident by annexin-V and propidium iodide (PI) uptake, PCNA and fibronectin status, modulation of caspase 8 mRNA level studied by real-time PCR. Affinity of annexin-V with phosphatidylserine was exploited to analyze PS externalization, an early indication of programmed cell death and PI uptake was used to quantitate hypodiploid cell numbers by flow cytometry. PCNA and fibronectin are important tumor markers suggested to indicate cancer cell proliferative states. mRNA of caspase 8 was analyzed by real time PCR which is an initiator caspase involved in extrinsic death receptor mediated apoptosis. There was significant ($P<0.05$) decrease in mRNA of caspase 8 in V and W transfected HeLa cells as compared to vector control.

MOLECULAR CHARACTERIZATION OF VP_2 GENE OF CANINE PARVOVIRUS FROM VACCINAL STRAINS

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Canine parvovirus-2 (CPV-2) has been considered to be an important pathogen of domestic and wild canids. The disease condition has been complicated due to emergence of a number of variants namely CPV-2a, CPV-2b and CPV-2c over the years. The present study was undertaken to genotype CPV vaccinal strains using six commercial vaccines available in Indian market. The VP_2 gene of CPV was amplified by using three sets of primers (*51/R1, F1/R2* and *F2/R3*), which yielded three overlapping fragments of 708 bp, 719 bp and 736 bp respectively, cloned in pTZ57R/T vector and transformed into *E.coli* (DH5α) strain. The clones which were having correct insert were selected for plasmid extraction and then used for sequencing. For each vaccine, three fragments were compiled to get six complete VP_2 gene sequences each of 1755 bp. The consensus VP_2 gene sequences were obtained by SecScape software along with the reference sequence CPV-2 (Accession No. M38245). The obtained consensus sequences (GenBank Accession number JN625219, JN625220, JN625221, JN625222, JN625223 and JN625224) were compared by ClustalW with the CPV reference CPV-2, reference CPV-2a, reference CPV-2b, reference CPV-2c and reported vaccine sequences from different countries viz. South Korea (CPVint vaccine, CPVpf vaccine), China (Vac1VP2, Vac2VP2, Pfizer vaccine), Thailand (VAC M primdog, VAC_P Vanguard vaccine) and field isolates from India, Italy, USA, Taiwan and France. Nucleotide differences (with the CPV-2 reference) shown by Vac1, Vac2, Vac3, Vac4, Vac5 and Vac6 were 7, 5, 7, 4, 12 and 8, and the amino acid differences were 3, 4, 2, 4, 11 and 6 respectively . Vac5 showed maximum number (12) of nucleotide change as compared with reference CPV-2.Vac5 showed maximum (9) unique positions. The 426th position in VP_2 amino acid sequence showed remarkable variability with three different amino acids (E, N and D). The sequences of five vaccine samples Vac1, Vac2, Vac3, Vac4 and Vac6 showed AAT codon at 4064 position of the VP_2 gene, which codes for Aspargine at 426th position indicating them as of CPV type 2, while Vac5 showed GAT codon at the same position which codes for Aspartate indicating it to be of type 2b. Phylogenetic analysis of nucleotide sequences revealed that, Vac5 showed close relation to field samples from Taiwan, France and Ref-CPV2a, Ref-CPV2b, Ref-CPV2c falling under the same cluster. Vac2, Vac3, Vac6 and Vac2VP2 from China, CPVpf, Vac_P (Vanguard), Vac_M (Primdog) and Pfizer vaccines were showing more similarity with each other making one group. Out of six vaccines the maximum distance was observed between Vac1 and Vac5. Field strains from India, Italy and commercial vaccines Vac4, Vac1VP2, CPVint showed close relation while reference CPV-2 strain showed significant genetic distance with all other strains evolved subsequently the world over. Phylogenetic analysis based on amino acid sequences also revealed that, out of six vaccine samples four samples i.e Vac2, Vac3, Vac4 and Vac6 were grouped together, with Vac3 and Vac4 subgrouping together. Vac5 was placed in cluster I, while Vac1 in cluster III, indicating their more genetic distance with the other vaccines.

MOLECULAR CHARACTERIZATION OF VIRULENCE ASSOCIATED GENES OF *Streptococcus agalactiae* ISOLATES OBTAINED FROM BOVINE SUBCLINICAL MASTITIS CASES

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In the present study, 27 *Streptococcus agalactiae* isolates obtained culturally from bovine subclinical mastitis cases were confirmed by PCR. The template DNA from 27 *Str. agalactiae* isolates were obtained by boiling method which yielded good results and all the 27 isolates were confirmed as *Str. agalactiae,* as they yielded an expected amplification product of 586bp using *Str. agalactiae* primers Sag 432/Sag 1018 specific for the 16S rRNA. After confirmation, molecular characterization of virulence associated genes viz. *bca, scp*B, *rib*, *lmb* and *cyl*E were studied using gene specific primers. The expected product size for *bca, scp*B, *rib*, *lmb* and *cyl*E genes were 183, 255, 369, 572 and 248 respectively. The study revealed that out of 27 isolates of *Str. agalactiae,* none of the *Str. agalactiae* isolate was found to carry *bca* gene. However, 8 isolates were found positive for *lmb* gene, 7 isolates for *rib* gene and 6 isolates for *scp*B and *cyl*E genes each.

CAPSULAR TYPING OF *Staphylococcus aureus* ISOLATES FROM BOVINE MASTITIS CASES BY TRIPLEX PCR

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Bovine mastitis is an infectious disease associated with massive financial losses to the dairy sector. *S. aureus* is the most prevalent and contagious pathogens of intra-mammary infections in dairy cattle. One of the virulence factors of *S. aureus* is the capsular poly-saccharide (CP). Although 11 CP types are described, only two (CP5 and CP8) are reported to be clinically relevant from various geographic locations. The present study was carried out to identify the CP types of 231 *S. aureus* isolates from bovine mastitis cases from different geographical locations in India, by PCR. Subsequent to confirmation of the isolates as *S. aureus* by biochemical tests and PCR, uniplex followed by triplex PCR were standardized for the identification of CP1, CP5 and CP8 types, using previously published (CP5 and CP8) or newly designed (CP1) primers. The triplex PCR differentiated each of the CP types as verified when tested against reference type strains of *S. aureus,* coagulase-negative staphylococci and non-staphylococcal species including *Streptococcus agalactiae* and *E. coli*. Among the bovine mastitis-associated *S. aureus* isolates tested, 213 (92.2%), 14 (6.06%) and 4 (1.79%) belonged to CP5, CP1 and CP8 types by triplex PCR, indicating the predominance of CP5. This finding emphasizes the need for targeting CP5 types for the development of therapeutic or prophylactic measures for the control of bovine mastitis in India.

DEVELOPMENT OF MULTIPLEX PCR FOR THE RAPID DETECTION OF MASTITIS-ASSOCIATED *Staphylococcus aureus*

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Staphylococcus aureus is the major pathogen associated with bovine mastitis, one of the most economically important infectious diseases of dairy cattle worldwide. Since DNA-based assays for species-specific identification are rapid, we explored and standardized a multiplex PCR in order to improve the accuracy and the rapidity of tests for the diagnosis of *S. aureus* infections of the udder. To this end, conventionally identified isolates were reconfirmed as *S. aureus* by PCR for fragments of *23S rRNA*, *fibrinogen binding protein* (*fib*) and *thermonuclease* (*nuc*) genes. The multiplex PCR was validated to be specific to *S. aureus* by testing the DNA from more than 350 staphylococcal strains, including reference and wild coagulase-positive and -negative strains isolated from bovine mastitis samples as well as non-staphylococcal species. Further, this easy and rapid method allowed unambiguous identification of *S. aureus* from cultures mixed in the laboratory. We are in the process of evaluating the application of this multiplex PCR for detecting the presence of *S. aureus* by direct analysis of milk samples. This test should help in the rapid diagnosis and reconfirmation of bovine mastitis infections by *S. aureus*.

STUDY OF IMMUNE RESPONSE IN BUFFALO PERIPHERAL BLOOD MONONUCLEAR CELLS BY BOVINE HERPES VIRUS 1

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Bovine herpes virus 1, a member of *alphaherpesvirinae*, is the aetiological agent of infectious bovine rhinotracheitis, infectious pustular vulvo-vaginitis, abortions and fatal neo-natal systemic infections and it also predisposes animals for secondary bacterial infections leading to pneumonia and death. After primary infections, animals elicit immune response through production of various cytokines. Cytokines play essential role in limiting the virus infection, its spread and killing of virus infected cells through a cascade of mechanisms. In this experiment, peripheral blood mononuclear cells (PBMCs) of seronegative buffalo were infected with 10 MOI of live and mock virus. At different time intervals, various pro-inflammatory molecules and other cytokines were studied by real time PCR. At early infection, expression of pro-inflammatory molecules like Rantes, TNF-α and IL-1β was higher in virus infected PBMCs. The expression of IL-12p40 was also high and IL-12p40 probably induced $CD4^+$ Th0 cells and differentiated it into $CD4^+$ T_h1 cells for expression of IFN-γ and IL-2. The expression of IL-2 and IFN-γ remained higher till 48 hrs. Expression of IL-10 was also studied since IL-10 is a marker of Th2 type of immune response. Its expression was found down regulated in virus infected cells till 48 hrs. So this cytokine did not affect the immune response mediated by macrophages. As a whole, dominance of Th1 type of immune response over Th2 type was generated in *in vitro* to PBMC for clearing virus infection.

ELUCIDATION OF PEPTIDE STEREOCHEMISTRY BY CIRCULAR DICHROISM SPECTROSCOPY

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The design and conformation of Peptides has assumed considerably interest in the area of Biotechnology in recent years. Peptides segments of native functional proteins, can like play a significant role as that of parent molecule resulting in different technologies such as delivery systems required to transport proteins and particularly nucleic acids. Later molecules as DNA/RNA essentially require delivery systems as they can not evade cell membrane wall to execute their function as DNA vaccine or antigene therapeutics. A peptide RATH which has cell penetrating ability was designed and synthesized by Fmoc-chemistry and purified by RP- HPLC. In order to derive the co-relation with the functional ability of this peptide to deliver the cargo molecules its structure in solution needed to be elucidated, which was investigated using circular dichroism spectroscopy in different solvents such as water, trifluoroethanol(TFE)in water, phosphate buffer and SDS solution. Self assembling of peptide to form nanoparticles was also observed in near-UV CD spectra of peptide as a function of concentrations. Interaction of peptide with cargo DNA was observed in changes of DNA duplex CD spectra on titration with increment of peptide concentrations. As such peptide adopted predominantly â structure in water and biological buffers and with increasing concentrations of TFE in water resulted in inception of helical conformation as seen in far-UV CD spectra. Under membranogenic environment provided by TFE the peptide could adopt helical conformation required to cross the cell membrane. The quantitation of different secondary structure of RATH peptide revealed maximum â type (70%) of conformations in water. At 40% TFE concentration it has 3% á helical content and in 60% TFE concentration â content dropped to 67.5%. The environment like TFE (with decreased polarity) suggests that peptide when experience non polar environment like membrane in cell can adopt á helical structure necessary for transmembrane domain to traverse across the cell membrane. The mechanism of penetration across the cells is poorly implicit so that necessitate to study the active conformational changes in peptide which was studied by CD spectroscopy that provides information on solution structures qualitatively as well as quantitatively to draw correlation with exhibited function of peptide so that improvement in the function of molecules can be undertaken by analogs designing of delivery technology.

ANTI-FLAGELLIN ANTIBODY RESPONSES ELICITED IN MICE AGAINST *SALMONELLA* TYPHIMURIUM

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Salmonellosis is the disease complex of man and animals caused by various serovars of *Salmonella enterica* subspecies *enterica. Salmonella* spp. are facultative intracellular pathogens capable of causing localized and systemic disease of significant morbidity and mortality. In present study we investigated the immune response elicited in mice following s/c immunization with purified flagellin derived from *Salmonella enterica* serovar Typhimurium. Humoral immune response in mice was analyzed by indirect ELISA resulted in a significantly greater antibody response in flagellin immunized group than in the control group. It was further confirmed by western blotting which showed the immunoreactivity against sera obtained from flagellin immunized group on 21st day post immunization. Bacterial colony count in spleen of mice vaccinated with flagellin and challenged i.p with *Salmonella* showed complete clearance of the bacteria. Thus, it could be concluded form the above study that bacterial flagellin has the capacity to effectively trigger a protective immune response after s/c immunization but further efforts are needed to explore the complete picture of immunity against bacterial flagellin.

IDENTIFICATION OF BOVINE HORN CANCER SPECIFIC HOMING PEPTIDE BY PHAGE DISPLAY TECHNIQUE

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The wealth of genome/proteome data gathered in recent time helped in realizing the potential of peptide and nucleic acids as new generation therapeutics for cancer. However, the bottle necks remains with effective intelligent delivery of these molecules to have the therapeutic action at desired site, without losing them in biological pathway. To circumvent this obstacle there is a need to identify homing peptides which can act as a ligand on which the new generation therapeutics can be attached either covalently or non-covalently to achieve a targeted site specific drug delivery. Phage display library consists of approximately $2.8x10^9$ random sequences i.e. $2.8x10^9$ individual phages which are genetically engineered to express 7 mer amino acid sequences on their coat protein are widely used as a tool to identify tissue specific ligand. In this study, PhD-7 phage display library was used to identify bovine horn cancer specific ligand. For this, subtractive panning was conducted on different nontargeted bovine cell like PBMC and MDBK and buffalo liver, kidney and spleen for the generation of specific homing peptide for horn cancer cells. Three rounds of positive biopanning were conducted on horn cancer cell to enrich specific phages which are homing to horn cancer cells. Conserved amino acid sequence after 3rd round of panning can be considered as putative homing peptide. In this study we have identified four peptides i.e. GVQIMGRGGG, ALAHRILGGG, YSSKHIAGGG, STFTKSPGGG with 72.8%, 18.75%, 6.25% and 3.12% occurrence respectively (n =32). These peptides were FITC labeled at N-terminal end to test their homing ability to horn cancer cell by flow cytometry (FACS). These FITC labeled peptides were specifically binding to horn cancer cells (upto 70%) in comparison to nontargetd bovine cells (less than 15%) which indicates their homing ability to horn cancer cells.

NDV INDUCED APOPTOSIS OF HeLa CELLS IS MEDIATED BY INTRINSIC (MITOCHONDRIA) PATHWAY OF APOPTOSIS

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NDV, an avian virus is classified in the genus Avulavirus of Paramyxoviridae family. NDV is an enveloped virus containing negative-sense, single stranded RNA genome which codes for six structural and two non-structural proteins. NDV infection also results in immunosuppression in birds due to induction of apoptosis in lymphoid cells. Apoptosis is a genetically controlled process involved in embryonic development and has been implicated in the pathogenesis of many infectious diseases caused by viruses. NDV induces the activation of caspase dependent extrinsic and intrinsic apoptotic pathways. To elucidate the molecular mechanism involved in NDV induced apoptosis, HeLa cells grown to 80-90% confluency were infected with NDV and cells were harvested at 24, 48h and 72h p.t. and various cytochemical assays were performed to detect the role of various apoptotic pathways and proteins. The results of flow cytomety analysis to detect the change in mitochondrial membrane potential using JC-1 dye, western blot to detect efflux of cytochrome c. Flow cytometry, western blot and IFAT to detect activation of caspase 9 (initiator caspase of intrinsic pathway) and flow cytometry and western blot to detect activation of caspase 3 revealed that the apoptosis of HeLa cells induced by NDV is mediated by the intrinsic/mitochondrial pathway.

DIFFERENTIAL EXPRESSION OF SIX TOLL-LIKE RECEPTORS (TLRs) mRNA IN TISSUES OF *CYPRINUS CARPIO* (KOI CARP)

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All organisms possess some form of defense mechanism against infection, although innate immune system relay on a set of pattern recognition receptors (PRRs). Toll-like receptors (TLRs) act as first line host defense response to conserved molecular patterns broadly shared by microbes known as Pathogen-associated molecular patterns (PAPMs) serves as a trigger for the immune system and end in up regulation of proinflammatory cytokine and modulation of antigenic-specific adaptive immune responses. Although the expression profiles of TLRs have been extensively studied in zebra fish (*Danio rerio)* and puffer fish (*Takifugu rubripes),* information on TLRs and their expression in tropical fishes is scanty. Hence, this study was undertaken to identify TLRs (TLR2, TLR3, TLR4,TLR7, TLR9 and TLR22) and also determine the expression profiles of TLR mRNA by semi-quantitative reverse transcriptase PCR (RT-PCR) in various organs of koi carp *(Cyprinus carpio)* namely, skin, gill, brain, intestine, liver, spleen and kidney. Our partial sequences of the TLRs had 98-93% nucleotide identity with the fish TLRs sequences available in GenBank. Semi-quantification of the expression levels of the TLR mRNA was done using densitometry analysis of band intensities. All the TLR mRNAs (TLR2, TLR3, TLR4, TLR7, TLR9 and TLR22) were expressed in high amount in liver, intestine and skin. The apparently normal expression pattern and levels of each TLR mRNA in different tissues studied, confirming that it is the first line of defense that protects fish against the pathogens in the microbe rich aquatic environment.

CLONING AND EXPRESSION OF F GENE IN EUKARYOTIC EXPRESSION VECTOR

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Newcastle disease (ND) is one of the important contagious disease affecting chickens worldwide. NDV belong to *Avulavirus* of the family paramyxoviridae in the order of Mononegevirales. It is an enveloped RNA virus having non segmented, single stranded, negative sense, 15186 nucleotides long RNA genome which encodes six structural and two non structural proteins. The viral envelope is composed of a lipid bilayer and two surface glycoproteins, namely HN (haemagglutinin-neuraminidase) and F (fusion). Aim of present study was to clone and express F gene and use as a recombinant DNA vaccine. Total RNA was extracted from cell culture fluid by trizol method. Then cDNA was synthesized by using random hexamer primer. The 1675 bp of F gene was amplified using specific primers by RT-PCR and cloned into pTZ 57R/T cloning vector. The clone was confirmed by restriction digestion analysis using *Kpn*I and *Apa*I restriction enzymes and orientation was confirmed by colony PCR using T7 promoter sequencing and gene specific forward primers. Further, the gene fragment was subcloned in eukaryotic expression vector pcDNA 3.1(+) and confirmed by restriction digestion as described above. The recombinant pcDNA 3.1 having F gene was checked for its ability to express IFN-γ *in vitro* in cell culture and expression was confirmed by RT- PCR in total RNA as well as protein level using IFAT, IPT and western blot using NDV specific antiserum. All these experiments confirmed that F gene cloned in recombinant in pcDNA3.1.nd.F is functionally active. The recombinant construct is being evaluated as DNA vaccine against ND.

A COMPARISON OF IMMUNE RESPONSE TO ADJUVANT AND CARRIER (BCG-PPD) COMBINED VACCINE WITH THAT INDUCED BY ALUM ADJUVANTED VACCINE AGAINST *Pasteurella multocida*

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Haemorrhagic Septicaemia (HS) is an infectious disease of cattle and buffaloes caused by *Pasteurella multocida* serotype B:2. The disease causes huge economic losses worldwide. The present study was conducted for comparison of immune response induced by the adjuvant-carrier (BCG-PPD) combined HS vaccine with that induced by Alum adjuvanted HS vaccine against *Pasteurella multocida* infection in rabbits. On day 7th, 14th, 21st, 30th, 45th, 60th, 90th, 120th, 150th and 180th, the mean $\log_{10}$ values of antibody titres in rabbits sera by microagglutination test (MAT) were 1.2±0.17, 1.5±0.17, 1.8±0.17, 2.1±0.17, 2.4±0.17, 2.505±0.0, 2.50±0.0, 2.2±0.0, 1.92±0.0, and 1.62±0.0, respectively in case of adjuvant-carrier (BCG-PPD) combined HS vaccinated and 1.10±0.17, 1.40±0.17, 1.7±0.17, 2.0±0.17, 2.3±0.17, 2.505±0.0, 2.40±0.17, 2.05±0.21, 1.75±0.21 and 1.45±0.21, respectively in case of Alum adjuvanted HS vaccinated rabbits. Differences in $\log_{10}$ values of antibody titres on these intervals were found to be nonsignificant by unpaired't' test. The IHA mean $\log_{10}$ titres in rabbits on day 7th, 14th, 21st, 30th, 45th, 60th, 90th, 120th, 150th and 180th day were 1.2±0.17, 1.5±0.17, 1.8±0.17, 2.02±0.17, 2.3±0.17, 2.505±0.0, 2.505±0.0, 2.204±0.0, 1.902±0.0 and 1.60±0.0, respectively in case of adjuvant-carrier (BCG-PPD) combined HS vaccine and 0.89±0.17, 1.20±0.17, 1.47±0.15, 1.90±0.0, 1.90±0.0, 2.20±0.0, 2.51±0.0, 2.20±0.0, 1.90±0.0 and 1.45±0.21, respectively in case of Alum adjuvanted HS vaccinated rabbits. Differences in mean $\log_{10}$ values of antibody titres on these intervals were non significant by unpaired 't' test except on day 45th where it was significantly ($p<0.05$) higher in case of BCG-PPD vaccinated animals. Since IFN-γ secretion is an indicator of lymphocyte activation, and has important consequences for the course of immune response, the levels of IFN-γ in blood plasma of vaccinated calves were tested. The mean concentrations (pg/ml) of IFN-γ on day 0, 7th and 14th after vaccination in calves were 0.4±0.06, 81.11±21.62 and 198.89±49.36 in case of adjuvant-carrier (BCG-PPD) combined HS vaccinated and 0.44±0.15, 84±21.5 and 387.78±59.11 in case of BCG vaccinated calves, respectively. The differences were non-significant on day 0 and 7th but significantly ($p<0.05$) higher in case of BCG-PPD vaccinated animals on day 14th by unpaired 't' test. The mean concentrations (pg/ml) of IFN-γ in Alum adjuvanted HS vaccinated cow calves on day 0, 7th and 14th after vaccination were 0.44±0.08, 47.33±15.8 and 99.67±35.4, respectively which was non significantly different on day 0 but significantly ($p<0.05$) higher on day 7th and 14th in adjuvant carrier (BCG-PPD) combined HS vaccinated animals by unpaired 't' test. Hence the adjuvant carrier (BCG-PPD) combined HS vaccine gives an immune response comparable to that of alum adjuvanted HS vaccine. In addition, helper immune response is found to be higher in adjuvant carrier (BCG-PPD) combined HS vaccinated animals than the alum adjuvanted HS vaccinated animals alone.

INCIDENCE OF *Johne's* DISEASE IN MEHSANI AND SURTI GOATS OF GUJARAT

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Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of chronic granulomatous bacterial infection in animals known as Johne's disease (JD) or Paratuberculosis. Diagnosis of Paratuberculosis in goats was carried out by applying different conventional and serological methods. A total of 219 goats were screened. Percent positivity by faecal smear examination, rectal pinch, Delayed Type Hypersensitivity (DTH) was 9.2% (7/76), 0% (0/27) and 21.9% (27/123), respectively.

TEARS PRODUCE HIGHER ANTIBODY TITRE THAN SERUM FOLLOWING NDV VACCINATION

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The present investigation was carried out to see the effect of NDV"F" strain vaccination on antibody titre produced in serum and tears separately. Forty day- old chicks were divided into two groups of 20 chicks each. One group of 20 chicks was vaccinated intra ocularly on day 7 with NDV "F" strain vaccine by putting one drop of vaccine in each eye while the other group of 20 chicks was kept as unvaccinated control. Serum and tears samples were collected from 4 chicks each from both the groups on days, 0, 3, 5, 7 and 10 post vaccination. The chicks were sacrified immediately after blood collection and their trachea and conjunctiva were collected for virus isolation. It was interesting to record that the antibody titre in tears was 2 to 3 times higher than in the serum on day 5, 7 and 10 post vaccination. Similarly virus could be recovered from trachea only up to day 5, where as virus persisted in the conjunctiva up to day 7 post vaccination. None of the unvaccinated chicks yielded any antibody or the virus. Thus tears and the conjunctiva could be considered as a good specimen to be collected for the diagnosis of NDV infections.

DEVELOPMENT OF A TAQMAN PROBE REAL TIME MPCR FOR QUANTIFICATION OF BUFFALO X- AND Y-CHROMOSOME BEARING SPERMATOZOA IN SORTED SEMEN

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Semen sexing, involving the separation of X- and Y-chromosome bearing sperms, implies its application in assisted reproductive technologies (ART). At present, the only proven method for semen sexing is by flow cytometry, based on difference in DNA content. An accurate and easy method for finding the relative percentage of X- and Y- chromosome bearing spermatozoa in semen is essential for reanalysis of sorted semen. Here a new method for quantification of X- and Y- bearing spermatozoa in buffalo semen sample using TaqMan probe multiplex PCR is presented. Primers for sex specific genes SRY (Y- chromosome specific) and PLP (X-chromosome specific) were designed from the conserved regions of reported sequences. Initial standardization of real time PCR was done using SYBR Green chemistry. TaqMan Probes for real time PCR of SRY and PLP genes were designed. TaqMan real time assays were done using male genomic DNA and female genomic DNA in separate reactions. The primer efficiencies were found to be 104.4% for SRY and 93% for PLP genes. The Y- chromosome specificity of SRY gene and X- chromosome specificity of PLP was established as there were both SRY and PLP amplification in tube containing male genomic DNA (XY karyotype) and only PLP amplification in tubes containing female genomic DNA (XX Karyotype). The percentage of X- and Y- chromosome in 10 fold serially diluted genomic DNA from male buffalo blood and unsorted semen was carried out and was found to be ~50%. Thus it is clear that the new method based on TaqMan probe real time multiplex PCR can be accurately used for the quantification of X- and Y-chromosome bearing spermatozoa in buffalo semen. The assay can be directly used without the standard curve just by calculating the % spermatozoa corresponding to the change in Ct values of *PLP* or *SRY* amplification which are correlated with the X- or Y-chromosome bearing spermatozoa in unsorted and sorted semen.

STUDIES ON MOLECULAR HETEROGENEITY AMONG THE FIELD ISOLATES OF *Pasteurella multocida* FROM BOVINES

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The knowledge of clonality and heterogeneity of *Pasteurella multocida* strains is essential for efficient vaccine deployment and to trace epidemiological relationships of the outbreaks. Therefore, validation of different techniques and then typing of the isolates from clinical as well as outbreak cases is required. The present study was undertaken for molecular characterization of 19 *P. multocida* bovine isolates and P52 vaccine strain using random amplified polymorphic DNA (RAPD) analysis, repetitive extragenic palindromic (REP) sequences analysis and single strand conformation polymorphism (SSCP). RAPD analysis using random primers (OPG 10 and OPG 13) could differentiate the isolates into 7 different types each whereas using REP analysis the isolates could be grouped into 8 different types. SSCP analysis produced 10 different types from the amplified *mul* gene of the isolates. The calculated discriminatory index of RAPD OPG 10, OPG 13, REP and SSCP were 0.85, 0.75, 0.86 and 0.89 respectively. The SSCP analysis had the higher discriminatory ability as compared to RAPD or REP analysis. The results indicated that the molecular typing techniques could be employed to differentiate the bovine strains of *P. multocida* for epidemiological studies.

CYTOKINE PROFILE OF BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) IN RESPONSE TO *Pasteurella multocida* B:2 STRAIN P_{52}

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The present study was planned to comprehend the molecular mechanism and immune response entangled in pathogenesis of disease caused by *Pasteurella multocida* B:2 by quantitation of cytokine gene response in bovine PBMCs *in vitro* using real time PCR assay. The expression of genes encoding the cytokines IL-1α/β, IL-2, IL-4, IL-8, IL-10, IFN-γ and TNF-α was monitored in cells stimulated with *Pasteurella multocida* organism in dose dependent manner while unstimulated cells served as control and mRNA was extracted from both stimulated and non stimulated cells. The efficiency sensitivity and linearity of Q-RT-PCR reactions were examined using pooled cDNA samples of different concentrations ranging from 500ng to 32.5ng. The relationship between Ct and log copy number of cDNA for all genes was linear with R2 ranging from 0.96-0.99, indicating that Ct values changed proportionally to serial dilutions of the samples. The study indicated the significant upregualtion of pro-inflammatory cytokine TNF-α, IFN-γ, IL-8 and IL-1α/β in treated groups of PBMCs. The significant elevation of IL-8 transcriptional level most likely indicates the important role in development of characteristic neutrophilia in disease progression. The mRNA expression of IFN-γ was found to be significantly increased in PBMCs treated with whole cell bacterium.

MOLECULAR CHARACTERIZATION OF INCLUSION BODY HEPATITIS-HYDRO PERICARDIUM SYNDROME VIRUS IN BROLIER CHICKEN

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IBH-HPS is an important, recently emerged disease of poultry, particularly of 3 to 6 week of broilers birds, characterized by its sudden onset with high mortality. In present study attempts were made to molecular characterization of Avian Adenovirus causing inclusion body hepatitis-hydropericardium syndrome in chicken. During the study 6.65% mortality was recorded around Anand district, Gujarat due to inclusion body hepatitis-hydropericardium syndrome in broiler chicken. Samples for virus characterization were collected from field cases of broiler birds presented at department of pathology for PM examination. Liver and heart revealed lesion of inclusion body hepatitis. Two Liver sample were collected in glycerol saline from the birds came from Aman and Janki poutry farm for the postmortem in the Departmentof pathology,Veterinary college, Anand (Gujarat). Extraction of viral DNA from infected liver tissues was done as per the method of Meuleman et al., (2001) with minor modifications. The amplified PCR analyzed by agarose gel electrophoresis indicated a DNA fragment of approximately 890 bp using the primer hexonA forward and hexonB reverse. PCR assay revealed presence of IBH-HPS virus. DNA Sequencing was carried out of the PCR product obtained from the sample of AMAN and JANKI poultry farm and using the blast programme of NCBI. The obtained nucleotide sequence of both samples was found to have 94% nucleotide sequence identity with fowl adenovirus 11 isolate 1047 and Fowl adenovirus 11 isolate FAdV-11/Brazil/2006/USP-0. Also Fowl Adenovirus D of different isolates show 94 to 95 percentage of identity with both the sample. On phylogenetic analysis using Clustal W program showing 3 major group like upper, middle and lower respectively. In the minor branch of upper group the AMAN and JANKI isolates were found to group with Fowl adenovirus 12 strain 380 and Fowl adenovirus 11 strain C2B. AMAN and JANKI isolates indicating a new fowl adenovirus genotype.

DETERMINATION OF DNase PRODUCE BY *Staphylococcus aureus* ISOLATES FROM CLINICAL CASES OF MASTITIS

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Deoxyribonuclease (DNase) is known to catalaze depolymerization of DNA. The presence of DNase activity is often used as surrogate marker for the identification of *Staphylococcus aureus* particularly in milk samples. For DNase production, test was carried out using DNase agar medium as describe by Deighton & co-worker in 1988. DNase was found in 65 (81.25) isolates out of 80 (20 isolates each from sheep, goat, cattle and buffaloes) *Staphylococcus aureus* isolates. DNase was found in 14(70%), 16(80%), 17(75%) and 18(90%), out of 20 *Staphylococcus aureus* isolates each from sheep, goat, cattle, and buffaloes, respectively. Higher percentage of DNase positive isolates were found in buffaloes and lower percentage of DNase positive isolates were found in sheep.

EARLY DIAGNOSIS OF *Peste des petits ruminants* BY VIRAL ANTIGEN DETECTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Peste des petits ruminants (PPR) is an acute and highly contagious transboundary viral disease of small ruminants. In present study, PPR viral antigen was detected from peripheral blood mononuclear cells (PBMCs) using SYBR green based real-time PCR targeting Nucleoprotein (N) gene of PPR virus. Goats were experimentally infected with PPR vaccine (Sungri/96 strain) and virulent (Izatnagar/94) viruses separately. Unclotted blood samples were collected on alternate day up to 21 days post infection (dpi) or till survival of animals. As expected, a typical signs of PPR were observed from day 6 post infection in virulent virus infected goats; whereas no signs could observe in vaccinated goats. Blood samples were subjected for PBMCs isolation using density gradient centrifugation. Isolated PBMCs were subjected for N-Gene based semi-quantitative real-time PCR against known recombinant plasmid of the same gene. PPR viral antigen could be detected from day 3rd to 12th with maximum copy number on day 7th post infection. Unlike virulent infected goats, no antigen could trace out in PBMCs of vaccinated goats. It has supplemented the previous finding that PBMCs are one of the replication sites for the virulent PPR virus. N-gene based real time-PCR is proved to be highly sensitive and suitable alternative over other known tests for early detection of viral nucleic acid from the samples. This study tells us how early we can detect the PPR infection even before onset of clinical symptoms. In addition to that, using this method one can determine the extent of viral load or infection in animal. Therefore, with the help of this test, infected/suspected animals can be detected at the earliest in face of outbreak, resulting in better control of disease.

RETROSPECTIVE STUDY ON COMBINED PREVALENCE OF *Peste des petits ruminant* AND BLUETONGUE IN SMALL RUMINANTS

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Peste des petits ruminant (PPR) is an acute and highly contagious viral disease of sheep and goats whereas; bluetongue (BT) is an infectious, non-contagious, arthropod-borne viral disease primarily of sheep but it may affect all domestic and wild ruminants. Both PPR as well as BT are endemic in India and are major constrain of small ruminant health and production. In the recent past there were several instances wherein simultaneous prevalence of both PPR and BT was noticed in affected flocks. Cases of mixed infection of PPRV and BTV are now becoming increasingly common in small ruminants and are being tested positive for antibodies against both PPRV and BTV. In this study, prevalence of both PPRV and BTV antibodies were undertaken to address the mixed infection. A retrospective study on mixed PPRV and BTV infection using serum samples tested by respective ELISA for antibody was carried out. A total of 471 serum samples were screened between 2003-2009 from serum repository of Rinderpest laboratory, Division of Virology for prevalence of mixed infection. A total of 243 samples were found positive for both PPR and BTV antibodies by PPR competitive and bluetongue indirect ELISA respectively. The percent positivity was more than fifty percent (51.59%) which indicated that, the animals from which samples were taken had infection of both the viruses. The cases of mixed infection of PPRV and BTV are presumably rising because of the endemicity of both the diseases. The exact synchrony of these diseases and the interactive sequelae are yet to be proved especially the rise in the number of clinical cases of BT in small ruminants results probably due to immunosuppressive effects of PPR.

ULTRASONOGRAPHIC EVALUATION OF FEW PARAMETERS FOR UTERINE INVOLUTION IN POSTPARTUM MEHSANA BUFFALOES

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Eighteen pluriparous Mehsana buffaloes calved recently and maintained at Livestock Research Station of university were selected randomly to study the effect of PGF2α on uterine involution by using real time ultrasonography consisting of trans-rectal probe of 7.5 MHz. The animals were allotted to three groups; Group-I (control), consisted of recently calved buffaloes; Group-II also included recently calved buffaloes but received intramuscular injection of Iliren 5 ml on day 5th postpartum, whereas, Group-III had the animals completed 2 months postpartum without treatment. The ultrasonic evaluation of uterus and cervix was done at 2 days interval from day 5 onwards up till day 21 postpartum, and subsequently followed at 5 days interval up to day 40 following parturition in animals of Group-I and II. The ultrasonography of cervix, uterus, middle uterine artery and caruncles during the period of uterine involution depicted the images of different echogenicity. The echogenicity of involuting cervix was recorded as hyper echoic wall with anechoic lumen; similarly uterus was imaged as hyper echoic wall with anechoic lumen having hypo echoic spots. The diameter and thickness of wall of cervix reduced during the period from 5-40 days postpartum in Group-I and II, but at day 40 postpartum it remains significantly ($p<0.05$) more in these groups in comparison to Group-III. Similarly, the gravid and non-gravid uterus in Group-I and II reduced in diameter and thickness in its wall, however, the differences were non-significant for diameter while thickness was demarkably greater on day 5 and 30 postpartum for the gravid horn. On the other hand, the non-gravid horn reduced in thickness at faster rate in Group-I on day 5, 7, 9, 11, 13, 15 and 17 postpartum. In comparison to Group-III, the uterine diameter was significantly ($p<0.05$) less and uterine thickness was greater at day 40 postpartum in buffaloes of Group-I and II. The middle uterine artery and caruncles did not show any demarkable change from day 5 to 11 postpartum in Group-I and II except the caruncular width which remained more in buffaloes treated with Iliren. The findings envisage that the treatment of Mehsana buffaloes with Iliren advocated intramuscular at the dose rate of 25 mg on day 5 postpartum didn't enhance the rate of uterine involution.

SEROPREVALENCE OF BTV ANTIBODIES IN BUFFALO OF GUJARAT STATE

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Bluetongue (BT) is an infectious, non-contagious, arthropod borne viral disease of domestic and wild ruminants and has been reported from most of the tropical and subtropical regions of the world. A serological survey for Bluetongue antibodies was carried out by c- ELISA in Buffaloes in North and South Gujarat regions. Location wise, area wise, age wise and breed wise seroprevalence was studied. Out of 110 serum sample screened from buffalo, 65(59.09%) showed the positive reaction to Bluetongue virus antibodies. Sera were tested from 2 different locations of two Regions. Maximum Seroprevalence of 67.77% was recorded at Livestock Research Station Sardarkrushinagar Followed by Livestock Research Station Navsari(20.00%). North Gujarat Region showed highest Seroprevalence of 67.77 per cent followed by South Gujarat (20.00%). Maximum Seroprevalence of 68.75 per cent was found in the age group of > 6 years followed by 3-6 years (58.13%), 1-3 years (57.14%) and the 1year (50.00%). Maximum Seroprevalence of 67.77% was recorded in Mehsani breed followed by Surtibreed (20.00%).

THE HYPO-OSMOTIC SWELLING TEST : AN ASSAY OF SPERM CELL MEMBRANE INTEGRITY AND QUALITY OF COCK NEAT SEMEN

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A total of six 38-weeks old healthy Gramapriya hybrid cocks were kept in individual California layer cages (three tier systems) and fed *ad libitum* with layer mash and water. The temperature was between 8.7 and 35.2 °C, relative humidity varied between 18.6 and 87.6 % and no additional light was supplied throughout the study. Semen from all the experimental cocks was collected by abdominal massage method two times a week in morning between 8:30 a.m. to 9:30 a.m. during November 2008 to February 2009. Hypo-osmotic swelling test was use to check out the sperm cell membrane integrity which helps in viability of sperm. A total 114 neat semen samples from six healthy Gramapriya hybrid cocks was subjected to Hypo-osmotic swelling test (HOST) incubating at 37°C for 60 minutes in a Sodium citrate fructose solution with an osmolarity of 100 mOsm/kg. 0.1 ml semen sample for each test and control were used and rest of used for other physical characteristics. Smears were viewed under oil emulsion lens (100 X) of a simple light microscope, a proportion of cells with intact membrane were found swollen characterized by coil tailed and twisted head. The mean value of plasma membrane intact spermatozoa (HOST reactive spermatozoa) was 92.98 ± 0.26 percent. Statistical analysis of the data showed highly significant ($P < 0.01$) and negatively correlated with abnormal spermatozoa count and ejaculate volume while significant ($P < 0.05$) and positive correlation of HOST was found with sperm concentration and individual motility. A characteristic feature observed, was the twisting and swelling of head of the spermatozoa in HOST test.

MOLECULAR CHARACTERIZATION OF BLUETONGUE VIRUS ISOLATES FROM GOATS AND *Culicodes* VECTOR

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Bluetongue is an infectious, non-contagious arthropod born viral disease of ruminants which occurs almost worldwide between latitudes 35°S and 50°N. The causative agent Bluetongue virus (BTV) is a prototype virus of an orbivirus belonging to the family Reoviridae. The biting midges of the genus *Culicoides* is the vector of BTV. RNA-PAGE is used for analyzing the segmented RNA genome of BTV after staining with Silver Nitrate Stain. The present study was undertaken for molecular characterization of Bluetongue virus isolated in BHK-21 cell line from two aborted goat foetus spleen, *Culicoides* from Livestock research station and Jasdan by using RNA-PAGE and RT-PCR. These isolates were processed for RNA extraction using TRI Reagent. RNA-PAGE revealed a classical 10 segments migration pattern in all 4 isolates. The viral RNA was reverse transcribed with M-MuLV-RT to generate cDNA template. RT-PCR was performedusing NS1 gene specific primer. The amplified products were electrophoresed on 1.5 per cent agarose gel and visualized using gel documentation system (DNR Bio-imaging systems). The size of the PCR amplicons was analyzed by comparing them with that of the 100 bp DNA molecular weight marker using Gel quant computer software. All isolates revealed 274 bp amplicons with NS1 gene specific primer. Similarly, nested PCR also yielded a DNA band of 101bp size.

SEROPREVALENCE OF BTV ANTIBODIES IN SHEEP OF GUJARAT STATE

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Bluetongue (BT) is an infectious, non-contagious disease of domestic and wild ruminants. Bluetongue virus (BTV) causes severe disease in sheep, which is transmitted by insect vector belonging to *Culicoides* spp. It is particularly a viral disease of sheep, occasionally affecting cattle, buffaloes, goats, camels and other wild ruminants. A serological survey for Bluetongue antibodies was carried out using c- ELISA in sheep in North Gujarat, Kachchh and Saurashtra regions.Out of 499 sera samples tested from sheep 211(42.28%) showed positive reaction to Bluetongue virus antibodies. Sera were tested from11 different location of three Regions. Maximum Seroprevalence of 68.96 per cent was recorded at Patan Panjarapole followed by sheep breeding farm, Naliya (60.00%), GSWDC Jasdan (47.82%), GSWCD Lyza (47.16%), GSWDC Aseda (46.66%), Sheep breeding farm, Patan (46.42%), GSWDC Mankuva (38.88%), Idar panjarapole (35.48%), Dantiwada Panjarapole (30.30%) and Livestock Research Station Sardarkrushinagar (19.60%).Out of three regions under study, Saurashtra Region showed highest seroprevalence of 47.82 per cent. Maximum seroprevalence of 46.09 per cent was found in the age group of 3-6 years followed by the 1 year(42.62), 1 to 3 years (41.48) and 38.27 per cent in>6 years of age groups.

PCR BASED DETECTION OF X REGION OF *spa* GENE IN *Staphylococcus aureus* ISOLATES

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The gene encoding protein A (*spa*) is composed of functionally distinct regions : Fc binding region, X region and C termius required for cell wall attachment. Twenty isolates of *staphylococcus aureus* (five isolates each from sheep, goat, cattle and buffalo) where investigated by PCR for gene polymorphism in the X region of protein A. Three different amplicons of approximately 254, 270 and 298 bp were observed. On the basis of the size of the corresponding PCR product, 8-10 repeates were supposed to be present in the region X of *spa* gene investigated. The isolates of sheep, goat, cattle and buffaloes showed 8,9,10 and 11 repeates most frequently i.e 20.39%, 15.53%, 14.16%, and 16.50%, respectively. It showed that more than seven repeates were found in 66.98% isolates, while seven or less repeats were found only in 33.02% of isolates.

DETECTION OF POLYMORPHISM IN *spa* GENE AND *coa* GENE OF *Staphylococcus aureus* ISOLATED FROM MASTITIS CASES

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Staphylococcus aureus is an adaptable, opportunistic pathogen causing a wide spectrum of diseases.It produce and secretes many proteins which could contribute to the ability of organism to establish disease in the mammalian host. *Staphylococcal* protein A is a bacterial cell wall product that binds immunoglobulin G. The gene encoding protein A i.e.,*spa* gene is composed of functionally distinct regions such as Fc binding region, X region and a sequence required for cell wall attachment at C terminus. The repetitive X region of the *spa* gene includes a variable number of 24-bp repeats.The *coa* gene that codes for coagulase protein is highly polymorphic because of variable sequence at it's 3' coding region.The size of PCR products of *coa* gene normally ranges from approximately 440-1050bp. In the present study, a total of 85 mastitic milk samples were collected from different parts of Gujarat. Out of the 40 isolates, 20 were selected randomly of which each ten isolates were from cattle and buffaloes. The isolates obtained were identified as *Staphylococcus aureus* by mannitol fermentation, catalase test, phosphatase test, coagulase test, DNase and slime production. For PCR reaction, DNA extraction was carried out using Genei TM spin Genomic DNA Preparation kit method.Out of the 40 positive samples, 3(7.5%), 8(20%) and 29(72.5%) samples shows amplification at 267bp, 270bp and 296 bp, respectively in case of *spa* gene. In case of *coa* gene 4(10%), 6(15%), 6(15%), 7(17.5%) and 17(42.5%) samples shows amplification at 500bp, 550bp, 600bp, 636bp and 670 bp respectively.

DOWN SYNDROME WITH ROBERTSONIAN TRANSLOCATION – CASE STUDIES

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Trisomy 21 or Down syndrome is the most common type of autosomal chromosome abnormality, with an incidence of one out of 700 live births. Down syndrome is associated with psychomotor delay, characteristic facial features, and sometimes, cardiac, digestive and ocular malformations. Peripheral Blood Lymphocyte Culture (PBLC) was done, followed by GTG banding to study the chromosomal abnormality. More than hundred metaphase plates are evaluated for karyotyping. Two clinically Down syndrome cases were observed with Robertsonian translocation. The first case was a Down syndrome male with karyotype 46,XY,+21, rob (21;21) (q10;q10) and the second case was a Down syndrome female with karyotype 46,XX[70]/46,XX, rob (21;21) (q10;q10) [46]. Karyotype revealed Robertsonian translocation in both the cases. While in the first case the translocation was *de novo*, the second case showed mosaicism with 70 cells showing the normal karyotype and 46 cells showing the translocation.

MULTIPLEX PCR TO DETECT SEROTYPE 1 AND SEROTYPE 3 VIRUSES OF MAREK'S DISEASE

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Marek's disease, a contagious and oncogenic lymphoproliferative disease of chickens is caused by the Marek's disease virus belonging to the genus *Mardivirus*. MD still remains as a disease of major economic importance throughout the world, due to the evolving and virulent nature of the pathogen. Based on its molecular and serological differences, MDV has been classified into three serotypes. Serotype 1 (MDV 1) includes all the oncogenic and attenuated strains of MDV, serotype 2 (MDV 2) consists of the non-oncogenic strains of MDV and serotype 3 (MDV 3). In India, HVT vaccine (serotype 3) is extensively used as vaccine to control MD infection in poultry and serotype 1 MD vaccine is not available in India. Recently there are reports on the presence of serotype 1MDV in India. Presence of vaccine virus along with the field virus increases the virulence of the field virus. Hence, the objective of this paper is to develop a multiplex PCR to identify simultaneously serotype 1 and 3 viruses present in the MDV suspected samples. Primers were designed to amplify *meq* gene of serotype 1 field viruses and *sorf* gene of serotype 3 (HVT vaccine). PCR was done to amplify the *meq* gene of serotype 1 MDV (180 bp) and *sorf* gene of serotype 3 MDV (380 bp). PCR products were cloned into TA cloning vector separately. The sensitivity of the PCR to detect recombinant plasmids (pMDV-1 and pMDV-3) was by serially diluting the pMDV-1 and pMDV-3 DNAs. The detecting limits for pMDV-1 were 100 copies and 10 copies respectively. Similarly, the sensitivity of the multiplex PCR was assessed by adding the serially diluted DNAs of pMDV-1 and pMDV-3 in a single reaction and the results showed that the multiplex PCR could also detect similar copies of MDV-1 (100) and MDV-3 (10) respectively. One hundred and seventy three samples (organs, blood samples and feathers) collected during MDV outbreaks in 2006-07 from three states of India, Tamil Nadu, Karnataka and Andhra Pradesh were used for this study. DNAs extracted from the samples were subjected for multiplex PCR and found that five samples were positive for both serotype 1 and serotype 3 MDVs and 24 samples were positive for serotype 1 MDV and 8 samples were positive for serotype 3 MDV. Results indicated that the presence of serotype 1 MDV was more prevalent than the serotype 3 MDV. The interaction between the serotype 3 vaccine virus (HVT vaccine) and the serotype 1 field viruses have to be explored.

T_3, T_4 AND CORTISOL CONCENTRATION OF KANKREJ COWS UNDER DIFFERENT HOUSING SYSTEMS

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Eighteen lactating Kankrej cows of Livestock Research Station, Sardarkrushinagar were subjected to three housing systems. Each group was randomly allotted to one of the three treatments viz., RCC shed (T_1), Thatched roof (T_2) and Tree shelter (T_3). Serum concentration of T_3 (Tri-iodo thyronine), T_4 (Thyroxin) and cortisol hormones were estimated once in a season for one year. The serum concentration of Tri-iodothyronine recorded under T_1, T_2, and T_3 was 0.96 ± 0.01, 0.89± 0.02 and 0.90 ± 0.02 respectively. T_3 hormone did not differ significantly due to treatment as well as season. While serum concentration of Thyroxine hormone was significantly ($P < 0.05$) higher in winter (37.30 ± 1.0) followed by monsoon (27.03 ± 1.0) and summer (13.65 ± 0.70) season. The Serum concentration of cortisol also did not differ significantly.

STUDY OF SOME OF ATYPICAL CHARACTERS OF *E. coli* ISOLATES OBTAINED FROM CASES OF SEPTICAEMIA IN POULTRY AND DIARRHOEIC CASES

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E. coli is a member of family Enterobacteriacae causing colisepticemia in poultry and diarrhoea in calves. A typical *E. coli* isolate is motile and H_2S negative. Study was conducted for assessing motility and H_2S production by *E. coli* isolates. Samples were collected from colisepticemic cases of poultry at, in and around the Palanpur Taluka of Banaskantha District of Gujarat state and from diarrhoeic cases of calves at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, and also from college clinics. Seventy Five *E. coli* isolates (25 isolates from poultry and 50 from calves) were obtained by isolation on MacConkey and Eosin Methylene Blue (E.M.B.) agar and confirmed by IMViC (Indole test, Methyl Red test, Voges-Proskauer test, Citrate test) reaction. Motility and H_2S production activity of isolates were carried out using Motility Sulfide medium. Out of total 75 *E. coli* isolates, 59 (78.67%) isolates were found positive for motility test. Among them 20 (80%), out of 25 poultry isolates and 39 (78%), out of 50 Calves isolates were found positive for motility. Out of 75 *E. coli* isolates, 23 (30.67%), were found positive for H_2S Production. Among them 20 (80%), out of 25 poultry isolates and 3 (6%), out of 50 calves isolates were found positive for H_2S production. This study shows prevelance of some of the *E. coli* isolates showed atypical characters *viz.,* motility and H_2S production activity of *E. coli.*

COMPARATIVE EVALUATION OF SEROLOGICAL, MOLECULAR AND CONVENTIONAL METHODS FOR DIAGNOSIS OF JOHNE'S DISEASE IN CATTLE

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Paratuberculosis (Johne's disease) is a chronic granulomatous bacterial infection caused by *Mycobacterium avium subspecies paratuberculosis* (MAP). It is one of the serious infectious diseases of ruminants for over a century and has become endemic in most countries. In present study different serological, molecular and conventional diagnostic tests were applied for diagnosis of Johne's disease. Sample from total 23 highly suspected cattle were subjected for comparison by fecal smear examination, rectal pinch smear examination, ELISA and PCR No. of positive cattle found positive by fecal smear examination were 20 (86.95 %), by rectal pinch smear examination were 20 (86.95 %), by i-ELISA were 23 (100.00 %) and by PCR were 16 (69.56 %) respectively. Out of 23 cattle, 16 (69.56 %) were found positive in all four tests. No animal was found negative by all four tests. 3 (13.04 %) were found positive only by fecal smear examination and ELISA while found negative by PCR and rectal pinch smear examination, 3 (13.04 % %) were found positive by rectal pinch smear examination and ELISA while found negative by PCR and fecal smear examination and 1 (04.34 %) was found positive by fecal smear examination, rectal pinch smear examination and ELISA while found negative by PCR.

CPK LEVEL IN NON SYMPTOMATIC (CARRIER) FEMALES IN DUCHENNE MUSCULAR DYSTROPHY

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Muscular dystrophy refers to a group of hereditary and non-hereditary, muscle diseases that weaken the musculoskeletal system and hampers locomotion. Muscular dystrophies are characterized by progressive skeletal muscle weakness, defects in muscle proteins, and the death of muscle cells. There are nine types of muscular dystrophies of which Duchenne muscular dystrophy (DMD) is the most common pediatric neuromuscular disorder that affects 1 in 3500 live male birth. DMD is a recessive, fatal, X-linked disorder caused by mutations in the dystrophin gene at Xp21. This gene is responsible for synthesis of dystrophin protein, which is a rod-shaped cytoplasmic protein found at the inner surface of muscle fibers. It is a vital part of a protein complex termed dystrophin-glycoprotein complex (DGC), which bridges the inner cytoskeleton and the extra-cellular matrix to provide strength to the musculoskeletal system. In DMD affected families males are seen as a symptomatic while the female are as non symptomatic Because of the way the disease is inherited, males are more likely to develop symptoms than are women. Elevation of CPK activities is reflecting a degeneration of muscle membrane. Our investigation documented higher levels of CPK in carrier females. Objectives are To identify carrier females in DMD families with help of Biochemical indices (CPK).Methodology: CPK levels were analyzed using standard protocol on Cobas integra.Results: Data revealed that the CPK levels were higher than the normal value in non symptomatic females.Conclusion: This preliminary study revealed that the CPK level is very useful marker in diagnosing non symptomatic females as a carrier for future management and counseling.

IDENTIFICATION OF RAPD MARKERS FOR CYTOPLASMIC GENIC MALE STERILE AND RESTORER LINES OF PIGEONPEA

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The cytoplasmic-genetic male sterility (CMS) system is considered to be feasible approach to develop hybrids in pigeonpea. Identification of CMS lines and their putative restorers using molecular markers in the early stage of growth is important and economical in long duration pigeonpea. The RAPD analyses of genomic DNA were carried out using 80 RAPD primers in ten different genotypes each of A and R lines of pigeonpea. Seventy six of the 80 RAPD primers used, produced clear and unambiguous banding pattern among A and R lines. Out of these, 72 were polymorphic and 4 were monomorphic. A total of 702 bands were amplified, out of which 544 were polymorphic. This evinced on an average 7.55 polymorphic bands /primer; though the average number of amplified bands per primer were 8.77. The number of amplified loci varied from two each in OPC2, OPC12, OPB1, OPB5, OPB6 and OPB9 to twenty two in OPA 1 and OPA19. The size of amplified bands ranged from 100 bp to 2850 bp. The polymorphic 1.1kb fragments amplified by OPC1 were specific only to fertile (R) lines. The other specific fragment that was confined only to sterile (A) lines was of 1.5 kb size was amplified by OPA11. However, this requires confirmation through linkage studies. The said RAPD primers are dominant in nature and would be more useful if they are converted in sequence based primers like SCAR. SCAR markers being co-dominant in nature, can also be used for grow out test of the hybrids since they are specific to each parental line.

COMPARATIVE EVALUATION OF COMPETITIVE ENZYME LINKED IMMUNOSORBENT ASSAY (C-ELISA) AND INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY (I-ELISA) FOR DETECTION OF BTV ANTIBODIES IN SHEEP SERA

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Bluetongue (BT) is an infectious, non-contagious disease of domestic and wild ruminants which is transmitted by insect vector belonging to Culicoides spp. It is particularly a viral disease of sheep occasionally affecting cattle, buffaloes, goats, camels and other wild ruminants. The severity of disease varies from mild to severe degree leading to death, depending on the strain of the virus, breed of sheep, environmental factors related to climatic conditions. In the present study, performance of the c-ELISA and i-ELISA for the detection of BTV group specific antibodies in sheep was compared & Cross tabulation of c-ELISA and i-ELISA considering c-ELISA as reference test was recorded as per method described by Martin (1977) to determine relative sensitivity and specificity of i-ELISA. A total of 382 sera were tested for the detecting BTV antibodies in sheep sera from various places of Gujarat using c-ELISA and i-ELISA kit made available by courtesy of Dr. M. M. Jochim, President, Veterinary Diagnostic Technology Incorporation, USA and Bluetongue virus laboratory, IVRI, Mukteswar respectively. Out of 382 serum samples tested from sheep 150 (39.26%) and 130 (34.03%) were positive respectively by c-ELISA and i-ELISA. Considering c-ELISA as the reference test, relative sensitivity and specificity of i-ELISA to c-ELISA was 86.67 and 100.00 per cent, respectively. Overall agreement between both the tests was 94.76 per cent. c-ELISA proved to be the most sensitive in detecting BTV group specific antibodies than i-ELISA as it detected 150 samples positive as against 130 samples detected positive in i-ELISA.

EFFECT OF IVERMECTIN ON HAEMATO-BIOCHEMICAL PROFILES IN DONKEYS NATURALLY INFECTED WITH GASTRO-INTESTINAL PARASITES

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Monitoring of haemato-biochemical alterations during parasitic infections in several host-parasite systems is an essential criterion for precisely assessing the drug's efficacy at pre and post anthelmintic treatment. Present study was conducted in Banaskantha district of North Gujarat on 40 naturally infected donkeys with helminthes. Selected donkeys were administered single dose of 1 % solution of ivermectin (Neomac inj., INTAS) @ 0.2 mg/ kg. b.wt, subcutaneously. Haematological profile revealed significant increase in Hb, TEC, PCV and lymphocyte; significant decrease in eosinophil and non-significant decrease in TLC and neutrophil with progress of treatment. With progress of days after treatment biochemical profile revealed significant increase in glucose, calcium, phosphorus, total protein, albumin, globulin, iron and copper concentration; significant decrease in cholesterol, ACP, AST and ALT concentrations, where as non-significant increase in TIBC, zinc and magnesium and non-significant decrease in triglyceride, AKP and creatinine.

DETECTION OF BTV GENOME FROM FEW RANDOMLY SELECTED ANTIGEN POSITIVE SAMPLES USING RT-PCR

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Bluetongue (BT) is an infectious, non-contagious, arthropod-borne viral disease, principally of sheep, occasionally affecting cattle, buffaloes, goats, camels and other wild ruminants. Etiological agent of the disease belongs to the genus Orbivirus in the family Reoviridae. Nucleic acid based assays have been proposed as suitable tools for detecting BTV directly in clinical specimens because of their exceptional sensitivity and specificity. BTV sequencing and hybridization studies have suggested that the viral genome segment 6 encoding non structural protein (NS1) has 97-100 % nucleic acid sequence homology with other serotypes. Thus this gene segment is highly conserved. So, RT-PCR assays have been described for the detection of BTV in animals. Present study was intended to detect BTV nucleic acid from the clinical as well as spleen samples and pooled Culicoides by using the using NS1 gene specific RT-PCR. A total of 35 samples (31-blood, 3-spleen and 1-pooled Culicoides) were processed which included 30 s-ELISA positive and five s-ELISA negative samples. s-ELISA positive samples, comprised of 29 blood samples and one spleen sample, whereas five s-ELISA negative samples, included two spleen, two blood and one pooled Culicoides. A standard strain of BTV (BTV-23) procured from BTV lab, IVRI, Mukteshvar are included as control. Out of 35 samples, eight s-ELISA positive blood samples and BTV-23 produced approximately 274 bp amplicons with NS1 gene group specific primer. Detection of BTV nucleic acid by NS1 gene based RT-PCR was possible in blood. Samples which were positive in s-ELISA but were found negative in NS1 gene based RT-PCR, might be due to the presence of PCR inhibitors or degradation of viral RNA in these samples.

CLONING AND EXPRESSION OF RECOMBINANT FLAGELLIN (FLIC) IN PROKARYOTIC EXPRESSION SYSTEM

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Vaccination remains the most efficacious and valuable tool in the prevention of infectious diseases. Subunit and new generation vaccines need the help of adjuvants for inducing efficient immune response. Flagellin as an adjuvant is recently emerging and effective. It is a pathogen-associated molecular pattern (PAMP) recognized by the innate immune system through Toll-like receptor 5. In this study, we have successfully expressed the flagellin protein in prokaryotic expression system. The genomic DNA has been isolated from *Salmonella* Typhimurium, using specific designed primers PCR amplification of fliC (1.5 kb) was done and cloned in a TA-cloning vector. Following confirmation of the recombinant plasmid subcloning of the FliC was done in PET 32a vector. The recombinant PET 32a-FliC was transformed into BL 21 strain of *E. coli* cells and the positive clone was induced to express the flagellin protein followed by purification using affinity chromatography. The purified flagellin was analyzed by SDS-PAGE and confirmed by immunoblotting. In future this recombinant flagellin protein can be used as an adjuvant and use of recombinant FliC protein will avoid handling live bacterial cultures some of which are zoonotic in nature.

PREVALENCE OF *Rhodococcus Equi* IN EQUINE ENVIRONMENT OF ARID ZONE BASED ON 16s RNA RIBOTYPING

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Rhodococcus equi (*R. equi*) is an important animal pathogen with equines being the most susceptible host. This facultative intracellular pathogen is quite stable in nature and contaminates equine environments. Such contaminated stables can lead to contamination of skin and upper respiratory tract which may further cause systemic infection of foals and dams. The infections caused by *R. equi* are mostly subclinical for a long period of time. The clinical manifestations range from respiratory tract infections to osteomyelitis and lymphadenitis where there is high temperature. When the symptoms are expressed the foals become untreatable. The present study was conducted to observe the normal prevalence of *R. equi* in apparently healthy animals in the arid region of Bikaner. Out of 202 samples processed for the present investigation 132 were from the non organized farms and 70 were from animals of organized farm. These samples were subjected to culturing for isolation and preliminary identification. Out of 202 samples processed, 54 (40.91%) samples from non-organized farm and 21(30.00%) samples of organized farm had colonies that were moist, slimy and pale yellow in colour with earthy odour. The preliminary identification tests on these colonies showed Gram-positive cocci to rods, catalase positive, oxidase negative organisms that were either unreactive or gave oxidative reaction in oxidation fermentation test on prolonged incubation. These isolates were further subjected to genotypic characterization using 16S rRNA as the target gene specific for *R equi*. The 16S rRNA acts as a critical component of bacterial cell function and the gene coding for 16S rRNA has been found to be highly conserved. A total of 37 isolates could be confirmed as *R equi*. The overall prevalence of R equi was 18.31 percent. Although no significant difference was found in the isolation rate, higher isolation of *R. equi* was observed from non organized animals (19.70%) as compared to that of the organized farm animals (15.71%). The percent isolation of *R. equi* was higher in summers i.e. 31.94% and 17.50% than that in winter season i.e. 5% and 13% in non organized and organized farms respectively.

GAIN AND RETENTION OF KNOWLEDGE REGARDING CLEAN MILK PRODUCTION THROUGH MULTIMEDIA-EFFECTIVENESS STUDY OF EXTENSION TOOL

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The present study was conducted in the Gujarat State of India to measure the effectiveness of multimedia on gain and retention of knowledge regarding clean milk production of livestock owner of selected villages of tribal and non tribal talukas on exposure of multimedia.The results indicated that the knowledge level before exposure of the multimedia was correlated with some of the personal characteristics of livestock owner while no such correlation was observed with the level of knowledge after exposure of the multimedia. Similar result was also observed at the level of retention.

DEVELOPMENT AND STANDARDIZATION OF A POLYMERASE CHAIN REACTION ASSAY BASED ON OUTER MEMBRANE PROTEIN GENE *OMP31* FOR RAPID DIAGNOSIS AND DIFFERENTIATION OF *Brucella spp.*

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Brucellosis is a worldwide zoonosis, caused by various species of genus *Brucella*. The gold standard for antigenic diagnosis of brucellosis i.e. bacterial culture is comparatively less sensitive, time consuming and technically demanding. It also requires specialized laboratory facilities and may be hazardous. Therefore, there is immense need to develop an efficient assay for rapid and sensitive detection of this pathogen. The present study was aimed to develop and evaluate a PCR assay based on immunodominant outer membrane protein gene *omp31* for rapid diagnosis and differentiation of *Brucella* species. For this, *omp31* gene sequences available for various species of genus *Brucella* were downloaded from the NCBI database and multiple sequence alignment was carried out using MEGA 4.0 software. Seven primers were designed from the aligned sequences of the OMP31 gene sequences and were used in four different combinations (viz. Brucella Diag OMP31 F1 and Brucella Diag OMP31 R1; Brucella Diag OMP31 F2 and Brucella Diag OMP31 R1, Brucella Diag OMP31 F3 and Brucella Diag OMP31 R2, Brucella Diag OMP31 F4 and Brucella Diag OMP31 R3). Standard *Brucella* species isolates were procured from Indian Veterinary research Institute Izatnagar (Bareilly) and some clinical isolates were also included in the study. Cell lysates were prepared by hot-cold lysis method. PCR amplification using three sets of primers (Brucella Diag OMP31 F1 and Brucella Diag OMP31 R1; Brucella Diag OMP31 F2 and Brucella Diag OMP31 R1 and Brucella Diag OMP31 F4 and Brucella Diag OMP31 R3) resulted in amplicons of 172, 176 and 516 bp, respectively in all *Brucella* species strains tested under the study. PCR amplification using primer pair Brucella Diag OMP31 F3 and Brucella Diag OMP31 R2 resulted in amplicon of 513bp in *Brucella abortus* and 477bp in *Brucella melitensis* while both 513bp and 477bp amplicons were present in other species of *Brucella*. All the four primer pairs resulted in specific amplification even at annealing temperature of 68°C. High temperature of annealing (65°C) reduced the total time required for complete PCR run by minimizing the ramping time as well as reduced the chances of non specific amplification leading to high specificity in results. Although, the primers may not differentiate all the reported species of genus *Brucella* and various vaccine strains utilized throughout the globe, the simplicity of the PCR assay using only one pair of primers clearly indicates their utility for diagnosis of the brucellosis as well as for differentiating various species of *Brucella* commonly causing human and animal illness in India. However, further studies need to be carried out to test these primers on a battery of strains of *Brucella* species as well as vaccine strains so that their applicability in clinical diagnosis of brucellosis and differentiation at species level can thoroughly be evaluated.

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A NOVEL DEOXYRIBONUCLEASE (DNase) FROM *Salmonella enterica* subspecies *enterica* serovar Gallinarum

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Deoxyribonuclease (DNase) is a toxin produced by a number of Gram positive and Gram negative bacteria and its role in the pathogenesis is well established. The present study aimed at determining the DNase production potential of *Salmonella enterica* ssp *enterica* serovars bearing animal, human and zoonotic significance. A total of 119 strains representing 78 serovars, isolated from different parts of India were included in the present study. DNase activity was evident in six *S.* Gallinarum strains (E-76, E-4045, E-2179, E-2451, E-2455 and E-2118) as detected by marked discoloration around the stabbed bacterial culture on DNase test agar medium. One of the Gallinarum strain (E-76) producing maximum activity was further studied for characterization of its DNase. Although, DNase activity could not be detected in the culture supernatant; sonicated crude cell lysate had detectable DNase. DNase activity could not be detected in the proteins precipitated out from the culture supernatant but those precipitated out from the sonicated crude cell lysate by salt precipitation method retained the DNase activity. However, solvent precipitation methods failed to reproduce the activity. The precipitated DNase caused visible discoloration in ~0.5 µg protein and was active ever after. The activity was maintained after heat treatment at 50°C for 15 min but lost after treatment of 80°C for 15 min or at higher temperatures. On fractional salt precipitation, (0-30%, 30-50%, 50-70% and 70-95%), out of the four fractions, DNase activity was restricted to only two fractions viz. 30-50% and 50-70%. To further characterize the DNase of *S.* Gallinarum and its role in pathogenesis, experiments needs to be carried out as it appears to be the first report of the frank deoxyribonuclease (DNase) production by any *Salmonella* serovar to date.

Trends in veterinary viral vaccine research

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Vaccines can be considered as one of the most effective agents in protecting humans and animals against various infectious diseases. Human medicine demonstrated that small pox can be eradicated from the globe and the veterinary profession hailed its victory over rinderpest. These achievements were possible only by the use of efficacious vaccines. Both the professions are now contemplating to eradicate diseases such as polio and PPR respectively. The vaccinology has undergone metamorphosis and the recent research is directed towards the development of safe and efficacious vaccines The current areas of virus vaccine research include sub-unit vaccines, DNA vaccines, viral vectors, virus-like particles (VLPs) and plant derived vaccines. A brief introduction to the various platforms will be discussed. The talk will cover also diagnosis, diagnostics and vaccine research and development for two important diseases namely Rabies and Foot-and-mouth disease. Diagnosis and molecular epidemiology of rabies helps in the confirmation of the disease and in understanding the relationship of viruses isolated from different species of animals. Human monoclonal antibodies against rabies virus have been generated and are being evaluated for its efficacy. Safe and efficacious rabies vaccines for humans and animals are available. Current vaccine research is directed towards the development of vaccine using baculovirus expression system and also a live attenuated vaccine for oral immunization. Indian Immunologicals Limited is involved in various FMDV research projects [(CIDLID (BBSRC/DFID) and DISCONVAC (EU FP7)] which include vaccine efficacy, diagnosis, molecular epidemiology and transmission studies in cattle, buffaloes, sheep and goats and development of tests for various in-process controls in vaccine manufacturing and development of new vaccines. New diagnostic methods, diagnostics and vaccines for FMD will be discussed.

DEVELOPMENT AND STANDARDIZATION OF A POLYMERASE CHAIN REACTION ASSAY BASED ON OUTER MEMBRANE PROTEIN GENE *lipL32* FOR RAPID DETECTION OF PATHOGENIC LEPTOSPIRES

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Leptospirosis, caused by a spirochaete belonging to genus *Leptospira* is a globally emerging zoonosis. Traditionally, the disease was considered to be prevalent in several south Indian states and Andman and Nicobar Islands, however, in the recent past, cases have started been appearing in considerable number from various north Indian states including Punjab, Haryana and Uttar Pradesh and Delhi. Therefore, the disease is considered to be emerging zoonosis in Northern India. The conventional methods used for diagnosis of leptospirosis including culture, silver staining, dark field microscopy etc. are comparatively less sensitive, time consuming and technically demanding. Therefore, there is immense need to develop an efficient test for rapid detection of this notorious pathogen. The present study was aimed to develop and standardize a PCR assay based on outer membrane protein gene *lipL32* which has been reported to be evolutionary conserved only in pathogenic leptospires. For this, *lipL32* gene sequences available for various serovars of *Leptospira* were downloaded from the NCBI database and multiple sequence alignment was done using clustalw software. Three sets of primers (named Lep Diag LipL32 F1 and Lep Diag LipL32 R1; Diag LipL32 F2 and Lep Diag LipL32 R2 and Lep Lep Diag LipL32 F1 and Diag LipL32 R2) were designed. *L. interrogans* serovar Canicola was procured from Regional Medical Research Centre, Port Blair, Andaman and Nicobar Islands and cell lysate was prepared using standard protocol. PCR amplification using these primers resulted in amplicons of 175, 294 and 430 bp, respectively. All the three primer pairs resulted in specific amplification even at annealing temperature of 68°C. High temperature of annealing (68°C) reduced the total time required for complete PCR run by minimizing the ramping time as well as reduced the chances of non specific amplification leading to high specificity in results. *In silico* studies using these newly developed diagnostic primers also resulted in specific amplicons of desired size in pathogenic serovars only (no amplicon in non pathogenic/saprophytic ones). This study clearly indicates the utility of the *lipL32* gene based primers for diagnosis of the leptospirosis as well as for differentiating pathogenic and non-pathogenic leptospires. However, further studies need to be carried out to test these primers on a battery of serovars of *Leptospira* including non-pathogenic ones so that their applicability in clinical diagnosis of leptospirosis can thoroughly be evaluated.

Poster Presentation

CELLULAR IMMUNE COMPONENTS OF COLOSTRUM

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Colostrum is a rich source of humoral as well as cellular immune componenets. Immunoglobulins (Ig) are the main immune component present in the colostrum. In addition to Ig, colostrum contains viable cells including macrophages and neutrophils which secrete a range of immune related componenets into the milk and colostrum. Immune factors play an important role through providing protection against pathogenic organisms. Somatic cell count (SCC) and Differential Leukocyte count (DLC) are used as an asset to determine the mammary health and quality of milk and colostrum. Twenty five colostrum samples were collected 3 days post partum and subjected to SCC and DLC. It has been observed that SCC was significantly decreased from day-1 to day-3. Highest count was recorded in day-1 i.e. 6.44±0.28 lakh/ml followed by 5.42±0.21 lakh/ml on day-2 and the lowest count was recorded in day-3 (4.08± 0.15 lakh/ml). Number of neutrophil significantly decreased in colostrum samples with highest 35.27±0.40 % in day-1. The number went down in subsequent days reaching 32.97±0.19 % and lowest 27.66±0.37 % on day-2 and 3, respectively. A significant increase was found in lymphocyte percentage from lowest 51.07±0.24 % on day-1 to 57.40±0.32 % by day 2 reaching highest 61.67±0.38 % on day-3 among colostrum samples of different days after calving. A significant increase was observed in number of macrophage from day-1 (6.47±0.18 %) to successive days but there was non- significant increase in day-2 (7.85±0.28 %) to day-3 (8.40±0.37 %). Lowest was observed in day-1 followed by of day-2 and the highest within day-3.

DETECTION OF *Mycoplasma mycoides subspp. capri* BY PCR IN NASAL SWABS COLLECTED FROM SICK GOATS

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Thirty eight nasal swabs were collected from the nasal tract of the sick goats showing the sign of respiratory discharge. The swabs were collected in the broth medium (MBHS-L) directly and transported on ice. After subsequent transfer to the fresh medium within one hour, the samples were incubated for around seven days under 5% CO_2 tension. The growth in the broth medium from samples were inoculated on solid media (MBHS-A) and incubated subsequently under 5% CO_2 tension and humidity at 37 ºC for 10 days. The growth in broth was also subjected to DNA extraction and PCR. PCR was performed using 16S rRNA based *Mycoplasma* genus and *Mycoplasma mycoides subsp. capri* specific primers viz. GPO/MGSO and P4/P6. After PCR, specific amplification products of 715bp and 194bp were obtained with primers GPO/MGSO and P4/P6, respectively in only one sample. The results of PCR were also substantiated by the cultural isolation of *Mycoplasma* on solid medium. Therefore this study confirms the *Mycoplasma mycoides* subsp. *capri* as one of the major pathogen related to respiratory tract infections and shows the significance of advance molecular techniques, like PCR in the detection and diagnosis of the pathogens which are difficult to isolate.

PREVALENCE OF VIRULENT *Listeriae* IN MEAT

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Meat samples (n = 92) collected from retail market at Nagpur. Samples were examined for the presence of *Listeriae* following enrichment and plating on selective agar. The isolates of *Listeriae* were subjected to PCR assay for detection of virulence marker genes (*hly*A, *plc* A *act*A, *iap* and *prf* A). After processing of 92 meat samples , 49 isolates of *Listeriae* (poultry meat 26, Chevon 11, Fish 5 and pork 7) were recovered. Virulence genes, *hly*A, *act*A, *iap,* and *prf* A were detected in three strains, *hly*A, *act*A and *iap* in twenty strains, *hly*A, *iap,* and *prf* A was detected in a strains. Two genes , *hly*A and *iap* and *act* A and *iap* were present in one strain each, Single gene *iap* and *hly*A was present in 13 and seven strains, respectively, whereas, *plc*A, *prf*A and *act*A was present in a strain respectively. Prevalence of virulent *Listeriae* in meat would contribute to food borne infection in human.

In vitro MYOSTATIN GENE SILENCING IN CHICKEN EMBRYONIC MYOBLAST CULTURE BY SHORT HAIRPIN RNAS

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Myostatin (MSTN), a member of transforming growth factor-â (TGF- â) superfamily, is a negative regulator of the skeletal muscle growth as it suppresses the proliferation and differentiation of myoblast cells. Dysfunction of MSTN gene either by natural mutation or genetic manipulation (knockout or knockdown) has been reported to interrupt its proper function and increase the muscle mass in many mammalian species. RNA interference (RNAi) mediated by small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) has become a powerful tool for gene knockdown studies. In the present study, transient silencing of MSTN gene in chicken embryo myoblast cells was evaluated using two shRNA expression constructs. We report 10 and 25% silencing of myostatin mRNA using these shRNA constructs in transiently transfected myoblast cells ($p< 0.05$). At the same time we also observed high level of myostatin silencing resulted in 5.5 and 14.25 fold induction of interferon responsive gene (OAS1) ($p< 0.05$). Interestingly, the shRNA with maximum silencing effect exhibited minimum interferon response. To increase the muscle mass in the transgenic animals, it will require further work to study stable expression of anti-myostatin shRNA with minimum interferon induction.

TISSUE SPECIFIC TEMPORAL EXOME CAPTURE FOR DETECTION OF CANDIDATE SNPS IN INDIAN BUFFALO GENOME

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Though it is proposed to sequence a genome in thousand dollars, it is yet to come true. Whole genome sequencing of buffalo is yet to complete, and in near future it may not be possible to identify exome (coding region of genome) by designing probes to capture exome. The present study was designed with main objectives of exome capture, tissue specific gene expression and SNP detection in coding region of Indian buffalo genome. In the present study, we employed 'in solution hybridization' for sequencing 'Tissue Specific Temporal Exome' (TST exome) in buffalo. We utilized cDNA prepared from buffalo muscle tissue as a probe to capture TST exome from buffalo genome. This resulted in prominent reduction of repeat sequences (up to 40 %) and enrichment of coding sequences (up to 60 %). Enriched targets were sequenced on 454 pyro-sequencing platform generating 101,244 reads containing 24,127,779 high quality bases. Obtained sequencing data revealed 40,100 variations, of which 403 were indels and 39,218 were candidate SNPs containing 195 non synonymous candidate SNPs in protein coding regions. The study has revealed that 80% of the total genes identified from capture data were expressed in muscle tissue. The present study is first of its kind to sequence TST exome captured by use of cDNA molecules for candidate SNP finding in coding region without any prior sequence information of targeted molecules. The result obtained in present study shows that this strategy for TST exome sequencing can very well be utilized in other important farm animals as well in which high density capture arrays are still not available.

THE STUDY OF THE COINCIDENCE OF *ROTAVIRUS* AND *E. coli INFECTION* IN NEONATAL CALF DIARRHOEA

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The present study was undertaken to find out the prevalence of Rotavirus and *E .coli* associated with neonatal calf diarrhoea. A total of 52 faecal samples were collected from diarrhoeic calves aged one to four week. These samples were processed for detection of Rotavirus antigen by Latex Agglutination Test (LAT) in which seven (13.46%) samples were found positive. Further, they were tested for detection of *E.coli* through standard culture technique; yielded thirty eight (73.07%) positive isolates were confirmed by Polymerase Chain Reaction (PCR) using Eubacterial universal primer. Rotavirus was detected in association with *E. coli* in five (9.66%) cases while Rotavirus and *E. coli* alone were detected in two (3.84%) and thirty-six (69.23%) cases of diarrhoeic calves, respectively.

SCREENING FOR BOVINE LEUKOCYTE ADHESION DEFICIENCY (BLAD), BOVINE CITRULLINAEMIA (BC) AND FACTOR XI DEFICIENCY (FXID) IN *Bos taurus X Bos indicus* CROSSBRED CATTLE BY PCR BASED TEST

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Autosomal recessive disorders are breed specific in cattle, some of them are Holstein specific which include mainly Bovine leukocyte adhesion deficiency (BLAD), bovine citrullinaemia (BC) and factor XI deficiency (FXID). As imported Holstein-Friesian bulls or their semen doses were extensively used in India, it became mandatory to screen farm-born HF and HF crossbreds for autosomal recessive disorders before they are used in breeding programmes. Although the initial incidences of carriers for autosomal recessive disorders are low, the actual number of carriers could be substantially higher in the future if the animals are not regularly screened for these diseases. In present study, 89 HF crossbred bulls from different co-operative dairies of Gujarat (India) were screened for BLAD, BC and FXID genotypes to obtain an indication on the importance of these defects in HF crossbred bulls. Genomic DNA was obtained from blood and semen samples. PCR was carried out for all three BLAD, BC and FXID gene fragments using reported primers. PCR products of BLAD and BC were digested with TaqI, AvaII restriction enzymes respectively and Factor XI deficiency was checked only by PCR. Out of 89 animals two animals were detected carrier for BLAD, no carriers found in case of BC and FXI.

INTRODUCTION TO METAGENOMICS: BIODIVERSITY AND PHYLOGENY

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Metagenomics is the study of genomic material obtained directly from the environment, instead of from culture. Previously in microbiology, bacteria needed to be cultured in a lab for researchers to understand the organism; those that would not grow in lab conditions are considered unculturable. From these unculturable organisms, the collection and analysis of their genetic material is the study of metagenomics. Two approaches, function-driven and sequence-driven are used to obtain a metagenomic library. Bacterial symbionts are an example of a function-driven approach while rRNA is primarily used for sequence driven analysis. Many biochemical techniques are currently used in metagenomics including: stable isotope probing, suppressive subtractive hybridization, differential expression analysis, PCR for amplification, RT-PCR and microarrays. Metagenomics led to many novel discoveries of proteins, organisms and phylogeny studies. Metagenomic analysis involves using culture independent techniques to identify genome sequences of a community of organisms inhabiting a common environment. Metagenomic analysis can be done using the Roche Genome Sequencer FLX (454) System. The main source of genetic material used to study evolutionary relationships is the 16S rRNA subunit. Slight changes over millions of years of evolution can then be observed in the rRNA sequence. These differences or similarities in the rRNA sequence can then be looked at between organisms in sequence alignment software to determine how close there evolutionary origins.

ELISA BASED SERODETECTION OF *TRYPANOSOMOSIS* IN CATTLE

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Trypanosomosis ,caused by *Trypanosoma evansi* ,is an important haemoprotozoan disease of livestock in India. It is very much prevalent in India. Cattle sera procured from different farms of Kerala were screened using native antigen of *Trypanosoma evansi*. The organism were raised in laboratory mice from the cryopreserved stock kept in the laboratory. The mice were regularly screened for paracetemia and were euthanized at the timing level of paracetemia. The blood was collected from heart in heaparanized syringe. The blood was purified using DEAE cellullose chromatography so as to obtain only Trypanosomes. The purified organism were repeatedly sonicated in order to obtain native whole protein. This protein was used for the serodetection purposes. For optimum reactivity in ELISA the concentration was worked out as 15mg/ml. The ELISA plates were coated by native whole protein over night and standard ELISA procedure was followed. The field cattle sera in 1:100 dilution in PBS were used. Known positive and negative controls were used in each plate. A total of 320 samples were screened out of them 62 samples were found to be positive (19.38%) with the native whole protein.

IN-VITRO EXPRESSION OF RECOMBINANT PROTEIN COVERING THE CLEAVAGE SITE OF THE F GENE OF THE VIRULENT BAREILLY NEWCASTLE DISEASE VIRUS STRAIN FOR PRODUCTION OF MONOCLONAL ANTIBODIES

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Newcastle Disease Virus, an enveloped virus, initiates infection through attachment to susceptible cells and subsequent membrane fusion, through processes directed by two genomic glycoproteins: the hemagglutinin-neuraminidase (HN) attachment protein and the fusion (F) protein. In this study, the variable 675bp fragment covering the cleavage site of the F gene of NDV bareilly strain was amplified and cloned into prokaryotic expression vector pET 32a(+) at NcoI and *XhoI* restriction sites. The primers were designed from the consensus sequence flanking the cleavage site of the F gene in lentogenic, mesogenic and velogenic strains and can be used to amplify the variable cleavage region in all the strains. The region covering the cleavage site was found to be epitopic on in-silico analysis. The recombinant plasmid was transformed in BL21 pLysS (DE3) *E.coli strain*, and induced with 1.5mM IPTG for expression. The expressed recombinant protein was purified and confirmed by western blot after identifying a specific band with predicted molecular weight of approximately 45 kDa. The fusion protein was purified on Ni-NTA agarose resin columns and injected into Balb/C mice. The serum raised was confirmed by western blot and studies to produce specific hybridoma against the Virulent Bareilly Newcastle Disease Virus strain are underway.

PCR BASED IDENTIFICATION OF MAJOR BACTERIAL PATHOGENS ISOLATED FROM SUB CLINICAL MASTITIS OF COWS

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A total of 349 quarters of 89 lactating cows affiliated with Anand Agricultural University, Anand were screened for subclinical mastitis. A total of 126 isolates of five different microbial genera were recovered from 107 quarters of 47 cows including 19 quarters having mixed bacterial infections by cultural examination. PCR based identification of major bacterial pathogens was carried out after conventional cultural identification. Out of 68 Staphylococci isolates tested for identification of *S. aureus* by PCR, 30 isolates were identified as *S. aureus* by obtaining amplification product of 1318bp using *S. aureus* specific primer for 23S rRNA. Out of 30 PCR positive *S. aureus,* 18 (60%) were positive and rest were negative for coagulase test. All the 27 Streptococci isolates were identified as *Str. agalactiae* by amplifying 586bp product using *Str. agalactiae* specific primer for the 16S rRNA while, none were amplified for *Str. dysgalactiae* (401bp) and *Str. uberis* (94bp) based on primers specific for the 16S rRNA and 23S rRNA respectively. All the six *E. coli* isolates yielded 232bp amplified product using *E. coli* specific primer targeting DNA sequence coding for the 23S rRNA. In this study, the identification of pathogens causing mastitis is important for disease control and epidemiological studies.

CLONING : AN EMERGING TECHNOLOGY IN ANIMAL BIOTECHNOOLOGY

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A cloning is an entity, which is hereditarily identical with another entity. Cloning is a form of asexual reproduction that is widespread in nature. In animal husbandry, the production of clones of procreation stock promises to get better animal performance and quality while at the same time reducing production costs. It is likely that cloning techniques will add further credence to existing trends in optimizing the performance potential of livestock, i.e. high-performance animals. With regard to the questions of genetic improvement and diversity in animal breeding, selection for unambiguous performance characteristics can have the goal of breeding livestock with the help of cloning, and hence unavoidably breeding livestock generally. The resulting genetic status quo is accordingly very probably tied to a reduction in genetic diversity. Although there is in principle still great need for research on more exact documentation of the status quo, appropriate measures should be taken now to limit artificial production of increasing numbers of offspring of individual animals. This relates to techniques ranging from current routine artificial insemination through to cloning. Overall, the application of cloning using nucleus transfer in animal husbandry requires careful consideration of the advantages and disadvantages. Cloning has opened many doors that could lead to extraordinary therapeutic advancements but, as with all new technologies, it will be accompanied by ethical and social dilemmas. Now day successes will lay concrete on the road to improving efficiencies and help add to the basic understanding of our cells. Even Dolly's creator, Ian Wilmut, is focusing less on sheep and more on understanding the mechanism of reprogramming our heritable fabric. Finally, it can be concluded that animal cloning technique is play significant role in many Research opportunity like stem cell production, duplication of genetically privileged animals, and upkeep of in danger of extinction species, and safeguarding of bionetwork.
KEY WORD: Animal Husbandry, Cloning Technology, Genetics

EUKARYOTIC EXPRESSION AND CHARACTERIZATION OF BHV-1 GLYCOPROTEIN D (GD) AS A POTENTIAL DIAGNOSTIC ANTIGEN

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BHV-1 causes Infectious bovine rhinotracheitis/Pustular vuvlvovaginitis in cattle. Glycoprotein D of BHV-1 represents a major component of the viral envelope and is a dominant immunogen. gD encoding gene was expressed in a baculovirus-insect cell system. Viral genomic DNA extracted from BHV-1 grown on MDBK cell monolayer was used as a template for PCR amplification of gD gene (1255bp) using a self designed set of primers. Gel purified gD gene was used for directional cloning into pENTR/SD/D Directional TOPO vector to produce entry clone. Recombinant plasmids were screened by PCR and RE digestion for gD gene insert. The endotoxin free purified plasmids were then subjected to LR recombination reaction with Baculovirus linear DNA. LR recombination mix was transfected into Sf-9 cells and observed for appearance of cytopathic effects (CPE). The recombinant virus was serially passaged for 3 more generations and the 4th passage viral stock was used to infect fresh Sf-9 cells for gene expression study. The recombinant gD protein was immunoprecipitated and when subjected to SDS-PAGE and western blot analysis protein band of ~70kDa was detected consistently. The recombinant gD protein was further confirmed by dot-ELISA indicating its potential as a coating antigen in gD-based diagnostic ELISA.

NICKEL CHLORIDE AND POTASSIUM DICHROMATE INDUCED GENOTOXICITY IN WISTAR RATS

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Environmental pollution is on global agenda since it has become major threat to the very existence of mankind on the planet. One of the major mechanisms behind heavy metal toxicity has been attributed to its genotoxicity. The present study was carried out to assess the genotoxicity by measuring the comet cell percentage in Wistar rats orally intoxicated with heavy metals Viz., nickel chloride and potassium dichromate by conducting two different experiments. In nickel chloride and potassium dichromate induced toxicity, a total of fifty adult Wistar rats were divided uniformly into five equal groups for each study separately. Group I rats received only deionised water and served as control for both experiments. In nickel chloride experiment, Group II, Group III and Group IV rats were given nickel chloride @ 5.25 mg/kg, 10.5 mg/kg and 21 mg/kg body weight, respectively through drinking water for 28 days. In potassium dichromate induced toxicity, Group-II, Group-III and Group IV rats were given potassium dichromate @ 0.625 mg/kg, 1.25 mg/kg and 2.5 mg/kg body weight, respectively, orally through drinking water for 28 days. Group V served as positive control for genotoxicity in which rats were administered with Cyclophosphamide @ 20 mg/kg body weight. by intraperitoneal route prior to sacrifice in both of the experiments. In nickel chloride genotoxicity study, there was increase in the mean comet cell percentage in group II, III and group IV rats. Group V which was pretreated with cyclophosphamide showed the highest (44.20%) comet cells. Group IV rats treated with high dose showed significant increase comet cell percentage (26.20%) along with the increase in comet tail length followed by Group III (19.20%) and Group II (9.20%) as compared to control Group I rats. In Potassium dichromate toxicity, there was dose dependent increase in the mean comet cell percentage in Group I, II, III and IV. Group V rats which served as a positive control and treated with Cyclophosphamide showed the highest comet cells. Group IV rats treated with high dose showed significant ($P>0.05$) higher comet cells followed by Group III and Group II as compared to control Group I rats.

A COMPARATIVE EFFICACY OF DIFFERENT PCR PRIMER PAIRS FOR DETECTION OF *Brucella*

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Brucellosis is a chronic infectious disease caused by genus *Brucella* that may affect a range of different animals. There are number of biochemical, serological and molecular techniques for disease diagnosis and identification of *Brucella*. Polymerase chain reaction (PCR) based techniques are specific, highly sensitive, rapid and efficient. Identification of *Brucella* isolates with PCR assay targeting different genes has been developed. In present study total of three primer pairs amplifying three different fragments (i) gene encoding a 31 kDa immunogenic *bcsp*31 (primer pair B4/B5), (ii) a sequence 16S rRNA of *Brucella abortus* (primer pair F4/R2) (iii) a gene encoding *omp*2 (primer pair JPF/JPR) were compared for their efficiency for detection of *Brucella* from samples of aborted fetal stomach contents, uterine discharge and vaginal mucus from cattle and buffaloes. Primer B4/B5 could produce the desired amplification of 223 bp in 15 out of 76 samples tested. However, primer pair F4/R2 could produce amplicons of 905 bp in 10 out of 76 samples. A total of 12 samples were found positive by primer pair JPF/JPR with amplification of 193 bp. All the samples found positive by primer pair F4/R2 were also found positive by primer pair B4/B5. Thus variation found between primers in detection of *Brucella* DNA might be due to the presence of large amounts of bovine genomic DNA and low proportionate presence of *Brucella* DNA. This could be as a result of competitive non-specific hybridisation of the large amounts of bovine genomic DNA with these primer pairs.

STUDY for detection of COAGULASE positive *Staphylococcus aureus* IN PYOGENIC CONDITIONS

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Staphylococcus aureus is an adaptable, opportunistic pathogen causing a wide spectrum of diseases.It causes pyogenic conditions in various parts of the body like skin, wounds, ears & joints. *Staph. aureus* produces coagulase,an extracellular enzyme that binds to protein to form a complex with thrombin-like activity which converts fibrinogen to fibrin.Detection of coagulase production carried out by two different methods,one is growth on Vogel Johnson Medium & other is Tube Coagulase Test.The formation of black coloured colonies with yellow colour zone around indicates positive in VJ medium.Any degree of clot formation,in which the plasma get converted into stiff gel so that it may remain in place when tube was tilted or inverted is considered to be positive in case of Tube Coagulase test.Plasma remained as wholly liquid or showing only a flocculent or ropy precipitate is considered to be negative in case of Tube Coagulase test.In the present study,a total of 85 pus samples (38 from cattle & 47 from buffaloes) were collected from different pyogenic conditions *viz.*,horn cancer,abscesses,post operative wound *etc.* from Gujarat.Of this, 40 isolates of *Staphylococcus aureus* were obtained;20 from cattle & 20 from buffaloes.On inoculation, 20(100%) isolates from cattle & 19(95%) isolates from buffaloes showed positive reaction on VJ medium.In Tube Coagulase test, 19(95%) isolates from cattle & 18(90%) isolates from buffaloes showed positive reaction. In short,out of 40 isolates,39(97.5%) isolates showed positive in VJ medium & 37(92.5%) isolates showed positive in Tube Coagulase test.

DETECTION OF *Mycobacterium avium subspecies paratuberculosis* IN MEHSANI AND SURTI GOATS OF GUJARAT USING MULTIPLE DIAGNOSTIC TESTS

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Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of chronic granulomatous bacterial infection in animals known as Johne's disease (JD) or Paratuberculosis. Diagnosis of Paratuberculosis in goats was carried out by applying different conventional, molecular and serological methods. A total of 219 goats were screened which were categorized in three groups, Group-I consisting of 123 Mehsani goats, Group-II, 76 Surti goats and Group-III, 20 Non descript goats. Percent positivity by faecal smear examination, ELISA, AGID and PCR was 9.2% (7/76), 43.3% (95/219), 10.9% (24/219) and 12.5% (5/40), respectively.

HEAVY METAL INDUCED OXIDATIVE STRESS IN WISTAR RATS

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Heavy metal induced toxicity is very well reported in the literature. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. The present study was carried out to assess the oxidative stress by measuring the activity of lipid peroxidation (LPO) and Superoxide Dismutase (SOD) in erythrocytes of Wistar rats orally intoxicated with various heavy metals Viz., lead, cadmium, chromium and nickel by conducting four different experiments. The sub acute oral toxicity was induced by oral administration of lead acetate, cadmium chloride, potassium dichromate and nickel chloride in Wistar rats by gavages in different doses. In lead acetate induced toxicity, a total of forty adult Wistar rats were divided uniformly into four equal groups. Group I rats received only distilled water and served as control. Group II, Group III and Group IV rats were given Lead Acetate @ 250 ppm, 500 ppm and 750 ppm respectively through drinking water for 28 days. In cadmium chloride induced toxicity, Group II , Group III and Group IV rats were given Cadmium chloride @ 100 ppm, 250 ppm and 500 ppm respectively through drinking water for 28 days. Chromium toxicity was studied by oral administration of potassium dichromate @ 0.625 mg/kg (Group-II), 1.25 mg/kg (Group-III) and 2.5 mg/kg (Group-IV), respectively orally for 28 days. Nickel toxicity was induced by oral administration of Nickel chloride @ 5.25 mg/kg (Group-II), 10.5 mg/kg (Group-III) and 21 mg/kg(Group-IV), respectively orally by gavage for 28 days. The dose in each metal was calculated on the basis of known oral LD50 value in rats. Lipid peroxidation (LPO) activity in terms of Membrane peroxidative damage in erythrocytes was determined as Malondialdehyde (MDA) production by the method suggested by Rehman (1984), whereas, SOD activity was estimated as per the method described by Madhesh and Bal Subramanian (1998).In lead acetate, cadmium chloride and potassium dichromate induced toxicity, a significant ($P < 0.05$) rise in LPO activity was observed in all the experimental groups as compared to Group I control. There was significant ($P < 0.05$) decrease in SOD values in all the experimental groups as compared to Group I control rats. In nickel chloride toxicity, a significant ($P < 0.05$) dose dependant rise in Lipid peroxide level was observed in nickel treated rats as compared to Group I control, whereas significant ($P < 0.05$) decrease in SOD values were observed only in Group IV rats receiving the high dose as compared to Group I control rats. To conclude, lead, cadmium, chromium and nickel generated free radicals and led to oxidative stress as shown by increase in LPO activity and decrease in SOD activity in erythrocytes of Wistar rats at mentioned doses.

PESTICIDE INDUCED OXIDATIVE STRESS IN WISTAR RATS

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Pesticide-induced oxidative stress as a possible mechanism of toxicity has been a focus of toxicological research for the last decade. The present study was carried out to assess the oxidative stress by measuring the activity of lipid peroxidation (LPO) and Superoxide Dismutase (SOD) in erythrocytes/tissues of Wistar rats orally intoxicated with various pesticides Viz., Quinalphos, Carbofuran, Chlorpyrifos and Carbaryl by conducting four different experiments by sub acute oral toxicity. In quinalphos induced toxicity, a total of twenty four adult Wistar rats were divided into four equal groups. Group I rats received only groundnut oil @ 1mg/kg B.W. and served as control. Group II, III and IV rats were intoxicated with quinalfos @ 0.689, 1.378, 2.756 mg/kg body weight, respectively, for 28 days. In Carbofuran induced toxicity, rats were orally administered with carbofuran @ 0.35 (II), 0.70 (III) and 1.40 (IV) mg/kg body weight. In Chlorpyrifos induced toxicity, Group II, III and IV rats were orally administered with chlorpyrifos @ 4, 8 and 16 mg/kg body weight. In Carbaryl induced toxicity, Group II, III and IV rats were given carbaryl @ 15.58, 31.15 and 62.30 mg/kg body weight. Lipid peroxidation (LPO) activity in terms of Membrane peroxidative damage in erythrocytes/tissues was determined as Malondialdehyde (MDA) production by the method suggested by Rehman (1984), whereas, SOD activity was estimated as per the method described by Madhesh and Bal Subramanian (1998). In Carbofuran and Carbaryl induced toxicity, a significant ($P < 0.05$) rise in LPO activity was observed in all the experimental groups as compared to Group I control. There was significant ($P < 0.05$) decrease in SOD values in all the experimental groups as compared to Group I control rats. In Quinalphos, a significant($P < 0.05$) dose dependant rise in Lipid peroxide level was observed in Group II and IV rats as compared to Group I and III , whereas significant($P < 0.05$) decrease in SOD values were observed in Group II and IV rats as compared to Group I and III rats. In chlorpyrifos induced toxicity, a significant ($P < 0.05$) rise in LPO activity was observed in all the experimental groups as compared to Group I control, whereas significant($P < 0.05$) decrease in SOD values were observed in Group III and IV rats as compared to Group I and II rats.The oxidative stress was also measured in liver and kidney and brain of rats induced with chlorpyrifos and carbaryl. There was significant rise in LPO activity and decrease in SOD activity in all the experimental groups as compared to Group I control rats. To conclude, quinalphos, carbofuran, chlorpyrifos and carbaryl led to oxidative stress as shown by increase in LPO activity and decrease in SOD activity in erythrocytes/tissues of Wistar rats at mentioned doses.

EVALUATION OF A RAPID MOLECULAR METHOD FOR DETECTION OF *Listeria monocytogenes* DIRECTLY FROM BROTH CULTURE

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L. monocytogenes is responsible for severe food-borne infections in humans and can also cause invasive disease in many different animal species including farm ruminants viz. cattle, buffalo, sheep and goats. The present study was carried out to know the lowest detection limit of *Listeria monocytogenes* by PCR. The quantification of organisms was done by colony forming unit (CFU) counting on potassium tellurite agar from ten folds serially diluted broth culture. Then PCR based amplification of *inlA* gene fragment was performed from each dilution. The PCR could detect as low as 20 (2×10^1) organisms indicating the lowest detection limit of PCR. Thus, lowest number of *L. monocytogenes* detectable by PCR is a low-cost and rapid procedure that can be appropriated for the detection in real time of low *L. monocytogenes* levels in naturally contaminated food and is suitable to implement in the food industry.

International symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" and XVII Annual convention of Indian Society of Veterinary Immunology & Biotechnology

29-31 December, 2011

Recommendation of different Technical sessions

1. Biotechnological interventions are required to reduce global warming by formulating modified feed, and manipulations of the genes responsible for methane production.
2. There is need to protect fauna in the Thar desert through biotechnological approaches.
3. Development of new generation diagnostics for early disease diagnosis, immuno-adjuvants and vaccines, including thermo-resistant and combination vaccines, and value addition of animal products for ensuring food security is required.
4. There is need to energise the animal health system in the country by reporting and enhancing diagnostic facilities of state animal health department.
5. There is need to develop and refine diagnostics to make cost effective, easy to use penside tests.
6. Herd Immunity against Haemorrhagic Septicemia, Foot & Mouth Disease and other diseases in livestock need be monitored nationwide to assess the level of protection offered by current vaccines.
7. Studies on efficacy of NDV vaccines and development of more efficient and thermostable vaccines using molecular techniques, using local isolates are required.
8. Establishment of quality control labs for animal product export in any veterinary universities and establishment of salmonella typing laboratory
9. Study on immuno-adjuvants including TLRs in all livestock species and development methods for enhancing response to vaccine.
10. Genotype II and IV of New Castle Disease Virus should be included in the surveillance programme.
11. Research on blue tongue be augmented to prevent the entry newer serotypes in the country
12. More emphasis should be given to DIVA (Differentiation of Vaccinated and Infected Animals) technology for important livestock diseases.
13. With the available expertise in the molecular biology effort should be made to develop recombinant based diagnostics for diseases diagnosis.
14. Molecular based epidemiological studies be directed towards disease forecasting.
15. For the diagnosis of animal diseases, a combination of conventional assays and molecular biological tools be used.
16. Molecular characterization including developing gene maps of important indigenous breeds is required to understand genetic elements associated with adaptation, production and resistance.
17. Biodiversity conservation of indigenous livestock breeds through effective utilization of best genotype for production and disease resistance traits and better managemental practices.
18. There should be more emphasis on Artificial Reproductive Technologies for enhanced livestock productivity.

SUBJECT INDEX

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Gujarat Livestock Development Board

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Podium Level, Gandhinagar
Phone No. 23244618, 23257916, Fax No. 23244618.
E-mail: gldb.gandhinagar@gmail.com

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Please contact
Veterinary Dispensaries, Animal Husbandry wing of Jilla Panchayat or GLDB - Gandhinagar for more details.