International Symposium and 10th Convention of ISVIB

on

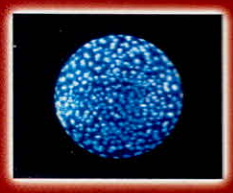
Biotechnology - Production, Productivity, Health and Value Addition

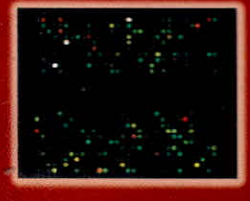
COMPENDIUM

18th - 20th Dec. 2003

at

MADRAS VETERINARY COLLEGE
Chennai - 600 007, INDIA





Organized by

Indian Society for Veterinary Immunology and Biotechnology

and

Tamil Nadu Veterinary and Animal Sciences University

ORGANIZING COMMITTEE

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Dr. R. Kadirvel	Vice-Chancellor, TANUVAS
Dr. Manju Sharma	Secretary, Department of Biotechnology, New Delhi.
Dr. V.K. Taneja	DDG Animal Sciences, ICAR, New Delhi.
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Dr. K.G. Tirumurugaan

Legends for the pictures on cover page

1. Care of pet animals.
2. ELISA kit for quantification of EDS virus-specific antibodies
3. Sheep embryo stained with Hoechst 33342 showing individual blastomere cells
4. Micro array technique for detection of pathogens

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and

Tamil Nadu Veterinary and Animal Sciences University

P.S. RAMAMOHAN RAO

Raj Bhavan
Chennai - 600 022

MESSAGE

I offer my felicitations to the Tamil Nadu Veterinary and Animal Sciences University and the Indian Society for Veterinary Immunology and Biotechnology(ISVIB) for organizing an International Symposium and the 10th Annual Convention of the ISVIB from 18 to 20 December as part of the Centenary celebrations of the Madras Veterinary College.

The theme of the Symposium is both relevant and timely. If one can find satisfactory answers to the moral issues involved, biotechnology can go a long way in enhancing and upgrading the contribution of animals to the quality of human life and living. I hope the Symposium will identify the important strands of the strategy for use of biotechnology in animal sciences for the betterment of the human society.

I extend my best wishes for the success of the Symposium.

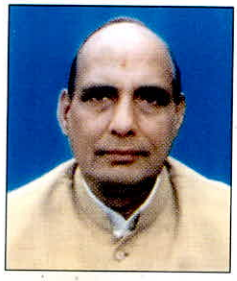
(P.S. Ramamohan Rao)

Dy. No. 1501/[redacted]/AM/2003

राजनाथ सिंह
RAJNATH SINGH

सत्यमेव जयते

कृषि मंत्री
भारत सरकार
कृषि भवन
नई दिल्ली-110 001
MINISTER FOR AGRICULTURE
GOVERNMENT OF INDIA
KRISHI BHAWAN



MESSAGE

I am very happy to know that Indian Society for Veterinary Immunology and Biotechnology and Tamil Nadu Veterinary and Animal Sciences University are jointly organizing an international symposium on "Biotechnology – Production, Productivity, Health and Value Addition" during December 18-20, 2003 in continuation of the Centenary Celebrations of Madras Veterinary College. It is a pleasure to learn about the past history of Madras Veterinary College and its achievements. I congratulate the teachers, scientists, students and other faculty members for putting their untiring efforts to add another feather in the cap of this great institution.

The theme of the symposium is very relevant to our country in the 21st century. I wish the organizers of the above event a grand success.

(RAJNATH SINGH)

हुक्मदेव नारायण यादव
HUKUMDEO NARAYAN YADAV

कृषि राज्य मंत्री
भारत सरकार
कृषि भवन
नई दिल्ली-110 001
MINISTER OF STATE FOR AGRICULTURE
GOVERNMENT OF INDIA
KRISHI BHAWAN
NEW DELHI-110 001

19 NOV 2003

MESSAGE

It is great pleasure for me to know that a century old Madras Veterinary College is organising an International Symposium on **"Biotechnology – Production, Productivity, Health and Value Addition"** in collaboration with Indian Society for Veterinary Immunology and Biotechnology and Tamil Nadu Veterinary and Animal Sciences University during 18-20 December, 2003. I felicitate Madras Veterinary College for its centenary celebration and again deem it a privilege to wish the faculty, scientist and other staff a grand success in organizing the International symposium.

This International Symposium, will certainly be a good platform to discuss all the important issues and exchange of biotechnologies available globally which will provide impetus and also drew the attention of large number of students and scientists including industries involved in various discipline and enable them to interact with each other to generate new ideas and innovations in this regard.

I wish all the success to the participants and the organizers of this International Symposium.

19/11

(Hukumdeo Narayan Yadav)

P.V. DAMODHIRAN
MINISTER FOR ANIMAL HUSBANDRY
& Pro-Chancellor,
TANUVAS.

SECRETARIAT
CHENNAI - 600 009

Date..... 01.12.2003 .....

MESSAGE

I am greatly pleased to note that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) are jointly organizing an International Symposium and 10th Convention of ISVIB on "Biotechnology - production, productivity, Health and Value addition" during the month of December 2003, at Madras Veterinary College, Chennai.

The theme of the Symposium rightly lays emphasis on updating our knowledge with latest scientific achievements in the fields of vaccination and disease prevention. The Madras Veterinary College celebrated its Centenary in the presence of Hon'ble Chief Minister Dr. Puratchi Thalaivi "**AMMA**" and is poised for further growth and international fame under the able guidance of our beloved Hon'ble Chief Minister "**AMMA**".

I fervently hope that the ideas that emanate from the deliberations at the Symposium are extended to the field to actually benefit the farmers and livestock for whose welfare, our Hon'ble Leader "**AMMA**" and her Government are striving all the time.

On this occasion, I extend my heartiest greetings on behalf of our Government for the successful conduct of the Symposium bringing greater international glory to the Madras Veterinary College and the University.

P.V. Damodaran
1.12.2003

(P.V. DAMODHIRAN)

सत्यमेव जयते

डॉ. मंगला राय
सचिव एवं महानिदेशक

DR. MANGALA RAI
SECRETARY & DIRECTOR-GENERAL

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद्
कृषि मंत्रालय, कृषि भवन, नई दिल्ली - 110 001

GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE, KRISHI BHAVAN, NEW DELHI - 110001
Tel. 2338 2629; Fax 91-011-2338 7293; E-mail: mrai@icar.delhi.nic.in

MESSAGE

It is indeed a matter of great pleasure to know that Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai has entered its centenary year. It is heartening to see that to commemorate this historic occasion, TANUVAS and Indian Society for Veterinary Immunology and Biotechnology (ISVIB) are jointly organizing International Symposium on "Biotechnology – Production, Productivity, Health and Value Addition" during 18-20 December, 2003 at Madras Veterinary College, Chennai.

I learn that the participants would deliberate on application of biotechnological tools in ETT, IVF, value added products, vaccines, diagnostics, biotech based epidemiological methods in the surveillance of animal disease, etc.

I convey my heartiest congratulations to Madras Veterinary College for completing 100 years of existence and convey my best wishes for the success of the Symposium.

(MANGALA RAI)

Dated the 25th November, 2003
New Delhi

LAKSHMI PRANESH, I.A.S.
CHIEF SECRETARY

SECRETARIAT
CHENNAI - 600 009

MESSAGE

Dt: 3-12-2003.

I am glad that ISVIB & TANUVAS are jointly organizing an International Symposium and 10th Convention on "Biotechnology - Production, Productivity, Health and Value Addition" during 18-20th December, 2003.

Madras Veterinary College has been in the forefront in the field of Veterinary Science, Research and Education. I am glad that spirit of developing and disseminating modern technology continues among the veterinary fraternity of Madras Veterinary College. Biotechnology is the need of the hour. I am happy to know that Veterinary Faculty of TANUVAS is also ahead in this area.

I convey my best wishes to all the faculty members who are involved in organizing this International Symposium.

2-12-2003

(LAKSHMI PRANESH)

Phone 2567 15 55 Fax 2567 71 28, 2567 23 04

VISHWANATH SHEGAONKAR, I.A.S.,
Secretary to Government.

Animal Husbandry & Fisheries
Department
Secretariat,
Chennai 600 009.

(O) 044-2567 2937
® 044-2487 2857
Fax: 044-2567 7590

Dt: 13-11-2003

Message

I am happy to learn that the Indian Society for Veterinary Immunology & Biotechnology and Tamilnadu Veterinary and Animal Sciences University (TANUVAS) have jointly decided to organize an International Symposium and 10th Convention of ISVIB on **"Biotechnology-Production, Productivity, Health & Value Addition"** during 18th -20th December, 2003 to commemorate the centenary event of the Madras Veterinary College of Chennai which has a long inning of rendering services to the development of Animal Husbandry and Fisheries sector. I have no doubt that the deliberations of this symposium would be of immense use to all the concerned to achieve higher growth rate in the sector by introducing new bio-technological skills and information. I take this opportunity to wish the symposium all success.

(VISHWANATH SHEGAONKAR)

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
MADRAS VETERINARY COLLEGE CENTENARY CELEBRATIONS
(1903 - 2003)

Dr. R. KADIRVEL, Ph.D.
Vice-Chancellor

Madhavaram Milk Colony campus
Chennai - 600 051, Tamil Nadu, India

Date : 5-12-2003

MESSAGE

I am immensely pleased to know that the Indian Society for Veterinary Immunology and Biotechnology is organizing an International symposium jointly with Tamil Nadu Veterinary and Animal Sciences University on "*Biotechnology- Production, Productivity, Health and Value addition*" during 18-20 December 2003. It is heartening to note that the scientists of this society are converging at this historic college to participate in the international symposium in commemoration of 100 years of excellence of Madras Veterinary College in the service of innumerable dumb animals and for the upliftment of downtrodden society.

India is a country with vast genetic diversity and provides ample scope to tap strategic genetic potential for improved production. Our country is bound to witness a demand driven revolution in livestock food production to meet the growing needs of the ever expanding human population and to comply with international obligation in the present process of globalization.

In order to achieve desirable and palpable changes in our production system a paradigm shift in research priorities by forging frontier technologies at appropriate levels for factory farming is essential to accelerate productivity per unit cost.

I hope this symposium will throw light on assisted reproductive technology for the improvement of fertility in farm animals, biotechnological tools on biosecurity, global trends in nanobiotechnology that can alter the cardinal characters of livestock and poultry and novel product development to compete with world market.

I am sure that this International symposium will play significant role to achieve these objectives. I extend my wholehearted wishes to the organising committee for successful conduct of this scientific convention.

(R. Kadirvel)
VICE-CHANCELLOR

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E.Mail : rkadirvel@yahoo.com; tanuvas@vsnl.com; vetvc@md3.vsnl.net.in; Website : www.tanuvas.com

M.S. SWAMINATHAN RESEARCH FOUNDATION

M.S. SWAMINATHAN
Chairman

MESSAGE

India is rich in animal genetic resources. The ownership of farm animals is more egalitarian than the ownership of land. Therefore livestock play a critical role in strengthening household nutrition and livelihood security. India today occupies the first position in milk production in the world. This has become possible because of the contributions of nearly 60 million women who manage the decentralised and small-scale dairy industry, as well as due to the growth of dairy co-operatives which confer the power of scale on small-scale producers.

Biotechnology will help further to improve the productivity, profitability and sustainability of our livestock enterprises. I therefore wish the International Symposium much success.

M S Swaminathan

3rd Cross Street, Taramani Institutional Area, Chennai (Madras) - 600 113, INDIA.
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E-mail : msswami@mssrf.res.in

Indian Society for Veterinary Immunology and Biotechnology

(Regd. Society No.409/1990,Chennai Central, Tamil Nadu)

K.S. PALANISWAMI, M.V.Sc., Ph.D.,
President, ISVIB and
Chairman, Organising Committee
e-mail: kspswami@yahoo.com

MESSAGE

Greetings. A most important objective of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is the dissemination of technical knowledge and information. Annually organized ISVIB meetings with premier institutions always have a few definite themes. On this occasion, these include focus, development and application of gene based technology for improving animal production and health.

The joint organisation with Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai is with a purpose. TANUVAS has a favoured destination for national and international funding for prudent research as it claims the honour of first veterinary university in India. Any action at TANUVAS will have a serious exercise towards research convergence with urge to be innovative.

This society feel proud and is privileged to have this International Symposium, jointly organised with such great organization. ISVIB's appreciation is also due to the excellent support from ICAR., DBT., DST and all other sponsors and well wishers.

The papers to be given in the symposium will cover all important topics of the theme and will be very constructive and enjoyable by the delegates from India and abroad.

I extend my personal thanks, to the authors, members of the organizing committee and the authorities of the University for the immense amount of work and support that was necessary to have this symposium, with elegance and excellence.

10.12.03

K.S. PALANISWAMI

सी सी एम बी
कोशिकीय एवं आणविक जीवविज्ञान केन्द्र
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्)
उप्पल रोड, हैदराबाद - 500 007, भारत

CCMB
CENTRE FOR CELLULAR AND MOLECULAR BIOLOGY
(Council of Scientific & Industrial Research)
Uppal Road, Hyderabad - 500 007, India

Dr. Lalji Singh
Director

MESSAGE

No doubt, there is an upsurge of biotechnology all over the world during the last two decades. To reassert the supremacy in the biotech arena, different states in India have taken initiatives for the promotion of biotechnology. However, it requires increased attention from political as well as bureaucratic leadership along with proactive approach from research and academic institutions, industries apart.

About a month back, the nation celebrated former Prime Minister Pandit Jawaharlal Nehru's Birthday as "Biotechnology Day", keeping with the increasing impact of biotechnology. Production of superior livestock, designing of vaccines, molecular diagnostics, controlled farm breeding through ETT, IVF, etc. are no more dreams. However, it is imperative that such technologies become an integral part of the development strategies essential for the uplift of common man all over the world in general, and our nation in particular.

I am extremely happy that ISVIB and TANUVAS have jointly come forward to organize this Convention which is not only timely but also most appropriate for creating the awareness of various avenues available in the area of biotechnology for researchers, teachers, entrepreneurs and students as well.

I am pleased to heartily extend my best wishes to the Convention. I wish the event a grand success.

L. Singh

[Lalji Singh]

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तार : बायोसेन्टर
Telegram : Biocentre

Prof. B. B. Mallick, D.Sc., F.A.I., F.N.A.V.S.

Formerly :
Vice-Chancellor, W.B. University of Animal and Fishery Sciences.
Director, Indian Veterinary Research Institute, Izatnagar.
Director, Central Institute of Research on Goats, Makhdoom.
Professor of Eminence, Indian Council of Agricultural Research.
Advisor, Andhra Pradesh Agriculture University.
Station-in-Charge, Indian Veterinary Research Institute, Mukteswar-Kumaon.
President, Medical and Veterinary Science, Indian Science Congress Assoc.
President, Indian College of Allergy and Applied Immunology.
President, Indian Society for Veterinary Immunology and Biotechnology.

Fellow, Indian College of Allergy and Applied Immunology.
Fellow, National Academy of Veterinary Science (Indian).

&
Founder President, ISVIB

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Phone : 359-8282
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E-mail : biswas_a@yahoo.com

Date .....

25.10.2003

Dear Prof. Nachimuthu,

I am glad to know that the Society for Veterinary Immunology and Biotechnology in collaboration with Tamilnadu Veterinary and Animal Sciences University is organising the 10th Convention of this Society at Madras Veterinary College, Chennai on the occasion of Centenary year of this great institution. It is also heartening to know that an International Seminar on **Biotechnology – Production, Productivity, Health and Value Addition,** during 18 and 20 December 2003 is also being organised during this convention and a large number of delegates from the country as well as from abroad will be participating in this International Seminar.

The society has many credits in the past and has established reputation in the country through scientific deliberations on important subject of Veterinary Immunology and Biotechnology. This international seminar is going to add another laurel to the achievements of this society. I am glad that the authorities have rightly decided to organise this international seminar on the occasion of the **Centenary Celebration** of the **Madras Veterinary College**, one of the pioneer institution in this country.

I wish a Grand Success of the 10th Convention and the international symposium.

Yours sincerely,

(B. B. Mallick)

Indian Society for Veterinary Immunology and Biotechnology

(Regd. Society No.409/1990, Chennai Central, Tamil Nadu)
TANUVAS, Madhavaram Milk Colony, Chennai - 600 051. India.

Prof. Dr. K. Nachimuthu, Ph.D.,
Organising Secretary & Director of Research

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tanuvasisvib@yahoo.com

Date : 08.12.2003

REPORT FROM THE ORGANISING SECRETARY'S DESK

Indian Society for Veterinary Immunology and Biotechnology (ISVIB) was formed in 1990 and since then it has been the torchbearer for the cause of Biotechnology education and research by holding symposia annually at different places in India. For the first time in the history of ISVIB, an international symposium is being organised at Chennai in commemoration of the centenary year of Madras Veterinary College.

Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) and ISVIB are jointly organising the symposium on the theme Biotechnology – Production, Productivity, Health and Value addition. The symposium would focus on the contemporary areas of Biotechnology and Immunology with the aim to kindle the interest of the young scientist and students to help them understand the cutting-edge technologies used in their respective fields.

There has been overwhelming response by the scientists from USA and India to participate in the symposium. There are five sessions aptly designed to focus on the imminent areas of Biotechnology and Immunology. Ten American delegates have accepted to address the gathering and around 120 Indian delegates are expected to participate in the symposium. The special feature of the symposium is an interface session of academics and industry to address the challenges faced by livestock and poultry industry in the context of WTO. This meet would help to bridge the existing wide gap between academic institution and the Industry and identify the collaborative roles of Industry and Institutions.

The financial support extended by TANUVAS, ICAR, DBT, DST, TNS & T, Corporate bodies and institutions for the conduct of the symposium is greatly acknowledged. I thank all the delegates who have responded to our invitation and making the symposium a grand success.

Last but not least I would like to thank Dr. R. Kadirvel, Vice-Chancellor, TANUVAS who has been the source of inspiration and guidance for the successful conduct of the international symposium.

8/12/2003

(Dr. K. NACHIMUTHU)

PROGRAMME

THURSDAY, 18TH DECEMBER, 2003

09.00 – 10.00	**Registration**		
10.00 – 11.00	**Inauguration of the Symposium**		
11.00 – 11.30	Coffee Break		
11.30 – 12.00	**Dr. Richard Masillamony Oration Awards (2002 & 2003)**		
12.00	**Session I – Biotechnology in Animal Production**		
12.00 – 12.30	Prof. P.B. Seshagiri	Indian Institute of Science, Bangalore	Transgenic Animal Production – Perspectives and Prospects.
12.30 – 13.00	Dr. Muthu Periasamy	Ohio State University, USA	The genetic manipulation of animals to improve organ function.
13.00 – 13.30	Dr. N. Balaraman	National Dairy research Institute Karnal	New Vistas in Biotechnology
13.30 – 14.00	Lunch		
14.00 – 16.30	**Session II – Biotechnology in Animal Production (contd)** Research papers presentation		
16.30 – 16.45	Coffee Break		
16.45	**Session III – Biotechnology in Animal Health Care**		
16.45 – 17.15	Dr. Polly Roy	London School of Hygiene & Tropical Medicine, UK	Genetically engineered structure-based vaccine
17.15 – 17.45	Dr. Sri. S. Srikumaran	University of Nebraska, USA	Emerging concepts in Vaccinology
17.45 – 19.00	Research papers presentation		

FRIDAY, 19th DECEMBER, 2003

09.00	**Session IV – Biotechnology in Animal Health Care (contd)**		
09.00 – 09.30	Dr. M.S. Shaila	Indian Institute of Science, Bangalore.	Plant-based oral delivery vaccine for livestock.
09.30 – 11.00	Research papers presentation		
11.00 – 11.15	Coffee Break		
11.15 – 13.00	Session IV – (contd)		
13.00 – 14.00	Lunch		
14.00 – 16.30	Session IV - Biotechnology in Animal Health Care (contd)		
16.30 – 16.45	Coffee Break		

16.45	**Session V – Biotechnology in Pet Animal Care**		
16.45 – 17.15	Dr. S. K. Ray	College of Vet.Sci, Orissa	Novel vaccines and diagnostics for pet animal care.
17.15 – 17.45	Dr. Pulok K. Mukerjee	Jadavpur University, Kolkata	Botanicals used in Veterinary Medicine
17.45 – 18.15	Research papers presentation		

SATURDAY, 20th DECEMBER, 2003

09.00	**Session VI – Biotechnology Products and Value Addition**		
09.00 – 09.30	Dr. Mahadev Murthy	National Institute of Health, USA	Biotechnology in India: Beginning of a New Era.
09.30 – 10.00	Dr. P.K. Sehgal	Central Leather Research Inst. Chennai.	Biomaterials and Devices for Health care.
10.00 – 11.00	Research papers presentation		
11.00 – 11.15	Coffee Break		
11.15 – 12.00	**Session VII Interface Session : Scientists Vs Industry Meet**		
	Dr. A.T. Venugopalan	TANUVAS	Challenges facing the poultry industry
12.00 – 13.00	Session VII (contd)		
13.00 – 14.00	Lunch		
14.00 – 15.00	**Young Scientist Award Institution and Award of Fellowships of ISVIB**		
15.00 – 16.00	**Plenary Session**		
16.00 – 17.00	**Valedictory Function**		

CONTENTS

KEY NOTE ADDRESSES

KN-2

Genetic Manipulation of the heart using embryo gene transfer Studies on SR calcium ATPase pump

*Gopal J Babu and Muthu Periasamy**

Department of Physiology and Cell Biology and Davis Heart and Lung Research Center,, The Ohio State University, 304 Hamilton Hall, 1645 Neil Ave, Columbus, OH 43210
Running Title: SERCA2 transgenic mouse models
**Address for Correspondence: Phone: 614-292-2310,Fax: 614-292-4888, Email: periasamy.1@osu.edu*

Abstract

Transgenic technology has been widely used in experimental research to study the role of specific genes in normal physiology and their relevance to disease mechanisms. To date a number of genetically altered mouse models are available for complex human disease including cancer, heart disease and neuronal disorders. Research in our laboratory has been focused on understanding the mechanisms contributing to human heart failure. We and others have discovered that alteration in expression and activity of sarco (endo) plasmic reticulum Ca^{2}+ ATPase (SERCA) is associated with end stage heart failure in human and in experimental animal models of heart failure. To directly address the importance of SERCA pump in regulating intracellular calcium and cardiac contractility, we have genetically altered the SERCA protein expression levels using transgenic and gene targeted knockout mouse technology.

Key words : SR Ca^{2}+ ATPase, cardiac specific gene expression, transgenic, knockout, mouse

Introduction

Intracellular calcium acts as a signaling molecule in regulating the most complex cellular functions such as contraction, secretion, fertilization, and metabolism. In cardiac muscle, the sarcoplasmic reticulum (SR) membrane network plays a central role in beat to beat function of the heart by regulating the intracellular Ca^{2}+ concentration (1-4). Release of Ca^{2}+from the SR initiates muscle contraction, whereas reuptake of Ca^{2}+ by the SR results in muscle relaxation. The rate of muscle relaxation is primarily determined by the Ca^{2}+ uptake function of a Ca^{2}+ transport pump, the sarco (endo) plasmic reticulum Ca^{2}+ ATPase (SERCA) (1-4). SERCA is a transmembrane protein of ~110 kDa and belongs to a family of P-type ion pumps (2, 5). SERCA protein isoforms are encoded by a highly conserved family of genes, SERCA1, SERCA2 and SERCA3 (6, 7). SERCA1 gene encodes two alternatively spliced transcripts SERCA1a and SERCA1b and is exclusively expressed in fast skeletal muscle. The SERCA2 gene encodes SERCA2a and SERCA2b isoforms which differ only in the carboxyl-terminus. SERCA2a has a short C-terminus with four unique amino acids whereas SERCA2b has 49 amino acids at the C-terminus (6, 7). SERCA2a is the primary isoform expressed in the adult atria and ventricle (8, 9).

A number of studies conducted on animal models of heart failure and human failing hearts suggest that alterations in SR function, including decreased SR Ca^{2}+ store and Ca^{2}+ release, elevated diastolic Ca^{2}+, and reduced rate of Ca^{2}+ removal as hallmarks of the failing heart (10, 11). It has been documented that these changes are due to reduced expression and activity of the SR Ca^{2}+ ATPase. This phenomenon could account for diastolic (increased diastolic calcium levels) and systolic dysfunction (decreased availability of SR calcium) and thereby contribute to the progression of heart failure (10, 11). Although importance of SERCA pump in cardiac physiology is clear, the degree to which normal cardiac function and long-term health are dependent on the appropriate levels of SERCA2 in heart is uncertain. Genetically modified mouse models have proved to be extremely useful for understanding the regulation of many gene products and the role of specific genes in complex biological process. The ability to knockout genes

and to overexpress transgenes specifically in the heart made it possible to address the significance of SERCA isoforms and the physiological relevance of altered SR Ca^{2}+ pump expression. In this chapter, we describe the methods that we have used to generate transgenic mouse models with altered SERCA levels.

Cardiac specific overexpression of SERCA pump

Transgenic animals with increased SERCA pump levels were generated by a number of investigators (6, 12). In our laboratory, transgenic mouse models overexpressing different SERCA pumps 1) the cardiac isoform SERCA2a, and 2) the fast twitch-isoform SERCA1a were generated. The cardiac α – MHC promoter based vector system which has been used by several investigators (13-18) was used to target the ectopic expression of SERCA isoforms in the heart muscle. The -MHC promoter is cardiac specific and is developmentally regulated allowing gene expression perinatally. The cloning strategy for creating the recombinant vector is shown in Figure 1. The recombinant plasmid construct was injected into the male pronucleus of fertilized mouse oocytes derived from super ovulated FVB/N females (19). Injected eggs were implanted into the oviduct of pseudo-pregnant females for the production of transgenic mice.

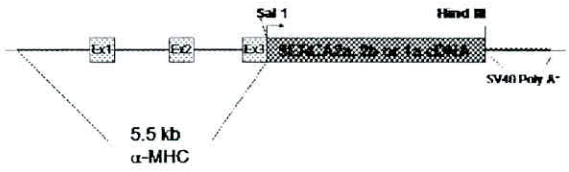

Figure 1: Schematic representation of the - MHC/SERCA transgene construct

Using the above technology, we have generated two independent mouse lines for SERCA2a overexpression which showed a 4 to 8 fold increase in SERCA2a mRNA levels and 30 to 50% in protein levels (20). Maximal velocity of SR Ca^{2}+ uptake was increased by ~37% in these animals. However the apparent affinity for Ca^{2}+ was not altered in SERCA2a transgenic hearts despite of an increase in pump level. Studies using isolated work performing heart showed increased rates of contraction (+dP/dt) and relaxation (-dP/dt).

We also developed a mouse model overexpressing the skeletal muscle isoform SERCA1a which has faster kinetics of Ca2+ transport (21). Over expression of SERCA1a in the hearts resulted in a ~2.5 fold increase in total SERCA protein levels (Fig. 2). Interestingly the endogenous SERCA2a level decreased to ~50% in the SERCA1a transgenic hearts. The maximal velocity of Ca^{2+} uptake and the steady state level of SERCA phosphoenzyme intermediate were significantly higher in the transgenic hearts. Functional analysis revealed that the hearts were able to generate significantly higher force and contractile function. Our studies also demonstrate that the fast twitch muscle isoform, SERCA1a can functionally substitute endogenous SERCA2a in the heart (21-23) without resulting in any form of cardiac pathology. These studies provided a crucial step towards using SERCA gene therapy as a therapeutic approach to rescue heart failure (24).

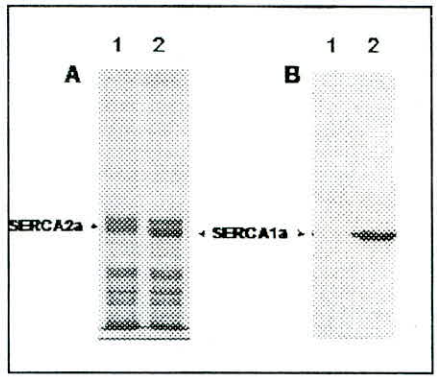

Figure 2: Western blot analysis of SERCA1a expression

Equal amounts of total protein isolated from non-transgenic and SERCA1a transgenic hearts were resolved on a 6% SDS-PAGE and stained with Coomassie blue (Panel A) or transferred to nitrocellulose membrane and immuno blotted with SERCA1a specific antibody (Panel B).

Lane 1- Non-transgenic heart and Lane 2- SERCA1a transgenic heart

Generation of a SERCA2 gene knockout mouse model

To gain a better understanding of the physiological function of SERCA2 in vivo, a mouse carrying a null mutation in the SERCA2 gene was developed. To disrupt the SERCA2 gene, the transcription initiation site, the first two exons and part of the third exon were replaced with a neomycin gene under the control of phosphoglycerate kinase (PGK) promoter in reverse orientation (Fig. 3 and ref. 25). The linearized construct was electroporated into mouse sVJ/129 embryonic stem cells. ES clones resistant to neomycin were screened for correct targeting by southern blot analysis. Targeted clones were injected into C57BL/6 blastocysts. Chimeric males were mated to B6 females and germ line transmission of the targeted allele was detected by Southern blot as well as by PCR analysis.

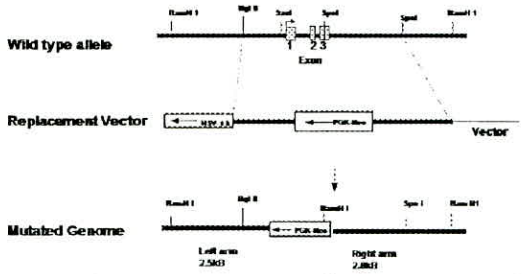

Figure 3: Gene targeting strategy for the generation of SERCA2 knockout mice

Breeding of chimeric animals led to the generation of heterozygous mutant ($SERCA2^{+/-}$) mice, but homozygous null mice for SERCA2 were not observed as expected. When compared with the wild type littermates, $SERCA2^{+/-}$ mice were indistinguishable with respect to growth, appearance and behavior. These data indicated that the loss of one copy of the SERCA2 gene did not cause serious perturbations of embryonic development or viability. To determine levels of SERCA2a mRNA and protein, RNAse protection and Western blot analyses were carried out. RNAse protection analysis showed that SERCA2 mRNA in heterozygous was reduced to ~45% (Fig.4). Western blot analysis and Ca^{2+} uptake studies showed that SERCA2a protein and activity was reduced by ~35%. Measurements of in vivo cardiac function revealed reductions in heart rate, mean arterial pressure, systolic ventricular pressure and both maximal rates of contraction and relaxation. However $SERCA2^{+/-}$ animals did not develop cardiac pathology contrary to expectations (25). We therefore reasoned that $SERCA2^{+/-}$ hearts were able to meet the functional requirements under normal conditions. However, it was not known if $SERCA2^{+/-}$ hearts would be able to adapt well to a pathological stress such as increased hemodynamic load. Our recent studies indeed demonstrate that heart failure occurred more rapidly with reduced SERCA2 levels in conjunction with pressure overload (26).

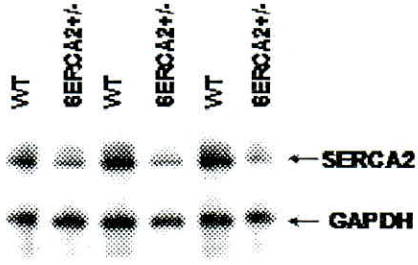

Figure 4: RNase protection analysis of SERCA2 mRNA expression in SERCA2+/- knockout hearts. Note that RNA levels for SERCA2 are reduced to ~ 50% in the heterozygous knockout mice hearts.

Conclusions

In conclusion, our studies showed that both cardiac muscle contractility and relaxation were impaired by the reduction in SERCA activity in the heterozygous mutant mice and correlated well with the increased contractility and relaxation observed in SERCA overexpressing mouse hearts. These results clearly demonstrate that the levels of SERCA pump affect the contractility by controlling the amount of calcium available for release from the SR and affect relaxation by controlling the rate at which calcium is retaken from the cytosol. Our overexpression studies also demonstrate that it is possible to increase the SERCA pump level in vivo and the long-term overexpression of SERCA is not pathological. Further, results from transgenic and knockout mouse models strongly support that SERCA gene transfer can effectively be used to enhance cardiac contractility and rescue contractile function in failing human hearts. These transgenic approaches described here were very important towards better understanding functional importance of individual genes.

Although it has been relatively easy to apply these techniques in mouse models, application of this technology in larger animals are somewhat difficult. There is an increasing effort to produce transgenic livestock with improved quality and as "biopharms" (Reviewed in Ref. 27). Transgenic farm animals have been produced by a number of techniques including 1) retroviral gene transfer, 2) microinjection of genes into pronuclei of ova, 3) injection of ES cells into blastocysts 4) sperm-mediated exogenous DNA and 5) nuclear transfer with ES or embryonic germ cells. The disadvantageous like limitations of DNA size, lower frequency of integration of foreign DNA into the chromosome and low success rates limits the use of some of these transgenic techniques in making transgenic farm animals. Birth of first cloned piglets generated by nuclear transfer using adult or fetal cells as nuclear donors promising the use of nuclear transfer technique in the field of animal biotechnology. Further, sophisticated strategies for controlled tissue or cell specific gene expression that are being currently being examined in transgenic mice could be extended to the generation of transgenic livestocks

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Genetically engineered structure-based vaccine for bluetongue disease

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Abstract

Recent protein expression technology has provided novel approaches for development of intrinsically safe vaccines. The technology involves the synthesis of immunogenic proteins and particles that elicit highly protective immune responses. Successful vaccine development requires systems where the engineered products mimic the authentic proteins, not just in terms of their primary amino acid sequences but specifically in terms of their three dimensional structures, i.e., the products must be as authentic as possible. We have generated and utilized one such protein engineering systems to develop vaccine for an insect-borne animal disease caused by a structurally complex virus, Bluetongue virus. By appropriately designing and manipulating viral genes we have synthesised multilayered, multiprotein complex structures at a high level. These synthetic particles essentially mimic the virus particles, but completely lack genetic materials hence no opportunity to breakthrough mutation. The immune responses of these non-replicating empty particles (virus-like particles, VLPs) have been tested animals (BTV susceptible sheep) in a series of clinical trials using 50 to 200 sheep in each trial. The results from these trials are highly encouraging which demonstrated that as little as 10 µg VLPs in each sheep afforded long lasting protection (at least 15 months, maybe longer) against bluetongue disease when challenged with the same strains of virulent virus as the vaccine strains. Moreover, the protective immune responses were sufficiently strong to protect the vaccinated animals against related heterologous strains (there are total of 24 types of BTV) of the virus indicating that not types of VLPs would be required to combat the infection caused by 24 serotypes. Such complex subunit vaccines offer particular advantages as potential vaccines over other systems.

Introduction

Most of the current viral vaccines are prepared using attenuated or inactivated virus. This approach, although useful in many cases, has certain drawbacks including vaccine breakthroughs or disease caused by incompletely inactivated vaccine. Viruses with multiple serotypes, as well as segmented genomes, particularly pose challenging tasks for development of safe vaccines as gene exchange between viruses may occur randomly. This may cause generation of infectious virus with mixed genes. Such live virus vaccine production has to be undertaken in containment laboratories providing added costs both for the production and the safety and efficacy testing of vaccine lots.

Bluetongue virus (BTV) is the type member of the genus *Orbivirus*, in the family *Reoviridae*. This is one of the largest families of viruses and includes major human pathogens (e.g., rotavirus) as well as other vertebrate, plant and insect pathogens. Orbiviruses are distinct from other members of the *Reoviridae* in a number of ways; they multiply in arthropod and vertebrate cells, causing often severe disease and high mortality. BTV is transmitted by *Culicoides spp.*, causing disease in ruminants in many parts of the world. To date, some 24 different BTV serotypes have been identified (BTV-1, BTV-2, etc) from different parts of the world (1). In sheep, the disease is acute and mortality may be high, whereas in cattle and goats the disease is usually milder (1). In a typical case in

sheep, the onset of the disease is marked by high fever lasting about 5-7 days. By 7-10 days, distinctive lesions appear in the mouth and the tongue can be severely affected, turning blue. In contrast to sheep, infected cattle experience prolonged viraemia and infection during pregnancy can often cause teratogenic defects in calves and abortion of the foetus.

Other orbiviruses infect a variety of animals and also cause significant diseases, for example the recent outbreak of African horse sickness virus (AHSV) in Spain and Portugal. The spread of *Culicoides* insects from endemic to non-BTV and non-AHSV regions in the past highlights the concern that these viruses are a threat to areas that are presently free from viral infection. As a result of its economic significance, BTV has been the subject of extensive molecular, genetic and structural studies and now represents one of the most well characterised viruses (2). BTV virions are architecturally complex structures composed of 7 discrete proteins (Fig. 1) in a specific but non-equimolar ratio that are organised into two shells, the inner core and outer capsid (2).

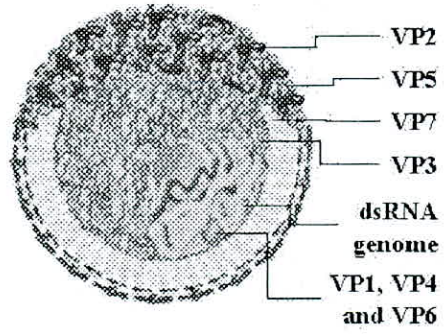


Fig. 1 *BTV particle: A schematic of BTV particle showing the organization of 7 BTV proteins and the RNA genome.*

The virion contains a genome of 10 double-stranded (ds) RNA segments. The outer capsid is composed of two major structural proteins (VP2 and VP5) and is involved in cell attachment and entry of virus during the initial stages of infection (3). Shortly after infection, BTV is uncoated (VP2 and VP5 are removed) to yield a transcriptionally active core particle that is composed of two major proteins (VP3 and VP7), three minor proteins (VP1, VP4 and VP6) and the dsRNA genome (2). While the four major proteins (VP2, VP3, VP5 and VP7) form the bulk of the virus capsid, the 3 minor proteins, together with genomic RNA form the virus replication complex. In addition to the structural proteins, non-structural (NS) proteins NS1, NS2, NS3 and NS3A are made in BTV-infected cells which are involved in virus replication and assembly (2).

Of the ten BTV proteins, only the two outer capsid proteins, VP2 and VP5 that are responsible for virus entry into the susceptible host cells (3, 4), are variable from serotype to serotype (24 serotypes), although close phylogenetic relationships are easily detectable indicating that the genome mutations may have played a major role in generating multiple serotypes (5). In contrast to VP2 and VP5, all five core proteins and the three non-structural proteins are highly conserved (5). Both by *in vitro* (tissue culture) and *in vivo* (animal) studies we and others have shown that BTV is highly capable of reassorting the RNA segments between different serotypes (2). Therefore, potentially, the whole virus vaccines might play a significant role in the generation of endemic strains.

In the past, live attenuated Orbiviruses have been employed as vaccines in those regions of the world where BTV or AHSV cause epidemics of disease in livestock. Although reasonably effective, since vaccine strains are replication competent and since orbiviruses have segmented genomes there is some concern over the use of live attenuated virus vaccines. Live virus vaccines may aid virus maintenance in nature, and facilitate genome segment reassortment and the generation of new viral genotypes. Moreover, since there are multiple serotypes of BTV and AHSV, it is likely that not all vaccine strains would have required level of attenuation.

In order to develop rationally designed BTV vaccines, over the past few years we have been developing an understanding of the structural and functional relationships of the BTV genes and gene products and the assembly pathway for the formation of virions. Recent advances in gene manipulation have made it possible to express foreign genes in heterologous systems. The productivity and flexibility of insect baculovirus expression vectors and the ability of the baculovirus genome to incorporate (and express) large amounts of foreign DNA in *Spodoptera frugiperda* insect cells have permitted this system to be used for the expression of not only a single gene, but also for the simultaneous expression of dual and multiple genes. To accomplish this, several expression vectors have been developed based on the resident promoters of the nuclear polyhedrosis virus of *Autographa californica* (AcNPV), (6), (7), (8). Using these various expression vectors, we have expressed all ten genes of BTV either individually or in various combinations using single, dual, triple and quadruple expression vectors and have analyzed the

structure-function of each gene and gene-product (5). Information gained from these studies has served as platform for rationally designed safe vaccine for BTV and AHSV. Extensive clinical trials have been conducted using these proteins and protein structures, some of which are discussed below.

Results

Three-dimensional structures of BTV virion and core

To design the VLP vaccine it is necessary to understand the structural organisation of BTV capsid. Therefore, the three-dimensional structure of cores and virions were determined by cryoelectron microscopy and computer image reconstruction methods which allow to visualize the individual protein organisation in large particles such as viruses. The major advantage of cryoelectron microscopy over conventional electron microscopic techniques is that the biological molecules are observed in a frozen hydrated state in amorphous ice, which closely resembles the native aqueous state. The problems associated with heavy metal stains, fixatives and dehydration are thus avoided.

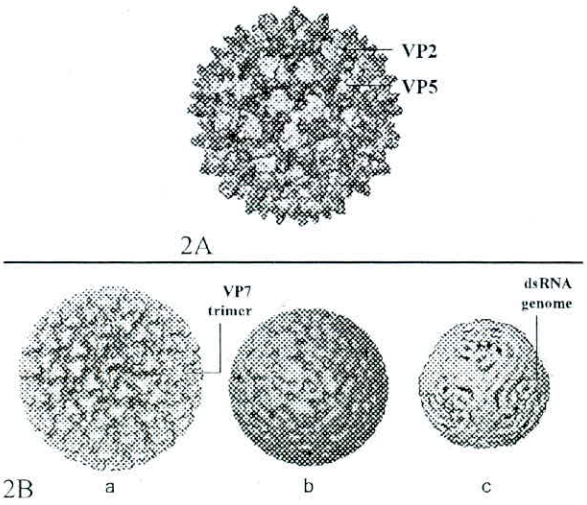


Fig. 2 Surface representation of a cryoelectron micrograph of BTV. (A) An icosahedral complex whole virus particle viewed along a two-fold axis, showing the topography of the two outercapsid proteins, one globular-shaped, VP5 and the other sail-shaped VP2 protruding for 4nm above the surface of the particles.

***(B)** BTV core viewed along the icosahedral three-fold axis (a) showing the protrusions of 260 VP7 trimers in the outer layer (b) showing the smooth inner VP3 layer formed by 120 molecules (c) RNA genome and replication enzyme complex formed by VP1, VP4 and VP6 (9).*

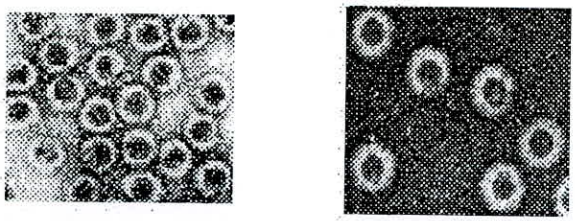
The surface of the core serves as a foundation for deposits of the two outer capsid proteins, VP2 and VP5. Cryoelectron micrographs (Cryo-EM) analysis has revealed a wellordered morphology of the virion outer capsid. This is in contrast to the morphology deduced by negative-staining methods, which indicate that the outer capsid of the complete BTV particle has a fuzzy appearance (2). The capsid has an icosahedral configuration and the two proteins of the capsid have distinctive shapes, one globular and almost spherical, the other sail-shaped (Fig. 2A) (9, 10) . The globular proteins, 120 in number, sit neatly in the channels formed by each of the six-membered rings of VP7 trimers. The sail-shaped spikes, which project 4nm beyond the globular proteins, are located above 180 of the VP7 trimers and form 60 triskelion-type motifs that cover nearly all the VP7 molecules.

These spikes are the viral haemmagglutinating protein VP2, which also contains the virusneutralizing epitope, and that the globular proteins are VP5. The two proteins are attached to underneath VP7 layer and together they form a continuous layer (outer capsid) around the core except for holes on the fivefold axis. The structures of these two outer capsid proteins indicate that the formation of VP7 layer is essential for deposition of the outer capsid.

It was therefore necessary to examine the core structure. Cryo-EM analysis of BTV cores revealed that the core has a diameter of 69 nm and that the surface exhibits icosahedral symmetry with a triangulation number of 13 (Fig. 2B) (11). The core structure is divided into two concentric layers of protein enclosing the RNA and minor proteins. The surface layer of the core is made up of clusters of VP7 trimers, which bear prominent knob-like protrusions and which are organized into pentameric and hexameric units with channels between them (Fig. 2B panel a). There are a total of 780 VP7 molecules per particle, 132 channels and 260 trimers or knobs at all the threefold axes. The smooth scaffold for the VP7 trimers is made up by 120 molecules of the second major protein VP3 (Fig. 2B panel b) which is closely associated with the VP7 trimers. The remaining 3 minor enzymatic proteins, VP1, VP4, VP6 that are responsible for replication of viral genome occupy the innermost components genomic RNA (Fig. 2B panel c).

Assembly of BTV core-like and virus-like particles by baculovirus expression systems Since VP2 and VP5 together induced protective immune response in sheep it is likely that the immunity would be enhanced if these proteins could be presented in a similar manner as in

native virion particles. It would be rewarding if virus capsid structures without the genetic materials could be synthesised. The flexibility of baculovirus expression vectors and the capacity of the baculovirus genome to accommodate large amounts of foreign DNA allowed us to exploit the system for the simultaneous expression of multiple BTV genes in a single insect cell. Since 3D studies indicated that it might be possible to obtain a stable scaffolding core structure consisting only the VP3 and VP7 which may allow eventually assembling VP2 and VP5 on the surface, we prepared dual and multigene baculovirus vectors system.



CLPs **VLPs**

Fig. 3 Electron micrographs of negatively stained, baculovirus-expressed core-like particles (CLPs) consisting of VP3 and VP7 and virus-like particles (VLPs) consisting of VP2, VP3, VP5 and VP7.

To assemble the VP3 and VP7, a dual baculovirus expression vector was utilized to express the coding sequences of the L3 (VP3) and S7 (VP7) genes of BTV (12). Recombinant baculoviruses synthesising both proteins were isolated and indeed core-like particles (CLPs) were produced and distributed throughout the infected insect *Spodoptera* cells. Gradient-purified CLPs were similar in size and appearance to cores prepared from BTV (Fig. 3).

VP3 and VP7 were the only protein components identified in the expressed particles and the molar ratios of these two proteins were similar to those of VP3 and VP7 derived from infectious BTV. The CLPs appeared to lack nucleic acids when analysed by phenolchloroform extraction and alcohol precipitation.

Subsequently we constructed baculovirus multigene vectors to co-synthesise up to four BTV proteins in the same cell (7) (8). Two different expression cassettes were generated; one that expressed VP2 and VP5 simultaneously and the other that expressed VP2, VP3, VP4 and VP7 proteins in a single cell (7, 13). The expressed proteins from the quadruple vector assembled into virtually homogenous double-capsid particles (Fig. 3). Coinfections with two dual expression vectors (namely VP3 & VP7/VP2 & VP5) gave VLPs that contained different amounts of the outer capsid proteins, depending on the experiment (13). The formation of complete VLPs in the absence of non-structural proteins or the internal minor proteins implies that these proteins are not necessary for the assembly of these double-capsid particles or for CLPs. VLPs express high levels of hemagglutination activity, similar to that of BTV virions. Further, antibodies raised to the expressed particles gave high titers of neutralising activity against the homologous BTV serotype (13).

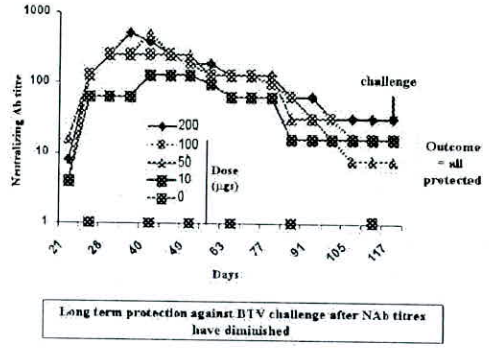


***Fig. 4** Vaccination trials of sheep with BTV-10 VLPs. 2-4 sheep were vaccinated with various doses of VLPs (1ml) in presence of 50% ISA-50 (1ml). Neutralizing antibodies of sheep at various intervals after vaccination with bluetongue are indicated. VLPs and their protective responses following challenge (after 4 months) with homologous virulent bluetongue viruses.*

When 3D structure of CLPs and VLPs were analysed by electron cryo-microscopy clearly both type of particles were comparable to that of authentic cores and virions and exhibited essentially the same basic features and full complement of the two or four proteins (14), (10). VLPs synthesized by recombinant baculoviruses were also characterized further at biological and immunological levels and compared to that of the native virion. VLPs exhibited high levels of haemagglutination activity similar to those of authentic BTV. Further antibodies raised to the expressed particles contained high titers of neutralizing activity against the homologous BTV serotype emphasizing their authenticity at functional level (13).

VLPs as Vaccine

Since recombinant baculovirus derived VLPs elicited strong neutralizing antibodies in guinea pigs, it can be anticipated that VLPs should elicit protective responses in sheep against bluetongue viral infection. Consequently, a number of experiments were performed to examine the protective efficacy of VLPs in sheep. In

each experiment, BTV-susceptible, 1-year old Merino sheep (BTV-free) were divided into groups, and each group was immunized subcutaneously with purified VLPs in saline containing the various amount of protein suspended in 50% Montanide Incomplete Seppic Adjuvant (ISA-50, Seppic, Paris). Each animal received 2 ml of the mixture. For each concentration of protein, a minimum of two sheep were used. For control experiments, one group of sheep received only saline. Each vaccinated animal was boosted with the same amounts of protein on day 21.

From day 21 to the day of challenge, serum from each animal was collected at intervals and virus neutralization tests were performed by plaque reduction neutralization last. Sheep that received VLPs developed demonstrable neutralizing antibodies, albeit to different levels (15, 16) . The levels of neutralizing antibodies depended on the amount of VLPs administered (Fig. 4). Significant levels of neutralizing antibodies were elicited with all concentrations of VLPs and persisted throughout the study. The control sheep inoculated with saline remained sero-negative. All sheep were challenged by subcutaneous inoculation of 1ml of infective sheep blood containing virulent BTV-10 (SA strain) at day 117 (Fig. 4). The clinical reactions (CRI) of the animals and the viraemia were monitored from 3 to 14 days post-challenge (17). The challenged sheep developed neither clinical signs nor viraemias, indicating suppressed replication of BTV. The post-challenge blood samples of the sheep that only received saline were viraemic and these sheep developed high neutralizing antibody titers indicative of a primary infection. In summary, protective immunity to BTV disease was obtained by vaccinating sheep with doses of 10 µg or more of BTV VLPs as well as with high doses of outercapsid protein VP2 or VP2 and VP5.

To analyse further the protective effects and duration of VLP vaccination, a similar protocol was employed for VLPs (10 µg or 50 µg per sheep) representing BTV-10 and BTV-17 (16) . The neutralizing antibody titers of the vaccinated sheep were determined at weekly intervals and over a sixty week period after the booster. Both types of VLP elicited (to various levels) antibodies that neutralized the homologous virus. In almost all cases these neutralizing titers remained high throughout the 60 week period. The neutralizing antibody titers for the animals that received 50 µg doses of VLPs were not significantly higher than those that received the 10 µg doses (Fig. 5). Sheep vaccinated with the mixture of the two types of VLPs induced antibodies that neutralized both types of virus as well as some related heterologous viruses (eg, BTV-4) when tested by plaque reduction assays. As expected, the control sheep that were inoculated with saline remained sero-negative.

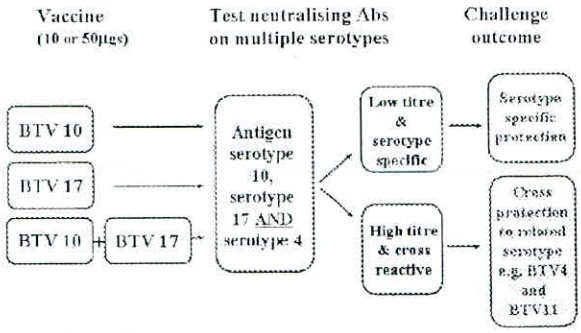


Fig. 5 VLP vaccination trials of sheep with homologous and heterologous virulent virus challenge: A group of sheep received 2 doses of mixtures BTV-10 (10 µg) and BTV-17 (10 µg) or 2 doses of mixtures of BTV-10 (50 µg) and BTV-17 VLP (50 µg). Neutralizing antibodies of sheep at various intervals after vaccination were determined and protective responses following challenge (after 14 months) with homologous or heterologous virulent viruses were assessed as described in Figures 2 and 4.

All the sheep were challenged 14 months after the booster vaccination by the subcutaneous injection of virulent BTV. The animals that were challenged with the homologous viruses (BTV-10, BTV-17) were completely protected and showed no clinical reactions, even those that received 10 µg doses of VLP (Fig. 5).

Also, no viraemias were detected in these animals after challenge. In addition, some animals with 50 µg VLPs were also protected in low level when challenged with heterologous virus. By comparison, the control animals developed high or moderate signs of disease (BTV-10, CRI: 7.1-8.0; BTV-17, CRI: 1.6-2.7) and produced viraemias.

Similar vaccination trials with a cocktail of 5 different BTV VLPs (see Fig. 6) representing 5 different serotypes were also undertaken. When these vaccinated animals were challenged with heterologus types that were not included in the cocktail, very encouraging data was achieved. There were clear indication of the cross protection and such protection was depended on both the amount of the vaccine dose as well as the sequence variations of the outer capsid proteins.

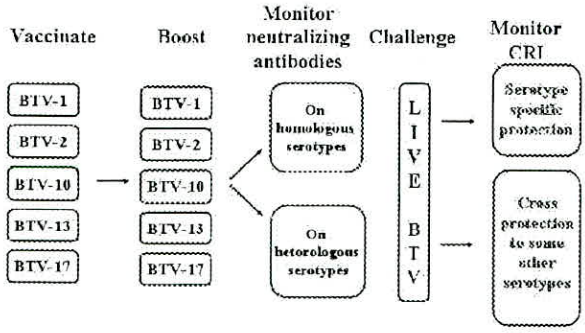


Fig. 6 VLP vaccination trials with a cocktail of BTV-2, -10, -11, -13 and -17 VLPs (10 µg each) in 50% ISA-50 given in each step, boasted after 21 days. The protection against various virulent virus challenges were determined as in Figures 2 and 4.

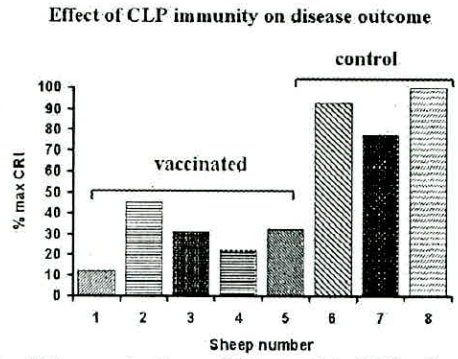


Fig. 7 Immunisation of sheep with CLPs showing partial protection against heterologous virus challenge.

In summary, the data showed that long-lasting protection against homologous BTV challenge was provided by vaccination with VLPs. Some preliminary evidence was obtained for cross-protection, depending on the challenge virus and the amounts of antigen used for vaccination (16).

Protection Afforded By Cores

BTV cores are conserved across the 24 serotypes. Therefore it would be highly beneficial if synthetic core-like particles could afford any protection against the viral infection. The question of whether CLPs containing the two conserved proteins VP3 and VP7 would provide a measure of homologous and heterologous BTV protection by cell mediated mechanism was therefore investigated. For initial studies two groups of five sheep each were used. One group of five sheep inoculated with 100 µg BTV-10 CLP in ISA-50 boosted on day 21 was challenged with BTV-10 two weeks later.

All post-challenged sheep developed viraemias and neutralizing antibodies (18). However, with the exception of fever, the vaccinated sheep developed only slight clinical reactions whereas controls showed characteristic mouth and feet lesions in addition to fever. The average CRI of the vaccinated sheep was 3.5 whereas that of the control sheep was 9.0 (data not shown). In summary, partial protection against BTV challenge was afforded by CLP vaccination.

Discussion

Bluetongue has been known to be associated with disease and mortality in sheep and cattle for decades. Despite the fact that this can have serious economic impacts, not only in terms of animal health but for some countries import and export regulations for sheep and cattle, only live attenuated vaccines have been developed in South Africa and in the United States. In South Africa, sheep are vaccinated with pentavalent live attenuated virus vaccine at 3-week intervals. In the United States, although five BTV serotypes have been identified (BTV-2, -10, -11, -13 and -17), a modified live virus vaccine is available only for BTV-10. Conventional live attenuated virus vaccines have certain inherent disadvantages. In the case of bluetongue, such virus vaccines can cause infection in the fetus with teratological consequences. When used as a polyvalent vaccine, interference occurs between the component BTV serotypes, resulting in the development of incomplete immunity. Moreover, live attenuated vaccine strains may be neutralized passively by the antibody in maternal colostrums.

Recent developments in biotechnology have made it possible to synthesize doubleshelled BTV-like particles, mimicking authentic virions but lacking the harmful genetic material and viral replicating machinery. Therefore, these particles are as safe as subunit vaccines, and potentially as effective as "whole" virus vaccine. A number of vaccination trials in BTV susceptible sheep were undertaken. The results clearly demonstrated that VLP vaccines are highly efficacious. A very small amount (10 µg) of VLPs (10-20% of the VLP mass is VP2 i.e., 1-2 µg of VP2 per dose) in the presence of appropriate adjuvant protected the sheep against the disease. There are several possible explanations. First, the conformational presentations of the relevant epitopes on VP2 probably mimic those on the authentic virus. Second, both VP2 and VP5 are present. Third, the VP3 and VP7 may provide a necessary scaffold for VP2 and VP5 antigen presentation. Fourth, any

of the four BTV proteins might have a direct role in eliciting cell-mediated immunity induced by the BTV VLPs. It can also be anticipated from the results obtained that this technology has much to offer for development of vaccines for both veterinary disease and human diseases.

VLPs offer particular advantages as potential vaccines over other systems. Additional advantage is that large quantities of these particles (BTV proteins) can be produced due to the high expression capabilities of baculovirus vectors (produced in serum-free medium), and purified using a one-step generic protocol based on the physical proprieties of the particle.

We have yet to determine the minimum amount of VLP needed for complete protection and the duration of the immunity conferred by these particles. It is also essential to perform similar vaccination trials in cattle, since they are a major reservoir host of BTV. Another important aspect of vaccine development is the role of adjuvants. Our data demonstrate that Montanide ISA-50 enhanced the neutralizing-antibody responses in sheep more effectively than incomplete Freund's adjuvant.

Our previous studies involving cDNA-RNA hybridization experiments, as well as complete sequence analysis of cDNA clones of viral RNA species, have demonstrated that both outer capsid proteins VP2 and VP5 are among the most variable proteins of different BTV serotypes. Depending on the serotype, they exhibit sequence relationships to other BTV serotypes (5). Data indicating that antigens of one BTV serotype (19) could neutralise other BTV serotype. There is every reason to believe that it should be possible to make vaccine chimaeras representing different BTV serotypes (e.g., involving the expression of several BTV VP2 genes), as well as chimaeras containing protein sequences representing other pathogens (e.g., chimaeric genes involving VP2, and/or VP5, and/or VP3, and/or VP7 sequences and selected sequences of viral bacterial, fungal, or protozoan pathogens). This is an exciting prospect for the future.

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Emerging Concepts in Vaccinology : Epitope-based Vaccines against Viral Infections

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The paradigm that the best vaccine is the one that most closely mimics the natural infection may not apply to all viral infections. Furthermore, immunization with live viruses, even after attenuation, may result in immunosuppression and/or latent infections with subsequent reactivation and shedding of the virus, as in the case of herpesviruses[1]. Therefore, research in the past few years has focused on alternatives to modified live virus (MLV) vaccines which has led to the emergence of the concept of *new generation vaccines*. Epitope-based vaccines constitute an important component of this new group of vaccines.

Epitope-based vaccines are comprised of immunogenic epitopes of the pathogen which elicit humoral and cell-mediated immune responses. Neutralizing antibodies (Abs) present at the portal of entry of the virus into the host are an important element in the prevention of virus entry into the cells. Although other cellular defenses such as antibody-dependent cellular cytotoxicity and natural killer cells may also play a role, virus-specific cytotoxic T-lymphocytes (CTLs) are the vital components of the host defense against viruses in general, and herpesviruses in which intracellular spread occurs earlier than extracellular spread, in particular. Therefore, an ideal epitope-based anti-viral vaccine should consist of neutralizing Ab epitopes and CTL epitopes. Inclusion of T-helper epitopes as well, would further enhance the efficacy of this vaccine.

Mapping of Ab epitopes, and their incorporation into an epitope-based vaccine is relatively simple. However, development of CTL epitope-based vaccines for use in an outbred population of animals faces three major challenges: 1. mapping of the CTL epitopes; 2. major histocompatibility complex (MHC) polymorphism exhibited by the outbred population, and 3. necessity to direct CTL epitopes to the MHC class I antigen presentation pathway because, unlike Abs, the majority of CTLs recognize antigenic peptides only in association with the MHC class I molecules. At this point, it is appropriate to briefly discuss the current knowledge on the generation and presentation of viral peptides by MHC class I molecules to the CTLs, and identification of CTL epitopes.

The majority of the CTLs are of the $CD8^+$ phenotype, and recognize antigens in the form of short peptides presented by the MHC class I molecules. These peptides are generated by proteasomal processing within the cytoplasm, and transported into the endoplasmic reticulum (ER). The class I molecules, consisting of the heavy chain, β_2-microglobulin (β_2m) and the peptide, are assembled in the ER, and expressed on the cell surface after maturation in the Golgi[2]. Stable cell surface expression of the class I molecules requires the presence of peptides. The minimal peptide sequence that elicits a CTL response defines a CTL epitope. The classical method of identification of CTL epitopes consists of the following steps: 1. identification of the viral protein(s) recognized by the CTLs; 2. location of the region on the viral protein recognized by the CTLs by using recombinant fragments of the protein; 3. identification of the minimal epitope by the use of peptides with N- and C-terminal truncations of the longer peptide that sensitizes targets for CTL-mediated cytotoxicity. This method is very laborious.

Sequencing of the pool of self-peptides bound by a particular class I allelic product (allomorph) has led to the development of a relatively easier method of

identification of CTL epitopes called *reverse immunogenetic* method[3,4]. This method involves the identification of the so-called *allele-specific peptide motif* (ASPM) which defines the anchor residues and their relative positions in the sequence of the peptides bound by an allomorph. Potential CTL epitopes can be predicted by scanning the viral proteins for sequences that conform to the ASPM of an allomorph. Cytotoxicity assays with targets pulsed with synthetic peptides representing the potential epitopes could identify the veritable epitopes[4]. ASPMs have been identified for several class I molecules of humans and mice, and used successfully to identify the CTL epitopes. Employing the ASPM of the murine class I allele H-2D[d], our laboratory has identified three CTL epitopes of bovine herpesvirus (BHV-1) in the murine system[5]. However, the situation is very different in cattle and other farm animals. Only a few class I alleles have been characterized. Due to the poor characterization of class I alleles, identification of CTL epitopes of livestock pathogens have lagged behind. Even when the ASPM of a class I allomorph is known, the lack of availability of suitable antigen presentation and target cell systems (such as Epstein-Barr virus transformed autologous B cell-lines in humans, and syngeneic B cell and macrophage cell-lines in mice) renders the mapping of CTL epitopes difficult. Thus, reports of cell-mediated immune response to livestock pathogens in the literature discuss only T cell proliferative responses and cytokine measurements. In our studies with BHV-1, we determined the ASPM of bovine class I allele, BoLA-A11, and used it to map the CTL epitopes of BHV-1[4]. We were able to narrow down the CTL specificity to a pool of three candidate epitopes. We could not identify the minimal CTL epitope because of the difficulty in sustaining the *in vitro* growth of CTLs long enough. Upto our knowledge, not a single CTL minimal epitope of any bovine or porcine pathogen has been identified yet.

Even when CTL epitopes of the pathogens are well defined, the problem of MHC polymorphism makes it difficult to develop epitope-based vaccines that will be effective in a population with diverse MHC background. It is necessary to incorporate at least one epitope presented by each of the alleles expressed in the population for which the vaccine is targeted. Attempts are made in the human system to incorporate a few CTL epitopes conforming to the so-called *super motif*, which might bind to, and be presented by, several alleles which are collectively referred to as a *supertype*[6,7]. However, the success of this approach remains to be proven.

In view of the problems discussed above, it is important to investigate the feasibility of developing an epitope-based vaccine that does not require the prior identification of CTL epitope sequences, and a means of channeling the epitopes to the class I antigen presentation pathway. Recent studies in several tumor model systems, and a few viral systems have revealed that certain heat shock proteins (hsps) and the associated peptides present such an opportunity.

Heat shock proteins are a group of highly conserved proteins, which are present in cells of all living organisms. Although the expression of hsps can be induced by a variety of stresses including heat (hence the names stress proteins and heat shock proteins), deprivation of nutrients, viral infections, and other stresses, they are constitutively expressed in normal cells in abundant levels[8]. Based on sequence homology and molecular weight, hsps have been grouped into different families: hsp110, hsp100, hsp90, hsp70, hsp60, hsp40 and others. Hsp90, an 83kDa protein, is the most abundant cytosolic protein in eukaryotic cells with a homolog in the ER. The ER-resident homolog of hsp90 is induced by glucose starvation, and hence is referred to as the glucose-regulated protein. It is commonly called as gp96. Similar to other ER-resident proteins, gp96 has an N-terminal signal sequence, and a C-terminal ER retention signal. Studies in the past decade revealed that hsps and their close relatives serve as molecular chaperones that are involved in various steps in protein biogenesis.

Gp96 binds a wide array of peptides in the ER. The size of the peptides range from 400 to 2000 kDa[9]. The hsp molecules and MHC molecules share some common properties, such as the ability to bind peptides, high degree of phylogenetic conservation, ubiquitous tissue distribution and inducibility by interferon-(. These common properties, and similarities in the structure of the "$_1$ and "$_2$ domains of human MHC class I molecules and C-terminal domain of hsp70 have led to the suggestion that hsp molecules are involved in the transfer of peptides from the transporter associated with antigen processing (TAP) to the class I molecules. Although experimental evidence to support this claim is still lacking, other studies have provided convincing evidence that some hsps are involved in antigen presentation, and hence can be used to induce specific immune responses against tumors and infectious agents[10]. Further studies have revealed that peptides derived from intracellular proteins that are associated with hsps are responsible for the specificity of the immune response induced by

hsps. Hsps from which peptides were removed did not induce tumor-specific immunity. More importantly, this hypothesis has been validated by demonstration of induction of specific CTLs in several tumor systems[11].

The success of hsp immunization in the induction of protective immunity against cancer prompted the investigation of this approach in viral systems. Immunization of mice with gp96 isolated from a BALB/c cell line transfected with the nucleoprotein of influenza virus (BC-NP) resulted in CTLs specific for BC-NP, but not for a syngeneic cell-line not transfected with flu NP[11]. Similar success was also demonstrated in the SV40 viral system. Studies in the vesicular stomatitis virus (VSV) system further expanded the scope of gp96 immunization to induce virus-specific CTL response[12]. In these studies, mice of the H-2^b haplotype were immunized with gp96 isolated from VSV-infected cells of the H-2^b or H-2^d haplotype. Gp96 isolated from cells of either haplotype was able to induce VSV-specific CTLs in H-2^b mice, demonstrating that gp96-associated peptides isolated from a cell are not limited to the peptides that are presented by the MHC class I molecules expressed by that cell. This concept of *cross-priming* was further demonstrated by experiments in which gp96 isolated from cells of either the H-2^b haplotype or the H-2^d haplotype were equally efficient in sensitizing macrophages of the H-2^b haplotype for recognition by previously established CTLs of the H-2^b haplotype. The presentation of the peptide by the macrophages was inhibited by brefeldin A (which inhibits the passage of the peptides from the ER), but not inhibited by chloroquine (which inhibits processing in an acidic environment, such as the endosomes). Taken together, these results suggested that the gp96-chaperoned peptides are processed internally, and re-presented by the MHC class I molecules of the macrophages.

Further studies in the influenza virus system demonstrated the induction of influenza virus-specific CTLs in mice by immunization with gp96 isolated from a hamster-derived cell-line, BHK-21, transfected with the influenza virus NP gene[13]. These studies thus extended the concept of *cross-priming* by gp96-peptide complexes demonstrated in the allogeneic system (H-2^b and H-2^d), to a xenogeneic system (hamster and mice).

The successful use of hsp-peptide complexes in the induction of CTLs and Abs in several tumor models and virus systems prompted us to investigate the potential of the hsp gp96-peptide complexes as immunogens against livestock pathogens. Bovine herpesvirus 1 is an ideal virus model system to test the concept of the use of hsps as molecular chaperones, and the associated peptides as immunogens because of the following reason. The use of MLV vaccines against BHV-1 has several problems, the most important of which are the ability of the virus to establish latent infections, and its ability to induce immuno-suppression. Not only the wild type virus, but also the MLV vaccine strains can establish latent infections. Subsequent reactivation and shedding of the MLV strain may lead to infection of naive animals, thus perpetuating the virus rather than eliminating it. Furthermore, studies from our laboratory have revealed that BHV-1 down-regulates the expression of MHC class I molecules which could result in defective CTL priming[14]. In addition, BHV-1 infects CD4+ T-cells resulting in considerable loss of CD4 expression followed by apoptotic death of these cells. Thus, it is very clear that vaccination with live BHV-1 compromises the development of CTL response both by interference with the production of cytokines that promote the development of CTLs, and by the down-regulation of MHC class I molecules.

We were able to induce BHV-1-specific CTL response in mice by immunization with gp96-peptide complexes[15]. In earlier studies, we identified three H-2D^d-restricted CTL epitopes of BHV-1 in the murine system[5]. A group of BALB/c mice was injected with these three murine CTL epitope peptides of BHV-1 complexed *in vitro* with bovine gp96 (gp96-peptides). Three other groups were injected with either the peptides alone, gp96 alone, or the peptides complexed with BSA. CTLs from mice immunized with gp96-peptides specifically lysed the peptide-pulsed syngeneic targets, as well as BHV-1-infected targets. CTLs from the other three groups did not lyse these targets. To further evaluate the utility of this approach, groups of BALB/c mice were immunized with gp96 isolated from a syngeneic cell-line transduced with BHV-1 glycoprotein D (BC-gD). Mice immunized with gp96 from BC-gD developed CTLs as well as Abs specific for BHV-1 gD. Furthermore, *in vitro* stimulation of naive bovine PBMCs with gp96 from BC-gD resulted in CTLs specific for BHV-1. These results demonstrate the feasibility of using gp96-peptide complexes isolated from cells expressing BHV-1 proteins to induce CTL and Ab responses against BHV-1, without the prior knowledge of the CTL and Ab epitope sequences.

The above experiments were repeated in cattle. However, we could not demonstrate CTLs in immunized cattle. One of the reasons for this failure might be the inadequate amount of gp96-peptide complexes used as immunogen. Isolation of adequate quantities of gp96-peptide complexes from transduced cells, for immunization of large animals such as cattle, is laborious and expensive. Therefore, we determined the feasibility

of inducing CTL response against BHV-1, by co-immunization with plasmids encoding BHV-1 gD (pELVSgD) and bovine gp96 (pS-Btgp96). One group of mice was injected intramuscularly with equal quantities of pELVSgD and pS-Btgp96. The second and third groups were injected with pELVSgD and pS-Btgp96, respectively. The group of mice co-immunized with pELVSgD and pS-Btgp96 developed a strong CTL response against BHV-1. As expected, the control group immunized with pS-Btgp96 did not develop anti-BHV-1 CTLs. However, the CTL response of mice co-immunized with pELVSgD and pS-Btgp96 was not significantly higher than that of mice immunized with pELVSgD alone. Co-immunization may not always result in the entry of both plasmids into the same cells. In order to rule out this scenario, we developed a single plasmid encoding both the cytosolic version of BHV-1 gD, and S-Btgp96. Preliminary results indicate that the mice immunized with this plasmid developed a better BHV-1-specific CTL response than those immunized with the plasmid encoding BHV-1 gD alone[16]. Further evaluation of this plasmid is currently underway.

In conclusion, epitope-based vaccines have potential for successful use in livestock. However, characterization of more MHC alleles is a prerequisite for the development of such vaccines. Until such time, other strategies such as hsp-peptide complex- and DNA-immunization should be explored.

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KN-6

Plant-Based Oral Delivery Vaccines for Livestock Diseases

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Recently, transgenic plants have been investigated as an alternative means to produce and deliver biopharmaceuticals, antibodies and vaccines. The use of plants as bioreactors for the production of immunogenic, protective antigens have been increasingly used. They are potentially an inexpensive source of antigens that could be used as edible vaccines. Viral proteins have been expressed.

The production of transgenic plants includes the control of transcription of the foreign gene by an efficient plant promoter. In most cases the gene coding for the foreign antigen is put under the control of the cauliflower mosaic virus (CaMV) 35/2 promoter, which ensures its constitutive transcription in different parts of the plant. The limitation, in most cases is that the amount of the foreign antigen expressed in different parts of the plant. Gene transfer into a plant genome could be mediated by a plant-infecting bacterium, *Agrobacterium tumifaciens*, which contains a low copy number of Tumour inducing (Ti) plasmid. When agrobacteria appears in conjunction with a wounded plant cell, the Ti plasmid transfers part of its DNA (T-DNA) into the plant genome. The T-DNA is flanked by repeated sequences (left and right borders) which are required for integration into the plant genome. Thus, when the foreign gene is incorporated between left and right borders, it can be transformed into the plant cell followed by regeneration to obtain transgenic plants.

Few important food plants for which transformation have been reported are – tomato, potato, banana, alfalfa, maize and wheat. Plant model systems such as tobacco, engineered to express a recombinant vaccine protein is not an ideal material for oral delivery because of high alkaloid and nicotine content in tobacco. The main disadvantage with potato tubers and tomato is that the main disadvantage with potato tubers and tomato is that they are not high in protein content; the level of recombinant protein must be higher on a percentage total protein basis. Economical use of plants as a production system requires maximum transgene expression and reasonable levels of protein accumulation. Augmenting protein stability, mRNA stability and translatability and enhancing the promoter strength can increase yields of recombinant proteins. A strategy based on tissue-specific expression of vaccine antigen involves that accumulation of recombinant protein only in tissue to be fed as a vaccine. An important issue in the expression of vaccine antigens is post-translational modifications such as glycosylation as carbohydrate groups confer immunogenicity. Plants also glycosylate proteins but the rules for N- and O-linked glycosylation in plants are different.

The surface protein antigen A (SpaA) from *Streptococcus mutants* produced in transgenic tobacco was the first description of an antigen being produced in plants, which appeared in a European patent. The concept of edible vaccine was first proposed by Arutzen and Mason in 1992. In 1995, Haq and colleagues reported in Science, the successful demonstration of expression of immunologically active *E. coli* enterotoxin LT-8 thus providing first proof for edible vaccine concept. Oral feeding of mice with transgenic potatoes expressing LT-B resulted in oral immune responses and mice fed with transgenic tubers were partially protected against challenge with holotoxin LT.

A large number of investigations have followed, encouraged by this novel and cheap method of providing subunit vaccines, mainly for combating diseases affecting

humans. Notable among them are the development of transgenic potato expressing Norwalk virus capsid protein, Respiratory syncytial virus fusion protein in tomato, Measles virus hemagglutinin protein, Rabies virus glycoprotein in tomato and potato, in tobacco rotavirus NSP4 protein in potato.

Oral delivery of protective antigens to target animals to combat animal viral diseases is more feasible as compared to use of transgenic vaccine plants as human foods. Thus, many investigators have tested the feasibility of using plants as delivery vehicles of future vaccines for Livestock diseases.

The VP1 protein immunogenic epitope 135-160aa as well as the full length protein from Food and Mouth Disease virus has been expressed in transgenic alfalfa and protective immune responses have been shown in a mouse model. The S protein of Transmissible Gastro Enteritis Virus (TGEV) has been expressed in transgenic Arabidopsis and corn. The TGEV S protein expressed in corn showed 50% protection in piglets fed with a meal made of corn and other additives, when challenged with virulent virus. This study represents the first example of an animal used in conventional food husbandry acquiring protection from a major disease through ingesting an edible vaccine.

We have generated transgenic peanut plants stably expressing the Hemagglutinin-Neuraminidase protein of Peste des petits ruminants virus (PPRV) and have tested into immunogenicity, both in a mouse model as well as in sheep. Oral immunization, without any adjuvant elicits high levels of neutralizing antibodies in circulation as well as secretory IgA. Further, T cell responses have also been detected. These results have provided a strong basis for generating transgenic plants expressing not only the HN protein, but plants expressing a poly epitopic protein consisting of both B and T cell epitopes of HN & F proteins of PPRV, thus providing an easy and cheaper means of mass vaccination against PPR disease in developing countries.

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KN-7

Novel vaccine and diagnostics for better pet animal healthcare through application of biotechnological tools

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The advancement of molecular biological methods has noticeable impact and great potential for better production and productivity from livestock and offers excellent opportunity for more reliable means of producing novel vaccines and diagnostics for animal healthcare. The history of advances in molecular biology dates back to 1950 when the structure of nucleic acid was elucidated by Waston and Crick. The discovery of cell fusion and the development of immortal hybrid cells by Kohler and Milstein for production of monoclonal antibodies (MAbs) added a new dimesion to the application of biotechnology. In the last one and half decade, India has witnessed application of molecular biological tools and hybridoma technologies for development of improved diagnostics and effective vaccines against some economically important diseases in animals such as rinderpest, bluetongue, bovine herpes virus 1 infection, infectious bursal disease, clostridiosis, salmonellosis and pasteurollosis (Butchaiah, 1997). Though the breakthrough in pet animal healthcare in India through quick and reliable diagnostic procedures and effective novel vaccine production by application biotechnological tools is very little, but there is tremendous scope for application of molecular biology techniques for better healthcare of dogs and cats with the growing population of pet animal lovers who can afford for quick and reliable diagnostic tests, and reliable vaccines.

Novel Vaccine Development

Vaccination plays an important role to protect the animal from suffering of many important and life threatening viral and bacterial diseases, and has become more than a routine in pet animals, particularly in pedigree dogs. Even, most of the kennel clubs has made vaccination mandatory for active participation in the competition. Though conventional vaccines are mostly used at present, but they are associated with many problems. Those include contamination of pathogens, poor keeping quality and reversion of virulence. Biotechnological tools provide an excellent opportunity to develop vaccines that are free from limitations associated with conventional vaccine. Genetically engineered vaccines using synthetic peptides, empty capsids, deleted mutants, chimeric gene products and DNA vaccines have helped in controlling some of the diseases like Blue tongue and Rinderpest in developed countries (Reddy and Natarajan, 1999). However, limited work has been done in this direction in India, particularly to develop vaccines against pet animals. Also, the safety and potency requirements should be consistent for genetically manipulated live vaccine before they are being used in mass scale for animals.

Molecular biological tools provide opportunity for *gene deletion* responsible for virulence, leaving virion that can be used in live virus vaccine with reduced or no virulence. Even deletion of a set of gene of an infectious agent that express a particular protein in the host, aids in differentiation between vaccinated animals from animal naturally exposed to infectious agent. In the former the protein expressed by the deleted gene will be missing unlike the later.

Another scope is development of vaccinia vectored vaccines. A plasmid is constructed containing the DNA of donor virus. Then the plasmid is inserted in to a vaccinia virus genome and the vaccinia virus can be used

for vaccination of animals. Besides vaccinia virus, baculo, retro and other viral systems are being used to produce the immunogens. Some other recent approaches for development of novel vaccine include (1) use antisense RNA which will bind with mRNA for the virus or bacteria thereby inhibiting the translation of viral or bacterial genome, responsible for virulence, (2) inhibiting the receptors and thereby stopping the virus into the succeptible cells and (3) vaccine with enhanced mucosal immunity (Reddy and Natarajan, 1999).

Production of multivalent vaccine has been attempted using chimeric gene products. Genes of one virus are introduced into another virus and the chimera formed will be protection for both the viral diseases. Expression of viral antigens in leaves and stems of plants are also been explored for livestock diseases like FMD.

Development of diagnostic for pet animal use

The molecular biological tools have great potential for rapid and reliable diagnosis of infectious diseases including diseases of parasitic origin. They not only diagnose the disease with overt clinical signs, but also helps in identifying the etiology in carriers and animals with subclinical disease state, facilitate reliable and prompt diagnosis and eliminates human eye errors. For example, diagnosis of protozoan diseases in dogs and cats through microscopy, though a "gold standard test", is labour intensive and requires well trained microscopist for accurate identification and interpretation, particularly for parasites that are morphologically similar, very small in size or present in very small numbers. The diagnostic skill of the investigator also plays an important role for diagnosis and leads to variations in results from laboratory to laboratory. Often, there is chance of misdiagnosis or the disease remains undiagnosed. Further, the *in vitro* cultivation of infectious agents is often costly and often very slow. Immunodiagnostic tests have the limitation of cross-reaction with other microorganisms and are not sufficiently specific to distinguish closely related serotypes (Morgan and Thompson 1998). The application of biotechnological tools for diagnosis of diseases in pet animals overcomes the above said limitations and has the advantages of rapidness and reliability. The molecular diagnostics use the biological techniques for detection of DNA (Deoxyribo Nucleic Acid), protein or antibodies in the biosamples to pinpoint the disease etiology and also provide information on molecular epidemiology (Gorham and Knowles 1995).

A. Detection of DNA

1. Diagnosis by restriction fragment length polymorphism (RFLPs)

This technique, otherwise know as nucleic acid fingerprinting, maps the genome of a microorganism and can detect the difference in the genomes of closely related microbial species. The DNA is extracted from the samples and clipped into fragments at specific nucleotide sequences by restriction enzymes. The resultant DNA fragments are then separated in agarose gel by electrophoresis and visualized with ethidium bromide. The fragments can then be identified with complimentary DNA (cDNA) tagged with 32P to determine the difference or similarities in the genomes. This technique provides clue for molecular epidemiology and traces to the origin of the isolate. Regional variants of the rabies virus in Canada were identified by RFLP analysis which showed consistent differences in the viruses from distinct geographical regions. DNA hybridization, RFLP and random amplified polymerase DNA (RAPD) has been discussed in detail for molecular diagnosis of parasitic nematodes in dogs (McKeand *et al.*, 1998)

2. Polymerase Chain Reaction (PCR)

This molecular biology technique is used to amplify the desired sequence of DNA from a complex mixture of heterogeneous sequences using specific primers (short strand of DNA complementary to both the strands of DNA). The target DNA is denatured to separate the two complementary stands. Specific primers are then annealed at low temperature and extended with DNA polymerase at an intermediate temperature utilizing the target DNA as a template. This cycle is repeated to produce the target DNA sequence in a geometric progression. With RNA viruses, a cDNA copy of the RNA must be made for PCR amplification of desired sequence (RT-PCR). The identity of PCR product is confirmed using DNA probes or the product is sequenced to characterize the genome. A second set of primers are used to amplify the sub-fragment of target DNA sequence to increase the specificity and the method is called nested-PCR. PCR is very useful for diagnosis of chronic persistent infections and is a sensitive procedure for detecting infectious agents in host tissues and vectors.

Reithinger *et al.*, (2003) evaluated PCR as diagnostic tool for mass screening of dogs to detect Leishmania spp. PCR detected Leishmania infection in

total of 90 of 1066 (8.4%) dogs. Howver, they advised for restricted use of PCR protocol for diagnosis of active cases and to parasitological monitoring of patients, unless more sensitive PCR protocols are developed. Nested-PCR was used as a molecular biology tool for diagnosis of canine corona virus infection through sampling of faecal samples and the results were compared with electron microscopy and viral isolation and n-PCR was found to be most rapid and sensitive for diagnosis of corona virus infection in dogs (Pratelli *et al.*, 2000). Primer sets prepared against a short genomic segment that is only found in feline corona virus (FeCov) that are associated with feline infectious peritonitis (FIP) could be of great help in its specific diagnosis and management of FIP (Telford *et al.*, 1997). PCR was used for early detection of Canine parvo virus infection (Meerarani *et al.*, 1996). PCR has also been used for molecular detection of parasitic protozoa (Morgan *et al.*, 1998).

3. DNA probes

Use of DNA probes along with PCR is a very good diagnostic tool of diagnosis of viral and bacterial agent. DNA probes search the tissue of an animal or an insect for the complementary nucleotide sequence of a pathogen. Denatured DNA can be freed from the specimen and applied to a solid support system such as nylon or nitrocellulose membranes and the probe can be applied on it. The binding of the probe (hybridization) to the PCR product, denatured DNA in the samples or on support system can be verified by tagging radioactive ^{32}P and can be detected by X ray film exposure. Alternatively, radioactive tags are replaced with sensitive and non-radiolabeled tags. In this case the DNA probe is labeled with biotic and is detected by streptavidin which is linked to horseradish peroxidase or alkaline phosphate which yield colour in presence of their substrate. Verger (1988) discussed the importance of DNA probe as a tool for disease diagnosis.

B. Detection of Protein

1. *Immunohistochemistry*

Immunohistochemistry is rapidly becoming a standard tool for rapid diagnosis of antigens associated with viral, bacterial and protozoal microorganism. The detection of antigen in fixed tissue with the use of monoclonal antibodies offers a number of advantages over other diagnostic techniques.

2. Western Blotting (Immunoblot)

This technique is performed mainly to identify desirable protein antigens in complex mixture. The protein is separated by SDS polyacrylamide gel electrophoresis and transfered to nitrocellulose membrane. After incubation, protein band are visualized with peroxidase conjugated protein and coloured reagent.

3. *Antigen Capture*

This test has ability to detect antigen direct on pathogens directly from an animal prior to or during clinical disease. Antigen from the test samples are first captured by a specific monoclonal antibodies on a solid phase support and their presence is detected through the use second radio- or enzyme-labeled monoclonal or polyclonal antibody.

C. Detection of Antigen

1. *Competitive ELISA*

Monoclonal antibody based ELISAs have tremendous potential for use in diagnosis of animal pathogen, particularly for those disease for which diagnostic antigen can not at be produced in cell culture. The procedure is used for screening large number of sera sample. The components of C-ELISA include a monoclonal antibody and the corresponding epitope. The monoclonal antibody must posses an appropriate affinity such that polyclonal antibody can replace it in the competitive reaction. The desirable epitope characteristic include linear peptide composition immunodominance and conservation among isolate. Since the specificity of the C-ELISA depends entirely on the monoclonal antibody used, the C-ELISA is well suited for use with recombinant antigens.

2. *Production of Antigens by recombinant DNA technology*

Recombinant DNA technology offers an opportunity to continuously produce test antigen *in vitro*. It eliminates expensive, time consuming production of antigen through cell culture or other *in vivo* methods. The antigen of potential diagnostic significance is identified by studying the antibody response of the host to the proteins of the organism. The immunodominat antigens, that arouse highest antibody titer, is identified and monoclonal antibody or nonspecific polyvalent sera is generated for use in screening recombinant gene libraries for the protein of interest. Recombinant libraries can be produced from the genomic DNA of the organism or by cDNA synthesis using organism messenger RNA (mRNA) as a template. The genomic DNA or cDNA is molecularly cloned into a prokaryotic or eukaryotic expression system, and the gene library screened for expression of the desired protein.

Therefore, it is concluded that the recent developments in molecular biological tools has tremendous potential and enormous scope for early and rapid diagnosis and for development of novel vaccines with minimal side effects. But, the products are required to be validated before they are being used for healthcare of pet animals.

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KN-8

Botanicals used in Veterinary Medicine - Biomarker Profiling and their Standardization

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Introduction

The use of herbals in veterinary medicine has been reflected from our ancient heritage dating back to the Mahabharata. The herbal medicines were used with predictable results during the famous war of Mahabhaarata fought some time in 5000 BC, when thousands of animals afflicted with wounds and diseases were successfully treated under the able guidance of the Pandava prince, Nakul. Those useful herbal remedies have been mentioned in 'Nakul Samhita' the first treatise on treatment of animal with herbs, which was written during that period. So it is essential and useful to vindicate the claims of these herbal remedies on modern scientific lines to clearly assess their value. Thus from the ancient times the herbs are being used for the treatment of animals, exploitation of which on the aspect of quality and safety is the need of the day [Mukhopadhyay, 1994; Mukherjee, 2003].

So many plant materials being used veterinary medicine are mostly based on the ethno medicinal claims as well as from folklore practices. Though their therapeutic efficacies has been established through experiences from years together and also their scientific validation has been made to a certain extent but the aspects on their quality perspectives has less been studied than in practice. There has been a resurgence of interest on plants and plant derived products as source of human and veterinary medicine in the last few decades as the herbal products has occupied a major part in curing different human ailments, but what about the quality of those products? Certain of these drugs have been known and are being used by man for many centuries, while others are still being isolated and evaluated. Herbal drugs have become one of the totems in this era of phytotherapy. Along with the increased interest in herbal medicines there has been an explosion in the amount of literature on the subject and quality control is of utmost essential in this respect world over. In the herbal boom worldwide it is estimated that high quality phyto-medicinals will provide safe an effective medication.

Alternative systems of medicine in animal care

Alternative systems of medicines are not only becoming more popular for people, but also for our furry, feathered and scaly friends. Over the last few years as interest in alternative and complementary medicine for humans has grown, there has been a corresponding growth in interest in their use in animals. All alternative modalities that can be used in humans can also be used in animals. At many Veterinary Hospital, exclusively Alternative Veterinary Medicine is offered for the treatment of animals. In India, Ayurveda like Traditional Chinese Medicine, incorporates the use of herbs in the treatment of animals. The history of use of this type of medicine in treating animals in much longer than that of "conventional" medicine. Traditional healers who specialized in animal treatment were described in Egypt around 2000 BC. The use of herbal medicine in animals was designated a specific branch of Traditional Chinese Medicine around 1000 BC. It was only in the 19th century, that the discovery of pathogenic organisms and the promise of medications that could treat or prevent them led to the decline in the popularity of more traditional and holistic medical systems. Although Ayurveda has mainly evolved as a medical system for humans, its focus on living in harmony with nature can be applied to every living creature, including pets. In both humans and

animals. Ayurvedic treatments involve diverse strategies to restore optimal function and balance.

Apart from alternative systems in India, there are several other systems of medicines worldwide where herbs are used. Bach - Flower Therapy for Animals is a method of treatment could be compared to a combination of herbology and homeopathy, where it uses extracts of various flowers that have been diluted. However dilution is not carried as far as with homeopathic medications. Bach Flower remedies are believed to work by acting on the emotional state of the pets. The most common use in Veterinary medicine is the relief of stress in animals that have a tendency to be high-strung or anxious. Aromatherapy and Bach Flower Therapy are both related to Herbal Therapy but have not yet achieved widespread use in veterinary medicines. Aromatherapy uses volatile essential oils from plants and its primary use in animals has been used to modify behavior (e.g. to reduce stress-related barking in boarding kennels). Bach Flower Therapy uses various flower's essences, and is used mostly in small animals) for treatment of stress, behavior problems; and certain chronic neurological problems.

Nutritional therapy is one of the alternative treatments where herbs are major constituent of the treatment. Lot many research has been performed in identifying the nutritional requirements of healthy animals, but the area of therapeutic nutrition - treating disease by altering or adding supplements to the diet - has received relatively little attention in mainstream veterinary medicine. Nutrients are used in holistic veterinary medicine to boost the cancer patient's appetite and energy supply, reduce toxicity and side effects of cancer therapy, speed recovery from surgery and boost the animals immune system. Nutrients used can range from specific amino acids and enzymes to vitamins and supplements as common as garlic (www.selkirkvet.com).

The fundamental requirement of Industry and other organizations dealing with botanicals so much so herbal products is their quality assessment and evaluation. The fact must be taken into account that the plant material to be examined has a complex and inconsistent compositions based on its contents of secondary metabolites. Therefore the analytical limits are not as precise as for the single chemical entity. Adequate standards using biological, chemical, instrumental and physiochemical methods required to be developed [Frasworth, 1998; Mukherjee 2002].

Traditional Chinese herbs - and herbs from around the world - are used in veterinary herbal medicine. The use of specific herbs and plants for medicinal purposes has been practiced for centuries all over the world. Herbs have healing powers that are capable of balancing the emotional, mental, and physical dimensions of animals. In Traditional Chinese Medicine (TCM), the flow of 'Qi' (or energy) can be nurtured by the use of certain herbs.

Herbal potential in animal care

Various herbs either in the form of polyherbal formulation or as a single herbal extracts are reportedly used in different ailments and disorders in animals. In anemia, autoimmune diseases, epilepsy and seizure disorders, ashwagandha (Withania somnifera) is used along with other plants and polyherbal preparations like triphala. The combination of Ashwagandha and Boswellia serrata has been very effective in treating osteoarthritis and Hip Dysplasia. Ashwagandha's anti-inflammatory properties, along with its content of steroidal lactone help to build up lost muscle mass due to diseases. Ashwagandha is also calming and helps discomfort and pain. It allows animal to sleep better, and to waken with less stiffness and soreness. Boswelya's anti-inflammatory properties, as well as its chondro protective actions, make it an effective herb in treating both osteoarthritis and hip dysplasia. In various skin disorders Neem (Azadirachta indica) products, both orally and topically, along with Omega-3 and -6 oils (i.e. flax and fish oils) and Boswellia serrata are useful. Neem leaves extract or Neem oil shampoo and washes are quite common veterinary products in the market. All kinds of liver problems related to infections, including toxic overload, cholangio hepatitis, hepatic lipalosis are greatly helped by diet modification, supportive therapies and supplementation with Ayurvedic products like Phyllanthus amarus containing preparations. Allergies, bronchitis and conditions like asthma are reportedly treated with combinations like Tylophora, Triphal and Ashwagandha.

In veterinary treatment the herbs are mostly used in the form of polyherbal formulations. A formulation of ginseng, cimicifuga rhizome, bupleurum, tangerine, saussurea, amomum fruit, white atractylodes rhizome, pueraria, angelica root, rhubarb, peach kernel, orange fruit, Cerydalis yanhusua rhizome, coptis, pinellia root, pulsatilla root, balloon flower root, lotus seed is used in the treatment of animals suffering from inflammatory bowel disease. A supplement designed for animals suffering from skin conditions due to inhalant and contact allergy contains Schizonepeta, siler root, bupleurum, bitter orange, moutan root, platycodon, ligusticum, dahurican angelica root, poria, mint,

honeysuckle, scutellaria, cicada, silkworm, licorice root, zaocys. A formulation containing angelica root, frankincense, myrrh, Cerydalis yanhusuo rhizome, tangerine, tumeric, angelica du hou root, Dahurican angelica root, cinnamon, drynaria, teasel root, ox knee root, clubmoss, dragon's blood, Atractylodes lancea, clematis, astragalus, white atractylodes rhizome, aconite is used for treating the animals suffering from hip and knee arthritis. Like these and many more formulations are used in veterinary treatment where the herbs are main component of the medicament

Quality control and standardization of Botanicals through Marker

In the herbal boom worldwide it is estimated that high quality phyto-medicinals will provide safe an effective medication. For the development of the quality and standardization parameters of botanicals and thereby to assist the herbal drugs manufacturers engaged in production, the manual responds to the growing use of medicinal plants, the special quality problems they pose, and the corresponding needs for international guidance on reliable methods of quality control and development of standardization parameters is of great importance. Highlights are being made on various approaches of biomarker profiling of herbals to establish the quality control approaches with chemo profiling techniques with the lead from some therapeutically potent medicinal plants [Anonymous, 2000]. Biomarker profiling of a maximum number of medicinal plants being used in therapy required to be established to highlight the quality control development based on this new emerging techniques which is being utilized by the people through out the globe for drug development from natural resources.

Quality evaluation of crude drugs and herbal preparations is a fundamental requirement of industry and other organizations dealing with Ayurvedic and herbal products. Unfortunately this requirement is often not possible to meet with usual Pharmacognostical tests such as macro and microscopical evolution, ash value and extractive value etc. Directives on the analytical control of crude drugs must take account of the fact that the material to be examined has a complex and inconsistent compositions, therefore the analytical limits are not as precise as for the single chemical entity based on chemoprofiling through marker analysis (Mukherjee 2002a, Mukherjee et al 1998).

The use of indicative substances for development of quality control profiles has been considered as the thrust area for standardization of botanicals globally. They can be selected among the constituent substances of the plant, irrespective of whether these are active or accompanying substances. They should however be characteristic of the drug plant under investigation and easily demonstrable. Analytical data other than those obtained by thin layer chromatography can also be used for identification and quality assurance (De Smet 1992; Mukherjee 2002; Mukherjee and Verpoorte 2003).

Based on experience attempts have been made here to develop the marker profile of Phyllanthus amarus, using WHO guidelines for quality control as the basic data required for evaluation of the extract. In India the plant is often used in traditional system of medicine for a variety of ailments including flu, dropsy, asthma, bronchial infections, and diseases of the liver. In Ayurvedic system of medicine it is used in problems of stomach, genito-urinary system, liver and kidney. Apart from the major bio active lignan constituents of the plants Phyllanthin and Hypophyllanthin, the plant is reported to contain Amariin, geraniin, corilagin, 1,6-digalloyl-glucopyranoside, rutin and quercetin-3-O-glucopyranoside (Mukherjee 2002a, 2002). To make standardization profile for the Phyllanthus amarus, various parameters prescribed in Indian Herbal Pharmacopoeia (IHP) were considered. The macroscopical characteristics like color, appearance, odor and taste were studied and noted as per requirement of Indian Herbal Pharmacopoeia. Parameters like test for extraneous material and physio-chemical analysis were carried out on the basis of protocol prescribed by WHO [Annonymuos 1998; 1998a]. Successive extractive values, quantitative analysis of extract for the presence of Phyllanthin and Hypophyllanthin were carried out by HPLC. Phyllanthin and Hypophyllanthin were used as standards for HPTLC finger printing and to analyze the extract qualitatively. Marker profiles of several other plant materials like Boswellia serrata, Accorus calamus etc that are being used in veterinary medicine for years together for treatment of various ailments has been made and will be highlighted in the presentation.

Conclusion

It is an accepted fact that qualitative and quantitative analysis of major bioactive chemical components (marker components) of crude drug constitute an important and reliable part of quality control protocol as any change in quality of the drug directly affects the constituents. HPTLC densitometric scanning of the extract as well as reference standard compound indicated the use of Phyllanthin and Hypophyllanthin in Phyllanthus amarus,

boswellic acid in Boswellia serrata, beta asarone in Accorus calamus as the biomarker. The plant sample was found to comply with the parameters like test for extraneous material, physico-chemical analysis. Successive extractive values were also performed to comply the same with the specific requirements with different solvent systems like petroleum ether, chloroform and methanol respectively. Chromatographic profiles were developed for various extracts of Phyllanthus amarus. Boswellia serrata, Accorus calamus for different biomarkers.

Qualitative and quantitative analysis of biomarker not only helps to control the quality of herbal material used, but also can be used to evaluate the quantity of biologically active chemical entities required to produce specific pharmacological activity. Thus can be used to monitor the therapeutic activity with their pharmacokinetic and pharmacodynamic data (Mukherjee 2002, Mukherjee 2001). Quality control of the plant drugs has always been a problem because of its complex constituents. When demand for the herbal therapy is booming, it is required to have proper tools to ensure the quality of the plant products supplied in global market and to remain in the competition. In our laboratory we are developing the quality control protocols for various plants used Indian Medicine, thus to help to have compiled information on individual plants based on there biomarker profiles.

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KN-9

WTO - Countdown: Beginning of a New Era for the Indian Biotechnology Sector

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Summary

While India has benefited enormously from globalization in many trade/services sectors, there are still many which are either moving slow or face major constraints as India is ready to become a full compliant member of the WTO in 2006. Biotechnology is one sector that seems to be dominating the Indian policy makers and academics. Although India has well trained human capital in this sector, it is less competitive. Unlike IT, biotechnology sector requires intense R&D and heavy investment capital. This sector is also highly sensitive to the effects of economic, environmental, social, cultural and ethical issues. Other factors such as product development and payback processes, local government influence and the role that academics play in IP-based commercial R&D stages also affect competitive biotechnology growth. However, there are lessons to be learnt from developed countries (US, EU and Japan) where policies concerning regulations, investments and Intellectual Property Rights (IPR)-based R&D, have contributed to the robust growth of life sciences, which has been the engine of the economic activity. Given the challenges that India has in this sector, Indian academics, technocrats and policy makers are forced to re-think their strategies focusing vigorously on IP-based and translational R&D to remain competitive. IPR is the essential key driver to maintain global competitiveness in the long term, and this forces India to deviate radically from the past. IPR gives India considerable leverage for accessing global markets elsewhere for its products. Although India has committed to move forward in this sector through public sector efforts using the concept of bio-clusters, it needs stimulate private sector investments. Human and animal health (drugs, diagnostics, vaccines and animal production) appear to be most promising growth areas within the next few years compared to other biotechnology sectors.

Introduction

Globalization has brought significant improvements in both cost reduction and efficiency in many sectors, including transportation, communication, and production worldwide. This has undoubtedly improved inter-country competitiveness and broken down trade and cultural barriers among the countries. The growth in Information technology (IT) has provided new ways to exchange and diffuse information on a global scale and it has served as a major link in making globalization somewhat workable. In particular, India has benefited enormously from the IT-based globalization and it has been the key driver for its rapid economic growth and foreign exchange reserve. Despite these positive changes, there are concerns in the developing world on issues relating to jurisdiction and/or sovereignty in favor of international standards led by international/ regional trade-related organizations such as the World Trade Organization (WTO), which has been in place since 1995. Since its inception, it has been daunting for this world body to design ways to work around domestic political structures and policies, reforms of state-owned enterprises (through privatization/ competitive markets), standardization of labor and setting of internationally acceptable environmental standards, and generalized enforceable IPR of different countries. The new WTO rules are particularly hard on the developing countries in the short term as their infrastructure, trade and regulatory rules are not as

sophisticated as the developed countries although there is tremendous growth in consumerism, which is good for economic growth. In addition, urbanization, population and income growth in these countries are fuelling a massive demand for food of animal and vegetable origin, veterinary and human health drugs, transportation and water resources (urban and rural). Being the second populous country in the globe, India is forced to recognize and apply advanced biotechnologies to meet the growing domestic market needs as well as to compete in the global market. To be competitive, India will have to develop robust policies on regulations, IPR and R&D issues and investments. At this juncture, India does not have much time left to deal with the post-WTO era, particularly on the biotechnology sector, which is different from that of the IT sector.

World Trade Organization (WTO)

The WTO established on January 1, 1995, is a relatively new international organization. The WTO replaced the General Agreement on Tariffs and Trade (GATT), which dated back to 1948. This was a consequence of a decision taken by governments after seven and a half years of negotiations (the "Uruguay Round"), which ended in 1994. With the WTO's creation, the rules were expanded to new areas. While the GATT dealt with trade in goods only, the WTO covers trade in services and IPR as well. There are also some areas, such as textiles, agriculture and sanitary and phyto-sanitary measures, where the WTO goes beyond the GATT by having established specific trade rules. Under the WTO, the procedure for settling trade disputes has also been strengthened. The dimension of trade policy, subsidies, intellectual property protection and market access in services in relation to competition are always on the WTO agenda. However, there seems to be a greater focus now on the debate within the WTO as to whether there should be specific rules pertaining to national competition law and its enforcement.

Intellectual Property Rights (IPR)

According to Robert Hall and Charles Jones (Economists), primarily the institutions determine a country's long-run economic performance and government policies that make up the economic environment, within which individuals and firms make investments, create and transfer ideas, and produce goods and services. According to Douglass North, the 1993 Nobel Laureate, the invention of intellectual property (IP) and its protection caused an explosion in creativity that was the basic force behind the Industrial Revolution. As Jones observes, sustained economic growth is a very recent phenomenon and it began with the Industrial Revolution in Britain in the 1760s According to the thesis of Douglass North and several other economic historians, the development of IPR, a cumulative process that occurred over centuries, is responsible for modern economic growth. History suggests that it is only when the market incentives were sufficient that widespread innovation and growth took hold. Often industry proponents have argued that IPR protection serves as a major incentive for innovation and growth, which can benefit even imitators (such as generic drug companies). Imitators survive by copying innovators' inventions. If invention declines (e.g., due to weakening of IP protection), there is less for imitators to copy. Weaker IP protection makes it easier for imitators to dip into the pool of innovations, but it also shrinks the size of that pool; the latter effect may dominate the former. Imitators always favor weaker protection for innovations that have already been produced, but may benefit from stronger protection for innovations yet to be produced. The empirical evidence suggests that, across industries, a high average level of IP protection is economically beneficial. The benefits of strong IP protection are even greater for the biotechnology sector in the long run because these firms rely heavily on product patents than firms in other sectors. The IP categories such as: a) Patents of invention; b) Utility model patents; c) Industrial design patents; d) Trademarks, and e) Copyrights, are all important for developing countries under the new WTO system although some may be more important in the short term and some in the long term.

Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement)

The TRIPS Agreement marks a significant turning point in the international law on IPRs. The most far reaching changes in existing IPR protection have been made obligatory under this Agreement, especially in the area of patents. With product patents being accepted world wide, the question of tempering prices through procurement and import policies does not arise. Developing countries can only hope to reduce prices by increased product competition, which can only come with indigenous R&D. The strengthening of trademark regimes will help large biotechnology multinational enterprises (MNEs) to register and use their trademarks without any fear of restrictions on such use. Governments, however, would be free to promote the use of generic names by 'substitution

laws and other policies as long as they do not restrict registration or use of trademarks. The effect of the section on protection of undisclosed information, in so far as it concerns the protection of its data from "unfair commercial use", would depend largely on the interpretation of this clause of the TRIPS Agreement. It is as yet unclear as to whether national governments could rely on test data, originated by the first company, which requested marketing approval, for clearing the cases of subsequent applicants for the same product. If this is permitted, and is not considered as unfair commercial use, this clause would have no adverse effects on prices of pharmaceutical products. If, however, this were considered as prohibited, it would mean that market exclusivity would be granted to patent-expired and non-patentable products for an unlimited period, a situation with far graver implications for pharmaceutical prices than has been the case with product patents.

Us trade and commerce model and its impact on India

The US global trade and commerce model, which promotes no restricted economic boundaries, advocates more open markets and competition in the developing world, particularly in countries such as India and China where the potential access to huge markets benefit the US and other developed countries and thus, promote more job opportunities in their own countries. The US through WTO, has also been advocating this model to the biotechnology sector. However, the benefits vs. harm resulting from the application of WTO enforcement policies by 2006, particularly for the biotechnology sector, have fuelled a strong debate. The convergence of competition policies and their overall impact on trade and investments within the biotechnology sector remains contentious at best in countries like India and China. Although there are international trade agreements and organizations, antitrust regulations, investment and distribution systems, IPR laws and laws governing labor standards, India faces major constraints because of its slowness to make the quick transition on its regulatory and policy reforms in the biotechnology sector. Although WTO has structured dispute mechanisms, there are major concerns in areas such as agriculture, animal and human health and production.

Under the US Trade and Commerce model, there is ample evidence that global economic, investments and trade policies have increased economic prosperity in both developed and developing nations even though there are questions as to the overall impact on developing nations such as India where the gap between the rich and poor still remains high. However, it has spurred consumer spending, which has led to an overall economic growth. As a result, as to what is feasible or desirable and what is more fair and efficient in a global competition policy remains highly contentious in areas such as agriculture and health. With EU emerging as the lead regional force, it remains to be seen how US and EU would dominate in determining the direction of competition policies and antitrust regulations and what kind of influence China and India and other regional players would have on the overall system of global competition. In addition, differences in corporate governance, levels of economic development and, political and judicial sophistication, also factor in on India as it travels through the path of globalization in the biotechnology sector.

Three instruments that are critical to the success of US-Trade model include: a) Globalization and Convergence; b) Global Financing and Investments; and c) Globalization and Information Technology. To explain Foreign Direct Investment (FDI) determinants in the developing economies, Dunning and Roject proposed a four-fold typology that included: a) resource seeking, a) market seeking, c) efficiency seeking, and d) strategic asset seeking. According to Dunning, three necessary conditions are required to motivate FDI. These conditions include the following: a) ownership of knowledge-based assets; b) existence of locational advantage (e.g., tariffs, quotas, transport costs, cheap factor prices, access to customers); and c) benefits to producing internally rather than through licensing arrangements (e.g., corporate governance, transaction costs, imperfect information, bounded rationality, asset specificity, and incomplete contracts). Economic research on a number of industries (including biotechnology) has demonstrated that the rate of private R&D investment is very sensitive to expected returns. In his influential study of almost a thousand inventions in four different industries, the late economist Jacob Schmookler found that the expected profitability of inventive activity determined the pace and direction of industrial innovation. This seems to be valid for the biotechnology sector.

WTO and the biotechnology in India

Being the second most populous country in the world, India has huge market opportunities because if its sizable middle class with disposable income. In addition, India has well-trained and ready rich human

capital that can make the post-WTO transition in the biotechnology sector less painful compared to other developing nations. Unfortunately, the concept of bio-biotechnology clusters as a transition for commercialization being aggressively pursued by India still uses the public sector-funded model. The private sector investment (both FDI and Domestic) has lagged behind causing some concerns about India's ability to remain competitive in the post-WTO era. India still faces multiple constraints, including the short time it has to address R&D and IPR, competition policies, regulatory rules, investments (local and FDI), WTO violations, international market access problems, the potential for increased local drug prices, and the effect it is likely to have on the small/medium biotechnology companies after it becomes a full compliant member of the WTO despite assurances that India is committed in principle. to the product patent concept and its implementation by 2005. The data from the patent offices of the US (USPTO), EU and Japan, show that the US dominates in the number of patents and trademarks that it holds within the biotechnology sector, followed by Europe and Japan. Both India and China still fair at the bottom in the actual number of international patents and trademarks. There are several reasons for India to lag behind on IPR despite it's strong R&D infrastructure and well-trained personnel: a) IPR was not a priority for India due to its restricted trade policies, b) Scientists in the public sector (national research centers, universities, etc.) emphasize more on publishing papers rather than IPR-based innovation; c) Biotechnology sector (including pharmaceuticals) had enough strengths to imitate and/or produce products that met the local market needs without violating any international trade rules, d) Local companies did not have to compete with global companies, and e) Lack of robust investments by the private sector. During the last several decades, a significant proportion of the competent trained personnel has left the country to seek opportunities around the globe.

Given these constraints and the absence of robust venture capital, India has decided to pursue revenue earning business strategies for biotechnology while making changes to their trading system, which may have certain advantages in the short term but in the long run it does not provide a competitive strategy. India has adopted an IT sector model even for biotechnology despite major differences between the two. The biotechnology sector differs from the IT in many respects, which include: a) capital intensive, b) takes any where 5-15 years for product development, c) product development cost are very high (800 million dollars to develop a block buster drug in the US), d) country-driven regulatory controls (making it very difficult to access markets in developed countries even under new WTO rules), e) the failure rate is extremely high, f) Entry barriers are extremely high, g) IPR costs are high, h) markets are variable (domestic markets may be small compared to outside markets, particularly the US where drug prices are very high), and i) low FDI and domestic investments (absence of robust venture capital). Other constraints include: a) ability of many small/medium size companies who lack R&D infrastructure to remain in business in the post WTO-era due to their inability to compete with outside companies unless they can maintain competitive quality and price for their products, b) lack of full capacity to conduct well developed clinical trials, c) uncertain outcomes of vaccine research and trials, and d) long time that is necessary to increase the production of animals for human consumption. As shown in the Figure 1, the biotechnology sector uses a very similar paradigm through out the world other than the fact that it is highly sensitive to the regulatory controls.

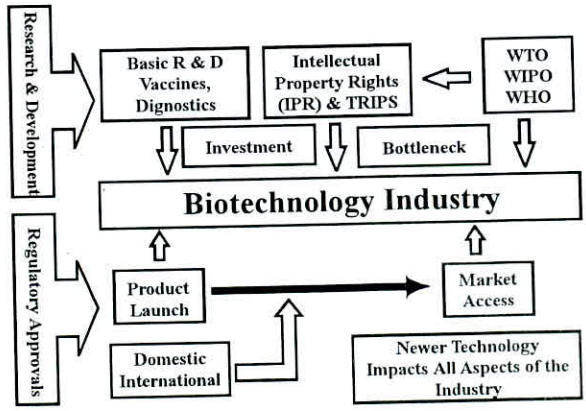

Fig. 1. Biotechnology IPR-Based Commercial Product Model

As seen in the western world, it is likely that there is going to be significant consolidation in the Indian biotechnology sector as well. Large Indian companies will have strengths to establish strategic partnerships with MNEs and they will aggressively pursue opportunities in both domestic outside markets. As to how this consolidation will hurt non-innovative small/medium biotechnology companies remains to be seen. It is also anticipated that the entry of outside companies beginning 2006 may result in increased prices for

human as well as veterinary drugs. In addition, vaccine research and development can be slow and outcomes can be uncertain. Given these constraints, India may be left with no alterative other than to pursue a revenue generating model using other avenues such as contract research, providing a base for R&D outsourcing and manufacture of bulk drugs & licensed products. Unless it is long term, pursuing bioinformatics strategy for India in the short term comes with significant risks as the expected returns can be unpredictable.

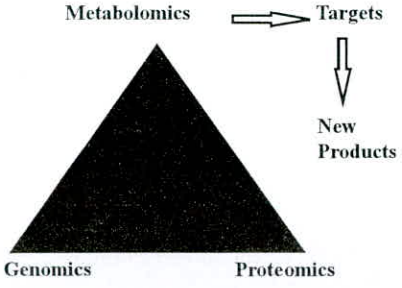

Fig. 2. Bioinformatics Model

Bioinformatics and its successful application in India to product inventions still faces major constraints as seen in the western world other than transient contract opportunities (Figure 2). As shown in the model, the development of successful products such as drugs, vaccines and diagnostics using this technology requires heavy capital; capacity for well developed clinical research and well developed databases; favorable regulatory controls; and longer time to be successful. Moreover, the failure rate is very high as seen in the western world. Given its constraints, India will be better served in the long run by pursuing IPR-based competitive strategies and commercialization model (US Global Trade Model) while using a transient revenue-generating model in the interim.

Swot analysis of the indian biotechnology sector: Based on the available reports, the biotechnology in India using a US-Global Competitive model can be summarized as:

Strengths (S)

- Trained manpower, knowledge base, language skills and quick adoptability;
- Potential for developing successful companies on many fronts, including, diagnostics and vaccines;
- Research infrastructure (public sector funded research laboratories);
- Potential for investment (FDI and Domestic) opportunities;

Weaknesses (W)

- Lack of capital and incentives;
- Relatively low R&D expenditure by the *industry;*
- Lack of IPR protection;
- Lack of strategic leadership in IPR-based R&D and its commercialization, and in translational research;
- Slow building of capacity for clinical/ drug testing protocols;

Opportunities (O)

- Access to other world markets;
- Domestic market expansion through newer innovative products;
- Access to Foreign Direct Investments (FDI) and domestic investments;
- Potential for opportunities for Diagnostics and vaccines in the animal health area.

Threats (T)

- Competition from outside companies;
- Weaker IPR regulatory policies.

In summary, globalization has fuelled economic growth/prosperity in India. Biotechnology sector is no doubt a growth area but whether India will be ready for the post-WTO era remains to be seen. The US global business model has worked extremely well for the US even though it has hurt much of its high cost manufacturing. However, the US's highly competitive biotechnology sector remains robust and unchallenged. About 10 years ago, Japan went through similar paradigm shifts as India going through now in biotechnology. After 10 years in the making, Japan is still facing constraints even though it has superb disciplined work force, better streamlined regulations, and infrastructure and competitiveness. Because of its economic clout in the G8 group, Japan has positioned itself well through a trilateral agreement with the US and EU on IRP-related issues in the biotechnology sector. There are lessons that India can learn from Japan's experience to lead its biotechnology sector so that it can position itself as a competitive force in the global market. The revenue-generating model alone similar to that of the IT in the absence of long-term multiple strategies may not work given the complexity of the sector. Investment capital is a huge bottle neck for India in order to grow this sector successfully. Based on reports, the flow of FDI into India has slowed down significantly in the past year because of China. India still needs to remain competitive in the region and it appears to be losing some grounds to China in this sector. There are significant opportunities in India itself for stimulating private sector investment opportunities, which has not been addressed well. India also needs to recognize both domestic and outside market opportunities although regulatory constraints may make it more difficult for Indian companies to penetrate the US and EU markets. India remains challenged on its domestic markets for biotechnology products as global companies enter the Indian market beginning 2006. Another 5-10 years from the WTO enforcement of IPR, would have benefited India tremendously. However, biotechnology sector should be a priority for India and hopefully, it will come out as a winner at the end.

Competitive Parameters between Developed Countries and India in the Biotechnology Sector

Competitive Parameters	Developed	India
1. R&D Infrastructure	Well developed	In India, there is good R&D infrastructure However, capacity for clinical research infrastructure still needs to be developed.
2. Intellectual Property Rights (IPR)	US, Japan and EU are aggressive in in their policies	IPR policies were almost non-existent in the pre-WTO era. During this period, India could copy and produce imitations. This will change beginning 2006. However, India is strong in the domestic generic industry.
3. Patents Trademarks Licensing, etc.	US, Japan and EU dominate in this area	International patents are very low for India. Thus, it does not have a competitive compared to US, EU and Japan.
4. Regulations It is a regulated Industry sector	Well streamlined Many changes have occurred in the last	India has not streamlined well. It is making changes but they are slow in coming in both public and private sectors decade
5. Co-operative Agreements	Tri-Lateral Agreement between US, Japan & EU	No such agreement exists now in the region
6. Foreign Direct Investment (FDI)	High	Moderate. China has dominated in FDI compared to India and Brazil
7. Public Health Infrastructure	Outstanding	Poor
8. Health Insurance System	Excellent	Poor

Competitive Parameters		Developed	India
9.	Health Care Delivery	Good to Excellent	Poor
10.	Generic Drug Industry	Good to Excellent	Good
11.	Information Technology	Excellent	Good (IT Infrastructure)
12.	Cost of Labor Force	High	Less costly
13.	Market Access	Developed Countries Eager to access markets in developing countries such as India and China They can access these markets readily.	On the contrary, India will have constraints even though it is eager to enter the US and EU markets. If not careful, India may lose its market because of its inability to compete with MNEs. It can be a double edge sword.
14.	Governance	Democratic & Stable	Often it can be difficult to work with because of ideology and political differences, which can slow down regulatory reforms leading to difficulties in being competitive in the long run with the developed countries.

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KN-10

Biomaterials and Devices for HealthCare

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Abstract

The dressed animal constitutes normally 30 – 40% of the weight of the animal and the remaining 60 – 70% of the animal constitutes animal by-products. Proper end uses of these materials contribute to considerable profit if they are properly collected and processed. In the last two decades, raw and fresh animal by-products are receiving considerable attention in India and some useful technologies have emerged from these materials. This is happening by the active involvement of concerned research institutions and adaptation of technologies by the industries. Central Leather Research Institute (CLRI) has the necessary expertise and is working on the development of biomaterials and devices for healthcare from the freshly collected tissues of slaughtered animals used for human consumption. Collagen derived products are emerging as valuable products of biomedical technology. CLRI has introduced technologies from collagen-based raw materials obtained from fresh bovine tissues. The conversion of bovine Achilles tendons into Reconstituted Collagen Sheet (RCS) and bovine intestine into Collagen Sheet (CS) are some of the examples of successful ventures developed at CLRI and transferred to industry. Both are currently produced and marketed in India and also exported to neighbouring countries. A number of other biomaterials and devices are in the pipeline. Development of all these products would ultimately bring India in the forefront of biomaterials and devices for the healthcare.

Introduction

India is a rich depository of livestock wealth. It possesses 210 million cattle, 90 million buffaloes, 120 million goats and 52 million sheep ranking first (except in sheep) among livestock holding countries (table 1 & 2).

The buffaloes, cows, goats, sheep and other domesticated animals are slaughtered for human consumption all over the world. Everything produced by or from the slaughtered except the dressed animal is considered a by-product. Proper utilisation and conversion of these products into high value products ensure their proper disposal with revenue from these waste materials. The main sources of animal by-products are abattoirs, fallen carcasses, meat processing plants, poultry farms, fish, prawn processing units etc. Large quantities of carcass by-products are available from these resources. The non-utilisation or under utilisation of these by-products causes environmental pollution and results in considerable loss of revenue to the country.

There is a general impression that meat is the main edible product and there is no by-product, which can be obtained in sufficient quantity from the abattoir. This is a misconception. Data given in this work illustrate the approximate yield of meat and by-products based on the live weight of the animal as shown in table 3.

Technology Package

Package of technologies for carcass by-products utilisation and establishment of related industries in India is practically non-existent. This is a very important area as it may play a major role in the availability of the raw material on continuous basis and also solve the disposal problem of a large quantity of bio-waste emanating from various sources such as slaughterhouses, meat processing plant, poultry dressing unit, fish processing plant, fallen carcass utilisation centres and tanneries. In Central

Leather Research Institute, Chennai and in some other institutes in the country concentrated efforts are being made to develop fully engineering technology packages and transfer of technologies. For developing full fledge packages following factors are important.

- Techno-economic viabilities of several processes
- Engineering aspects of unit process and operation of carcass by-products process.
- Development of component engineering organisations to implement these technologies on turnkey basis.
- Development of carcass by-products complexes, which can fully utilise the entire wastes.

Biomaterials & Devices for HealthCare

1. Biomaterials from Bovine Collagen

Technologies in the management of skin ulcers, wounds, burns have started relying on new group of biomaterials. Collagen derived products are emerging as valuable products of biomedical technology.

A major medical problem continues to be the clinical treatment of skin loss due to severe and massive burns and wounds. It is particularly critical that coverage of a damaged area occurs before the onset of infection. While management of small wound may not pose any problem, the management of large wounds and open raw areas, however is of serious concern to the medical profession. Certain categories of wound in particular such as those caused by burns pose formidable problems. One of the most common complications of burn is secondary infection and considerable fluid loss from the exposed burn areas. A superficial burn, for example with many of the deep epithelial cells preserved has the potential for good spontaneous healing within two weeks. Onset of infection however may interfere and delay the normal healing process resulting in deformities, contractures, cicatrix and scars. Similar problems may arise in traumatic wounds and other raw areas even though the problem in the wounds caused by burns may be comparatively more serious.

Coverage of the uninfected wound with autograft has been found to be the best for preventing fluid loss from the burn areas and controlling the infections from air borne bacteria. However, the availability of the autograft particularly when the area of skin loss is large is rather limited or at times not possible for various reasons. Moreover, autograft does not provide a satisfactory solution if the wound is already infected.

The next best solution is the coverage of the raw area with homograft. But here again availability of homograft particularly for large areas is rather limited. Like autograft, homograft also does not provide any satisfactory solution if the raw areas is already infected. In the absence of the availability of autograft and homograft in sufficient quantities, various other bio-dressings such as cadaver skin, porcine skin, amniotic membrane and synthetic skin have been used with advantage to cover extensive granulating surfaces during the last many years. However, the availability of these materials particularly in our country is rather limited. They also pose a serious problem in their preservation and sterilisation.

Hence there is a long felt need for the development of a suitable bio-dressing material readily available at reasonable price. CLRI has developed a process for the conversion of bovine Achilles tendons into Reconstituted Collagen Sheet (RCS) and bovine intestine into collagen sheet (CS). Both Achilles tendons and bovine intestine are available as by-products of meat industry.

Reconstituted Collagen Sheet (RCS) is prepared from the Achilles tendons of bovine animals. Highly purified collagen is prepared from Achilles tendons by solubilising the material using a patented procedure developed at CLRI. Sheets are prepared from the pure collagen in a sterile and dust free chamber. They are crosslinked with GTA and sealed in polypropylene sachets. The sachets are sterilised by, ethylene oxide fumigation or g- irradiation for extended shelf life.

Collagen Sheet is prepared from the serosa layer of bovine intestine by a series of chemical and enzymatic treatments. It is further stabilised by crosslinking with basic chromium sulphate (BCS) or glutaraldehyde (GTA) and finally sterilised by ethylene oxide treatment. The sterilised membranes are sealed individually in a sterile fluid for extended shelf life.

The sheets consist of mainly type I collagen with small percentage of type II and type V collagens. The sheets are now commercially available in India. Central Leather Research Institute. They are currently produced and marketed by M/s. Eucare Pharmaceuticals Pvt. Ltd., Chennai as Kollagen and NeuSkin. They are being exported to Sri Lanka, Thailand, Malaysia, Singapore, Kenya, Cambodia, Nepal and Bangladesh etc.

Both Collagen Sheet (CS) and Reconstituted Sheet (RCS) act as a temporary biological cover for second and third degree burns, open injury, wound ulcer etc. They are India's first natural non-immunogenic sterile

temporary biological skin covers for burns and open raw wounds. Both are ideal skin substitutes for the management of first and second degree non infected burns, raw areas caused by release of burns contractures, traumatic loss of skin cover, skin donor sites, amputation sites, chronic skin ulcers (stasis, arterial or trophic), shallow pressure sores and dermabrasion cases. In third degree burns they are useful as a temporary cover after escharectomy/ tangential excision.

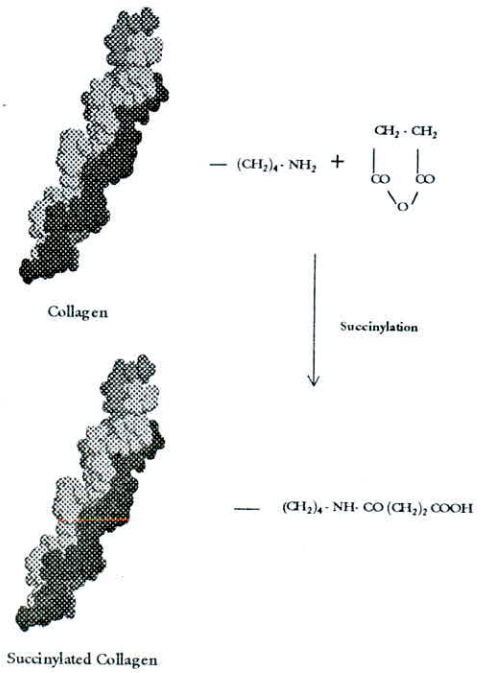
Among these, Collagen membranes have been widely established as an effective dressing for wounds especially the burns because of its easy commercial availability in sterile usable condition in various sizes, there is no threat due to HIV and Hepatitis-B infections and added with long shelf life under normal storage conditions.

Chemically, the bovine collagen is very similar to the human form. This is crucial, as the human immune system will reject everything that deviates too much from its own proteins. Because of all these reasons, collagen membranes are well pronounced for usage as an effective wound cover. Collagen as a natural biomaterial has unique properties like degrading in biologic environment, leaving no foreign residue, low antigenicity, ability to support cellular growth and is a haemostat. The scientists have been successful in bringing out this wonder material in different forms and shapes to handle problems in the health care industry, more specifically in the wound care segment. This commercial biomaterial is extracted from Bovine animal sources.

2. *Biomaterials From Chemically Modified Collagen*

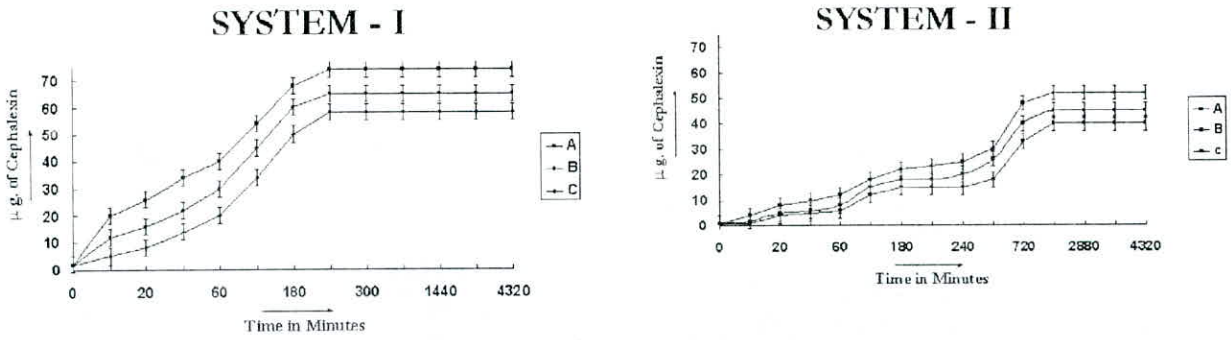
Enzyme Solubilised Type 1 collagen obtained from Achilles tendons of bovine animals was modified by succinylation. The succinylated collagen was converted into a bilayer dressing. The dressing was composed of a collagen membrane and collagen sponge, both modified by succinylation. The preparation of sponge was such that it has a rough surface on one side and a smooth surface on the other. The rough side of the sponge formed the bilayer system with collagen membrane having smooth surfaces on both the sides. Before converting into a bilayer dressing, Cephalexin - an antibiotic, at a concentration of $0.2 \times 10^{2} \pm 0.15$ mg/sq. cm was introduced in between the bilayer dressing. The bilayer system regulates the release of the drug in a controlled fashion. The dressing was stabilised by GTA cross-linking and was sealed in dry form using polypropylene packaging. The sealed dressing was sterilised by Ethylene oxide fumigation. This bilayer dressing can be applied directly on the wound. It is effective in the treatment of infected wounds.

This bilayer collagen drug delivery device in the form of a dressing showed *in vitro* release of antibiotic ciprofloxacin for 5 to 7 days. Now we are planning to test the antibiotic activity against gram positive and gram-negative organisms namely Staphylococcus *aureus* and Pseudomonas *aeruginosa* using agar diffusion method.



Succinylation of Collagen

Bilayer Collagen Dressing



A= 0.3 ´ 10^3 ± 0.15 mg/sq.cm
B= 0.2 ´ 10^3 ± 0.15 mg/sq.cm
C= 0.1 ´ 10^3 ± 0.15 mg/sq.cm

Cephalexin release in vitro from bilayer succinylated collagen drug release pattern

3. Use of Modified Collagen in Periodontal Regeneration

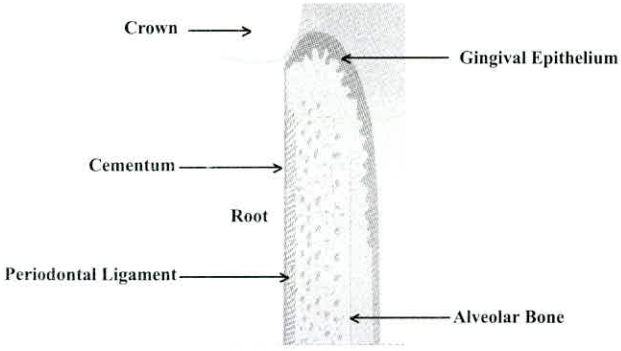
Guided Tissue Regeneration (GTR) defines as a procedure, which helps to regenerate lost periodontal structures through differential tissue responses. Barriers are employed in the hope of excluding epithelium and gingival connective tissue from the root surface in the belief that they interfere with regeneration. Objectives of GTR (Guided Tissue Regeneration) therapy are to exclude gingival epithelium and connective tissue from the root surface, which interferes with regeneration. It also helps to stabilize blood clot and maintain space in which regenerative tissues may form and allow time for new connective tissue attachment to become established on root surface.

Reconstituted type I bovine collagen membrane has been showing promising results as a wound cover in periodontal regeneration. Collagen is an ideal barrier material,

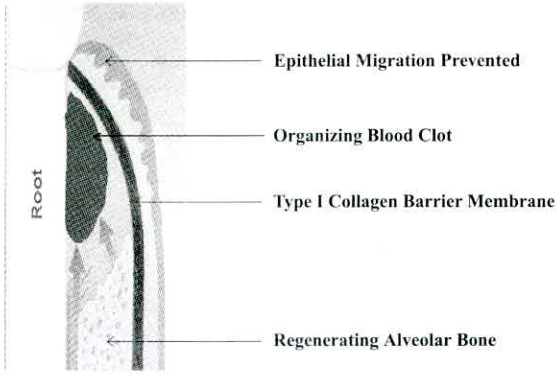
a natural component of periodontal tissues, non immunogenic and weakly antigenic, Malleable and semi-permeable. It supports cell proliferation & migration, chemotactic for fibroblast and facilitates wound stabilization. It also possesses haemostatic properties and bio-absorbable. The collagen membrane has been used in dentistry as in clinical conditions like gingival recession and infra bony defects. The treatment has been effective and the results have been very encouraging. In CLRI the collagen membrane (type I bovine collage) was specially prepared for its use in periodontal surgery and clinical cases of patients treated with this material. After periodontal therapy epithelium migrates first along the root surface followed by gingival connective tissue.

Barriers exclude epithelium and permit periodontal ligament and alveolar bone to regenerate.

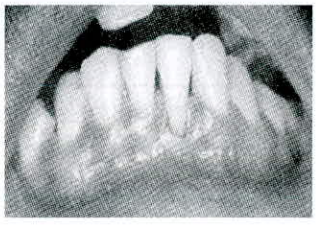
Healthy Periodontal Attachment



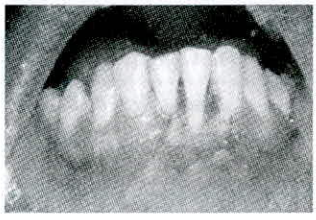
Biologic Principles of GTR



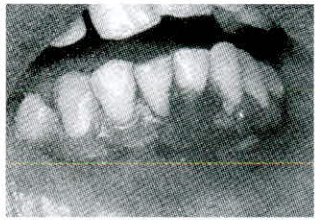
Treatment of Gingival Recession



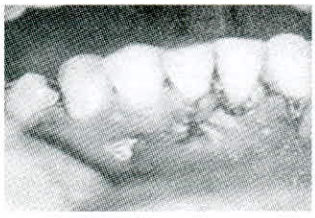
Pre-operative Condition



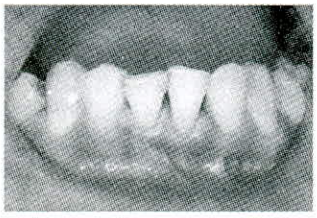
After Reflection of Gingival Flap



Type I Collagen Barrier Membrane in Place

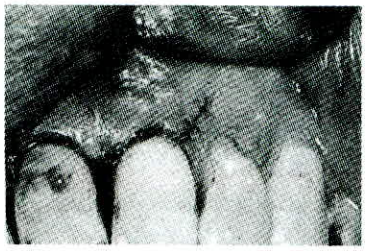


After Suturing

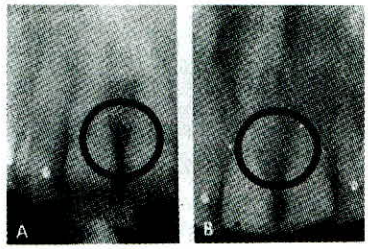


Postoperative Result After One Year

Treatment of Intraosseous Defect

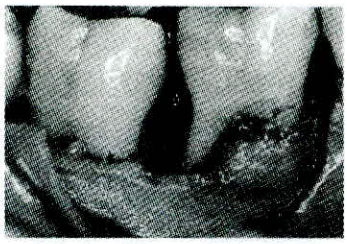


After suturing

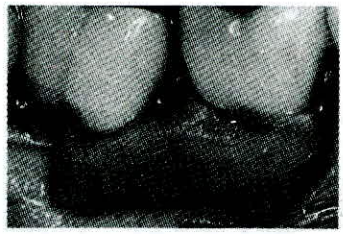


Pre and Postoperative
Radiograph Showing Bone Fill

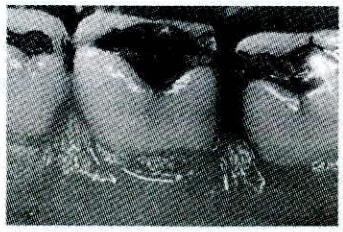
Treatment of Intrabony Defect



Defect Shown Clinically

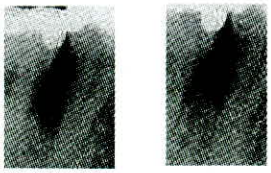


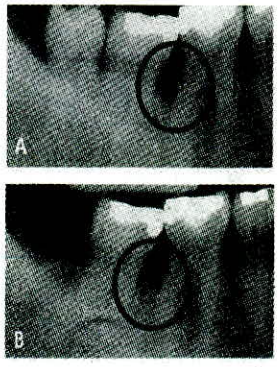
Collagen in place



Healthy gingival condition after one year

Pre and Postoperative Radiograph Showing Gain in Bone Height





From these photographs we see significant bone fill and dental regeneration in all the cases. More clinical trials have been planned in Dental colleges, Chennai. This will help to evaluate the efficacy of collagen material in treatment of dental defects, regeneration and bone fill.

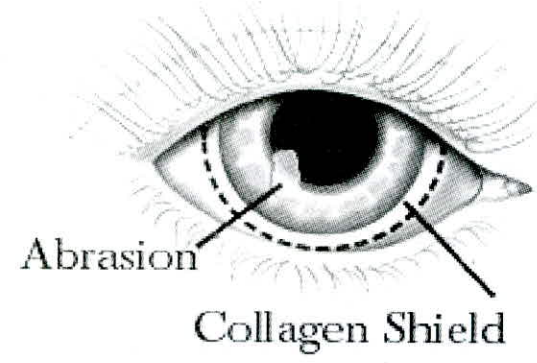
4. Use of Modified Bovine Collagen in Ophthalmology

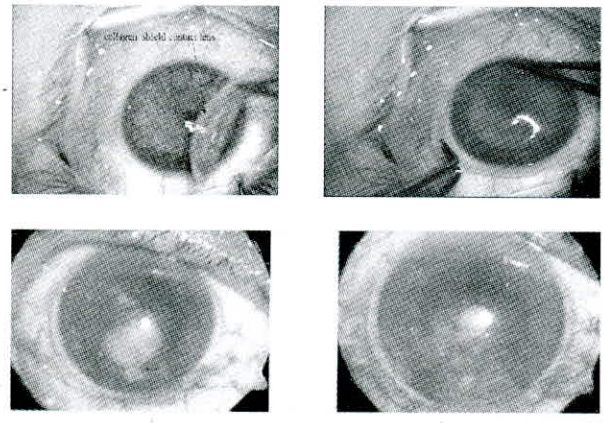
Chemically modified collagen, forms clear solution at physiological pH (7.4). Collagen shields prepared from the clear solution of succinylated collagen do not need any neutralisation before their application as ophthalmic inserts. Inserts were carefully modified, purified and blended to attain 100% transparency.

The modified collagen is evaluated by FTIR, DSC and SEM. They are showing potential application in cataract surgery i.e. during and after the removal of cataract lens. In cataract operation inserts were used to cover and protect the endothelial layer located on the inner portion of cornea facing the eye lens. The cataract lens is viewed, removed and replaced with intra ocular lens through the cornea and inserted during the surgery. The collagen inserts were used on 3 patients before phaco process. There were no iritis, no damage to central endothelium and no signs of inflammation during the surgery. The insert remains transparent and comfortable to the patient. There was some difficulty during insertion of the collagen insert and also it showed motility. Use of insert helps in protecting the cornea from inside and facilitates easy removal of the damage lens of the patient. Collagen insert (separate one) with or without antibiotic viz. Ciprofloxacin (use of antibiotic is optional) is subsequently used as a temporary cover on cornea for the post-operative healing of the incision wound. This helps in the faster recovery of patient without administration of any antibiotic for healing the incision wound.

The above experiment was done at Dr. Agarwal Eye Hospital, Eye Research Centre, Chennai. Collagen inserts have to be improved further, we are trying to make collagen insert less motile or non-motile. We are constantly interacting with the eye surgeons and as per their requirement the material is being modified. We hope to achieve good results in the near future.

Collagen Bandage Lens in Cataract Surgery

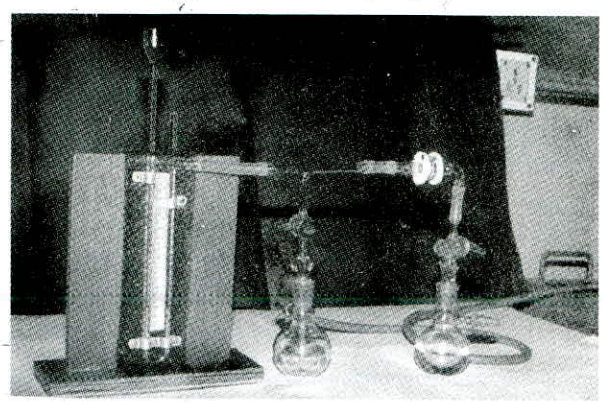




5. Oxygen Permeability Measurement Equipment

This is an equipment developed at CLRI to measure accurately the oxygen permeability of ophthalmic lenses and other membranes, which are optically refractive. Oxygen permeability of the ophthalmic lenses is a physical property and describes its intrinsic ability to transport oxygen. If the oxygen supply to the cornea is reduced, it may result in corneal edema by causing osmotic imbalance. A Novel, cost effective equipment has been designed to measure the oxygen permeability of ophthalmic lenses, oxygen Transmissibility and Equivalent Oxygen Percentage. This equipment could also measure oxygen permeability of any optical membrane not used as an ophthalmic lens, but finds its application elsewhere.

Oxygen Permeability Equipment



Conclusion

Individuals who suffer extensive loss of skin, commonly in fires, are in danger of succumbing either to massive infection or to severe fluid loss. Patients who survive these early threats must often cope with problems of rehabilitation arising from deep, disfiguring scars and crippling contractures. Here we have considered the physicochemical, biochemical, mechanical and biological properties to develop and purified reconstituted collagen sheet, to be used as a wound cover. Several polymeric membranes, differing widely in chemical composition, can conceivably qualify. They however lack the capacity to direct or regulate biological response. The specific choice of a reconstituted cross-linked collagen matrix, war arrived at for a number of reasons. Among the vast number of synthetic and natural polymers currently available, very few stand out as apparently suitable. Of these, the majority of polypeptides and polysaccharides are capable of being readily degraded by extra cellular enzymes or by simple hydrolytic scission into non-toxic products. Furthermore, these two classes of polymers are structurally related to the macromolecular components of the typical wounded surface. The latter consists primarily of proteins and polysaccharides in highly hydrated form. For these reasons, one expects that the wounded surface would be wettable by a skin substitute made up of a hydrated form of one or both of these tow classes of polymers.

By contrast poly (dimethyl siloxane), a very widely used biomaterial is clearly neither hydrophilic nor biodegradable. Polyimides such as Poly (hexamethylene adipamide) or saturated polyesters such as polyethylene terephthalate, also widely used biomaterials are weakly hydrophilic and poorly biodegradable. Poly (2-hydroxyethyl methacrylate), are relatively hydrophilic polymers but not biodegradable either. On the other hand, a polymer such as poly (n-butyl cyanacrylate) can wet the surface of several tissues adequately, and is biodegradable, but one of its degradation products is toxic. An inert dressing is highly undesirable. Synthetic dressing may lead to severe inflammatory reaction, followed by scarring and severe wound contraction.

Focussing on the biopolymers, collagen is compatible with human tissues. The three major characteristics, which make collagen-exciting biomaterial are

Physical : Referring to the high tensile strength of the collagen fibre and the fibriller nature of collagen allowing further orientation, weaving or knitting or the material.

Physiochemical : referring to the ability to get cross-linked with a variety of agents to give controlled and predictable changes of solubility, swelling and the rate of degradation. It is also known that collagen protein, having a high content of both diamino and dicarboxylaminoacids, serves an ion exchange in the body. This has been utilised when interacting collagen with various drugs in developing drug delivery systems.

Biological : The affinity of various cells for collagen substrate and the interaction of collagen with other macromolecules as in the blood clotting system and glycosoaminoglycans (proteoglycans). The platelet – collagen interaction is the best-known example of cell collagen interaction. Isolation of fibregenitic cells with collagen has been well documented. Adhesion of leucocytes and re blood cells to collagen has been suggested. All these suggest that collagen possesses chemotactic properties and the collagen implants does not behave as an inert material but actively promotes certain tissue reactions.

We note that collagen is a hydrophilic polymer, which is capable of resorption and integration with the body, in a controlled manner, through cross-linking. Even though its antigenicity is detected by immunological techniques, long clinical experience with collagen sutures and other collagen based medical devices, shows that collagen is a relatively weak antigen. It is amenable to a very high degree of purification, and in the form of reconstituted matrices, is sufficient tough to be useful in many surgical applications. The massive research effort expended on collagen make it one of the best understood polymers today and suggest its use as a biomaterial.

Table 1 - Livestock & Livestock Slaughtered (1996 to 2000)

					('000 no.)
Animals	**1996**	**1997**	**1998**	**1999**	**2000**
Cattle					
Stocks	208540	209541	210547	211558	212573
Slaughtered	13347	13411	13475	13540	13605
%Slaughtered	6.40	6.40	6.40	6.40	6.40
Buffaloes					
Stocks	87417	88239	89068	89905	90751
Slaughtered	9642	9733	9824	9917	10010
%Slaughtered	11.03	11.03	11.03	11.03	11.03
Sheep					
Stocks	55250	56427	57629	58856	60110
Slaughtered	16575	16928	17289	17657	18033
%Slaughtered	30.00	30.00	30.00	30.00	30.00
Goats					
Stocks	119485	120561	121646	122741	123845
Slaughtered	45381	45789	46201	46617	47036
%Slaughtered	37.98	37.98	37.98	37.98	37.98

Source : Basic Animal Husbandry Statistics 2002, Ministry of Agriculture, Dept. of Animal Husbandry & Dairying, Govt. of India.

Table 2 - State-wise Cattle and Buffalo Bull Population (1987 – 1992)

									('000 no.)
States/UTs	**Cattle**						**Buffalo**		
	Indigenous			**Crossbred**					
	1987	1992	% Growth	1987	1992	%Growth	1987	1992	% Growth
Andhra Pradesh	779	453	-10.28	22	10	-14.59	132	89	-7.58
Assam	404	505	4.56	6	7	3.13	77	102	5.75
Bihar	8276	8795	1.22	-	4	-	838	921	1.90
Gujarat	94	73	-4.93	3	3	0.00	24	28	3.15
Haryana	44	38	-2.89	9	12	5.92	47	78	10.61
Himachal Pradesh	32	62	14.14	6	5	-3.58	82	5	-42.29
Jammu & Kashmir	153	124	-4.12	26	26	0.00	11	12	2.43
Karnataka	132	180	6.40	10	31	25.39	36	88	19.67
Madhya Pradesh	654	1109	11.14	5	9	12.47	255	345	6.20
Maharashtra	338	322	-0.97	-	28	-	73	78	1.46
Orissa	4609	4996	1.63	52	59	2.56	629	646	0.54
Punjab	-	35	-	251	62	-24.40	-	47	-
Rajasthan	200	211	1.08	-	1	-	46	55	3.47
Tamil Nadu	161	257	9.80	19	42	17.19	51	9	-28.99
Uttar Pradesh	330	262	-4.51	75	67	-2.23	336	295	-2.58
West Bengal	4593	284	-42.69	48	23	-13.68	678	27	-47.45
India	**21014**	**18032**	**-3.01**	**570**	**431**	**-5.44**	**3382**	**2864**	**-3.27**

Source : Basic Animal Husbandry Statistics 1999, Department of Animal Husbandry and Dairying, Ministry of Agriculture (Govt. of India).

Table 3 – Percentage Yield of Meat and By-products

Name of the Product/By-product	Percentage Yield on Live Weight
Meat	35.00
Bones (Green)	25.00
Hide	6.50
Blood	4.00
Horns and Hooves	0.60
Small Intestines	1.00
Large Intestines	1.00
Stomach	2.00
Rumen Digest	11.00
Liver	1.20
Heart	0.35
Kidneys	0.20
Lungs	1.20
Brain	0.17
Spleen	0.15
Pancreas	0.07
Bile	0.06
Tongue	0.30
Hair, teeth and other	1.50
Cutting losses etc	8.70
Total	100.00

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KN-11

Challenges facing the Poultry Industry

Dr. A. T. Venugopalan

Retired Director, Centre for Animal Health Studies, TANUVAS, Chennai

Indian Poultry industry at a glance

The historical perspective of poultry industry is par excellence. From the backyard, the poultry industry made quantum jump units in a short period of time. The growth of poultry sector is noted here under

- ❖ Indian poultry population is 3% of worlds poultry population.
- ❖ Fifth in egg production.
- ❖ Twenty first in chicken meet production
- ❖ Seventeen in over all world's poultry production.
- ❖ Poultry meat constitute 25% of the total meat production in India.

1. To maintain this growth role sustainability of the poultry sector is a pre requisite. There is need for export since the purchasing power of our population is very much limited. It is well documented that out of Rs. 900/- as the capital expenditure on food only 1.75% (i.e.) Rs. 15.80 is expenditure on egg and chicken. This clearly illustrates the importance of creating demand for chicken and eggs besides processed products in the country.
2. The main problems faced by the exporters are here under.
   - Implementation of residue control programme as per directive 96/23 dated 29.04.1996 of European Union
   - Residue analysis as per standard protocol of European union vide decision 657 dated 12.08.2002.
   - Limit of quantification and minimum no required performance level of analytical equipments for residue analysis. FDA 2002 and EO 2003.
3. Further as per the requirement of European Union the certificate for export purposes should be issued only by laboratories accredited as per 17025 of ISO. In addition, such laboratories should implement methods of analysis based on validation and proficiency listing. As on date there is no laboratory in India with ISO 17025 accreditation. The exporters are sending samples to Europe at high cost and inordinate delay.
4. Further certain exports of animal products particularly egg powder now requires certificate of freedom from ND and FMD from India. India is endemic for ND and is not free from FMD. Under these circumstances there is difficulties in export areas.
5. For implementation of residue control programme legal basis for poultry sector.
   - Existing status and control acts the work "Animal" does not include poultry.
   - The Supreme Court of India gave a ruling that poultry is not coming under livestock.
   - The High court of Karnataka quashed in Hatchery control order on the plan that poultry does not come under Essential Commodities Act. As such there is no legal basis or protection for poultry sector.

6. The prevalence of non highly pathogenic influenza in India is confirmed by world reference laboratory as well as Indian reference laboratory. It is likely to mutate as highly pathogenic avian influenza at any moment in the near future. Apart from Bhopal laboratory standard methods of diagnosis is not available in any regional laboratories as per OIE norms.

7. Apart from export purpose there are import of livestock and livestock products. It is necessary to screen the same as per international norms such as OIE 2003 or WHO 2002. The non-availability of the following is the stumbling block.

   a. Certified reference material

   b. Standard for analysis as per OIE

   c. Analytical methodology as per OIE

Under these circumstances the role of university and industry interaction is of vital importance.

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BIOTECHNOLOGY IN ANIMAL PRODUCTION

AP-1

Sexed Semen, a Great Animal Biotechnology Tool, has it Become a Reality?

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The desire to control the sex of the offspring has been the dream of many in the world of livestock production. The different molecular/genetic make-up of X- and Y-bearing sperm has given an impetus for scientists and producers to seek ways to separate them before they are used for insemination thereby controlling the sex of the unborn. Many pioneering advances in this area have resulted in a few acceptable separation methods as confirmed by laboratory standards. However, many of these methods failed when attempting to repeat them, or to translate them into working models in field conditions. The reasons for these failures may not be known. Some promising works offered a clear direction for innovation-based industries to pursue and perfect laboratory methods for its application. However, these methods have not reached the stage of application. Difference in the DNA content of the two sets of sperm, coupled with the ability to sort them based on this fact by flow cytometry gave unparalleled momentum to scientists to make rapid progress in the art of sperm pre-selection in many livestock species. A few companies are marketing sorted sperm, while others are trying to perfect other methods. Considering the rapid changes that have taken place in animal agriculture over the past generation and the demands of the industry, changes in the market place are such that sex pre-selection for production systems has become imperative, and very real. Despite progress in this area, the main determinants to the commercial use of sexed sperm will be (1) pregnancy rate and (2) cost, two important variables that can dictate the usefulness of this technology. Until sorting efficiencies improve and costs decline, sales will be limited primarily to niche markets. Not withstanding these drawbacks, this technology will likely become commercially available in many countries within few years.

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AP-2

Development of Suitable Biotechnique to Overcome the Summer Fertility in Buffaloes

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An investigation was conducted to develop a suitable biotechnique through administration of $PGF_2\alpha$ and progesterone combination in 6 to 14 months post-partum anestrous buffaloes (1st to 5th lactation) maintained under rural management system during summer (ambient temperature 27°C to 42°C). All the animals were dewormed by anthelmintic (1.5 gm fenbendazole). Control internal drug release (CIDR) device containing 1.9 gm progesterone was inserted intravaginally in 30 buffaloes. The CIDR was removed on day 8 (10 buffaloes), 12 (10 buffaloes) and 14 (10 buffaloes). Prostaglandin

was administered (I/M) in each buffaloe 48 hrs before the removal of CIDR. Three out of 10 buffaloes inserted with CIDR for 12 and 14 days were detected in estrus between 45 and 55 hrs of CIDR removal. Eleven buffaloes received (I/M) 1st dose (1 ml) of Duraprogen, while 3 received (I/M) 1st dose (2 ml) of Duraprogen on day 10 of prostaglandin administration. One out of 11 buffaloes receiving 1 ml Duraprogen while 3 buffaloes receiving 2 ml Duraprogen were detected in estrus on day 11 and day 5 to 8 of Duraprogen injection. Out of 10 buffaloes receiving 1 ml of Duraprogen as first dose, 4 were injected with 2nd dose of 2 ml Duraprogen and 6 were injected with 1 ml of Duraprogen. Three out of 4 buffaloes were detected in estrus between 5 and 8 days of 2nd dose (2 ml) of Duraprogen. However, none of the buffaloes receiving 1 ml of Duraprogen as 2nd dose were detected in estrus. The daily milk yield in 60% of buffaloes during progesterone administration was decreased to 10-20%. The milk yield, resumed within 10 to 15 days after CIDR removal and 15 to 20 days after the last dose of I/M progesterone administration. The observation suggests that the summer infertility in buffaloes can be overcome either by intravaginal administration of progesterone for 12 days or by I/M administration of 500 mg progesterone.

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AP-3

Polymorphism of Three Microsatellites in South Indian Cattle Breeds

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Three microsatellites namely ETH225, HBB and ILSTS030 were analyzed for their polymorphic values in three South Indian breeds of cattle *i.e.*, Ongole, Kangayam and Umblachery. Among these microsatellites, ETH225 and HBB produced good polymorphism with PIC values of 0.891 and 0.831 respectively, whereas ILSTS030 was less polymorphic for these breeds and showed a PIC value of 0.519 only.

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AP-4

Efficacy of GnRH and hcG Treatment on Fertility in Estrus Synchronized Buffaloes

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Reproduction efficiency of a herd depends on the efficacy of estrus detection and timing of insemination. In buffaloes, silent estrus is a serious problem and this makes it difficult to obtain optimum reproductive efficiency. With widespread application of artificial insemination in cattle, it is

necessary to develop accurate, reliable and cost effective method to improve estrus detection and fertility.

A total of 28 Murrah buffaloes were selected and divided equally into 4 groups from District Livestock Farm, Orathanad, Tamil Nadu. In Group I, II, III synchronization of estrus was done with 15 mg of prostaglandin F_2 (Luprostiol) . Group IV served as untreated Control.

250 µg GnRH and 1500 IU hcG was administered in Group I and II respectively at the time of insemination which was done at 72 and 96 hrs after prostoglandin F_2 injection in first three groups. But in last group it was done at 12-18 hrs after the onset of natural estrus.

In all the groups onset, intensity, duration of estrus and conception rate were studied. It was observed that onset of natural estrus was during cooler part of the day, the average time taken for onset of induced estrus was 72.33± 0.53 hrs, weak estrus was more in natural estrus group while normal and intense estrus was more in induced estrus groups. The duration of estrus in synchronized buffaloes was found to be slightly longer than natural estrus. The first service conception rate of treated buffaloes were 57.14, 71.42, 57.14, and 42.86 percent in PGF_2 + GnRH, PGF_2 + hcG, PGF_2 and control groups respectively. Conception rate of prostoglandin F_2 + hcG group was higher than any other treated groups and comparable to that of natural estrus buffaloes.

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AP-5

Effect of Kappa Casein Variants of Cattle on Milk Yield, Composition and Technological Properties

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A study was conducted to analyze the genetic variants of kappa casein (κ-Cn) by polyacrylamide gel electrophoresis (PAGE), to identify polymorphism at κ-Cn locus by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), to study the effect of κ-Cn polymorphism with fat percentage, protein percentage, 305 days milk yield, total solids, casein percentage, rennin clotting time (RCT) and channa yield and to sequence the PCR products of genetic variants of κ-Cn variants in Red Sindhi, Cross breed, Jersey and Freisian animals.

The κ-Cn genotype frequencies of AA, AB and BB were 0.59, 0.32 and 0.09 in 95 Red Sindhi, 0.48, 0.34 and 0.18 in 60 Crossbred, 0.16, 0.36 and 0.48 in 50 Jersey and 0.64, 0.36 and 0 in 50 Holstein Friesian. The gene frequencies A and B of κ-Cn observed were 0.75 and 0.25, 0.58 and 0.42, 0.34 and 0.66 and 0.82 and 0.18 in Red Sindhi, Cross bred, Jersey and Holstein Freisian respectively.

Genomic DNA isolated from the different breeds of cattle were used for amplification of a 874 region between nucleotide Exon IV and Intron IV of κ-Cn gene and were digested with restriction enzyme Pst I. Genotype AA resulted in 460 bp and 414 bp fragments, genotype AB resulted in 874,

460 bp and 414 bp fragments and genotype BB resulted in 874 bp. Sequencing of these DNA also confirmed these results.

The effect of the polymorphic variants of κ-Cn on composition of milk, milk yield and technological properties of milk was assessed by Least Square Analysis. It was observed that BB genotype and Jersey breed had significantly higher fat%, protein%, casein % and total solids% than AB and AA genotypes and other breeds taken for this study. Both had lesser RCT and higher channa yield, the results were highly significant. BB genotype and Jersey had significantly higher milk yield.

PCR amplification and RFLP analysis presented in this study was found to be rapid and sensitive method for the identification of κ-Cn genotypes directly at the DNA level without the milk samples of lactating females. This PCR-RFLP, method could be used as a valuable tool for early selection of AI bulls and calves with desirable κ-Cn gene or genotypes for genetic improvement of dairy cattle.

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AP-6

Characterization of Insulin-like Growth Factor-binding Protein-3 (IGFBP-3) Gene in Buffalo

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Insulin-like growth factor binding protein-3 (IGFBP-3) gene is a structural gene associated with the growth and development of the animals. The present investigation was carried out to characterize IGFBP-3 gene in buffalo using nucleotide sequencing and polymerase chain reaction-restriction fragment polymorphism (PCR-RFLP) techniques. Genomic DNA was isolated from a total of 157 animals belonging to Murrah, Surti, and Jaffarabadi and Nagpuri breeds of buffalo. A region of IGFBP-3 gene spanning over a part of exon 2, complete intron 2, exon 3 and a part of intron 3 was amplified by using a set of forward (5'- CCA AGC GTG AGA CAG AAT AC –3') and reverse (5'- AGG AGG GAT AGG AGC AAG AT-3') primers. The length of amplified product was found to be of 655 bp in all the breeds of buffalo. The amplicons were digested with *Hae* III, *Taq* I and *Msp* I restriction enzymes. Digestion with *Hae* III yielded single restriction pattern (8 fragments) of sizes 201, 165, 154, 56, 36, 19, 16 and 8 bp in all the animals studied. Similarly *Taq* I and *Msp* I also revealed single restriction pattern yielding fragments of sizes 240 & 415 bp and 145 & 510 bp, respectively. This shows non-polymorphic nature of restriction sites in buffalo. Nucleotide sequencing of IGFBP-3 gene in Murrah buffalo was done and submitted to the GenBank (Accession No. AY304829). Sequencing of this gene revealed an addition of 4 bases in the intronic region as compared to cattle.

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AP-7

Genetic Polymorphism of Interleukin-10 Gene in Buffalo

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A study was undertaken to detect polymorphism at interleukin-10 gene in Murrah buffalo maintained at Cattle and Buffalo farm, IVRI, Izat Nagar, U.P and Buffalo farm, Lakhimpur-Kheri, U.P. Single strand conformation polymorphism (SSCP) was used to detect polymorphism at this locus. A fragment of IL-10 gene spanning over exon-1 to -3 (1.5 kb) was amplified with a pair of primers, forward 5'-CTGTCTGACAGCAGCTGTATC-3' and reverse5'-GGCAACCCAGGTAACCC TTAAA- 3'. The amplified product was digested with *Hinf*I enzyme and then SSCP was carried out. Eight types of SSCP patterns were detected for the animals of IVRI herd while nine SSCP types were observed for the animals of Lakhimpur-Kheri herd. These types have been reported as genotypes. The highest frequency of genotype was found for A_1A_2 of both IVRI as well as Lakhimpur-Kheri farm. The lowest frequency was observed for the genotypes A_2A_2, A_3A_3 and A_2A_4 of IVRI farm and A_2A_2, A_4A_4 and A_2A_4 for Lakhimpur-Kheri farm. However, four types of SSCP alleles namely, A_1, A_2, A_3 and A_4 were observed at both the herds. The highest allelic frequency was found for A_1 of IVRI and Lakhimpur-Kheri farm while lowest frequency was estimated for A_4 allele of both farms. Two half family study of both the farms was carried out for understanding inheritance pattern of alleles and the result was found as positive. The chi-square test indicated the true Mendelian segregation of genes at both the farms.

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AP-8

Prediction of Parental Genomic Proportion in BC_2population Using Microsatellite Markers

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The present study was designed to investigate the utility of microsatellite markers in estimating the parental genomic proportion, in a specialized resource population, developed for MAI programme. Two types of BC_2 populations were developed. Type A BC_2 population was the cross between BC_2 naked neck selected males with WLH females, while mating of BC_2 selected females with WLH males produced the type B BC_2 population. The panel comprising of 72 BC_2 (40 from Type A and 30 from Type B) progenies and the grand sire was genotyped with 10 microsatellite markers from chromosome 3. Out of 10 total rnicrosatellite markers, five markers i.e. MCW 40, LEI 166, LEI 65, LEI 113 and ADI 237 detected polymorphic alleles among BC_2 progenies and grand sire. The parental genomic proportion from the donor grand sire to the BC_2 progenies was estimated in terms of the genetic similarity calculated as average band sharing (B5) proportion between the grand sire and

BC_2 progenies. The BS estimates ranged from 0.44 to 1.00 in BC_2 population. The overall mean genetic similarity between the grand sire and BC_2 progenies in type A, type B and overall BC_2 populations was 0.693 ± 0.176, 0.671 ± 0.020 and 0.682 ± 0.013 from pooled over all Markers in type A, type B and overall BC_2 populations respectively. The differences were non-significant for BS estimates between both types of BC_2 populations. The genetic distance (D5) ranged from 0.00 to 0.81. The overall mean average genetic distances between the grand sire and BC_2 progenies were 0.379 ± 0.025 in type A, 0.412 ± 0.032 in type B and 0.396 ± 0.200 in overall BC_2 population. The differences were non-significant for genetic estimates between both types of BC2 populations.

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AP-9

Production of IVF Goat Embryos and Their Transfer to Synchronized Recipient Goats Using Laparoscopy

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The aim of this study is to produce *in-vitro* goat embryos and their transfer to recipient goats using laparoscopy techniques. Oocytes were released from slaughter house ovaries in oocytes collection medium (OCM) and isolated under stereo-zoom microscope. A and B grade oocytes were washed 4-5 times with oocyte maturation medium (Medium 199, 5µl/ml FSH, 100 µl/ml LH, 1 µl/ml estradiol-17ß, 10% heat inactivated goat serum, 5% gentamycin and 3% BSA) and placed in 100 µl drop in this medium at 15-20 oocytes per drop, in 35 mm dish covered with mineral oil. Dishes were kept in 5% CO_2 incubator with maximum humidity for 27 h. Semen was collected from bucks with AV and processed for capacitation of spermatozoa. For this, 50 µl semen was mixed in 5 ml of sperm TALP medium and centrifuged at 500 rpm for 5 minutes, sperm pellet was retained and the supernatant was discarded. This process was repeated and the pellet was finally suspended in fertilization-TALP medium containing heparin 50 µg/ml and kept in above incubator for 2h. *In-vitro* fertilization was performed with 27 h matured oocytes and capacitated spermatozoa. Cleaved embryos were isolated and cultured further in embryo development medium and on *in vitro* cultured oviduct cells. A total of 4671 goat ovary samples were collected and 11,247 oocytes were isolated from these ovaries. The oocytes were graded as A (21.20%), B (23.52%), C (25.31%) and D (29.95%) types oocytes depending upon the layers of cumulus cells around the oocytes and the visual appraisal of the cytoplasm. A total of 554 embryos were produced. Recipient goats were synchronized with two injection (15 mg /goat) of PGF_2 (Lutalyse®) 10 days apart and IVF goat embryos were transferred to the uterotubal junction of recipient goats using laparoscopy / laparotomy. From the present study it could be concluded that the simple method of laparoscopy is useful for transferring IVF produced goat embryos to synchronize recipient goats.

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AP-10

Polymorphism at Prolactin Gene Locus in Vechur and Other Dwarf Desi Cattle of Kerala Detected by PCR-RFLP

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The genetic polymorphism at the third exon of bovine prolactin (PRL) gene in Vechur and dwarf cattle of Kerala was analysed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Vechur (59) and other dwarf desi (33) cattle maintained at the Vechur Conservation unit of Kerala Agricultural University were used for this study. A 156-bp fragment enclosing the polymorphic *Rsa*I site within the third exon was amplified by specific primers. The digestion of the PCR product with *Rsa*I enzyme revealed two alleles; namely, allele *A* (156 bp) and allele *B* (82 + 74 bp). The frequencies of PRL-*A* and PRL-*B* alleles were 0.74/0.26 and 0.58/0.42 for Vechur and dwarf desi cattle, respectively. Milk fat percentage, peak milk yield, first lactation yield, and first lactation length were analysed for their association with the three PRL genotypes in Vechur cattle. However, the different PRL genotypes did not vary significantly ($p>0.05$) for the milk traits analysed.

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AP-11

PCR-RFLP Analysis of Bl-P 11 Region in Aseel and Broiler Chickens

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BL-β II region of MHC was anlysed by PCR-RFLP technique in Aseel-an indigenous breed and Synthetic Broiler Dam Line (SDL)-a parent line of high yielding broilers. Synthetic Broiler Dam Line (SDL) maintained at experimental broiler farm has been undergoing long-term selection for economic traits and Aseel birds are maintained at Desi Fowl unit under conservation programme. Divergent selection was practiced in SDL for response to sheep RBCs and high (HSRBC) and low (LSRBC) responding lines to sheep RBCs were produced. Response to sheep RBCs (0.5% suspension, i/v) was estimated by HA test after 5 dpi. Analysis of BL-b II region was carried out in Aseel birds randomly chosen from population and in extreme responders of HSRBC & LSRBC lines after second generation of selection. The DNA was isolated and purified as per standard procedures. PCR

amplification was done with individual DNA samples using specific forward and reverse primers and BL-b II region was amplified as 277 bp fragment. The PCR products were purified and digested with *Taql* enzyme as per recommendation of manufacturer. After overnight digestion the individual samples were run on PAGE and stained by silver staining. Genotypes AA and AC were observed on the gel. Genotypes AA was found, in all the samples of Aseel and LSRBC line whereas genotype AC was found in LSRBC line only. The polymorphism among Aseel and HSRBC and LSRBC lines substantiated the role of MHC in disease resistance. Aseel birds had much less history of selection for production traits and are much more resistant to diseases as compared to high yielding stocks. The monomorphic patterns obtained for Aseel showed that possibly the genes conferring resistance have been fixed in Aseel. The SDL line having higher response to Sheep RBCs also showed the pattern similar to that of Aseel, which indicated that this line might have better resistance to diseases. The results also indicated that selection for high response to sheep RBCs may provide higher resistance to diseases in high yielding broiler stocks.

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AP-12

Plant Enzyme for Clotting of Milk

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Preservation of milk as cheese has been handed down from generation to generation. Plants provide a good source for milk coagulating enzyme. In the present study the extract of the plant *Calotropis gigantea* was used in the preparation of cheese. Of the various methods of extraction viz. a) 5% NaCl, b) activated charcoal c) silica gel and d) 70% alcohol it was found that extraction obtained using 70% alcohol showed superior activity of milk clotting. The extract was then subjected to a column of Sephadex G-200. The chromatography of the extract revealed 5 fractions of which the second fraction showed higher clotting and lower proteolytic activity. The clotting activity and the temperature were directly proportional. The optimum temperature for the clotting activity was 70°C. The milk clotting activity of the extract was not affected by sunlight and UV radiation. The addition of 2% boric acid had no influence on the activity of milk clotting but 2 % formalin and 0.01N Iodine inhibited the activity. The extract lost its activity when stored at room temperature for more than 24 hours. Chemical composition and texture profile of cheese made with the extract of *Calotropis gigantea* were compared with that of direct acid cheese made with calf rennet. The composition of the experimental cheese showed 53% moisture, 15.4% fat and 24.3% protein. Relative to that made with calf rennet, cheese made with vegetable rennet was harder and scored lesser in flavour, body and texture and colour presumably because of the difference in chemical composition and physical characteristics between the cheese.

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AP-13

Genetic Characterization of Marwari Goat Population of Rajasthan

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Marwari is a medium sized and dual purpose (fibre/meat) goat breed of North Western region of Rajasthan (India). The name, Marwari, is derived from the "Marwar" region of Rajasthan, which is a natural habitat of the breed. The breed is found in Barmer, Bikaner, Jaisalmer, Jalore, Jodhpur, Naguar and Pali districts of Rajasthan. The breed constitutes 28% of the total goat population of its region. In the present study, an attempt was made to find out with-in-breed genetic diversity using microsatellite molecular markers. The blood samples were collected from 50 unrelated animals from its breeding tract. The molecular data were generated using PCR followed by allelic differentiation in urea PAGE and silver staining. Based on the guidelines of ISAG and FAO, a battery of 15 microsatellite markers viz. ILST008, ILSTS059, ETH225, ILST044, ILST02, Oar FCB304, Oar FCB 48, Oar HH64, Oar JMP29, RM4, ILST005, ILSTS019, OMHC1, ILSTS087 and ILSTS30 were selected to generate the data of the panel of 47 animals. Data analysis by POPGENE software showed an overall average heterozygosity of (0.66± 0.17) with maximum of 0.88 at locus ILST019 and minimum of 0.22 at locus ETH225. The maximum number of alleles (8) was observed at 3 loci viz. ILST008, Oar HH64 and Oar FCB 304 where as the minimum (3) was observed at other two loci viz. ETH 225 and ILST005. The maximum effective number (Ne) of alleles (4.39) and the minimum (1.43) was found with loci ILST030 and ILST044, respectively. The PIC was calculated and found maximum (0.79) and minimum (0.27) with loci Oar HH64 and ETH 225, respectively.

Besides obtaining with-in-breed diversity, the data generated will also be used in establishing genetic relationship among other goat breeds of Rajasthan as well as establishing their breed identity and genetic differentiation at molecular level. Furthermore, the information will be useful for goat conservation programmes of the region.

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AP-14

Genetic Resistance to Nematode Infection for Enhancing Productivity and Product Quality in Goats

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The development of drug resistance to many parasites is widespread and increasing. Resistance in target organism threatens drug efficacy and frequent application to control the nematodes is increasing the drug residues in milk and meat. Moreover, increased public awareness for the presence

of chemical residue in livestock products and their impact on environment has been stimulated in worm control methods. Genetic improvement of host resistance to nematode infection is one of the best alternatives that can enhance resistance level and reduce dependency on drugs. The faecal egg count (FEC) and packed cell volume (PCV) are used as an indicator trait for establishing resistance to gastrointestinal nematodes (GIN) in livestock.

An investigation was carried out in Jamunapari and Barbari goats with 10 half-sib families to analyze the genetic resistance to nematodes in response to natural infection. The FEC and PCV of kids have been taken with growth traits at 3,6 and 9 months of age.

Moderate to high heritability (h^2) of FEC and PCV were estimated to be 0.45 ± 0.25 and 0.59 ± 0.28, respectively at 9 months of age in goats, which is reliable and important for study to disease resistance in response to nematode infection. Sire had a significant ($P<0.01$) effect on FEC and PCV at 9 months of age. There was a significant ($P<0.05$) difference in FEC between progenies of resistant and susceptible sire families and also affected various production parameters such as body weight and body weight gain in kids. The difference between the progenies of resistant and susceptible sire families were 19.55 kg and 16.70 kg respectively in body weight and 50.53 g/day and 32.58 g/day, respectively, in body weight gain at 9 months of age. Selection of resistant animals within breeds or incorporating resistant breeds into breeding programme and identification of genes involved in regulating resistance will allow earlier selection of genetically superior animals and may increase the rate of selection for resistance.

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AP-15

Cytogenetic Analysis of Vero Cell Line

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The Vero cell line was established from the kidney of a normal adult African green monkey (*Cercopithecus aethiops*). The Vero cell line has been employed extensively in virus replication studies and plaque assays. The African green monkey has a diploid chromosome number of 60(2n). The cytogenetic analysis of vero cell line exhibited a moderately hypo-diploid chromosome count of 58 (32% of cells). The percentage of cells with varied chromosome number started from 40 to 60, 3 percentage of cells showed twice the modal count (2S) as there being the typical established cell line. The chromosomal structure of vero cell line (S=58) exhibited similar though not identical chromosomal structure. A typical modal structure comprising of 39 chromosomes with meta or submetacentric chromosomes and 19 chromosomes were telo / acrocentric chromosomes. Sex chromosomal pattern was not identified by conventional staining technique. Matching of chromosomes with homologous chromosome showed that some chromosomes without matching pairs. The cytogenetic characterization of the vero cell line stock apn162 revealed a modal chromosomal number of 58, indistinguishable from that of previous report.

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AP-16

In Vitro Development of Buffalo Embryos in Simple Medium Supplemented with Gelatin

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The experiment was conducted to find out the role of specific gravity of the *in-vitro* culture medium on the developmental rate of the buffalo embryos. The simple medium – modified synthetic oviductal fluid (mSOF) supplemented with 0.8% bovine serum albumin (BSA) was added with 1 mg/ml gelatin to increase the specific gravity from 0.9658 $\pm$ 0.009 to 1.0331 $\pm$ 0.013 g/cc. Follicles of the slaughter house ovaries were aspirated and the collected oocytes with cumulus oophorus complex (COCs) were cultured in TCM-199 medium supplemented with 10% FCS, 10% buffalo follicular fluid and 0.5 mg/ml FSH for 24hrs in 5% CO_2 incubator at 38.5°C. The maturation rate was observed by evaluating the cumulus expansion. Oocytes with degree 1 & 2 cumulus expansions were considered matured. The matured oocytes were then inseminated with frozen thawed buffalo semen processed in BO medium. After 18 hr of post insemination the oocytes were cultured in two groups :1) mSOF + 0.8% BSA (control) and 2) mSOF +0.8% BSA +gelatin (1mg/ml) (experiment). The result of this study shows that in control group, out of the 37 cleaved embryo, 28 (75.7%) reached to 8-16 cells stage of which 18 (64.2%) progressed to morula stage. Addition of gelatin (1mg/ml) did not increase significantly the rate of development of 8-16 cell stage embryo (61.8%) but the development rate of morula (80.9%) was significantly higher ($P<0.05$). The transferable quality embryos were however lower (40.1%) in experimental group than the control (48.7%). This study concluded that although the higher specific gravity of the media increases the developmental rate of morula stage embryo, decreases the quality of transferable embryos.

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AP-17

Effect of Supplementry Feeding of Germinated Wheat and Progesterone (P_4) Supplementation Augumenting True Repeat Breeding Syndrome in Crossbred Cows at Field Level

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A study was undertaken to determine the effect of 1 kg germinated wheat for one month and 125 mg of P_4 in seven equal divided doses, in improving repeat breeding in crossbred cows. Thirty one crossbred cows showing normal cyclic activity with failure of conception were selected. Group

I of 8 cows were supplemented with 1 kg of germinated crushed wheat as therapy once daily for one month, before artificial insemination and group II consisting of 8 cows were supplemented with 125 mg of hydroxy P_4 intra muscularly on alternate days, 3,6,9,12,15,18,21 after artificial insemination. In group III, 8 cows were subjected to feeding of germinated wheat @ 1kg/animal/day for 21 days and P_4 @ 125mg i.m./animal for seven divided days during artificial insemination and seven repeat breeding animals were kept as untreated control. All the animals were artificially inseminated with frozen semen twice at 1h interval during the oestrus and pregnancy was confirmed by per rectum on day 90. Among the animals conceived to supplementary regimens, the conception rates were 50, 62.5,75 and 12.5% in group I,II,III and IV respectively. From the result, it may be concluded that, though the conception rate was higher, the factors governing high percentage of conception needs investigation and requires further research at biotechnological levels which should be within the reach of the rural farmers.

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AP-18

Effect of Insulin and Epidermal Growth Factor on *in vitro* Development of Preimplantation Buffalo Embryos

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Mammalian embryos requires certain factors for development. They also secrete several growth factors and have receptors for various growth factors. The early mammalian preimplantation embryos develop in the oviduct which secretes various proteins and growth factors. It plays an important role in cellular proliferation and differentiation. However little information is available concerning their roles in the different stages of mammalian preimplantation embryo development. The effect of insulin and EGF on the development of IVM/IVF buffalo oocytes were investigated in this study.

A total of 165 oocytes were used to study the effect of 1 mg per ml insulin (Experiment I) and 165 oocytes for 10 mg per ml EGF (Experiment II) on *in vitro* development of buffalo embryos. Matured oocytes were inseminated with $2x10^6$ million spermatozoa per ml and were maintained for 8 days post insemination in CO_2 incubator. Observation were made for cleavage rate at 2-4 cell, 4-8 cell, 8-16 cell, morula and blastocyst formation.

Results show that insulin and EGF had no significant effect on different stages of embryo development. But it showed significant difference at 4-8 cell stage. However insulin and EGF increased the number of blastocyst formation.

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AP-19-P

Breed-Specific Alleles of Microsatellites in South Indian Cattle Breeds

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To review the possible use of microsatellites for identification of South Indian cattle breeds. Three microsatellites namely, ETH225, HBB and ILSTS030 were analyzed in three South Indian breeds of cattle, *i.e.*, Ongole, Kangayam and Umblachery. Certain alleles produced by these microsatellites that were specific for some breeds. ETH225 and ILSTS030 produced breed specific alleles in all three breeds whereas HBB produced breed specific allele in Kangayam only.

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AP-20-P

Genetic Characterization of Jakhrana Goat Population of Rajasthan

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Jakhrana is a large sized milch goat breed inhabiting the North Eastern region of Rajasthan (India). The breed derived its name from the village "Jakhrana" where it is found in the most purebred form. The breed is distributed in few other villages around Jakhrana near Behror in Alwar district of Rajasthan. According to a sampling estimate, not more than 6000 animals are found in the area. The breed is famous for milk production potential and used at many places for improving the other breeds for milk production. In the present study, an attempt was made to find out with-in-breed genetic diversity using microsatellite molecular markers. The blood samples were collected from 50 unrelated animals. The molecular data were generated using PCR followed by allelic differentiation in urea PAGE and silver staining. Based on the guidelines of ISAG and FAO, a battery of 15 microsatellite markers viz. ILST008, RM 088, ILSTS087, ILST005, ILSTS019, ILSTS058, Oar JMP29, Oar FCB304, Oar FCB 48 and ILSTS30 were selected to generate the date of the panel of 47 animals. Data analysis by POPGENE software showed an overall average heterozygosity of 0.63 ± 0.22 with maximum of 0.79 at locus ILST087 and minimum of 0.04 at locus OarJMP29. The

maximum number of alleles (10) was observed at OarFCB304 where as the minimum (3) was observed at locus OarJMP29. The maximum effective number (Ne) of alleles (6.19) and the minimum (1.09) was found with loci OarFCB 304 and OarJMP29, respectively. The PIC was calculated and found maximum (0.82) and minimum (0.08) with loci OarFCB 304 and OarJMP29, respectively.

Besides obtaining with-in-breed diversity, the data generated will also be used in establishing genetic relationship among other goat breeds of Rajasthan as well as establishing their breed identity and genetic differentiation at molecular level. Further more, the information will be useful for future goat conservation programmes of the region.

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BIOTECHNOLOGY IN ANIMAL HEALTH CARE

AH-1

Use of Recombinant Fimbrial Protein for the Specific Detection of *Salmonella enteritidis* Infection in Poultry

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Salmonella enterica subspecies *enterica* serovar Enteritidis *(S. enteritidis)* is perceived as an expanding pandemic and a significant threat to the human health worldwide. In the majority of the human food borne cases of *S. enteritidis* the consumption of contaminated eggs or egg products is implicated as the source of infection. Invasive strains of *S. enteritidis* silently infect adult chickens and contaminate eggs before shells are formed, causing egg products to be considered a major source of infection to humans.

We evaluated a recombinant rSEF 14 fimbrial antigen of SE for specific detection of SE infected birds in latex agglutination test (LAT) and ELISA. rSEF 14 antigen was highly specific in identifying birds infected with SE. The sera from birds infected with closely related serogroup-D Salmonella and other avian pathogens did not react with rSEF 14 antigen. The rSEF 14 antigen identified antibodies in serum of 88% of birds during first two weeks of infection and 100% of the birds subsequently. The SE specific antibodies were detected in egg yolk as early as 6 days post infection in rSEFI4-ELISA. Our results suggest that recombinant rSEF 14 based assays could be used as a screening tests for detection of SE antibodies and would overcome the cross reactions observed with existing serological tests.

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AH-2

Transcriptional Control of Lymphocyte Development and Function – Lessons from Gene Targeted and Transgenic Experimental Animal Models

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Generation of lymphocytes from hematopoietic stem cells is a highly coordinated process characterized by transcriptional control of cell type specific gene expression regulated by defined nuclear binding proteins. The Ets family of transcription factors share sequence similarity with the v-ets protooncogene of the avian retrovirus E26. E26 causes erythroblastosis and myeloblastosis in chicken. The Ets proteins are highly conserved during evolution and play important roles in regulating receptor mediated normal cell growth and differentiation in organisms ranging from

drosophila to humans. They are divided into subfamilies based on the structures of their DNA binding domains. The regulatory elements of many functionally important lymphoid specific genes contain Ets binding sites. Multiple Ets family of proteins such as Ets –1, Spi-B, Pu.1, Ets –2, Elf-1 and ERP are expressed in lymphocytes, suggesting a role for these proteins in the immune system. Although Ets family of transcription factors have been implicated in lymphocyte specific gene transcription *in-vitro*, their precise role *in-vivo* is not known. The precise role of each of these proteins in immune cells has been difficult to study using cell lines or primary cells. Recently, we have taken gene-targeting approaches in embryonic stem cells to generate and analyze mice deficient in Ets family of proteins in lymphocyte development and function. This approach, along with the conventional transgenic approach has identified a critical role for Ets family of proteins in B and T cell development and function. This presentation will address the strategies adopted in generation of transgenic and gene targeted animal models towards understanding the precise role of Ets family of transcription factors in immune system. A combination of genetic, biochemical and biological (whole animal) approaches to define the role of Ets family of transcription factors in the immune system and autoimmunity will be discussed. The outcome of this integrated approach will form a strong basis for our long-term objective of rationale design and use of therapeutic gene therapy reagents in human and animal diseases involving deregulated Ets family of transcription factors.

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AH-3

Development and Evaluation of Antigens for Sero-Diagnosis of Caprine Brucellosis

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Goat rearing and goat production have received much impetus in recent years. The National Livestock Policy envisages that the average carcass weight of goat (10 kg) be increased by 50% through improved reproductive efficiency and making availability of additional kids to be reared by reducing the losses due to mortality.

Brucellosis is one of the major causes of reproductive failure in goats leading to abortion in goats and losses of kids. The diagnosis of the infection in goats and thereby eventual control did not receive much attention earlier presumably due to the views embodied on goat rearing in the recommendation of the National Commission on Agriculture. Goats mostly suffer from *B. melitensis* infection. In view of difference in spatial distribution of A and M antigens on the surface of envelope of *B. abortus* and *B. melitensis* organisms, the Tube Agglutination Test with *B. abortus* antigen standardized with national or international standard anti-*Brucella abortus* serum might not yield desired result and *B. melitensis* antigen could give specific diagnosis. Further, according to World Health Organization Expert Committee on Brucellosis (1986) "the potential usefulness of Rose Bengal Test in developing methods for the eradication of brucellosis in sheep and goats warrants a comprehensive evaluation of its merit as either a screening or a

definitive test". Modification of cell concentration so useful for cattle, buffered saline and use of *B. melitensis* antigen were worthy of consideration.

In the light of above, different methods of production and standardization of tube agglutination test antigen and Rose Bengal Plate Test (RBPT) antigen with *B. melitensis* were tried and their utility in diagnosis on serum samples obtained at different time intervals from experimentally *B. melitensis* infected goats was studied. The observations are presented in this paper and their applicability in control of caprine brucellosis would be discussed.

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AH-4

Role of Cd38/CaDPR Signaling in Airway Inflammation

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The focus of ongoing investigations in the laboratory are: 1) regulation of CD38 activation in airway smooth muscle (ASM) cells; 2) role of CD38/cyclic ADP-ribose signaling in airway hyper responsiveness; and 3) the functional consequences of CD38 gene disruption using the cd*38*$^{-/-}$ mice. In ASM cells, cyclic ADP-ribose mediates Ca^{2+} release from the sarcoplasmic reticulum during endothelin-1 and muscarinic receptor, but not histamine H_1 receptor, activation. This agonist-specific activation of the cyclic ADP-ribose pathway of Ca^{2+} release suggests that CD38/cyclic ADP-ribose signaling in ASM cells results from stimulation of specific receptors. In ASM cells, the intracellular Ca^{2+} responses to agonists and contractility are altered during inflammation. Inflammatory cytokines and Th2 cytokines play a key role in the pathogenesis of asthma. Investigations from our laboratory have indicated that CD38 expression and ADP-ribosyl cyclase activity in ASM cells are elevated on exposure to these cytokines. An antagonist of cyclic ADP-ribose inhibits the cytokine-induced augmented intracellular Ca^{2+} responses to agonists. These observations provide evidence for a role of CD38/cyclic ADP-ribose signaling in ASM hyper responsiveness.

The functional consequences of cADPR-mediated Ca^{2+} elevation have been investigated in a number of different cell types and model systems using cells isolated from CD38 deficient (cd*38*$^{-/-}$) mice. In CD38 deficient pancreatic cells, glucose-induced insulin secretion is dependent on CD38 and cADPR-mediated Ca^{2+} release. Using bone marrow neutrophils isolated from cd*38*$^{-/-}$ mice, we have shown that CD38 and cADPR are necessary for chemotaxis to formyl peptide receptor ligands. We characterized the role of CD38 and CD38-dependent cADPR signaling in airway responsiveness using the cd*38*$^{-/-}$ mice. As the only contractile component in the airways, smooth muscle contractility should determine airway responsiveness and airway tone. Elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]$) in ASM is required for initiation of contraction and maintenance of airway tone. Therefore, we also determined the intracellular Ca^{2+} responses to contractile agonists in ASM cells isolated from airways of wild type and cd*38*$^{-/-}$ mice. The airway responsiveness to methacholine challenge, measured as increases in lung resistance or decreases in dynamic compliance, was significantly diminished in cd*38*$^{-/-}$ mice as compared to controls. The

intracellular Ca^{2+} responses to contractile agonists were also attenuated in ASM cells isolated from cd*38*$^{-/-}$ mice compared to the wild type controls. The results demonstrate that CD38/cADPR contributes to airway responsiveness and regulates airway tone through its effects on agonist induced intracellular calcium responses in ASM cells.

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AH-5

Bovine Immunodeficiency Virus Infection and its Effect on Immune Functions in Cattle

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Bovine Immuno deficiency virus (BIV) is a lentivirus and resembles human immunodeficiency virus (HIV). It can cause lymphocytosis, CNS lesions, progressive weakness and associated secondary diseases. An increased lymphocyte proliferation and a decreased CD4/CD8 ratio was demonstrated in cattle inoculated intravenously with BIV. Antibody response to bovine herpes virus and bovine virus diarrhea virus vaccines was delayed and significantly lowered in infected animals indicating immune suppression. BIV proviral DNA was detected in tissues of infected calves by PCR and PCR *in situ* hybridization and was predominantly present in neural tissues and lymphoid tissues. It also was detected in other tissues including lung, heart, esophagus, pancreas, muscles, and endothelial cells. The results demonstrated that BIV replicates in variety of bovine tissues *in vivo* and has a broad cell tropism. Recently a monoclonal antibody (MAb) against BIV Gag protein identified an epitope, which was absent in Jembrana disease virus (JDV), also a lentivirus that causes an acute and sometimes fatal disease of Bali cattle in Indonesia. The differential epitope was mapped to a 26 amino acid region of the Gag protein. The study will be valuable in distinguishing the two viruses on the basis of epitope specificity. It was determined that BIV is prevalent in about 18% of cattle population in Kansas, which approximates the U.S. national average. In addition, our result revealed that immuno-suppresion in cattle infected with BIV may be transient in nature during the course of one-year experimental infection. BIV proviral DNA in tissues was demonstrated without any clinical signs of the disease in cattle. In future studies, animals may be monitored for longer time to delineate the clinical nature of infection.

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AH-6

Biotechnology in Rabies Control and Prevention

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Rabies is a serious public health problem in India and other developing countries. Prevention is by vaccination using tissue culture vaccines. Biotechnology can be used to produce novel low cost vaccines and also to quantify the antigen in vaccine preparations. An economical vaccine

with the optimal quantity of antigen will encourage wide use of the vaccine and effective rabies control. In this presentation, novel vaccine strategies to manufacture low cost vaccine and in vitro antigen estimation methods for rabies vaccine potency will be discussed. A DNA vaccine against rabies was prepared by cloning the glycoprotein gene of the rabies virus in to a plasmid vector. Intramuscular inoculation of this plasmid resulted in the induction of neutralizing antibodies as measured by Rapid Focus Fluorescent Inhibition Test. However, the protection after challenge was not as good as tissue culture vaccine. Interestingly, addition of a small quantity ($1/625^{th}$ of the normal dose) of tissue culture rabies vaccine dramatically enhanced the potency of the DNA vaccine, paving way for a novel combination DNA rabies vaccine. These results indicate that a combination DNA rabies vaccine may be useful for rabies control in pet animals. We have also developed a sensitive immuno-capture ELISA for estimation of rabies antigen using monoclonal antibodies raised against the rabies glycoprotein. This test may be considered as a replacement for the *in-vivo* mouse potency test. Future strategies to produce novel vaccines and monoclonal antibodies

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AH-7

A Rapid Dot-Immunobinding Assay for detection of Egg Drop Syndrome - 1976 Virus

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Egg drop syndrome – 1976 (EDS-76) is an aviadenovirus of poultry causing about 40 per cent drop in egg production at their peak production time causing heavy economic losses. The disease syndrome is generally identified either by detecting the virus antigen by haemagglutination test (HA) or detection of antibodies by haemagglutination inhibition test (HI) that requires preparation of RBCs before the test which cannot be stored for long. A rapid dot-immuno binding assay (DIA) was standardized for detection of EDS-76 antigen in the samples. The allantoic fluid samples collected from EDS-76 virus infected embryonated duck eggs served as antigen samples. The dipsticks, in the form of nitrocellulose (NS) pieces of 0.34 mm pore size were coated with 2-5 µl of antigen sample and the free sites were then blocked with 2 per cent bovine serum albumin. The strips were then allowed to react with anti-EDS-76 antiserum followed by anti-chicken HRP conjugate. The colour was developed by diamino benzidine tetrahydrochloride-H_2O_2 substrate and development of brown colour was considered positive reaction.

Out of 82 samples tested, all samples were found positive with DIA as well as HA test. No HA negative samples gave positive results with DIA. The assay was found to be simple, rapid, specific and convenient also as compared to HA test. The antigen samples can be transported from field conveniently after adsorbing them onto NC strips and can be subsequently tested in the laboratory.

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AH-8

Immunomodulatory Effect of Amla *(Emblica officinalis)* on the Immune Responses to IBD Vaccine in Broiler Chicken

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** Professor & Head, Department of Vety. Microbiology, IGKVV, Durg.*

Amla has been used as a folk medicine since long. Ascorbic acid is believed to be the principal ingredient responsible for therapeutic activity. In the present study immuno modulatory effect of amla has been studied. Day old broiler chickens numbering 100 were divided into two equal groups. Birds in first group were given amla at 80 mg/bird/day till the termination of experiment (45 day of age) through feed, while the birds in other group did not receive amla and served as control. All birds in both the groups were vaccinated with IBD vaccine on day 10 of age, intraocularly. Blood samples were collected at 7, 14, 21 28 and 35 day post vaccination and sera were separated and subjected to QAGPT to evaluate the antibody titre to IBD vaccine. DTH was also determined using DNCB, PHA-P and PPD (tuberculin). Twelve birds each from both the groups were selected and challenged with virulent strain (field strain) of IBDV. Seven days post challenge, the birds were sacrificed to find the lesions, and the bursae were collected and subjected to AGPT for the presence of viral antigen. The results revealed a higher QAGPT titre (6.4 $\log_2$) in amla fed group than what was recorded in control group (3.8 $\log_2$). An improvement in DTH response was observed for DNCB, PHA-P and PPD (tuberculin). No lesion was observed in challenged birds. Absence of IBD virus antigen in bursae post challenge further supported the above findings. It was concluded that the amla may be used along with IBD vaccination to improve the antibody titre.

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AH-9

Immuno Potentiating Effect of Bhang (*Cannabis sativa*) on Immune Response to IBD Vaccinated Broiler Chickens.

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Bhang is an indigenous plant used for medicinal purpose by folklore. In the present study immuno-potentiating effect of bhang leaves has been evaluated. Day old broiler chickens numbering 100 were divided into two equal groups – birds in the first group was given bhang at 40 mg/ bird daily till the termination of experiment (45 day of age) through feed while the birds in control group did not receive bhang and served as untreated control. The birds in both the groups

received IBD vaccine (IV 95 strain) on day 10 of age intraocularly. The blood samples were collected from both the groups at 7, 14, 21, 28 and 35 day post IBD vaccination. The serum was separated and subjected to QAGPT to determine the antibody level to IBD virus. DTH were determined using DNCB, PHA-P and PPD (tuberculin) 24 days of age. Further the birds in both the groups were challenged with field isolate on 45 day of age and bursa:body weight ratio as well as presence of IBD virus antigen in the bursae were studied on 7 day post challenge. The results revealed a higher QAGPT titre in treated group (6.8 $\log_2$) than control group (3.8 $\log_2$). There was pronounced effect in DTH. The bursa:body weight ratio was also higher in treated group in comparison with untreated group. The IBD virus antigen was not detected in the bursae post challenge. The results suggested that the Bhang potentiates the immunity to vaccine.

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AH-10

Immune Enhancing Effect of *Carbo-Animalis* on Immune Response to Various Vaccines in Broiler Chickens.

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Carbo-animal is a homoeopathic antitumor drug. It has also been used as immunopotentiating agent to various vaccines. In this study the immunomodulatory effect of this drug was evaluated in broiler chickens. Day old broiler chickens, numbering 100 were divided equally into two groups. The birds in both the group were immunized by RD vaccine (F-strain) on 5 day of age and IBD vaccine (IV 95 strain) on 10 day of age. One of the group was given *Carbo animalis* (20 ml/100 birds) weekly through drinking water. Humoral immunity was monitored by determining HI antibody level to RD virus as well as QAGPT titre to IBD virus. The cell-mediated immune response was determined using PHA-P, PPD (tuberculin) and DNCB responses. Body weight gain and FCR were also determined at the end of experiment. The result demonstrated higher HI antibody titres to RD vaccine in treated group. Similar trends of higher QAGPT titres to IBD vaccine was recorded in case of *Carbo animalis* treated group when compared with untreated control. The DTH responses to PHA-P, PPD and DNCB were more pronounced in treated group than what was recorded in control group. The body weight gain and FCR were markedly improved in treated group at the end of experiment. The significance of *Carbo-animalis* in improving the vaccinal response in broiler chickens is discussed.

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AH-11

Immunopotentiating Effect of Tocopherol (Vitamin E) in IBD-Vaccinated Chickens

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Day old broiler chickens numbering 100 were divided into two equal groups – birds in the first group was given α-tocopherol (Vitamin – E) at 125 mg/bird orally till the termination of experiment (45 day of age) while the other group served as control. The birds in both the groups received IBD vaccine (IV 95 strain) on day 10 of age intraocularly. The blood samples were collected from both the groups at weekly interval till the termination of experiment. The sera were separated and subjected to QAGPT to determine the antibody level to IBD virus. The DTH was determined using DNCB, PHA-P and PPD (tuberculin). The body weight gain and FCR were calculated at the end of experiment. The result demonstrated a higher QAGPT titre (5.6 $\log_2$) in treated group than in control group (3.8 $\log_2$). The DTH responses to DNCB, PHA-P and PPD were more pronounced in treated group than what was recorded in control group. The result also demonstrated marked improvement in body weight gain and FCR in α-tocopherol treated group than in untreated control. The above finding points to the facts that vit- E may be employed along with IBD vaccination to improve the responses to the vaccine in order to ensure better protection.

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AH-12

Induction of Immune Response in Goats with a Experimental DNA Vaccine Encoding Omp31 Outer Membrane Protein of *Brucella melitensis* 16M

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In this study, we evaluated the ability of experimental DNA vaccine encoding the omp31 protein of *Brucella melitensis* 16M to induce cellular and humoral immune responses in goats. We constructed eukaryotic expression vectors called pTargeTomp31, encoding outer membrane protein (OMP 31) of *Brucella melitensis* 16M , and we verified that this protein was produced after transfection . pTargeTomp31 was injected intramuscularly three times, at 3-week intervals in a group of 10 goats of 6-12 months of age. pTargeTomp31 induced good antibody response in ELISA . pTargeTomp31 elicited a T-cell-proliferative response and also induced a strong gamma interferon and nitric oxide production upon restimulation with either the specific antigens or *Brucella melitensis* 16M extract. In this report, we also demonstrate that animals immunized with this plasmid elicited a strong and long-lived memory immune response which persisted even

after 4 months after the third vaccination. Furthermore, pTargeTomp31 elicited a typical T-helper 1-dominated immune response in goats, as determined by immunoglobulin G isotype analysis.

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AH-13

PCR-ELISA for the Detection of *Peste des petits ruminants* Virus (PPRV) in Experimentally Infected Goat Tissues

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Peste des petits ruminants (PPR) is a highly contagious viral disease adversely affecting the production of small ruminants throughout the country. Diagnosis of the disease at early or late phases of infection warrants a highly sensitive method for detection of the PPR virus in clinical materials. Amplification of N-gene of PPRV with specific primers was carried out to generate digoxigenin labeled PCR products, which were then detected by PCR-ELISA. This test offers advantages of detection of even very low amount of virus present in nasal and ocular discharges as early as 6th day and as late as 17th day after experimental infection in goats. The routinely used sandwich-ELISA could detect PPR virus in clinical materials from 7 to 12 day post infection, whereas the PCR-ELISA could detect for longer during duration (6 to 17th dpi) of the disease. This study proves the effectiveness of detection of PPRV by PCR-ELISA with high sensitivity. Details will be presented during the seminar .

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AH-14

Detection of Cytotoxins Produced by Avian Pathogenic *E. coli* and their Cytotoxicity Assay in CEF and Vero Cells

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In the present study it has been reported that the pathogenic strains of avian *E.coli* isolated from clinical cases of colibacillosis produces a cytotoxin that induces morphological alterations in CEF and Vero cells. For the detection of cytotoxins produced by avian *E.coli*, the isolates were grown in TSB medium. After incubation in shaking water bath for 18-20 hrs, the cell free culture supernatants (cytotoxins) were obtained by centrifugation (10000 rpm/min at 4°C for 30 min) and supernatants were filtered through 0.22 µm membrane filters. The cytotoxicity assay of toxins was assayed in 96 wells microtitre tissue culture plates.

Among 24 *E.coli* strains, 22 (91.7%) exhibited cytotoxic effect on both CEF and Vero cells indicating production of enterotoxins. The cell cytotoxic activity was observed a vacuolation,

degeneration and detachment of monolayer cells. Intracellular vacuolation was clearly evident after 24 hrs and by 48 hrs detachment of cells from the surface started.

After heat treatment (85°C for 30 min), all the toxins gave no reaction on both monolayers except cytotoxic effect indicating that these were Heat labile (Lt) toxin and none of the isolates produced Heat stable (ST) toxin. In contrast, only 2 (8.3%) serotypes produced a distinct cytotoxic response in Vero cells indicating production of Verotoxin (VT).

The titres of toxins assayed in terms of cytotoxic activity on CEF by various *E.coli* ranged from 1:4 to 1:1204, while on Vero cells it ranged from 1:4 to 1:64 and majority of the serotypes were having toxin titre of 1:16 in both the cell lines i.e. CEF and Vero.

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AH-15

Detection of Temperature Sensitive Haemagglutinin (Tsh) and Heat Labile (Lt) Gene in Avian Pathogenic *E.coli* (APEC) by Polymerase Chain Reaction (PCR)

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PCR was performed to detect the presence of temperature sensitive haemagglutinin (Tsh) and heat labile (Lt) gene in various avian *E.coli* isolates recovered from clinical cases of colibacillosis by using two different primers specific to Tsh and Lt gene.

For amplification of Tsh gene, the conditions for PCR were 95°C for 4 min for initial denaturation followed by 35 cycles at 95°C for 1 min (denaturation), 65°C for 1 min (primer annealing) and 72°C for 1 min (DNA synthesis). When these amplified products were visualized by standard submarine gel electrophoresis (80 V for 45 min) with 50 bp DNA ladder marker, eleven isolates (46%) out of twenty four were found positive for Tsh gene (420 bp) and none of the isolates were found positive for Lt gene.

Tsh gene is mostly present in avian *E.coli* associated with colibacillosis or air sacculitis and this gene is not encountered among commensals thereby suggesting that Tsh gene present in pathogenic *E.coli* isolates could be associated with virulence. Further, it has been suggested that most of the isolates having Tsh gene were found to be pathogenic to embryonated chicken eggs and one-day old chicks suggesting that Tsh gene helps in enhancing the virulence of the organism or increasing pathogenicity.

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AH-16

Humoral Immune Response of Subunit Protein (OMPS) Vaccines against Avian *E.coli*

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The *E.coli* outer membrane constitutes a typical asymmetrical bilayer studded with proteins. They act as receptors for various important cellular components needed for the survival of the bacteria besides giving mechanical protection against various components of the host immune system. These proteins are highly immunogenic in nature and could be exploited for its antigenic homogeneity against economically devastating diseases like colibacillosis in broiler chicks. The Avian Pathogenic *Escherichia coli* (APEC) serotype 078 was grown in both normal media and media with 2,-2' dipyridyl (a selective iron chelator) and the outer membrane proteins were extracted and purified. The purified protein at 50 mg/bird was injected with or without cytokines as an immunological adjuvant in 1 week-old broiler chicks. The humoral immune response was monitored at weekly intervals using an indirect ELISA.

The antibody levels started increasing at 1 week post vaccination (WPV) and reached the peak level at 5 WPV. Then it declined slowly till 9 WPV after which the experiment was terminated. The OMP vaccine injected with adjuvant showed maximum antibody titre levels compared to the groups vaccinated without adjuvant. The results indicated the superiority of adjuvanted vaccines over non-adjuvanted vaccines with higher antibody response and long lasting protection against heterologous serotypes also.

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AH-17

Comparative Studies on Immunogenic Polypeptides of Capripox Virus Isolates

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Capripox is a highly contagious viral disease of sheep and goats. Immunogenic polypeptides of pox viruses isolated from goat and sheep were compared. The goat isolate initially collected from natural field outbreak and Jaipur strain collected from sheep were grown in secondary kid testes cell culture and lamb testes cell culture, respectively and purified by sucrose density gradient. For the detection and comparision of viral specific polypeptides, purified viruses were resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis using discontinuous buffer system and transferred onto polyvinylidine fluoride membrane (0.45μm pore size) by semi dry method of electroblotting. The immunoblot analysis using rabbit antigoat HRPO conjugate

and donkey anti sheep HRPO conjugate revealed five common polypeptides in both the isolates. The molecular weights of these polypeptides were approximately 71kDa, 59kDa, 43kDa, 35kDa and 30kDa. Additional polypeptides of 97kDa, 85kDa and 26kDa were only detected in goat isolate.

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AH-18

Development of Indirect Immunofluorescence Test for the Detection of Fowl Adenovirus-4 Associated with Hydropericardium Syndrome

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Hydropericardium syndrome (HPS), after the first report from Angara Goth of Karachi, has emerged recently in India as a serious threat to the poultry sector. The disease is caused by fowl adenovirus-4 (FAV4) and occurs predominantly in broilers of 3-6 weeks of age accounting for 10-80% mortality. The disease is characterized by the accumulation of straw colored clear fluid in the pericardial sac. The present paper deals with the recent outbreaks of HPS and isolation of the causative agent in chicken kidney cell culture (CKCC). Filtered liver homogenate was prepared from the liver of affected chicks from natural outbreak. The presence of virus in liver homogenate was confirmed by serological tests like Agar Gel Immuno-diffusion (AGID) and Counter Immuno-electrophoresis (CIE) with reference anti serum and experimental transmission of disease. The virus was then inoculated in CKCC monolayer. The virus was adapted in CKCC by repeatedly passaging it up to 10th passage. An Indirect Immunofluorescence test (IFT) was standardized to detect the presence of viral antigen in infected CKCC monolayer. The test was found to be more specific and more sensitive in comparison to AGID test and CIE test, as it could detect the presence of antigen in 1:200 times diluted antiserum. The characteristic specific intranuclear immunofluorescence was detected in infected cells at 24 hour, 48 hour, and 72-hour post infection.

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AH-19

Bacterial Cellular and Humoral Response Following Intrauterine Infusion of Immunostimulants in the Treatment of Endometritis in Cows

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The objective of this study was to assess the changes in the total bacterial count, cellular component and level of immunoglobulins in uterine flushing of cows treated with lipopolysaccharide and plasma in endometritis and to improve the conception.

Twenty four cows with endometritis were taken and divided into three groups. Group I and II cows were treated with intra uterine infusion of Lipopolysaccharide of *E.coli* and autologous plasma respectively. Group III comprised of cows with endometritis kept as untreated control and Group IV comprised of apparently healthy cows with clear mucus kept as control cows.

The total bacterial count was significantly increased to 42.25 $\pm$ 2.14 ′ 10^6 CFU/ml, 44.00 $\pm$ 2.35 ′ 10^6 CFU/ml and 43.25 $\pm$ 2.56 ′ 10^6 on day 0 in group I,II,III respectively when compared to that of group IV.(0.12 $\pm$ 0.05 ′ 10^6 CFU/ml).The polymorphonuclear cells (PMN) were significantly increased to 47.00 $\pm$ 3.28, 46.75 $\pm$ 2.48, 47.88 $\pm$ 2.16 on day 0 in group I,II,III respectively, when compared to that of group IV (20.00 $\pm$ 1.56). The total Immunoglobulin was significantly increased to 16.80 $\pm$ 1.15 mg%, 15.94 $\pm$ 1.31 mg% 14.06 $\pm$ 1.65mg% on day 0 in group I.II.III respectively when compared to that of group IV. (6.72 $\pm$ 0.76mg%).

The recovery rate was 87.5% and 75% in groups I and II respectively. The conception rate was 62.5%, 50%, 12.5% and 37.5% in group I, II, III and IV, respectively. Both LPS and plasma were effective in controlling infection in endometritis. Further, LPS is economical, and it can be adapted as a remedial measure in cows with endometritis under field condition to augment fertility.

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AH-20

Microbial Status and Efficacy of Treatment on Equine Endometritis

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In equine breeding, reduced conception is attributed to microbial endometritis, which is largely responsible for equine infertility. This study was undertaken to assess the microbial status and to improve the conception by different treatment on endometritis.

Endometrial swabs were collected from mares with endometritis for isolation, identification of bacteria and antibiogram studies. The predominant isolate was *Klebsiella* sp. and the incidence was 41.66 percent. The incidence of *Pseudomonas* sp. and *E. coli* sp. was 29.16 percent and 16.67 percent respectively. Whereas, the incidence of *Staphylococcus* sp., *Proteus* sp. and *Bacillus* sp. was 4.17 percent.

The result of the antibiotic sensitivity test revealed 100 percent sensitivity with Enrofloxacin, Ciprofloxacin and Gentamycin and resistance to Metronidazole. Sensitivity to other antibiotics in descending orders were Chloramphenical (70.83%), Furamycetin (58.33%), Ticarcillin (41.66%), Trimethroprim (33.33%), Penicillin G (12.50%), Ampicillin (12.50%) and Sulphamethoxazole (8.33%).

Both bacteriological and cytological examinations, confirmed twenty-four mares with endometritis, which failed to conceive for two consecutive seasons, were randomly divided into four groups. Group I animals were treated with Gentamycin I/Ut, Group II animals were treated with Gentamycin I/M, Group III animals were treated with Gentamycin plus PGF2 alpha I/M and Group IV animals were treated with Gentamycin plus Oxytocin I/M.

The conception rates were 100%, 83.33%, 66.66%, and 50.00% in group IV, I, III, and II, respectively. From this study, it is concluded that Gentamycin plus Oxytocin I/M was most effective for treatment of equine endometritis and also in improving the fertility in Equine breeding.

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AH-21

Fractionation and Characterization of *Fasciola gigantica* Soluble Somatic Antigens

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With the aim of isolating immuno-dominant and species-specific antigen(s) of *Fasciola gigantica* for developing early serodiagnostic tool, column chromatographic techniques were used. On gel filtration chromatography (using Sephacryl S-200 column), two prominent peaks (GP1 & GP2) were obtained. By ion-exchange chromatography (using DEAE- Sepharose CL-6B column) these fractions were further resolved into P1D1, P1D2 & P1D3 and P2D2, P2D2 & P2D3 respectively. More sero-reactivity was observed in P1D1 than the other fractions as assessed by ELISA using sera of hyperimmune and experimentally infected animals. P1D1 was observed to contain four polypeptides of molecular weights 26, 28, 38 and 64 kDa as assessed by SDS-PAGE. Anti-Fasciola antibodies in experimentally infected animals were detected by this fractionated antigen (P1D1) as early as 2nd week post inoculation and showed its potentiality in early serodiagnosis of fascioliosis

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AH-22

Studies on Immuno-Modulatory Effect of *Caesalpinia bonducella* Flower Extract

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Caesalpinia bonducella is a shrub grown near the coastal areas of South Asian countries. This is widely used as anti-inflammatory drug in ethnopharmacology. The alcoholic extract of

the flower was found to be positive for flavonoids. Flavonoids are mostly responsible for immuno-modulatory activity. The present study was therefore carried out to reveal the immuno-modulatory activity of alcoholic extract of *C. bonducella* flowers by using Indirect ELISA and delayed type hypersensitivity reaction. Antigen used in this study was ovalbumin (OVA). Two groups of rats (6 animals in each group) were injected intramuscularly with 200 µg of OVA in FCA. One group of animals were treated orally with 100 mg/kg body weight of extract daily for 21 days, considered as treated group and other group served as negative control. All the animals were injected with 100 µg of OVA in FIA on day fourteen. Serum was collected at weekly intervals up to 21 days for ELISA. In DTH test, the animals were injected intradermally with 50 µg of OVA in PBS on right ear of each animal. The DTH response was measured at 0, 24, 48 and 72 hrs by observing the thickness at injected area of the ear. The antibody titre was 10 times higher in treated animals than in control animals on day 21 in Indirect ELISA. The animals treated with *C. bonducella* extract showed significant ($P<0.05$) increase in ear thickness (DTH) in comparison to control animals. It is concluded that *C. bonducella* flower extract has immuno-stimulant activity in humoral and cell-mediated immune response. Detailed investigations are required to explore the immuno-stimulatory potential of the extract.

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AH-23

Cloning and Sequencing of Fowl Adenovirus Serotype-4 Short Fiber Gene

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Fowl adenovirus-4, which causes Inclusion body hepatitis- Hydropericardium syndrome, is an emerging disease, producing heavy economic losses to Indian poultry industry. Currently available vaccines to check the disease, are either not very effective or costly. Targeting different structural genes and producing recombinant DNA vaccines may prove effective in preventing the incidence of the disease outbreak besides being cheaper and thermostable. Fiber gene product as evidenced, is a structural protein attached to the penton base. There are two fibers (long and short) present in all serotypes of FAV except FAV-8. The fiber consists of N terminal region attachment to the penton base, a shaft region and a knob that is involved in the attachment of the virus to the host cell. The knowledge of sequence of fibre gene is necessary to design/produce either subunit or DNA vaccine. The FAV-1 and FAV10 short fiber gene have already been sequenced and are available in Genbank. FAV10 displays more homology with FAV4. So the primers were designed from FVA10 short fibre gene sequence to amplify the full length short fibre gene of FAV4. The FAV4 DNA was purified from infected liver homogenate as per the standard protocol. The gene was amplified with standardized PCR reaction. The size of amplified product was equivalent to that of FAV10. The product was then purified by GenElute™ Gel Extraction kit (Sigma, USA) and cloned in pDrive (U/A) PCR cloning vector (Qiagen). Positive full length recombinant clone was confirmed by restriction enzyme analysis, with *Acc*651 and *Not* 1 double digestion, which released the product with approximate size of 1.6 kb and colony PCR. The clone

carrying full length short fiber gene of FAV4 was sequenced in the sequencing facility available in Delhi University, South Campus. Complementary strand was also sequenced for confirmation. The sequence was aligned with published FAV SF sequence by Gene tool programme. The sequence of FAV4 and FAV10 was found to be highly related to each other than that with FAV-1. The sequence was submitted (GenBank Acc. No. 340863).

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AH-24

Complete Genome Sequence of Foot and Mouth Disease Virus Serotype Asia-1 Strain IND 63/72

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Foot and mouth disease virus (FMDV) is the causative agent of a disease that constitutes one of the main animal health concerns, as evidenced by the devastating outbreaks that occurred in different areas of the world over the last few years. The virus occurs as seven serotypes (A, O, C, Asia 1, SAT1, SAT2 and SAT3) of which four (A, O, C and Asia 1) are circulating in India. Different serotypes of virus show marked genomic and antigenic variation through out the world. This variation is geographically linked and requires custom-made vaccines. Adaptation of a field isolate which has a maximum coverage to use, as a vaccine strain is a regular procedure for the selection of vaccine strain. However, it is a time consuming process. Construction of recombinant virus with the backbone containing genes for non-structural proteins and production of inactivated viruses may be an ideal approach to combat the problem.

In order to initiate such work on FMDV, Asia 1 circulating in India was selected; cDNA copy of the genome was synthesized and sequenced. The 8167 base pairs sequence and the deduced amino acid sequence were compared to published FMDV sequences. The 5' end of the genome upstream from homo polymeric poly (C) tract (S fragment) was 370 nucleotides in length and the remainder of the genome (L-fragment), including the poly (A) tail was 7797 nucleotides. The L-protease fragment contains a single open reading frame of 6902 nucleotides terminating at a UAA codon 95 bases from the 3' poly (A) sequence. The strategy used for the construction of full-length cDNA will be discussed.

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AH-25

Sequence Independent Amplification and Cloning of ssRNA Terminal Sequences of Positive Sense Viruses by Oligo Ligation

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Cloning full-length large ss RNA genome fragments from small amounts of RNA has thus remained problematic. Here, a sequence independent synthesis and amplification was perfected for 3' terminal sequences of foot-and-mouth disease virus (FMDV) and tailored for routine use to clone complete genome. 5' phosphorylated (P1) bt cDNA (poly A) NH_2 primer was ligated to a Trizol-purified viral RNA initially at 37° C for one hr and subsequently was incubated at 17°C for 12 hrs. cDNA was prepared using MMLV reverse transcriptase and reverse complementary primer (P2) at 37° C for one hr. The second strand cDNA was synthesized using Klenow DNA polymerase at 12°C overnight. Then 3' untranslated region (UTR) was amplified using nested primers and cloned. Sequencing of the terminal ends of the cloned gene was performed to establish whether these genes were specific to cDNA copy of the genome. The sequence was found to be conserved among all the serotypes with secondary structures. This is the first nucleic acid sequence data generated for the 3' UTR of FMDV serotype Asia 1. Our main motivation for developing this cloning method is to facilitate recombinant vaccine development for FMDV and construction of mini-genome using 3' UTR as a part of its component.

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AH-26

In Vitro Activity Study of RNA Dependent RNA Polymerase of FMDV Expressed in a Heterologous System

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Foot-and-mouth disease (FMD) is one amongst the highly contagious viral diseases of cloven-hooved animals having economic importance. Control of the disease by various means assumes a lot of importance in a developing country like India, where mass culling of affected and in contact animals is not possible due to social reasons. Besides a basic understanding of immuno-prophylaxis and inhibitors of FMDV replication have a scope in FMD control, studies on the inhibitors of viral replication would open up many avenues for both preventive and therapeutic measures against the disease. Central to the replication of the virus is the RNA dependent RNA polymerase encoded by 3D gene. Production of the 3D polymerase through recombinant DNA technology and development of an assay system for measuring the activity of

3D polymerase will help in designing the drug targets. Besides drug development, understanding the conditions needed for polymerase activity would help in developing replicase-based gene vaccine vectors.

The present work is aimed at expressing enzymatically active 3D polymerase of FMDV and recording its poly (U) polymerizing activity. The sequence encoding 3D polymerase was amplified, cloned in pBluescript KS+ and sequenced. The gene was subcloned in bacterial (pET32a) and yeast (pPIC9K) vectors for expression. The expressed proteins were characterized by SDS-PAGE and western blotting. The purified enzyme was assayed for poly (U) polymerase activity. Polio viral 3D polymerase was used as a positive control in the activity study. The expression of enzymatically active 3D polymerase and the standardization of the assay system hold promise for the future development of antiviral drugs for FMDV.

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AH-27

Association of Fowl Adenovirus Serotype-12 with Hydropericardium Syndrome of Poultry in India

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Fowl adenoviruses (FAVs), belonging to group I having 12 serotypes are extensively distributed among commercial chickens throughout the world and all except FAV-11 have been reported to be associated with inclusion body hepatitis (IBH). In India, earlier the sporadic IBH disease outbreaks were reported, but in the recent, past occurrence of IBH along with hydropericardium (HPS) accounting for severe economic losses to the poultry industry has been reported. FAVs recovered from outbreak of IBH-HPS occurring in different parts of the country have been typed as serotype-4 by serum neutralization test. During the present study eight indigenous FAV isolates obtained from different geographical regions of the country viz. 383/AD/97, 387/AD/97, 421/AD/98, 488/AD/98, 507/AD/01, 528/AD/01, 608/AD/02 and 617/AD/02 were serotyped, using rabbit anti-FAV reference sera against all the 12 serotypes. Results of neutralization test revealed that all isolates except 421AD/98 and 617/AD/02 belonged to serotype-4 as their infectivity could be neutralized by 1:400 dilution of anti-FAV-4 serum. The isolate 617/AD/02 was neutralized with anti FAV-12 serum only suggesting that it belonged to another serotype of FAV, which has not been reported from the Indian subcontinent till date. Isolate 421/AD/02, not neutralized by any of the twelve anti-FAV serum, appeared to be a mixture of different FAVs. PCR amplification of the hypervariable region of hexon gene of these two distinct isolates viz. 617/AD/02 and 421AD/98 and the subsequent RE analysis using *Alu* I enzymes confirmed the isolate 617/AD/02 to be of FAV-12, while with isolate 421/AD/98, a mixture of bands with FAV-4 and FAV-12 was observed indicating it to be a mixture of FAV's. In conclusion, the involvement of FAV-12 alone or in association with FAV-4 in precipitating IBH/HPS among poultry flocks in India was detected. This urges a thorough revamping of our disease monitoring, surveillance and control strategies for adenoviral infections in poultry.

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AH-28

Sequence Analysis of the Cleavage Site-Encoding Region of the Fusion Protein Gene of Newcastle Disease Viruses Obtained from Pigeon, Guinea Fowl and Quail

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Newcastle disease is a highly contagious viral disease of poultry, affecting different species of birds worldwide. The causative agent of the disease, Newcastle disease virus (NDV), has been classified as avian paramyxovirus-1 in the genus Rubula virus of the family *Paramyxoviridae.* Based on the virulence, NDVs have been classified into less virulent lentogenic, moderately virulent mesogenic and highly virulent velogenic strains. Five field isolates of NDV including two from pigeon, two from guinea fowl and one from quail have been characterized by nucleotide sequencing of the cleavage site (F2 – F1) of fusion gene. A portion of 454 basepairs (bp) containing the cleavage site of the fusion gene was amplified by polymerase chain reaction (PCR) and the purified PCR products were sequenced directly by cycle sequencing method manually. The sequences of all the isolates were aligned and analyzed in the laser gene software DNA STAR. The amino acids present in the cleavage site and the percentage of divergence at nucleotide and amino acid levels revealed that all the isolates were of velogenic pathotype having the amino acid sequence RRQK / RRF at the cleavage site.

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AH-29

An approach for the sequencing of typed and untyped bluetongue virus isolates

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Bluetongue virus (BTV) is the prototype virus of the Orbivirus genus in the Reoviridae family. It is a non-enveloped virus with concentric protein layers enclosing a dsRNA genome consisting of 10 segments. The outer capsid consists of two proteins, VP2 and VP5. The inner core exhibits icosahedral symmetry and is composed of five proteins, two major proteins (VP3 and VP7) and three minor proteins (VP1, VP4 and VP6). Ten segments of dsRNA are also located within the core (L1-L3, M4-M6, S7-S10) coding for seven structural proteins and three

nonstructural proteins. There are 24 serotypes and also there exists variations within the serotypes. More information on the BTV genome sequences will help in determining the degree of homology among various BTV isolates and also their phylogenetic relationship thereby facilitating the design of control programmes by suitably incorporating appropriate serotypes of BTV isolates in the production of BTV vaccines. The sequence information will also be useful in identifying the conserved and variable regions in genome segment which is useful in designing the molecular probes and diagnostics. Various software programmes for accessing the information about BTV gene sequences from the public domain data bases, alignment of sequences using clustalW, design of primers, PCR, p Draw 32 for cloning and sequencing will be discussed.

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AH-30

Occurrence and Phenotypic Expression of *Stn* Gene among *Salmonella* Serovars Isolated from Different Sources

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Occurrence and phenotypic expression of *Stn* gene among 60 different serovars of *Salmonella* isolated from various sources were examined. Detection of *stn* gene by polymerase chain reaction (PCR) was carried out with known primers. The results indicated that *stn* gene was present in all the 60 isolates of *Salmonella*, irrespective of the serotypes to which they belong. All the isolates yielded a 617 bp product in the *stn* gene segment. In order to assess the phenotypic expression of *stn* gene, dot-ELISA test was performed with all the isolates using polymyxin-B extract of the *Salmonella* as the source of Stn and standard anti-Stn rabbit serum. Of the 60 isolates tested, 53 (83.33%) were found to be positive for Stn production by dot-ELISA. Out of 11 isolates of *S. typhimurium* from piglets, 9 (81.82%) produced Stn, while the corresponding figures for *S.enteritidis* and *S. bareilly* were 15 (93.75%) out of 16 and 2 (66.67%) out of 3 respectively. Nine (90%) out of 10 *S. typhimurium* isolates and the lone isolate of *S. enteritidis* from calves also showed enterotoxigenicity in dot-ELISA test. The only isolate of *S.cholerasuis* was also found to be enterotoxigenic. Among the poultry isolates, 7 (87.50%) out of 8 strains of *S. typhimurium* and 9 (90%) out of 10 strains of *S. enteritidis* were found to produce *Stn*.

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AH-31

Cloning and Sequencing of LipL32 Gene of *Leptospira interrogans* Serovar *canicola*

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Leptospirosis is an important zooanthropozoonotic disease of worldwide prevalence. It may seriously affect humans and wild and domestic animals including cattle, buffaloes, horses, sheep, goats, dogs and swines. In India, the disease is endemic in the southern states especially Tamil Nadu, Karnataka, Maharashtra and Andamans. Of more than 250 pathogenic leptospiral serovars so far known, serovar *canicola* is more important as it is frequently transmitted from animals to man. In the present report, we describe the cloning and sequencing of an immuno-dominant envelope protein gene LipL32 from *Leptospira interrogans* serovar *canicola*. The LipL32 gene was amplified from *L. canicola* using designed primers that yielded a product of 820 bp. The PCR product was confirmed by nested PCR. The amplified LipL32 gene was then eluted from agarose gel and ligated to pDrive vector (Qiagen Inc., USA). The ligated mixture was used for transformation into *E. coli* DH5α competent cells and plated out into LB agar supplemented with ampicillin (50μg/ml), X-gal and IPTG. The recombinant clones were picked up and the gene insert was confirmed by restriction digestion of the plasmid using Pst*I* and Sal*I*. The LipL32 gene was then sequenced by dideoxy chain termination method. The sequence was submitted to EMBL database under the accession number AJ580493. Further work on expression of the gene in prokaryotic hosts is in progress.

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AH-32

Reverse Transcription-Polymerase Chain Reaction Amplification and Cloning of HN Gene of Newcastle Disease Virus

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Newcastle disease virus (NDV) is a causative agent of Newcastle disease in chickens, characterized by respiratory distress, depression, diarrhea, and circulatory disturbances and in chronic cases, impairment of the central nervous system. NDV has a genome of approximately 15 Kb size that encodes six viral proteins including haemagglutinin-neuraminidase (HN) protein. Major envelope glycoprotein, HN is responsible for the attachment of virus particles to the cell receptors and for the induction of protective antibodies. We have amplified 1.7 Kb fragment of HN gene that includes complete coding region, by reverse transcription-polymerase chain reaction. The amplified product was cloned in pGEM-T Easy vector. The cloning was confirmed by

restriction analysis with *Not*I enzyme and by end sequencing using T_7 promoter primers. The insert was then subcloned in eukaryotic expression vector pcDNA3.1 (+) at *Not*I site. The orientation of the gene was confirmed by restriction enzyme analysis using *Eco*RI that released 1200 and 450 bp fragments. Further studies are being carried out to express the HN gene in a suitable eukaryotic system.

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AH-33

Infectious Bursal Disease Virus Induces Apoptosis in Cultured Cells via Caspase-dependent Pathway

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Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease, an acute, highly contagious, immunosuppressive disease of young chickens. Infection of vero cells with the virus adapted to these cells, causes extensive cytopathic effects leading to extensive cell death. In this study, we have investigated the apoptosis process during IBDV infection and the underlying mechanisms. Our results show that IBDV induces apoptosis, characterized by chromosomal shrinkage, DNA fragmentation and formation of apoptotic bodies as evidenced by DNA fragmentation analysis, acridine orange/ethidium bromide staining and TUNEL staining techniques. We demonstrate, for the first time, that the addition of general caspase inhibitor z-VAD-FMK to the culture media inhibits hallmarks of apoptosis induced by IBDV in vero cells, confirming that IBDV induces apoptosis via caspase-dependent pathway in vero cells. Further studies are being carried out to completely elucidate the pathway of apoptosis induced by IBDV.

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AH-34

Amplified Fragment Length Polymorphic (AFLP) Analysis of *Pasteurella multocida* Strains from Different Animals and Avian Species in India

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Pasteurella multocida is associated with a variety of specific diseases viz. haemorrhagic septicaemia in cattle and buffaloes, pneumonic pasteurellosis in cattle, sheep and goats, fowl cholera in poultry, atrophic rhinitis in pigs, snuffles in rabbits and pasteurellosis in wild animal

species. Conventional methods of detecting variation within *Pasteurella multocida* serogroup-A are not efficient. AFLP analysis is a novel PCR-based typing technique in which adapter molecules are ligated to restriction enzyme fragments and subsequently used as target sites for primers in a PCR amplification process.

In the present study, single restriction enzyme approach was used to type a total of 50 strains of *P. multocida* serogroup-A obtained from different geographical regions of India. AFLP analysis of the strains belonging to *P. multocida* serogroup A resulted in patterns with significant difference and was characterized by the presence or absence of 1-4 bands. A total of 34 different profiles were observed. The variation in the profiles of strains from same as well as different somatic serotypes indicated the discriminatory power of the technique even though there could be up to 55% similarity in band sizes. AFLP analysis showed unique PCR fingerprints with maximum diversity in avian and sheep isolates from same as well as different zone of isolation. Similar AFLP fingerprints were also observed for some strains presenting identical capsular and somatic antigens.

The current study indicated that AFLP technique described here can be applied to *P. multocida* without the need for expensive equipment or reagents and its performance is relatively rapid when compared to ribotyping; technically simple, more informative and valuable typing tool as it analyses the variation throughout the genome; it is a valuable addition to the existing genotypic fingerprinting methods available for *P. multocida*.

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AH-35

Cloning and Sequence Analysis of *tbpA* Gene Encoding Transferrin Binding Protein (TbpA) from *Pasteurella multocida* B:2 (strain P_{52})

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Iron is an essential nutrient for most organisms because of its importance in metabolic electron transport chains. Several outer membrane proteins are produced exclusively under low iron concentrations, which are known to induce cross protection, one among them is Transferrin binding protein-A (TbpA). *Pasteurella multocida*, known to affect wide range of domestic as well as wild animal and avian species. In the present study, attempts were made to identify presence of *tbpA* gene in *P. multocida* serogroup B:2 (strain P_{52}).

A set of primers were designed to amplify a tbpA gene encoding Transferrin Binding protein from *P. multocida* isolates by PCR assay. An amplified PCR product of ~2334 bp was observed from all the serogroups of *P. multocida* originating from different species of avians and animals. The construct obtained from ligation of PCR amplified *tbpA* gene from *P. multocida* B:2 (strain P_{52}) and pDrive Cloning vector, was designated as type B:2. The positive clones were confirmed

by colony PCR and insert release following digestion were subjected to nucleotide sequencing. The sequence analysis of *tbpA* gene region encoding transferrin-binding protein (TbpA) revealed a GC content of 39.54% in 2334 bp ORF of *P. multocida* serogroup B: 2. The nucleotide sequence analysis of *tbpA* gene revealed 98.4 % homology and 1.5% divergence with the gene sequence of *P. multocida* serogroup A:1. Amino acid sequence alignment revealed 96.5% homology and 3.4% divergence between TbpA protein from *P. multocida* serogroup A:1 and B:2. The characteristics of TbpA analyzed by various protein prediction programmes revealed presence of 777 amino acids accounting for matured protein of 89.16 kDa molecular weight, which appeared to have a very high antigenic index, hydrophilicity and surface probability.

The study indicated that TbpA could be used as a potential candidate antigen in development of haemorrhagic septicaemia and fowl cholera vaccines.

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AH-36

Confirmation of Bluetongue Virus Serotype-I in Tamil Nadu

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This paper reports on the first report of Bluetongue virus (BTV) serotype -1 from Tamil Nadu. The prevalence of BTV Serotype-1 was documented in Gujarat, Haryana, Maharashtra and Rajasthan. However in Tamil Nadu, BTV serotype-I was not encountered on virus isolation even though antibodies against them were documented earlier.

Two hundred and sixty blood samples were collected in EDTA tubes during the course of suspected outbreak of BT in districts of south Tamil Nadu. The blood samples were haemolysed using sterile distilled water and sonicated at 80 cycles per minute and the samples were centrifuged. The supernatant was inoculated to embryonated chicken eggs and the samples were subjected to antigen capture -ELISA using chick embryo liver as the antigen source. From a total of 167 antigen capture ELISA positive samples, one isolate of Bluetongue virus was obtained. The isolate was adapted to embryonated chicken eggs (ECE), which revealed cherry red colouration of the embryo. A total of 5 passages were made in ECE and the embryo extract was adapted to BHK-21 cell line. A total of 15 passages were made in BHK-21 cell line and the infectivity titre ranged from log $10^{3.2}$ to $10^{4.5}$ $TCID_{50}$ from 5^{th} to 15^{th} passage.

The same isolate was subjected to RT-PCR analysis using NS3 gene specific primers. The amplified product was sequenced and the data was submitted to Gen Bank (Gen Bank Accession No: AY-359960). The isolate was also sent to Institute of Animal Health, Pirbright, UK and confirmed as BTV Serotype-1.

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AH-37

Partial Nucleotide Sequence of Hydropericardium Syndrome Virus

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Hydropericardium syndrome is an emerging disease of poultry caused by fowl adenovirus type-4. It mainly affects commercial broiler chicks aged 3-5 weeks old and is characterized by a swollen pericardial sac filled with straw coloured fluid severely affected liver and kidney. In this study, the genetic relationship between chicken isolates and Quail isolate of HPS obtained from different geographical areas were compared based on nucleotide sequence analysis. The infected liver homogenate was used as template for polymerase chain reaction. The variable region of hexon gene of HPS was amplified using specific primers. The expected amplicon size of 1219 bp was observed in all isolates. The PCR products were purified and subjected to automatic sequencing. The phylogenetic relationship of our local isolates of HPS virus were assessed by comparing with published FAV-4 sequences available in Genbank.

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AH-38

Partial Nucleotide Sequence Analysis of Capripox Viruses of Tamil Nadu

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The goat pox and sheep pox are diseases of goat and sheep characterized by pyrexia, generalized skin and internal pox lesions. The occurrence of disease cause considerable economic loss throughout the country in terms of mortality. The suspected scab material obtained from different outbreak areas of Tamil Nadu was processed and the presence of sheep pox antigen was initially confirmed by conventional test. Then the scab material was used as a template for polymerase chain reaction. The viral attachment protein gene was amplified using specific primers. The expected amplicon size of 192 bp was observed in three isolates. The PCR products of three SPV isolates were purified and subjected to automatic sequencing. The genetic relationship of our local isolates was assessed by comparing with other published capripox virus sequences available in Genbank.

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AH-39

Efficacy of Different Oil Adjuvants with Ranikhet Disease Live Vaccine in Day Old Layer Chicks

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The study was conducted with three oil adjuvants viz. commercial liquid paraffin, conventionally extracted groundnut oil and sunflower oil along with Tween 80 and Span 80 as aqueous and oil phase emulsifiers. The aqueous and oil phase ratios of 1:4 and 1:7 were used. Eight number of day old layer chicks divided into 4 groups viz.,

Group I : Live RD vaccine adjuvanted with liquid paraffin in the ratio of 1:4 and 1:7

Group II : Live RD vaccine adjuvanted with groundnut oil in the ratio of 1:4 and 1:7

Group III : Live RD vaccine adjuvanted with sunflower oil in the ratio of 1:4 and 1:7

Group IV : Unvaccinated control group

The chicks were vaccinated on 1st week and serum was collected at 2 weeks interval till 8th week of age. The antibody level was assessed by HI test. The results were compared and discussed upon cost effectiveness.

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AH-40

Characterization of FMD Virus Type "O" Isolates from Field Outbreaks by Liquid Phase Blocking ELISA

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Ten isolates of FMD virus type "O" from field outbreaks in different places of Assam were characterized to assess antigenic relationship with the type "O" IVRI reference virus strain and among the isolates by liquid phase blocking ELISA. All the isolates were found to be antigenically related to the reference virus strain and the 'r' values range from 0.25 to 0.5 in the unidirectional testing. The reference virus strain also gave 'r' values from 0.25 to 0.5 in reverse direction testing. No interstrain variation among the isolates was observed and the 'r' values range from 0.25 to 1. The percentage of relationship of the isolates to the reference strain and among the isolates was found to be 25 to 100. Asymmetric relationship of the isolates calculated based on D values revealed dominance of two field isolates over the reference virus strain and one of the isolates showed greater antigenic relationship to all the isolates.

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AH-41

Detection of Toxin Genes of *Escherichia coli* by Polymerase Chain Reaction

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Enterotoxins expressed by *Escherichia coli* have been known to play important role in the pathogenic manifestations in piglet diarrhoea. Fifty two strains of *E. coli* isolated from 38 apparently healthy and 14 diarrhoeic piglets were screened for the presence of four different types of toxin genes viz., *est*, *elt*, *stx*1 and *stx*2 by polymerase chain reaction using specific primers. All but two strains were found to be negative for all the toxin genes. One isolate from the diarrhoeic piglets were found to be positive for *est* and *stx*1 genes. Since the sample size in the study was relatively small, the study suggested detailed investigation regarding the role of these genes in piglet diarrhoea in a larger sample size.

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AH-42

RAPD Analysis of Virulent *Bordetella bronchiseptica* Isolated from Rabbits in Meghalaya

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Rabbits are reared for meat and fur production in addition to research purpose. Intensive rabbit production often suffers from the problem arising out of various infectious diseases with high morbidity and mortality. Respiratory infections in rabbits like bronchopneumonia and snuffles are very common and usually caused by *B. bronchiseptica* alone or along with toxigenic strains of *Pasteurella multocida*. The study was undertaken to isolate and to analyze the genetic diversity of *B. bronchiseptica* isolates by RAPD profiles. A total of 25 nasal swabs from rabbits with a history of respiratory infections were collected and processed in Bordet-Gengou media with selective supplements and Blood agar. Out of 25 nasal samples, fourteen *B. bronchiseptica* could be isolated and identified based on cultural, morphological, and biochemical characteristics. Four virulent isolates were studied for comparative genome analysis by using Random Amplified Polymorphic DNA (RAPD) profile. Twenty numbers of 10-mer synthetic oligo-nucleotide (KitG, Operon Technologies Inc, USA) were used. Of the 20 primers, only OPG 05 gave scorable and reproducible pattern for all the 4 isolates but only after re-amplification of the first amplicons. Four isolates were grouped in three clusters indicating the difference among the virulent isolated.

Thus, the results emphasize the importance of RAPD profiling of *B. bronchiseptica* in epidemiological studies and in selection of an isolate for vaccine development. However, further work with large number of isolates from both healthy and ailing animals would provide better interpretation.

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AH-43

Characterization of *Bordetella bronchiseptica* Isolated from Pigs Suffering from Respiratory Disease

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The cause of large number of death of pigs and piglets, suffering from chronic respiratory disease with unapparent atrophy of nasal turbinate in organized piggery units in Meghalaya was investigated. Based on cultural, morphological and biochemical characteristics, the predominant isolate was identified to be *Bordetella bronchiseptica*. *In vitro* antibiotic sensitivity of all the 11 *B. bronchiseptica* isolates revealed more than 50% of the isolates were resistant to ampicillin, doxycycline, erythromycin, chloramphenicol, gentamicin and ciprofloxacin. On hemagglutination test using 0.5% cattle, goat, pig, rabbit and duck RBC suspension, all the isolates showed hemagglutination activity only with rabbit RBC. A total of 23 – 27 major soluble proteins were detected in the molecular weight range of 13.4 –114 kDa in all the *B. bronchiseptica* isolates on analysis by SDS PAGE in 5 – 12.5% gradient gel. However, citrate negative strains showed major differences in the polypeptides in the molecular weight range of 35 – 38 kDa and 55 – 62 kDa. On Western blot analysis, most of the immunogenic whole cell proteins were in the range of 20 to 100 kDa. Very few immunogenic bands were also visible in the 100 to 200 kDa. Three types of plasmid patterns with numbers ranging from 1- 4 of 3.5 – 24.0 kb size were found. The only common plasmid was of 22.1 – 24.1 kb in size. Dendrogram of overlaid graphs arising from plasmid profiles revealed similarity ranging from more than 43 – 100% among the isolates. Even though, the band similarity as per molecular weight was found among the plasmids, but they differed markedly with respect to *EcoR I* restriction pattern. The study emphasizes the strain differences among *B. bronchiseptica* isolates and the need for genetic diversity analysis before finding out clinical importance of various isolates.

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AH-44

Seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in Northeast India

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Infectious bovine rhinotracheitis (IBR), a herpes virus infection (Bovine herpes virus type-1, BHV-1) of cattle and other animals has adiverse clinical manifestation and the disease is listed under List B disease of OIE. Although serological evidence of IBR has been reported from different parts of India, the occurrence of the disease has not been recorded from Northeast India. The present study reports on the seroprevalence of IBR in Northeast India as detected by Avidin Biotin - Enzyme Linked Immunosorbent Assay (AB-ELISA). Of the 431 samples examined, 109 (25%) samples were found to be positive for IBR antibodies. The overall seroprevalence of IBR was found to be 25%. The state-wise seroprevalence was 21% in Meghalaya, 52% in Mizoram, 13% in Nagaland and 20% in Manipur. The species-wise prevalence of IBR was 27% in cattle, 13% in Mithun and 34% in Goats. None of the buffaloes tested were found to be positive for IBR. The high seroprevalence of IBR in the region emphasized the urgent need of IBR control programme so as to prevent livestock industries from greater economic losses.

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AH-45

Dot-ELISA for Detection of Enterotoxin Production by *Salmonella* Isolates from Japanese Quail

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Dot-ELISA, Biken test and Co-A test were standardized and perfomed for detection of enterotoxin production by Salmonella isolates from Japanese quails in Mizoram. A total of 36 isolates of *Salmonella enteritidis* were made from forty dead birds, screened for enterotoxin production by Dot-ELISA, Biken test and coagglutination (Co-A) test. A total of 34 (94.4%) isolates were positive for Dot- ELISA and 30 (83.3%) by Co-A test and 28 (77.7%) isolates showed faint to marked positive band in Biken test. Dot ELISA may be preferred over Co-A and Biken test for assaying Salmonella enterotoxin production, as the test is less time consuming, sensitive and simple to perform.

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AH-46

Molecular Changes of Cell Culture Adapted Velogenic Newcastle Disease Virus

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Newcastle Disease Virus (NDV) is a significant avian pathogen with world wide distribution and is classified as a list A virus by the Office International des Epizooties. A velogenic NDV isolated from a commercial layer and typed as C1 by monoclonal antibody typing was adapted 50 times in primary chicken embryo fibroblast (CEF) culture and 63 times in vero cells. At every 10^{th} passage, the nucleotide changes were assessed by sequencing a 254 base pair portion of the fusion gene harboring the fusion protein cleavage site (FPCS). The nucleotide changes, which were present at the 10th passage, were consistent until the last passage in both CEF and vero adapted viruses. The amino acid variations were at a level of 22.6 % between the virulent and the adapted viruses. The degree of attenuation of the adapted viruses were assessed by various virulence characteristics such as Mean Death Time (MDT) in embryonated chicken eggs and Intracerebral Pathogenicity Index (ICPI) in day old chickens. It was found that as the passage level increased the MDT values increased, whereas there was a considerable reduction in ICPI values. The adapted viruses also elicited good antibody titers in 20 weeks old serum antibody negative birds indicating that the viruses could be good candidates for live vaccine production.

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AH-47

Recombinant Antigen-based Dipstick ELISA for Diagnosis of Leptospirosis

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Leptospiral outer membrane protein Lip L32 was evaluated as an antigen in Dipstick ELISA for the diagnosis of leptospirosis. Lip L32 was cloned and sequenced from the pathogenic *Leptospira interrogans* serovar pomona. The nucleotide and the amino acid sequence of the Lip L32 were found to be conserved among the pathogenic serovars of leptospira. The recombinant Lip L32 showed high levels of expression in prokaryotic expression system. The purified recombinant protein Lip L32 was utilized in Dipstick ELISA to facilitate its use in serodiagnosis of canine and human leptospirosis. This assay proved to be sensitive and specific when compared with the standard Microscopic agglutination test. These findings indicate that recombinant Lip L32 is a promising serodiagnositc antigen for the detection of all the pathogenic serovars of leptospira.

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AH-48

Diagnosis of Egg Drop Syndrome - 76 Virus Infection in Poultry by Dot - Blot Hybridization

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Egg drop syndrome-76 (EDS-76) virus, Indian isolate VN1, grown in duck embryos was purified by sucrose density gradient centrifugation. The DNA extracted by phenol: chloroform method showed a clear band of more than 30 kb in agarose gel electrophoresis. The viral genome when digested with BamHI cleaved into fragments of 16, 11, 4 and 2 kb. The 2 Kb fragment was cloned into pBluescript II ks+ vector at BamHI site and the recombinant plasmid DNA was labeled with a(^{33}P)dATP label by random primer extension. Partial sequencing of 2 kb fragment showed 98% homology with EDS fibre protein and 100% homology with pVIII protein by BLAST analysis, indicating conserved portion for EDS. In hybridization experiments proteinase K treated, phenol extracted and alkali denatured faecal samples as well as cloacal swabs of laying hens showed signals with radioactive probe within 24 h post infection. Probe was sensitive and specific in detecting EDS viral DNA up to 1.25 pg and did not react with DNA of hydropericardium syndrome virus.

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AH-49

Screening of Leptospirosis in Man and Animals in and Around Bangalore During 1999-2003

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A total of 2888 samples from different species and soil have been screened for leptospirosis during 1999-02 using different techniques like Dark field Microscopy (DFM), Polymerase Chain Reaction (PCR), Slot Blot Hybridization, Microscopic agglutination test and cultural isolation in EMJH medium. The details of the study and results will be presented.

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AH-50

Characterization of Genomic DNA of *Chlamidia psittaci* Isolates Using Restriction Endonuclease Analysis

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The study was designed to compare the genomic DNA of the local isolates of *Chlamidia psittaci* obtained from caprine and bovine abortion, employing restriction endonuclease analysis (REA) in relation to a reference isolate obtained from Himachal Pradesh. Chlamydial isolates obtained were propagated in Mc Coy cell line. DNA was extracted from elementary bodies harvested from the infected cell line, purified by urografin density gradient centrifugation. The extracted DNA was subjected to REA using *Eco RI, Hae III* and *Bam HI*. All enzymes were found to be useful in the differentiation of *Chlamidia psittaci* isolates as these enzymes produced variation as well as similarity in the restriction fragment sizes.

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AH-51

Development of an Enzyme Linked Immunosorbent Assay (ELISA) to Measure Growth Hormone Level in Serum of Lactating Buffaloes

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Growth hormone (GH) is considered to be the most important hormone for regulation and maintenance of lactation. The effects of somatotropin in lactating animals have been the subjects of many scientific investigations. To study the control of GH secretion or to monitor the level of GH, a simple and reliable assay is required. GH level in sera of different species have been measured by different techniques but in buffaloes it has been quantitated by RIA only. We now report the development of an ELISA for quantitation of GH in the serum of buffaloes. To develop an ELISA, antibodies against recombinant bovine somatotropin (rbST) (gift from NHPP, California) were raised in chicken and rabbits. Antibodies in immunized animals were detected by indirect ELISA and purified by ion exchange (DEAE- Sepharose) and affinity chromatography (rbST coupled to Sepharose 4B). The purity of antibodies were confirmed by western blot and SDS-PAGE. The purified anti rbST IgG antibodies (raised in chicken and rabbits were used to detect GH levels in serum by indirect sandwich ELISA. A checkerboard analysis was performed in order to determine the optimum concentration of antibodies against rbST. It was observed that 1:10 dilution of serum gave sufficient sensitivity and accepted as suitable for the assay. Calibration curve to measure GH level in serum and milk was made by diluting rbST (1mg/ml) in PBST from

100 ng/ml to 0.049 ng/ml. The curve showed a linear response over the range 0.195 ng/ml –12.5 ng/ml. Sensitivity of the assay was 0.1 ng/ml. To validate ELISA, tests for parallelism, cross reactivity, precision and recovery were performed which confirmed the accuracy and specificity of the procedure.

The level of GH in serum of buffaloes quantitated by ELISA ranged between 7-17 ng/ml.

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AH-52

The Enhanced Innate Immunity Compensates for the Suppressed Adaptive Immunity During Pregnancy in Goats

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The embryo expresses MHC molecules of paternal origin too and hence the successful mammalian pregnancy depends upon the tolerance of a genetically incompatible fetus by the maternal immune system. The maternal immune system appears to be more modulated than being suppressed during pregnancy to allow the allogenic fetus to thrive and develop. Hence an experiment was conducted to study the innate immune status of goats during pregnancy. Ten cyclic goats were synchronized for estrus using Prostaglandin F2α and bred naturally with fertile buck. Goats were divided into pregnant (n = 5) and cyclic (n = 5), after the pregnancy diagnosis by trans - abdominal ultrasonography. Phagocytic activity of the polymorphonuclear cells (PMNS), expressed as the percentage of PMNS that have engulfed *Staphylococcus aureus,* was 38.0 $\pm$ 1.22, 38.0 $\pm$ 0.83 and 37.6 $\pm$ 1.28 during first, second and third trimester of pregnancy, respectively, in pregnant goats. In cyclic goats, the PMNS showed phagocytic activity of 29.5 $\pm$ 1.02, which was significantly ($P<0.05$) lower than that of pregnant goats. Production of hydrogen peroxide (H_2O_2) by PMNs of cyclic goats was 40.8 $\pm$ 0.62 where as in pregnant goats, it was 49.1 $\pm$ 1.14, 47.9 $\pm$ 1.18 and 47.0 $\pm$ 1.01 nmol/min/ 2 ´ 10^7 cells in first, second and third trimester respectively. The increased phagocytic activity and hydrogen peroxide production by PMNs of pregnant goats shows that the innate immune response of goats will be enhanced during pregnancy. Since the adaptive immunity will be relatively suppressed during pregnancy, the activated innate immune system might assume an important role in the overall maternal immune defense mechanisms.

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AH-53

Detection of Canine Parvovirus (CPV) DNA by PCR Approach and its Prevalence in Dogs in and Around Kolkata, West Bengal

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Canine Parvovirus (CPV) is a member of autonomously replicating parvoviruses and is associated with enteritis and myocarditis in dog. CPV has become an issue of great concern to the pet owner, practicing veterinarians and the scientists. The disease spreads rapidly through the susceptible canine population. Hence, early detection of the disease is of paramount importance. Faecal samples of 306 dogs suffering from gastroenteritis in and around Kolkata from July 2002 to June 2003, were collected and DNA was extracted by phenol-chloroform. In the study, CPV vaccine strain was used as a positive control. PCR was carried out to amplify VP1/VP2 gene using a set of 19 mer primers (Forward : 5′ - ATG GCA CCT CCG GCA AAG A-3′; and Reverse: 5′ -TTT CTA GGT GCT AGT TGA G-3′). A PCR product of approximately 2.2 kb was generated with positive faecal samples and vaccine strain of CPV virus. After screening, it was found that 103 dogs were positive for CPV (33.66%), where no sex variation was found amongst the CPV positive cases. Dogs, between the age group of 0-6 months were mostly susceptible with highest mortality rate followed by 6-12 months and 12 months of age. Regarding seasonal variation, highest occurrence was observed during summer season followed by rainy and winter season.

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AH-54

PCR-based Diagnostics for Canine Parvovirus Infection

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Canine parvovirus was isolated from clinical cases of dogs showing characteristic signs of CPV infection. The virus was adapted to replicate in the cell lines of canine and feline origin. The polymerase chain reaction based diagnostics was developed for specific and rapid diagnosis of CPV infection in dogs. Fecal samples from different breeds, age and sex and suspected for CPV infection were subjected to PCR assay.

For isolation of CPV DNA, a simple boiling technique was standardized. Three different sets of primers were used to standardize the PCR assay and then to detect the CPV in fecal samples. The primers were designed from conserved region of VP2 gene of CPV. A commercially available CPV vaccine was used as positive control in the PCR assay. Expected PCR products

were generated in the positive control, and in the positive fecal samples using different sets of primers. The presence of CPV was also detected in cell culture supernatants at different passage levels. There was no amplified product in the negative control and in the negative fecal samples. The primer sets used to standardize this test were not from the conserved region of porcine parvovirus (PPV), thus differentiating CPV from PPV. The results of PCR assay were compared with other serological tests. A good correlation was recorded between the results of these tests.

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AH-55

Epidemiological Investigation of Brucellosis in Cattle and Buffaloes Using Various Serological Tests

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Brucellosis, a chronic contagious disease is one of the most important causes of abortion and stillbirth in cattle and buffalo population in India. Being a disease of zoonotic nature, it is recognized as an important public health problem and classified as disease of epizootic occurrence. Various reports on sero-epidemiology of brucellosis among domestic animals highlight the need for a continuous surveillance and monitoring program in India. In the light of these facts, present study was undertaken to study the prevalence of brucellosis in different villages of Udham Singh Nagar district of Uttaranchal State.

A total of 216 serum samples were collected, which included 161 from cattle and 55 from buffaloes. The serum samples were tested for the presence of anti-brucella antibodies using Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme-linked immunosorbent assay (ELISA). The overall prevalence of 22.36% and 20.0% were observed in cattle and buffaloes respectively using all the three serological tests. Among cattle, the percentages of sero-positive animals were 18.01%, 5.26% and 9.93% using RBPT, STAT and ELISA whereas in buffaloes the values were 14.54%. 1.81% and 14.74% respectively. Higher prevalence of brucellosis was noticed in female cattle and buffaloes (25.19% and 20.90%, respectively) belonging to the age group of 3-5 years. Similarly the higher prevalence of brucellosis was recorded among the cross bred cattle belonging to indigenous breeds.

The results of present study indicate the need for the development of suitable practices to check the production losses in livestock in terms of disease occurrence.

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AH-56

Cloning and Expression of Major Outer Membrane Protein (omp C) Gene of *Salmonella gallinarum*

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Salmonella gallinarum the causative agent of fowl typhoid (FT) causes major economic losses to the poultry industry in India. Currently available attenuated and killed fowl typhoid vaccines are not effective in controlling this disease. Outer membrane proteins of *Salmonella gallinarum* have been considered as possible immunogen for the induction of protective status against fowl typhoid. Under natural conditions, inability to generate large quantity of specific protein for preparation of vaccine remains the main limitation in conventional methods. The present study was carried out to clone and express the major outer membrane protein C (ompC) of *Salmonella gallinarum* in *E. coli* system. Primers were designed to amplify full-length ompC gene (1271bp), which codes for major outer membrane protein of *Salmonella gallinarum*. The full length PCR product was cloned in pDrive U/A cloning vector (pdo_3). The insert was released from the pdo_3 colony by double digestion with *BamHI* and *Hind III* enzymes. The released fragment of ompC was purified by gel extraction and subcloned in pPROEx HT_b and the recombinant clones were selected on Ampicillin plates. Then the clone was confirmed by restriction enzymes digestion. The clone was induced with 1 mM IPTG and aliquots were collected at hourly intervals for 6 h. The aliquots were analyzed for expression by SDS PAGE and Western blot. In Western blot analysis, ompC protein appeared at around 43 kDa. This expressed protein, after purification can be used as a vaccine against fowl typhoid.

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AH-57

Assessment of Infectious Bursal Disease (IBD) Intermediate Vaccine Response in Commercial Broiler Flocks

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A study was carried out to assess the antibody response induced by an IBD intermediate vaccine in commercial broiler flocks by using indigenously developed single serum dilution ELISA technique. Three commercial broiler flocks (Ross strain) were selected for this study. Birds in these flocks were vaccinated with an intermediate vaccine of IBDV (ABIC) at 12th day of age. Eighteen serum samples were collected randomly at 1st ,7th day of age followed by 7, 14, 21 and 28 days post vaccination(dpv). Serum samples were subjected to single serum dilution ELISA

and all the three flocks showed similar pattern of antibody response. Continual decline of maternal antibody was observed at 7 dpv and significant antibody response was evidenced only at 14th dpv. Vaccine response further increased by 21 and 28 dpv. The most susceptible period of IBD is 3-6 weeks. The age of birds at 14th dpv was 26 days. Hence, this study reveals that optimum time for assessment of vaccine response is by 14th dpv and challenge studies at 14th dpv will correctly assess the protective titer that birds carry during most susceptible period of the disease.

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AH-58

Effect of Experimental Virulent Fowl Pox Virus Infection on RSV- induced Sarcomas in Chickens

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A total of 73 colored broiler chicks, 21 days old, were infected with BS-RSV (RAV-2) at 2000 pk.f.u/0.2 ml/chick, s/c in the left wing web. On day 10-post RSV infection, out of 51 sarcoma positive chicks, 2 groups were made as per the tumor size distributed equally in both the groups. Group A chicks (25) were infected with virulent fowl pox virus (FPV) at $4x10^4$ pk.f.u/ o.2ml, intrafollicularly in both the thigh region and the right wing web. Sarcoma (+) FPV (+) chicks were reared in a shed distant from group B chicks under similar experimental conditions. Tumor sizes were measured every week and expressed as tumor volume in cu.cm (cm^3).

Observations revealed that by 36 hours of FPV infection, 95.65% chicks in group A developed mild to extensive swollen feather follicles. By d14 of FPV infection, 100% developed variable cutaneous pox lesions from extensive nodular swelling to typical pock lesions on wing, web, thighs, abdomen, around the beak and eye. By d25 post FPV infection, 55.55% birds became completely negative for cutaneous pox lesions and rest 44.45% birds still revealed mild follicle swelling with only 1-2 birds showing the pock scabs. None of the FPV- infected birds revealed diphtheritic pock lesions on necropsy examination.

Further, tumors were seen regressed in 9 out of 25 chicks (36%) in FPV- infected group A, as compared to 8 out of 26 chicks (30.8%) in FPV uninfected group. Regression of tumors occurred in FPV infected group A chicks during days 21-77 post RSV infection, as compared to days 28-42 post RSV infection in FPV uninfected group. FPV infected group A chicks could regress completely those tumors that had attained maximum tumor size of 56.83 cm^3 (d21 post RSV infection) and 40.06 cm^3 (d35 post RSV infection), as compared to FPV uninfected control chicks with maximum tumor size of 1.8-3.34 cm^3 (d21 post RSV infection) and 1.95 cm^3 (d35 post RSV infection). Tumors were seen progressing slow in 5 out of 25 chicks (20%) in FPV-infected group A with maximum tumor sizes 239.881 cm^3 (week 8 post RSV infection) and 274.297 cm^3 (week 17 post RSV infection), as compared to 7 out of 26 chicks (27%) in FPV uninfected control group with maximum tumor sizes of 311.978 cm^3 (week 16 post RSV infection) and 566.986 cm^3 (week 17 post RSV infection). Tumors were seen progressing fast in 11 out

of 25 chicks (44%) in FPV-infected group with maximum tumour size of 133.571 cm^3 (week 5 post RSV infection), compared to 11 out of 26 chicks (42.31%) in FPV uninfected control group with 174.488 and 258.94 cm^3 (week 5 post RSV infection).

Thus, the results revealed that experimental virulent FPV infection not only regressed those large sized tumors which would have shown progression as either fast progressor or slow progressor tumors, but also checked progression of tumors in both slow progressor and in fast progressor group of chicks.

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AH-59

Immune System – Eastern Vs Western Concepts

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The health depends on the immune system, which starts functioning even before birth. The normal functioning of the immune system depends on the invisible major charkas located in the inner aura around the physical body. Knowledge of functioning of the charkas will improve the quality of the treatment modality and diagnosis of underlying cause. Both diagnosis and treatment are done through charkas by using only the palms (touch) using cosmic rays. This is the eastern method of healing, but western system gives temporary relief and does not remove the root cause because modern medicine has its own limitations.

Combination of eastern system of healing with western method will produce faster results. In the former, cosmic rays with healing power is used, it eliminates the root cause and is cost effective while the latter produces instant relief but suppresses the cause. The health is restored by removing the imbalances among charkas.

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AH-60

Susceptibility of Two Different Synthetic Lines of Broiler Chickens to Hydropericardium Syndrome Virus Infection

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Fowl adenovirus serotype 4 was isolated from a disease outbreak of Hydropericardium syndrome in broiler chickens. The virus produced cytopathogenic effects in vero cell line after 4 serial passages. The virus specific antigen was demonstrated in infected coverslip culture by immunoperoxidase test. The pathogenicity of the virus was assessed in two different synthetic cell line of broiler chicken – White (B1) and coloured (B2) plumage broilers. B1 broilers were infected at 3rd, 4th and 5th week of their age. Mortality rates were 97%, 92.3% and 60%, respectively. B2 broilers were infected at 3rd, 4th and 6th week of their age. Mortality rates were 23.3%, 15.7%

and 0% respectively. Gross pathological lesions were observed in liver, kidney and heart of dead chickens. Microscopic lesions were observed in liver, kidney, Hardarian gland, brain, heart, lung, pancreas, spleen and bursa. Some of the hepatocytes contained basophilic intranuclear inclusion bodies. Difference in susceptibility of broiler chickens to HPS disease has been discussed.

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AH-61

Virulence Characteristics of *Salmonella* Isolated from Pigs

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Out of a total of 151 samples collected from apparently healthy, diarrhoeic or dead pigs, 19 (12.58%) yielded *Salmonella*, 12 (7.95%) yielded *S. weltevreden* and 7 (4.64%) *S. choleraesuis*. All but one isolate of *Salmonella* were isolated from pigs above two months of age. Antibiogram revealed that the isolates were most sensitive to gentamicin, chloramphenicol, nalidixic acid and norfloxacin (100% to each), followed by nitrofurazone (94.74%), co-trimoxazole and nitrofurantoin (89.47% to each), neomycin (84.20%) and streptomycin (73.68%). As many as 89.47 per cent isolates were resistant to two or more drugs. All the five isolates randomly selected for testing, produced rabbit skin permeability factor (RSPF) and were found to be enterotoxigenic in rabbit ligated ileal loop (RLIL) test. *Salmonella choleraesuis* isolates were observed to be more pathogenic (producing 100% mortality).

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AH-62

Cloning and Sequencing of Buffaloe β-defensin 4 (BNBD4) Gene: an Antimicrobial Peptide

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Beta defensins are antimicrobial peptides serving as effector molecules of innate immunity that provide a first line of defense against pathogens. In mammals, they are synthesized and stored in granules of phagocytic leukocytes and are also present in those sites that are exposed to microbial invasion, such as mucosal surfaces. The encoding genes of β-*defensins* form a multigene family in cattle comprising at least 13 members, but no studies have been made in buffalo. The present study describes cloning and sequencing of complete cDNA, encoding neutrophil b-defensin 4 (BNBD4) of Indian water buffalo (*Bubalus bubalis*). Total RNA was isolated from buffalo bone marrow cells using Trizol reagent (Gibco B.RL, Life Technology). Ten μg of total RNA was treated with 100 units of RNase free DNase and reverse transcribed using MMLV reverse transcriptase and oligo dT primers (Quiagen, USA). The first strand cDNA was used to amplify beta defensin

4 gene by using a set of designed primers, which yielded a PCR product of 192 bp. The RT-PCR product was then ligated to pTZ57R/T vector (MBI Fermentas,USA.) and transformed into competent *E.coli* DH5a cells. The positive cDNA clone was sequenced by dideoxy- chain termination method and the sequence was deposited in EMBL database (Accession No. AY392452). The sequenced data revealed 87.5% nucleotide homology and 95 % amino acid homology with cattle BNBD4. This is the first report of buffalo BNBD4 cDNA sequence which possesses high sequence homology with cattle and may help in developing an alternative non-antibiotic therapeutic agent.

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AH-63

Molecular Differentiation of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Using Polymerase Chain Reaction

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Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS), responsible for respiratory diseases and synovitis in poultry and turkeys, continue to be of primary concern to the poultry industry. The polymerase chain reaction (PCR) was used to detect MG and MS in samples (trachea, lungs and air sacs) collected from commercial and government poultry farms in different parts of Tamil Nadu, using species-specific primers. The PCR primers used in this study amplified a fragment of 530 bp product in case of MG infection and a fragment of 207 bp in case of MS infection. Of 138 samples tested, 13 samples were positive for MG and 10 samples were positive for MS infection. The specificity of PCR was checked with positive control MG and MS cultures.

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AH-64

Pathotyping of Newcastle Disease Virus Isolates from Pet Birds by Fusion Protein Cleavage Site Sequence Analysis

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Newcastle disease is a highly contagious disease caused by an avian paramyxo virus serotype-1, affecting more than 250 species of birds and it is likely that many more susceptible species exist but have not yet been identified. The objective of the present study was to pathotype Newcastle disease virus isolates from pet birds (pigeon and psittacine birds) based on fusion protein cleavage site gene sequences. Four Newcastle disease virus isolates (one each from pigeon, Lory, parrot, Love bird) obtained from field samples were characterized by both biological and molecular methods. All the isolates were identified as velogenic by biological characterization.

A 254 bp fusion protein cleavage site (FPCS) gene sequence was amplified by reverse transcription – polymerase chain reaction (RT-PCR) for all the four isolates. The FPCS amino acid sequence of all the four isolates were found to be ^{111}GRRQKRFIG^{119}. The molecular pathotyping also confirmed the velogenic nature of the isolates. A phylogenetic analysis based on the nucleotide sequence obtained, grouped the four isolates in two clusters, one cluster in which Lory was closely related to mesogenic 'Roakin strain' and 'K strain' whereas the isolates from pigeon, parrot and Love bird formed the second cluster with velogenic strains like Texas and Herts 33.

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AH-65

Lymphocyte Subset Distribution in Apparently Normal and Tuberculosis Positive Buffaloes Analyzed by Flow Cytometry

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Monoclonal antibodies (MAbs) against bovine lymphocyte surface antigens namely, MHC class I, MHC class II (DP,DQ and DR), CD3, CD4,CD8, gamma, delta TCR, WC1N1 and WC1N2, were tested for its reactivity on buffalo mononuclear cells prepared from spleen and peripheral blood. All the MAbs cross-reacted with buffalo lymphocytes. The mean (±SD) CD4: CD8 cell ratio in the peripheral blood of apparently normal buffaloes was 1.08 ± 0.049, while in the spleen, it was 0.90 ± 0.080. The lymphocyte subsets in the buffaloes positive for tuberculosis by the single intradermal (SID) test was found to be altered; the CD4 cells were reduced while the CD8 and gamma delta cells were increased. The mean CD4:CD8 ratio in the SID positive buffaloes was 0.37 ± 0.010.

These Mabs were also used on cryostat sections of buffalo spleen and lymph nodes to assess the distribution of the lymphocyte subsets. CD8 cells were seen in the periphery of spleenic white pulp diffusing from central arteriole. The gamma delta cells were scattered both in the cortex and medulla of spleen and lymph nodes.

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AH-66

Production and Characterization of Monoclonal Antibodies to an Indian Isolate of Infectious Bronchitis Virus

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An Indian isolate of infectious bronchitis virus (IBV - TN 1) confirmed as Massachusetts serotype by monoclonal antibody typing was propagated in chicken embryonated eggs. The

purified virus was used as an antigen for production of monoclonal antibodies (Mabs). A panel of ten monoclonal were produced and characterized. Based on enzyme linked immunosorbent assay (ELISA) and western blot analysis, these Mabs were grouped according to their specificity and isotype. Six of the ten Mabs directed against spike protein (S2) and IgG1 or IgG2a isotype; three were specific to M protein and IgG1 isotype; and one was specific to N protein and IgG1 isotype. All these Mabs cross - reacted with eight other IBV isolates of Tamil Nadu, a vaccine strain and a reference strain M41, thus confirming their group reactivity. These Mabs were tested in Dot- ELISA and Immuno Peroxidase Test (IPT) for antigen detection with high specificity and sensitivity. This confirms that the Mabs developed against an Indian isolate would serve as a valuable tool for specific diagnosis of IBV infection in poultry.

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AH-67

Genetic Characterization of Glycoprotein Sequence of Indian Rabies Virus Isolate

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Rabies continues to be a serious problem in both developed and developing countries due to wild life and domestic animals reservoir status of rabies virus. Field strains of rabies virus are characterized by their heterogeneous biological properties, variable replication in laboratory animals and cell cultures. The glycoprotein (G) that constitutes the spike projections of the viral envelope for phylogenetic analysis has provided a balanced evaluation of the rabies virus isolates. Brain samples were collected at postmortem from rabies suspected dogs brought to the Small Animal Clinics of Madras Veterinary College, Chennai-7. Impression smears of the samples were tested with antinucleocapsid antibody conjugated with FITC (Diagnostic Pasteur, France) and a panel of monoclonal antibody (Mab) conjugates W502-2, C15.2 and W422-5 (Centre for Disease Control, Atlanta). A dog brain sample that tested positive was used for total RNA extraction. Synthetic oligonucleotides were designed for use in PCR amplification of the full-length G gene. A pair of internal primers were also designed for use in nested PCR to confirm the initial amplification. The amplified full-length G gene was purified and cycle sequencing was performed with both the outer and inner primers. Computer analysis revealed only one long open reading frame extending from the first ATG codon at position 9 to the stop codon TGA at position 1581, which was able to code for a polypeptide of 524 amino acids. The complete nucleotide sequence of the messenger sense strand was submitted to GenBank (Acc. No.AY237121). The full-length sequence was compared with available full-length G sequences from GenBank. Thus, the knowledge of the glycoprotein sequence of the local isolate helps in the understanding the differences and similarities of the local isolate with other field rabies virus strains and vaccine viruses. It will also provide insights into the molecular epidemiology of rabies in India, from samples associated with humans and animals.

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AH-68

Serodiagnosis and Genetic Characterization of Bluetongue Virus

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Bluetongue virus (BTV), the etiological agent of the infection is worldwide in distribution with 24 different serotypes and is considered as a disease of economic importance. In the present study, BTV was isolated from an outbreak of Rajapalayam during 1997 and was serotyped as type 23 at the Instituteof Animal Health, Pirbright, UK and Onderstepoort Veterinary Institute, South Africa. BTV was also isolated from spotted deer in a reserved forest are and sheep near Chennai. The molecular protein profile of these isolates were studied by polyacrylamide gel electrophoresis (PAGE) and Western blotting. Reverse transcriptase polymerase chain reaction (RT-PCR) was carried out using primers targeted to the hypervaribale region 12-29 (TCGCTGCCATGTATCCG) and 246-263 (CGTACGATCCGAATGCAG) of VP2 cistron to amplify a product of 251 bp. The sensitivity of the test was checked with unrelated viral and cellular nucleic acids. The amplicons were purified and subjected to automated sequencing. The sequenced samples were analyzed by using capillary gel formation BAI prism 377 DNA sequencer and analyzer. The nucleotide sequences of these isolates were submitted to genebank and accession numbers were allotted and released to the public domain. Phylogenetic analysis of the obtained VP2 gene sequences was analyzed using Clustal W and Phylip software programmes. Nucleotide divergence of 0.2 to 0.4% was noticed among isolates. The amino acid sequences were aligned to mark the position of the conserved sequence. Although BTV has been the subject of extensive molecular and genetic studies, little is known about the intracellular assembly process and the stoichiometrics of the viral components in the morphological pathway of virion assembly, which needs to be eroded.

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AH-69

Differentiation of Indian Isolates of Chicken Anaemia Virus by Polymerase Chain Reaction – Restriction Enzyme Analysis

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The present study was carried out to find out the differences among the Indian isolates of chicken anaemia virus (CAV) by polymerase chain reaction (PCR) – restriction enzyme (RE) analysis, if any. The RE analysis of the 454 bp PCR products of the conserved genome region (819-1272 bp) generated from six field virus isolates (A, B, C, D, E and F) from different geographical regions of the country was carried out using six different restriction endonuclease

enzymes viz., *Hae* III, *Hind* III, *Mbo* I, *Nci* I, *Rsa* I and *Sty* I in order to find out similarity or any variation in their genome. Digestion of amplicons of the five field isolates A, B, D, E and F with all the restriction enzymes revealed similar pattern with that of published sequence of CAV strain (Cux-1 isolate). Whereas, field isolate–C revealed a different pattern with *Hae* III, *Rsa* I and *Sty* I RE enzymes. Digestion with *Hae* III yielded four fragments with similar pattern with five field isolates viz., A, B, D, E and F whereas only three fragments were observed with isolate-C. *Rsa* I restriction pattern was identical in the five field isolates with two sites, whereas isolate-C also had two sites but at different position. Digestion with *Sty* I yielded two fragments with same pattern in five isolates but DNA of isolate-C was not digested. *Hind* III showed no site in any of the field isolates digested reflecting similarity to the expected pattern with the published sequence. The emzymes *Rsa* I and *Sty* I have been used for the first time for the RE analysis of CAV-DNA. In conclusion, RE analysis revealed that one of the isolates (isolate-C) was found to be different from other isolates. These findings can be of great importance for future molecular epidemiological investigation to distinguish different CAV isolates circulating in the poultry flocks of the country. However, the existence of difference between the isolates can be further analysed in details by nucleotide sequencing.

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AH-70

Expression of VP7 Gene (Group Specific) of Bluetongue Virus in *E. coli*

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Bluetongue is an vector borne viral disease of ruminants which causes severe economic loss to the farming community by primarily affecting sheep. In India, the disease has established endemic status and is widespread specially in the Southern states. High mortality has been reported during outbreaks and loss is attributable to both direct and indirect losses. Early detection of the disease and the disease causing agent is important because of other clinically over lapping viral diseases. The disease in India is mainly diagnosed serologically by the simple Agar Gel Immuno Diffusion Test (AGID) or by ELISA kits imported from other countries. Recombinant proteins specific to the BTV group are very much useful for serology based techniques like AGID and ELISA. In the present study, the genome coding for the group specific VP7 protein was cloned initially into the pRSET Ban expression vector. However, for adjusting the frame it was subcloned into pUC19 for expression purpose. The cloned gene was expressed in E. coli (BL21 C lys) and the expressed protein was confirmed by dot blotting and western blotting using specific sera. The gene cloned into pRSET B vector was also used for the preparation of non-radioactive DIG probes for detection of BTV RNA in clinical samples.

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AH-71

Typing of Leptospiral Isolates by Randomly Amplified Polymorphic DNA (RAPD) Analysis

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Leptospirosis is an anthropozoonosis of ubiquitous distribution, caused by spirochaetes of the pathogenic *Leptospira* species. Over 250 serovars have been recognized. The genus was serologically divided into two species, *L. interrogans* and *L. biflexa*. Recently this has been replaced by genotypic classification including all serovars of both *L. interrogans* and *L. biflexa*. In the present study, a PCR- based Randomly Amplified Polymorphic DNA (RAPD) analysis was employed for sensitive genotyping for studying epidemiological problems. Four different oligos were tried along with an established primer set B11 and B12 (Gerritsen et al., 1995). RAPD profiles were obtained for 24 reference leptospiral serovars representing 17 serogroups and 6 genospecies. Four leptospiral isolates (W41, D7, D14, and H12) were subjected for RAPD typing with the available RAPD profile. The analysis was carried out using the software programme RAPDistance: Version 1.04. The isolates were found related to each other (0) and closely related (0.208) to the serovar *hardjo*, a pathogenic serovar. The isolate W41 (0.21) is closely related to *andamana* (0.234), a non-pathogenic serovar.

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AH-72

The Effect of Probiotics on the Performance of Broiler Chicks

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The probiotic effects of Lactobacilli sp., Saccharomyces sp. and Torulopsis sp. were studied in a series of feeding trials with broiler chicks. Feeding of day-old broiler chicks with Lactobacilli sp. alone did not result ($p \geq 0.01$) in increase in weight gain when compared to control chicks. However, Lactobacilli sp. feeding reduced the faecal coliform count with a positive nitrogen balance and glucose tolerence. But, a combination of Lactobacillus acidophilus 0.500, Lactobacillus salivarius 0.250, Saccharomyces sp. 0.125 and Torulopsis sp. 0.125 x 10^6 CFU per kg diet significantly ($p \leq 0.01$) improved weight gain by 12.2%, reduced faecal coliform count by 60.3%, increased intestinal amylase activity ($p < 0.01$), elevated the humoral immune response ($p \leq 0.01$) to New Castle disease virus and increased the height and width of villi ($p \leq 0.01$). It is concluded that the synergism that existed between Lactobacilli sp., Saccharomyces sp. and Torulopsis sp., were responsible for the positive response observed in this study.

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AH-73

Genotyping of Infectious bronchitis virus

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Infectious bronchitis is an acute, highly contagious viral disease of chickens affecting both broilers and layers. In layers and broiler breeders it causes severe decline in egg production, deterioration in shell and internal quality. In broilers the birds have reduced weight gain causing great production losses. In this study a total of 47 samples were screened for the presence of infectious bronchitis virus (IBV) genome by N gene nested reverse transcription polymerase chain reaction (RT-PCR) and 10 were positive. N gene nested RT-PCR had a sensitivity of 10^0 EID_{50} (one) virus. A part of S1 gene of all the 10 isolates were amplified and sequenced. The sensitivity of S1 gene RT-PCR was found to be $10^{4.0}$ EID_{50} of virus.

Seven of the 10 isolates sequenced belonged to Mass 41 genotypes, the other 3 isolates sequenced gave non readable sequence data. A vaccine strain (H120) was also sequenced and had 100% nucleotide homology with the already available sequence in the Genbank. The serotype of four of the isolates (008, 177, S3 and U4) was determined by cross neutralization test using homologous and heterologous sera. All were found to belong to Mass 41 serotype like the H120 vaccine strain. An *in vivo* challenge trial using commonly available vaccines (H120, Ma5 and Ma5 + clone 30) demonstrated complete protection against challenge with one field isolate (008).

In the present study, no variant IBV could be detected. However, the possible presence of IBV variants, not protected by the existing vaccines, looms large and surveillance needs to be considered.

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AH-74-P

Cultural Characterization of Defined *Salmonella typhimurium* Mutants Anaerobically

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Cultural characterization of some defined mutants of *Salmonella typhimurium*, avirulent for chickens, was carried out to assess the in vitro inter- and intra-generic growth characteristics. Bacterial strains like *S.typhimurium* F98, phage type 14, highly virulent for chickens, and its spontaneous antibiotic resistant mutants, viz., F98 Nal^r, F98 Spc^r were taken for comparing cultural characteristics of its defined mutants viz., *nuoG, cydA, uncH, dpp and ompD. Escherichia coli* strain P4 (pig isolate), which do not produce complete growth inhibition against *S.typhimurium* was used as control organism where necessary. It was observed from preliminary study that the multiplication of *E.coli* and *S.typhimurium* were not inhibited in culture filtrates of *S.typhimurium*

and *E.coli*, respectively. There was complete inhibition of growth of *S.typhimurium* F98 Spc^r in culture filtrate of its F98 sensitive strain. This indicated that the growth inhibition was genus specific. But, this genus-specific growth was not observed when *S.typhimurium* was grown in culture filtrate of its defined mutants (*nuoG, cydA* and *uncH*). One of the possible reasons is that mutation in these genes is pleiotrophic (pleiotrophic gene affect more than one, apparently unrelated, characteristic of the phenotype) and because they affect proton gradient, they may affect nutrient uptake. Thus, these mutants may be less efficient nutritionally than the parent strain. In stationary phase cultures of these mutants, nutrient may be less efficient nutritionally than the parent strain. In stationary phase, cultures of these mutants, nutrient may be present which the mutant cannot utilize under low redox conditions but which the parent strain can utilize. If this was the case, filtrates of such cultures, prepared under anaerobic condition, to prevent readmission of oxygen, would allow a fast rate of multiplication of the parent strains than would filtrate prepared from a culture of the parent strain. Above hypothesis was experimented as below. In vitro growth inhibition was tested by inoculating 24 h LB broth culture filtrates of the strains to be tested with the challenge strains (constant initial concentration in the 24 h broth culture of 10^3 CFU ml^{-1}). Culture of *S. typhimurium* and its defined mutants (*nuoG, cydA* and *uncH*) were grown in LB broth (shaking incubator) for 24 h. The cultures were taken back to anaerobic cabinet and filtered under a nitrogen atmosphere. The filtrates were inoculated with challenge strains. The viable cell counts (CFU) were done at 0, 2, 4, 8 and 24th, respectively. Subsequent filtrations were done on 2nd day followed by re-inoculation of the challenge strains (*S.typhimurium* and its defined mutants viz., *dpp* and *ompD*). Viable counts of all the strains were made at intervals on LB plain and LB with antibiotics. Growth of *S. typhimurium* F98 Spc^r and defined mutants viz., *dpp* and *ompD* were assessed in the culture filtrate of F98 Nal^r or the defined mutants such as *nuoG, cydA* and *uncH*. Genus-specific growth inhibition was not observed. *S. typhimurium* F98 Spc^r grew well in culture filtrate of its F98 Nal^r strain. Moreover, neither the defined mutants (*nuoG, cydA* and *uncH*) inhibited the growth of F98 Spc^r nor the F98 Spc^r inhibited the growth of defined mutants (*dpp* and *ompD*). They grew from 10^3 to 10^8 (app) CFU ml^{-1} after 24 h. It indicated that the reason for inhibition of growth in stationary phase was not due to nutritional requirement.

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AH-75-P

Effect of Invasive Intermediate Strain IBD Vaccination in Broiler Chicken

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The emergence of very virulent type IBDV (vv IBDV) in 1987 has changed the disease scenario of infectious bursal disease. To protect the birds from the havoc of virus invasive intermediate strain IBD vaccine was introduced which was capable of inciting immune response even in presence of maternal antibody. In this study invasive intermediate strain IBD vaccine (IV

95) was given to a group of 50 broiler chickens on 10 day of age. The blood samples were collected from representative birds; sera were separated and subjected to QAGPT. The cell-mediated immunity was evaluated by delayed type hypersensitivity to DNCB, PHA-P and PPD (tuberculin). On 25-day post vaccination, representative chickens from each group were challenged with IBD virus (field isolate). The birds were observed for 7-day post challenge to study the clinical symptoms and mortality. On day 7-post challenge, the birds were sacrificed, bursae were collected and subjected to AGPT to detect the presence of virus. The result revealed protective QAGPT titre (3.8 $\log_2$). Pronounced DTH response was observed, indicating the role of CMI. Neither any clinical symptoms nor any mortality were observed. Besides, there was absence of haemorrhages in breast and thigh muscle. AGPT was negative for presence of IBDV antigen. It was concluded that IV 95 strain vaccine incited both humoral and CMI responses and effectively protected the birds as evident from challenge study.

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AH-76-P

Diagnostic Potential of the Culture Filtrate Antigens of Iron Deficiency Grown Mycobacteria in the Screening of Cattle Serum for Bovine Tuberculosis

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Mycobacteria grown iron deficiency was found to express proteins that are repressed in cells grown with increased concentrations of iron. The culture filtrates of the cells grown under iron deficient and iron sufficient conditions were used as antigens to screen serum samples from cattle in the villages of Mahbubnagar District in Andhra Pradesh. Their potential as diagnostic agents is analyzed.

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AH-77-P

Influence of Iron Concentrations on the Expression of Catalases in Mycobacteria

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Catalases of Mycobacteria are important due to their role in oxidative stress. For pathogenic Mycobacteria, the conditions encountered within the macrophages expose them to a high concentration of free radicals and hydrogen peroxide. The influence of the media iron concentration on the activity of catalase and the expression of the different isoforms is studied in different Mycobacteria, including *M.bovis* and *M.tuberculosis*.

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AH-78-P

Expression of Iron Related Proteins (IRPs) in Leptospira: Studies with *L.biflexa* serotype *Patoc I*

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This is the first report on the expression of iron-regulated proteins (IRPs) in the genus Leptospira. Leptospira biflexa serotype Patoc I was grown in EMJH medium to which iron chelators ethylenediamine-n,n'-diacetic acid (EDDA) and ethylenediamine di-o-hydroxyphenylacetic acid (EDDHPA) were added to effect iron deprivation. Examination by SDS-PAGE demonstrated the production of at least four additional proteins when iron was limiting. These iron-regulated proteins were ascribed apparent molecular masses of 82, 64, 60 and 33 kDa. The presence of the 64, 60 and 33 kDa proteins in the detergent phase indicate that they are surface proteins. The 82 kDa protein was seen only in the aqueous phase. The synthesis of these iron-regulated proteins was switched off where iron was made available. The CAS agar plates show that these organisms elaborate mechanisms for acquiring the dye-bound iron, possibly by the production of siderophores.

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AH-79-P

Antigenic Characterization and Immunogenic Cross Reactivity of Membrane Proteins of Avian *E.coli*

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More than 20 *E.coli* isolates from different species of poultry like chickens quails, turkeys and guinea fowls isolated from clinical cases of colibacillosis, were confirmed on EMB agar and serotyped based on 'O' antigen. The organisms were grown in Luria Bertani broth overnight and their outer membrane proteins were extracted by sarcosyl, purified by ultra centrifugation and subjected to SDS-PAGE analysis. The results indicated that all the isolates had expressed 4-5 proteins ranging between 27-40 kDa. Lower molecular weight proteins of 22 kDa and 18.4 kDa were the common antigens present in all the isolates. The antisera raised against a single serotype reacted sharply with all other serotypes as evidenced by Western blot analysis thus, confirming the immunogenic property of these proteins. Hence a vaccine designed with outer membrane proteins, as a subunit antigen delivery system had conferred heterologous protection against multiple serotypes, as most of the lower molecular weight proteins were common amongst all serotypes.

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AH-80-P

First report on Identification of Culicoides Vector Transmitting Bluetongue in South India

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Bluetongue an arthropod borne orbiviral infection was found to be transmitted by Culicoides, the bitting midges. The wide spread of this devastating disease is attributed to the vector in particular. In this study Culicoides vector was collected from the nomadic sheep flocks using indigenous manually operated light trap and suction trap during early hours. The insect vector was processed and identified preliminarily. Isolation of Bluetongue virus from the Culicoides yielded one isolate of serotype-I which was confirmed by Institute of Animal Health, Pirbright (Accession No: A Y-359960). The Culicoides vector were identified to species level Culicoides Oxystoma, Culicoides orenials, Culicoides Pereghrians, Culicoides imicola, Culicoides anophelis with the help of the Arbovirallaboratory, Institute of Animal Health, Pribright. UK.

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AH-81-P

Usefulness of Polymerase Chain Reaction in Early Detection and Tissue Tropism of Fowl Adenovirus Antigen in Experimentally Infected Chicken

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Inclusion body hepatitis – hydropericardium syndrome (IBH-HPS) is an emerging disease of poultry caused by non-enveloped icosahedral, fowl adenovirus type 4 (FAV-4). The tentative diagnosis of HPS is based on post-mortem lesions and histopathological changes on the affected chicken but confirmatory diagnosis is routinely done by demonstration of FAV-4 antigen on suspected tissue material. A cost effective, rapid, simple and sensitive diagnostic test is highly needed to demonstrate FAV-4 antigen in infected tissue for routine diagnostic purpose. In the present study, the efficiency of polymerase chain reaction (PCR) in terms of sensitivity and specificity was compared with conventional agar gel immunodiffusion (AGID) test for the early detection of HPS antigen in liver tissues of experimentally infected chicken collected after different hours of post-infection.

Liver is the major organ identified for demonstration of HPS antigen and the antigen is not demonstrated in other tissues. Hence, a detailed study on tissue tropism of the virus in different organs was carried out in experimentally infected broiler chicks using AGID and PCR test. The results were analyzed and discussed.

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AH-82-P

A typical Blackleg in Cattle of Manipur Caused by *Clostridium perfringens* Type A

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Cases of acute fatal myonecrosis in cattle reportedly vaccinated with conventional black quarter vaccine in Manipur state were investigated for the etiological organism. From more than 95% of cases Gram +ve, anaerobic rod shaped organisms were isolated. The anaerobic bacilli were highly proteolytic, hemolytic, DNase, lecithinase and gelatin liquefaction positive. Cultural, biochemical, biological assays and PCR analysis revealed the isolates to be *Cl. perfringens* Type A. In multiplex PCR analysis *for cpa, cpb, etx, iA, cpe,* and *cpb2*, the predominant isolates were found to be only *cpa* toxin gene positive. This report is in contrast to earlier report on involvement of beta-2 toxigenic *Cl. perfringens* Type A in Atypical Blackleg. We hereby confirm the toxigenic *Cl. perfringens* Type A as the causative agent of Atypical Blackleg (black quarter like disease) of cattle in Manipur. RAPD analysis further confirmed the homogeneity among *Cl. perfringens* Type A isolated from Atypical Blackleg.

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AH-83-P

Diagnosis of Leptospirosis in Captive Wild Animals

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58 samples from various species of wild life were screened for leptospirosis by different techniques like Dark field microscopy (DFM), Polymerase chain reaction (PCR), Culture method, Microscopic agglutination test (MAT). Eleven (6.38%) samples were positive by Dark Field Microscopy, and all the 11 samples were screened by PCR and out of this 7 (63.63%) were positive. MAT was carried out using live leptospiral antigens for 54 samples, of these 8 (4.32%) samples showed positive titer. Out of 58 samples screened for leptospirosis by Culture method, 2(1.16%) samples gave growth of leptospiral like organisms in EMJH medium.

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AH-84-P

An Inactivated Bivalent Vaccine to Control Bluetongue in Sheep and Goats

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Bluetongue is an International Epizootics list A disease, which causes economically devastating infection primarily of sheep and also of other small ruminants including goats. Bluetongue was first described in detail in Africa in 1902 by Hutcheon, whereas in India, the disease was first reported in 1964 by Sapre and others from Maharashtra State. Since then, the disease is being reported from several parts of the country and moderate outbreaks of bluetongue occurred in 1987 with a mortality of 20-30% whereas, in 1997, severe outbreaks of bluetongue resulted in losses to sheep and goats upto 80% in several sheep growing areas of India. Protective immunization against BTV has been a difficult goal for past several decades. The antigenic diversity exhibited by the known 24 serotypes is the major impediment towards the control of the disease. However several researchers have worked on the development of live attenuated multivalent vaccines, inactivated vaccines and baculovirus expressed recombinant vaccines. In India, the research is being initiated on the development of vaccine to control bluetongue. Recently an inactivated monovalent vaccine incorporating serotype 23 has been developed at Vaccine Research Centre-Viral Vaccines, whereas in Andhra Pradesh, inactivated monovalent BT vaccine incorporating serotype 2 has been developed. Currently, at Vaccine Research Centre-Viral Vaccines development of inactivated bivalent vaccine containing BTV 1 and BTV 23 is under progress. Quality control protocols of vaccine production and seromonitoring results of experimental and field trials will be discussed.

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AH-85-P

Isolation and Identification of Chicken Anaemia Virus (CAV) in Indian Poultry Flocks and Reproduction of Disease in SPF Chicks

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In the present study attempts were made to isolate chicken anaemia virus (CAV) from suspected flocks of different geographical regions of the country. Tissues collected/received from chicken flocks suspected for chicken infectious anaemia (CIA) viz., thymus, bone marrow, spleen, liver and bursa were screened for CAV-DNA by polymerase chain reaction (PCR). Isolation and

adaptation of the virus was attempted in MDCC-MSB1 (Marek's disease virus transformed chicken lymphoblastic T-cells) cell cultures from cases found positive by PCR. Six tissue samples, out of fifteen samples tested from different states viz. Maharashtra, Tamil Nadu, Uttar Pradesh, Himachal Pradesh and Haryana, of the country were found positive for CAV-DNA by PCR. Subsequently, six CAV isolates (A-F) were recovered in MDCC-MSB1 cell cultures from tissues found positive by PCR. Virus multiplication in MSB1 cells with respect to each isolate was confirmed by indirect immunofluorescent technique (IIFT) at each passage level. These isolates produced characteristic cytopathic effects after 7-8 passages and were confirmed as CAV by PCR, IIFT and virus neutralization test employing anti-CAV reference serum. Infected MSB1 cell cultures were found positive by PCR for CAV-DNA from first passage onwards, whereas by IIFT positive cells were detected at 6^{th} passage level. All the isolates were found to be heat (70°C/ 15 min) and chloroform resistant. On inoculation into day-old SPF chicks, the isolate A produced characteristic signs and lesions of infectious anaemia and the virus was re-isolated from the experimentally infected chicks at 14 days post infection. The isolation of CAV from five states of the country confirmed the existence of the virus in poultry flocks of India, warranting the need to study the epidemiology of the disease and devise suitable control measures. This report of CAV isolation from different geographical regions of the country highlights the wide prevalence of the disease in the sub-continent.

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BIOTECHNOLOGY IN PET ANIMAL CARE

PAC-1

Fibrin - Gelatin Composite, a Bio-material for the Treatment of Wounds in Dogs

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A pilot study was conducted to evaluate the effect of 'Fibrin - Gelatin Composite', a bio-material for the treatment of wounds in dogs. The purpose of this study was to develop an ideal bio-material using fibrin and gelatin for the treatment of various wounds in dogs. Twelve wound cases of dogs reported to the Orthopaedic Unit of Madras Veterinary College Hospital were treated with 'Fibrin - Gelatin Composite' moistened in topical oxytetracycline liquid. The parameters studied were clinical signs, planimetric studies, haematological, studies, biochemical studies and gross pathological changes. The degree of wound contraction and wound epithelization were at a quick pace and complete healing was observed between 14-21 days in all the animals. The Fibrin - Gelatin composite helped in haemostasis, was biodegradable and bio-compatible and can be utilized as an ideal wound dressing material, for the treatment of various types of open wounds in dogs.

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PAC-2

Use of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in Canine Renal Failure

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The urine samples of thirty six clinical cases of renal failure and twelve normal dogs were subjected to SDS-PAGE. There was differences observed in respect of the molecular weights of protein bands recorded at the end of the gel electrophoresis of the urine samples. Twenty two cases of tubulo interstitial nephritis had a predominance of low molecular weight(LMW) proteins while nine cases with glomerulo nephritis had a larger fraction of high molecular weight (HMW) proteins.

The third group of clinical cases numbering five exhibited a mixture of both low and high molecular weight proteins indicative of simultaneous glomerular and tubulo interstial damage. Canines in early stages of renal insufficiency exhibited a larger fraction of low molecular weight proteins in six cases and a larger fraction of high molecular weight proteins in three cases, depending on the primary location of the lesion (i.e) either tubulo interstitial or glomerular. The PAGE of urine samples from twelve apparently healthy animals mainly yielded protein bands in the molecular weight range of albumins.

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BIOTECHNOLOGY PRODUCTS AND VALUE ADDITION

PVA-1

Medium and Conditions for Optimal Recovery of Injured Salmonella from Fermented Dairy Products

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In the absence of established equivalency of inspection and certification systems between exporting and importing countries, food or food ingredients will be sampled and tested for assurance of microbiological safety at the port-of-entry. Since both the statistical sampling plans and microbiological test methods have inherent limitations due to non random distribution of microorganisms and the difficulty in detecting injured pathogens in finished food, end product testing is of limited value in assuring safety. Specific pathogens such as Salmonella in foods are exposed to stress from heating, acidification / fermentation and freezing, consequently these organisms require special handling and resuscitation for successful detection. Injured *Salmonella* undergo further stress during preparation of food homogenate with the diluents for further incubation to revive injured *Salmonella*. We developed a nonselective differential agar medium and evaluated the influence of diluents(Peptone water PW, Butterfields phophate buffer BP,Maximum Recovery Diluent MRD and milk) on recovery of injured and healthy *Salmonella* in cultured buttermilk. Despite similar osmolarity milk diluent recovered higher numbers of *Salmonella* than the MRD. PW and BP with lowest osmolarity recovered the lowest number of *Salmonella*, one to two orders of magnitude differnce. Casein diluent with low osmolarity as PW and BP, recovered higher numbers than the MRD.Universal preenrichment broth was better than lactose broth in recovery of Salmonella from aged cheddar cheese.

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PVA-2

Comparison of an Indigenously Developed Competitive-ELISA Test with a Similar Commercial Kit for the Detection of Antibodies to PPR Virus

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Peste des petits ruminants (PPR) is an important viral disease of sheeps and goats. The disease causes huge economic losses due to high mortality and morbidity in susceptible animals. The disease needs to be controlled using a suitable vaccine available in the country. For the detection of antibodies to PPR virus (sero-surveillance and sero-monitoring), a monoclonal antibody-based competitive ELISA test has been developed. The test uses a monoclonal antibody to virus neutralizing epitope

against haemagglutinating protein of an Indian isoalate of PPR virus. Present study deals with the comparison of newly developed test with a similar imported commercial kit developed by the World Reference Laboratory of Rinderpest, Institute of Animal Health, Pirbright, UK. The present investigation was carried out using 2488 identical sheep and goat sera samples from the target population. Diagnostic sensitivity and specificity of newly developed test Vs Commercial kit was found to be 87.8% and 97.1%, respectively, as determined using two-sided contingency table. Dispersion of the individual samples, showed that both these tests clearly differentiate between PPR positive samples and negative samples. These assays have high correlation coefficient (r=0.83) based on per cent inhibition (PI) of identical samples. PI values in general were higher in commercial kit as compared to newly developed test. Details of the data will be presented during deliberations.

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PVA-3

Dimensions of Biotechnology in Livestock Production and Productivity

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Since Independence with judicious application of the tools of selection and breeding the Indian livestock improved considerably in many of the production traits such as in cattle the average milk yield increased from 160 – 200 kg to 1500-2000 kg per lactation, in sheep and goat average body weight increased from 25 kg in 18 months to 30 kg in six month and in Poultry average egg production increased from 60 eggs to 230 eggs per anum.

In the present era to meet all the animal products requirement of ever growing Indian population, can we be complacent with the achievements in the animal sector through conventional techniques? Can the same pace of development see us through the next century? The answer is obviously 'No'. The Biotechnology offers a solution through another revolution called gene revolution to halt the fatigue of animal production and productivity. The quantum jump in Biotechnology has opened up the exciting possibilities for rapid increase in livestock production and productivity. Biotechnology has the tools for revolutionizing livestock through tapping of potential areas which can be exploited in animal production and productivity :-

- Conservation and multiplication of genetically defined superior breeds and strain of livestock for meat, milk and egg production.
- Production of Transgenic animals through gene manipulation to promote Animal Pharming.
- Physiochemical and nutritional studies for optimal production and reproduction.
- Cheaper and user friendly diagnostics and vaccines for improvement of animal health.
- Improvement of reproduction by ETT and MOET techniques.

- Increase in feed conversion efficiency of livestock and poultry and use of animal waste-recycling as animal feed.
- System approach integrated with Bio-informatics, computer and environmental safety.

The paper describes the impact of biotechnology on animal production and productivity, its relevance in context of India and regulatory, administrative and ethical issues involved.

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PVA-4

Protein p43-45 in Uterine Secretions, a Possible Marker of Early Pregnancy in Cattle and Buffaloes, may also be an Immunosuppressive Factor of Seminal Plasma

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Studies on the significance of proteins from the reproductive tract secretions in immunological interactions during reproduction were conducted on samples of seminal plasma of 9 cattle bulls and 6 buffalo bulls, and uterine secretions of 6 pregnant, 7 non-pregnant and 7 repeat breeder cows, and 6 pregnant and 7 non-pregnant buffaloes, respectively.

Dot ELISA detected a strong reactivity of anti-seminal plasma serum with uterine secretions in a very significantly higher proportion of pregnant compared to non-pregnant females in both cattle and buffaloes. Possibly, some immunosuppressant proteins may be common to the seminal plasma of males and the uterine secretions of pregnant female bovines, which may help in the suppression of maternal immunity against the allogeneic sperms and semi-allogeneic concepts during reproduction.

Western blotting of uterine secretions with anti-seminal plasma serum revealed a cross-reactive protein of high molecular weight present in uterine secretions from pregnant animals but absent in non-pregnant animals. Interestingly, a protein p43-45 was found by native PAGE to be present in the seminal plasma of bulls and uterine secretions of pregnant females but conspicuously absent in uterine secretions of non-pregnant females in both cattle and buffaloes and repeat breeder cows.

There is a substantial immunodiagnostic potential of putative immuno-suppressive proteins from male reproductive tract secretions as possible markers of early pregnancy in uterine secretions particularly keeping in view the limited success with the conventional approach of investigating pregnancy markers in serum samples of female animals.

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PVA-5

Self-replicating Gene Vaccine for Foot and Mouth Disease

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Foot-and-mouth disease is an economically important viral disease of cloven-footed animals. Because of the existence of multiserotypes, it is a great challenge to us to control this viral infection. Even though conventional vaccines are good enough to induce immune response, they fail to provide long-lasting immunity, as the FMDV antigen is a poor immunogen. Moreover, production of virus in cell culture needs sophisticated infrastructure as is associated with the risk handling of virus in bulk. Alternatively, DNA vaccines have emerged as new tools for the control of many infectious diseases, which offer both humoral and cell mediated immune responses. Since the DNA vaccines require molecular immuno modulators for enhanced response, attempts are being tried to work on Alpha viral replicase based mammalian expression vectors as self-replicating gene vaccines. As there are no reports on the development of such vaccines for FMD, an initial attempt has been made to produce self-replicating vector for the expression of FMDV antigen at higher level.

Self-replicating DNA vector was constructed by cloning CMV promoter at 5'end of the alpha viral replicase gene. The structural gene of FMDV virus 'O' serotype (P1-2A polyprotein gene) was cloned downstream of the replicase gene to make a self-replicating gene vaccine. In vitro transcription of the linearized construct was done to confirm the RNA production. The DNA construct was transfected into mammalian cells along with the conventional mammalian expression vector (pcDNA 3.1+) containing the P1-2A gene separately as a control. There was a marked increase in the level of expression as compared to the control, which was confirmed by Western blot analysis. Animal experimentation in guinea pigs and cattle is in progress for the evaluation of immune response to gene vaccine. The results of which will be presented.

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PVA-6

Cytokine adjuvanted DNA Vaccine for FMD

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Conventional vaccines against FMD have been shown to provide solid protection, although of reduced duration coupled with the inherent risks involved in handling live virus.

In recent years, DNA vaccination has emerged as one of the most promising venues for recombinant vaccine. DNA vaccines against FMDV have seen through several modifications seeking to improve upon the immune responses elicited. Further, several reports ascertain the efficacy of cytokines as gene adjuvants. With this backdrop, it is proposed to undertake the task of developing

a cytokine adjuvanted DNA vaccine for immunoprophylaxis against FMD. Consequently, the genes coding for structural and non-structural proteins *i.e.*, P1-2A, 3C and 3D and the Bovine Gamma Interferon gene coding for the bovine IFN-n (BGIF) for its immuno-adjuvant action have been included in the DNA vaccine. The DNA vaccine constructs have been checked for expression in BHK-21 cells and subsequently inoculated, intra-dermal, into guinea pigs. Immunological analysis of the serum samples showed SNT titers of 1:64 in animals inoculated with DNA constructs containing P1-2A, 3CD and IFN genes, 1:32 being protective in guinea pigs. Forty days after the second booster the animals were challenged with homologous serotype 'O' virus. The animals were monitored for development of lesions at the end of 48 and 72 hrs after challenge. Conspicously, animals inoculated with the construct carrying P1-2A, 3CD and IFN genes were conferred 100% protection while those inoculated with P1-2A, 3CD alone were conferred 60% protection. However, the enhancement in protection offered by the BGIF in guinea pigs is still an enigma owing to the fact that cytokines are species specific. Final experimental trials of the DNA vaccine in cattle are awaited.

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PVA-7

Production of Sheep Pox vectored Vaccine for FMD

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Foot-and-Mouth disease is an endemic problem in our country leading to considerable economic losses. Only way of controlling the disease is by regular vaccination of the affected animals. Though vaccination of large animals like cattle and buffalo is being carried out, small animals like sheep and goat are a neglected lot and sheep are known to be acting as reservoirs for the FMDV and a potential source of antigenically altered virus variants, since continuous variations of the virus and selection of virus mutants take place in the animal during the carrier/reservoir status. Hence these animals have to be considered as a potential risk of infection. So, for complete eradication, the elimination of the carrier status is important more so in the context of the Indian scenario where mixed farming of small ruminants like sheep and goat with cattle is common.

Conventional inactivated virus vaccines can be used for regular vaccination. However, production and handling of the bulk virus involves the risk of disseminating the virus. Therefore alternative approaches are desirable. Keeping this objective in view we aimed at producing a new generation FMD vaccine for sheep using sheep poxvirus as a vector.

The TK gene of sheep poxvirus was cloned and sequenced. To facilitate introduction of foreign genes, a few restriction enzyme sites were introduced along with a marker gene GFP. The P1-2A gene of FMDV was introduced into this TK-GFP construct. The TK-GFP-P1 construct was

transfected into BHK-21 cells and the expression detected by western blot analysis. Introduction of this construct into the sheep poxvirus vaccine strain is being carried out. This recombinant virus will be put through vaccine trials in sheep to detect the level of protection against sheep pox and FMD and hence can be used as a dual vaccine.

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PVA-8

Lactobacillus acidophilus as a Probiotic Culture in Preparing Dietetic Shrikhand

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SHRIKHAND is a fermented and sweetened dairy product of pasty consistency is popular in the states of Western India. Tradionally, shrikhand is made from "Chakka" which is an intermediate product obtained from draining of dahi prepared from buffalo or cow or mixed milk. In this study specific starter cultures of therapeutic properties were used for preparing a Dietetic shrikhand. Shrikhand was prepared as per Sukumar De (1980) using pasteurized buffalo skim milk under aseptic conditions using 2% combined culture containing *S. salivarius* sp. *Thermophilus* 1% and *Lactobacillus acidophilus* 1%. The therapeutic properties of shrikhand prepared using specific lactic acid bacteria of therapeutic importance was done. Biological trials were conducted to evaluate the therapeutic value of the product.

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PVA-9

Studies on the Preparation of Fortified Skim Milk

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Non-fat skim milk as such does not find a good acceptance by the consumers due to lack of fat. However, palatability of the skim milk was improved by converting it into nutritious, cheap flavoured milk fortified with iron and vitamin A. Among the different iron salts used, ferric ammonium citrate was found to be the best for fortification up to the concentration level of 30 mg/100 ml whereas Vitamin A up to a level of 500 IU /100ml. There were no noticeable changes compared to control with regard to flavour and acceptability during storage at 5°C for 7 days. The loss of iron and vitamin A content were less than 1% and 3% respectively.

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PVA-10

Natural Color and Flavor Concentrate to Flavored Milk

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A pilot study on the preparation of flavored milk by adding the extracts of carrot (2%), beetroot, mint and coriander (1%) for color and cardamom (2%) and dried ginger (0.01%) for flavor in the pasteurized milk was conducted. It was observed that beetroot color and cardamom flavour was the best combination for preparation of flavored milk. Since it was a cumbersome process, an attempt was made to prepare readymade natural color and flavor concentrate (35 ml beetroot juice, 2 g Cardamom powder and 100 g sugar) Four grams of this concentrate is required to 100 ml of flavored milk and the shelf life was one week at room temperature (30°C), 3 weeks at refrigerator (5°C) and one year at deep freezer (-20°C). The cost of production is Rs.6.00/200 g.

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PVA-11

Induction of Immune Response in Goats with an Experimental DNA Vaccine Encoding Omp31 Outer Membrane Protein of *Brucella melitensis 16M.*

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This study was conducted to evaluate the immunogenicity of the *Brucella melitensis* omp31 gene cloned into the pTargeT mammalian expression system plasmid, which is driven by the cytomegalovirus promoter. Injection of plasmid DNA carrying the omp31 gene (pTargeTomp31) into goats elicited both humoral and cellular immune responses. Antibodies to the encoded omp31 included immunoglobulin GI (IgGI) lgG2a, lgG2b, lgG3, and lgM isotypes. Animals injected with pTargeTomp31 exhibited a dominance of lgG2a over lgGI suggesting the induction of a Thl response. Even after the 5 months of last injection of DNA, CTL response was monitored up to the level of 55% in immunized animals. Altogether, these data suggest that pTargeTomp31 is a good immunogen for the production of humoral and cell-mediated responses in goats and is a candidate for use in future studies of vaccination against caprine brucellosis. Details will be presented.

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PVA-12

Characterization of Antimicrobial Peptides from Buffalo Polymorphonuclear Cells

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Polymorphonuclear cells from bufflo blood were isolated by lysing of RBC from packed cell Volume using hypotonic shock. The PMN cells thus obtained were >90% viable and about 90% pure for neutrophils. PMN cell granules were obtained by ultrasonication followed by differential centrifugation and subjected to 10% acetic acid extraction to get the crude protein / peptide extracts. About 10 to 12 mg of cationic crude proteins were extracted from 1 liter of buffalo blood. The pooled extracts were desalted using Sephadex G-10 column elution. The buffalo PMN granular protein extract thus prepared was subjected to molecular exclusion chromatography using Sephacryl S-200 column using 0.2M acetate buffer which resolved the proteins into four different peaks (I,II,II and IV as per their increasing elution time). The fractions of each peaks were pooled separately, concentrated and were tested against *E. coli* K12 strain for their antibacterial activity. The proteins/ peptides of peak IV were having highest antibacterial activity against *E.coli.* The peak IV proteins from Sephacryl S-200 column were further subjected to analytical acid urea-PAGE (AU-PAGE), SDS-PAGE and RP-HPLC. The proteins in peak IV were separated into four distinct protein bands in AU-PAGE and two protein bands having approximate molecular weights of 3kD and 1.6 kD in SDS-PAGE where as RP-HPLC using RP-C18 column resolved them into four different peaks of proteins designated as BNP-I, BNP-II, BNP-III and BNP-IV. The isoelectric points of crude peptides were found to be ranging from 8.3 to 8.7. the peptides were found to be devoid of detectable amount of carbohydrates, lacked free sulfhydryl group and did not contain tryptophan in their structures. All thse PMN granular peptides were found to be microbicidal against the bacteria such as *Escherichia coli, Brucella melitansis, Brucella abortus, Staphylococcus aureus* and *Streptococcus pyogenes* as well as against the fungus *Candida albicans* and *Aspergillus fumigatus* which suggested that these peptides might be the major effectors of innate immunity in buffaloes.

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PVA-13

Influence of Packaging Materials on the Quality of Prepacked Meat

A. Jagadeesh Babu*, M. Sreenivas Reddy, K. Prabhakar and G. Ramakrishna Reddy
Department of Meat Science and Technology, College of Veterinary Science, Rajendra Nagar, Hyderabad - 30. Andhra Pradesh

Meat samples packed in polyethylene bags, aluminium foil and polystyrene trays were stored at 4 ± 1°C for five days. Percent weight loss significantly ($P < 0.05$) more and ERV was significantly ($P < 0.05$) less in polyethylene packed meat than polystyrene and aluminium foil packed meats. TBA

values were significantly higher in polystyrene and aluminium foil packed meat and TVN content was significantly higher in polyethylene packed meat. As storage period increased there was increased percent weight loss, TBA and TVN content and decreased pH and ERV was noticed. The colour, flavour, juiciness, tenderness and overall acceptability were significantly higher in meat packed in polyethylene bags.

★ ★ ★

PVA-14

Determination of Heavy Metals in Commercially Available Pork Products in Chennai

D. Santhi, K.T. Radhakrishnan and V. Venkataramanujam
Department of Meat Science and Technology, Madras Veterinary College, Chennai - 600 007.

The heavy metals were determined in commercial pork products in retail outlets of Chennai, by Atomic Absorption Spectrophotometry adopting dry washing method. The analyzed samples had cadmium level in the range of 0.038 to 0.545 mg/kg, chromium in the range from below detectable level to 2.244 mg/kg, copper in the range from below detectable level to 2.847 mg/kg, lead in the range from below detectable level to 6.290 mg/kg, zinc in the range from 6.927 to 144.575 mg/kg. Cadmium level in 95.83% of the samples exceeded the Maximum Permissible Level (MPL) as stipulated by FAO (0.100 mg/kg), whereas none of the samples had copper content exceeding MPL specified by MFPO, 1973 (20 mg), 25.0% of the samples had lead content exceeding the limit of MFPO (2.5 ppm) and 20.83% of the samples had zinc values exceeding the MPL of MFPO (50 ppm).

★ ★ ★

PVA-15

Effect of Postmortem Injection of Calcium Chloride on Mutton Quality

S. Ezhilvelan and K.T. Radhakrishnan
Department of Meat Science and Technology, Madras Veterinary College, Chennai - 600 007.

The effect of post-mortem injection of 100, 200 and 300 mM of calcium chloride ($CaCl_2$) at 5% by weight on quality of chiller (1 to 2°C) stored mutton at 0,1,3 and 7 days of ageing (4 stages) were studied. Significant difference ($P < 0.01$) existed in pH between treatments and ageing period. Water holding capacity was significantly ($P < 0.01$) reduced by $CaCl_2$ injection and also by ageing. Drip loss and cooking loss were significantly greater ($P < 0.01$) in the treated samples and with ageing. Fibre diameter significantly ($P < 0.01$) reduced with ageing but treatment did not have any significant effect on the fibre diameter. Sarcomere length was significantly ($P < 0.01$) shorter at '0'd and '1'd, but on '7'd was longer than control. Myofibrillar

fragmentation index was significantly ($P < 0.01$) greater in treatment groups than controls, and was greater with ageing. Significant ($P < 0.01$) reduction in shear force was observed in the treatment groups and ageing also reduced shear force value significantly. Sensory evaluation revealed that the treatment did not adversely affect the flavour, but improved juiciness and tenderness significantly. The results of the study clearly indicate that mutton can be tenderised by injecting 200 mM calcium chloride at 5 percent by weight and ageing for 24 hours in chiller (1 to 2°C) storage.

★ ★ ★

PVA-16

Enumeration of *Escherichia coli* and *Salmonella* Species in Fresh Marketable Table Eggs and Processed Eggs in Chennai City

P. Krishnamoorthy[1], W. Manohar Paul[2] and A. MahalingaNainar[3]

[1] PG Scholar, Dept. of Veterinary Pathology, [2] Associate Professor, Dept. of Veterinary Microbiology and [3] Professor and Head, Dept. Of Animal Biotechnology, Madras Veterinary College, Chennai – 600 007.

Egg is one of the most essential commodities to fill the protein requirement of human beings. It contains highly nutritious substances and more prone for bacterial spoilage. Hence a study was undertaken to determine Escherichia coli and Salmonella contamination in fresh marketable table eggs and processed eggs in Chennai City. Random samples of 15 numbers of eggs from 20 different centers like whole sale shops, retail shops and markets were collected. Samples of half boiled egg, full boiled egg, egg omelette each 5 numbers were collected from 10 different places like hotels, canteens, fast food restaurants in Chennai city. Assessment of Escherichia coli and Salmonella species was undertaken in fresh marketable table eggs and processed eggs. Escherichia coli was enumerated in 4-methyl ubelliferyl β-D-glucuronide (MUG) medium. Escherichia coli and Salmonella Sp. were detected in 75% and 30% in egg shell and 65% and 25% in egg yolk respectively. Half boiled egg, full boiled egg, egg omelette samples contained about 30%, 20%, 20% and 20%, 30%, 20% of Escherichia coli and Salmonella Sp. respectively.

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ADDENDUM

KEY NOTE ADDRESSES

New Vistas in Biotechnological Applications in Livestock Production in India

Dr. N. Balaraman
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Karnal - 132001, Haryana

Introduction

Biotechnology as a field of science encompasses all aspects of science and technology dealing with live material and biological products. It is multi-disciplinary in orientation involving basic and fundamental disciplines such as molecular biology, genetic engineering, cell/tissue culture, cytogenetics, microbiology and biochemistry. Technologies using living organisms or substances from living organisms to make or modify a product or a process to improve microorganisms, plants or animals for specified purposes fall within the broad scope of biotechnology.

The role of biotechnology in nation building can be viewed from the point of view of national priorities such as poverty alleviation, employment generation, education, knowledge and skill development, improvement in production and productivity and improvement in overall quality of life. Among the applied aspects of biotechnology, disciplines such as nutrition, physiology, breeding (conservation and multiplication), products processing and biomolecules production are predominantly emphasized.

The scenario in world food situation and production dynamics is swiftly changing. FAO has predicted that the consumption of livestock products in developing countries will be substantially increased. There will be a shift in livestock production from temperate and dry regions to wet and tropical regions of the globe. The livestock production emphasis will be market driven and the systems of rearing will be more intensive, commercial and on industrial scale than ever before. Quality criteria will determine the acceptability of the livestock products both at the domestic as well as at the global market. Developing countries, especially India are poised to participate considerably at the global level.

New breakthroughs

Several new and upstream breakthroughs have brought in sharper focus on biotechnology as a modern tool for rapid advancement in technology development . Recombinant DNA technology, monoclonal antibody technology, embryo manipulation techniques, somatic nuclear transfer, cloning, production of biomolcules, food processing and farm or plant hygiene are just a few key areas

worth mentioning in this regard. Aspects of super ovulation and exploitation of female potential are new thrust areas in livestock production. In vitro fertilization (which involves recovery of eggs, maturation and fertilization in vitro and its implantation into foster mothers) makes available embryos for cloning, transgenesis and production of elite superior germplasm for higher productivity. Transport of embryos is easier and more cost-effective than fully developed animals.

Biotechnology is nothing new as an approach to meeting the society's requirements. The conventional products such as beer, bread, wine, curd and cheese are products of conventional biotechnology.

Reproduction augmentation

Artificial insemination and exploitation of male potential is a widely adopted practice because of its multiple advantages of rapid progress in large animal development. Embryo transfer and associated technologies have advanced the scope of multiplication of superior germplasm for specific purposes and exploitation of female potential in a much larger way. Sperm sexing based on higher DNA content of X bearing sperms (Beltzville sperm sexing technology) can allow choise of the sex in the offspring at the farm level. Synchronization of heat for ETT is an ideal scientific practice to streamline farm operations for effective farm management. Farming based on sexed embryos has been reported to produce 50% more of preferred offspring than the traditional system. It has been shown to be more cost-effective in pigs and poultry and has a positive influence on efficiency of feed utilization.

Cloning

This can be achieved either by nuclear transfer or embryo splitting. Elite embryos can be cloned for higher performance. Near-extinct species can be revived through cloning. Cost-effective experimentation is possible with genetically identical animals. Such animals have been employed in the study of resistance to diseases such as trypanasomiasis. The availabílity of clones enables the differentiation of direct embryonic component from the maternal component made up of the reproductive tract of the mother.

Multiple Ovulation Embryo Transfer

It is a complex technology involving several steps such as super-ovulation, fertilization, embryo recovery, short term in vitro culture of embryo, embryo freezing and embryo transfer. In this technique, the elite female is used with superior males. In the Open Nucleus Breeding System (ONBS), a nucleus herd is established from a base population under controlled conditions to facilitate selection. The elite nucleus herd is formed by the best animals of the base stock. The elite female herd is served with superior sires and the embryos are carried by the females of the base stock.. ONBS can be used with ETT or AI.

Marker assisted selection

A genetic marker is a DNA segment which is associated with a trait and segregates in a predictable manner as the trait. Availability of large number os such markers has enhanced the possibility of detection of major genes influencing quantitative traits. MAS can accelerate the rate of genetic progress by increasing the accuracy of selection and by reducing

the generation interval. MAS benefit is greater with traits of lower heritability. It can be used in identifying animals with tolerance/resistance to diseases and environmental stress (strongylosis, haemonchosis, listeriosis).

Transgenesis

A transgenic animal is defined as one whose hereditary DNA has been modified by adition of DNA from some other new parental germplasm through recombinant DNA techniques. Animals trans-genetically exploited include mice, rabbits, sheep, pigs and cattle. The scope of transgenesis lies in the fields of animal reproduction and resistance to environmental stresses. Production of medically important biomolecules such as insulin, clotting factors in milk, immunoglobulins etc. is facilitated through transgenesis. The study of genes in the control of physiological processes is the fundamental aspect of transgenesis. Application of transgenesis in animal breeding procedures is still limited. Utilization of embryonic stem cell and primordial stem cell can enhance the efficiency of gene transfer in cattle and sheep.

Animal genomics

It is a novel approach to examine evolutionary development. Its special application is in the field of development of drugs and diagnosis of diseases as well. The priority areas of buffalo genomics include 1. Distance analysis and breed identification using mitochondrial DNA analysis. 2. Exploration of buffalo genome for markers with respect to unique characters 3. characterization of milk protein genes and 4. buffalo gene mapping.

Conservation of animal genetic resources

Both ex situ and In situ preservation approaches are important for conserving valuable animal genetic resources. Under newer technological purview, cryo-preservation of gametes, embryos, DNA segments etc. may help in the regeneration and conservation of the preferred species of animal in question. Economic aspects of conservation need to be borne in mind while prioritizing the species/breed for conservation. Conservation of indigenous animal genetic resources is of top priority in this regard.

Nutrition and physiology

Recombinant bovine and porcine somatotropins are already commercially available in the market. In the field of animal nutrition, break of lingo-cellulosic bond in crude fiber in roughage material is considered as an important priority area in which considerable work has been done. Phanerochaete chrysosporium has been observed to bring about depolymerization of lignin. Improvement of nutritive value of cereals by incorporating the essential amino acid components is an important area calling the attention of researchers. Removal of anti-nutritional factors such as tannins, lathyrogens and mimosine through microbial agents is another key area of the animal nutritionist. Use of transgenic rumen microbes, use of enzymes for ensiling of fodder crops and incorporation of the trait for better nitrogen utilization by rumen microbes are some of the specific points of biotechnological interventions from the point of view of animal feeding.

Dairy Processing

Food processing is emerging as an important, need based commercial enterprise in our country. Milk

representing the most important protective food and highly perishable, its processing represents the single largest processing measure of food processing. Over 45% of milk being converted to products, the importance of milk processing cannot be over-emphasized. With the closest association of microbial activity with milk and milk products and the nutritional and non-nutritional attributes which are influenced by the microbial action, the role of biotechnology in milk processing and products development becomes all the more important. The areas in which biotechnology can significantly contribute include pro- and pre-biotics (oligosaccharides), fermented food products (chymosine), bio-preservatives, bio-active peptides, genetically modified organisms and resultant milk foods (starter cultures), oral vaccines (LAB), biodetergents, nutraceuticals, recombinant enzymes and proteins, PCR based diagnostics, various types of functional foods, designer milks, and not the least, dairy waste management.

ADDENDUM

BIOTECHNOLOGY IN ANIMAL HEALTH CARE

AH-74

Rickettsial Diseases of Animals and Humans Caused by *Ehrlichia* species: Molecular Biology and Proteome Analysis

Vijaya K. Singu, C. Cheng, H. Liu and R. R. Ganta

Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, 1800 Denison Avenue, Manhattan, Kansas 66506, USA.

Ehrlichia chaffeensis and *Ehrlichia canis* are tick-transmitted rickettsial agents that cause human and canine monocytic ehrlichiosis, respectively. Multigene loci with up to 22 genes that encode for 28 kDa outer membrane proteins have been reported from these two closely related organisms. The locus generated considerable interest for its possible role in immune evasion because it shares structural similarity to antigenic variant surface antigen genes of closely and distantly related bacterial pathogens. We recently reported the detailed characterization of this locus from several isolates of *E. chaffeensis*. In that study, we uncovered isolate-specific gene expression differences. In the present study, we utilized proteome analysis to map the protein expression, specifically to identify host-specific protein expression differences. We evaluated *E. chaffeensis* and *E. canis* proteomes by two-dimensional gel electrophoresis, Western blotting and MALDI-TOF methods. The data revealed unique proteome maps for *E. chaffeensis* and *E. canis*. The proteomes differed significantly from those of the host cells in which the organisms were grown. Importantly, the analysis revealed unique protein expression in the pathogens grown in different host cells. Immuno blot and MALDI-TOF analyses revealed differential protein expression from the 28 kDa outer membrane protein gene locus in vertebrate and tick hosts. The proteome maps for several pathogenic isolates also were different. The implications of these data will be discussed relative to the pathogen evasion mechanisms and vaccine development.

★ ★ ★

AH-86-P

Bacterial and Cellular Response following different Treatments on Equine Endometritis

R. Kalirajan and R.C. Rajasundaram***

** Veterinary Assistant Surgeon, Thevaram, Theni Dst. ** Professor, Central University Laboratory, Madhavaram Milk Colony, Chennai-51.*

The objective of this study was to assess the changes in the total bacterial count and cellular components in mares with endometritis and to improve the conception by different treatment.

Based on both bacteriological and cytological examinations, confirmed twenty-four mares with endometritis, which failed to conceive for two consecutive seasons, were randomly divided into four groups. Group I animals were treated with Gentamycin I/Ut, Group II animals were treated with Gentamycin I/M, Group III animals were treated with Gentamycin plus PGF2 alpha I/M and Group IV animals were treated with Gentamycin plus Oxytocin I/M.

The bacterial count (10^6/ml of uterine fluid) in mares affected with endometritis before commencement of treatment were 287.03± 2.05, 274.37 ± 1.61, 268.12 ± 1.63 and 269.25± 2.06 and the values after treatment were 2.68± 2.12, 4.51± 1.36, 1.56± 2.37 and 1.09 ± 1.37 in group I, II, III and IV, respectively. Statistically there was significant ($P < 0.01$) reduction in all the groups after treatment.

The mean values of PMN before commencement of treatment were 46.35 ± 7.8, 47.13 ± 3.57, 51.11 ± 8.24 and 51.48 ± 4.36 percent and the values after treatment were 11.05 ± 5.45, 14.93 ± 7.3, 13.73 ± 5.6 and 7.16 ± 5.56 per cent in group I, II, III and IV, respectively.

The EC to PMN ration before commencement of treatment were 10:26.79 ± 8.18, 10:20.59 ± 2.50, 10:34.52 ± 10.62 and 10:25.93 ± 4.51 and after treatment values were 10:4.26 ± 2.01, 10:5.46 ± 2.77, 10:4.56 ± 1.96 and 10:2.51 ± 2.00 in group I, II, III and IV respectively. Statistically the percentage of PMN and EC to PMN ration was significantly ($P<0.01$) reduced in all the groups after treatment.

The conception rate was 100%, 83.33%, 66.66% and 50.00% in group I, II, III and IV respectively.

From this study it is concluded that the reduction might be due to the ecbolic effect of oxytocin and the action of antibiotic. Gentamycin plus Oxytocin I/M was more effective for treatment of equine endometritis and to improve fertility in equine breeding.

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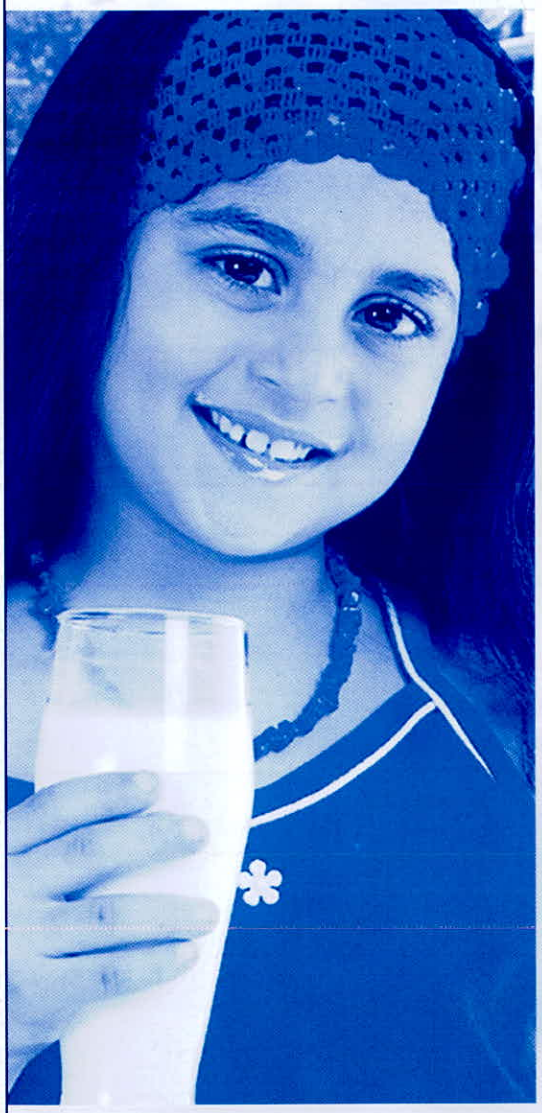

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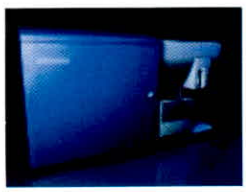

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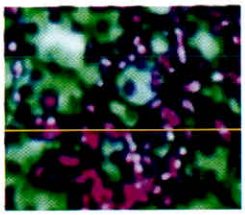

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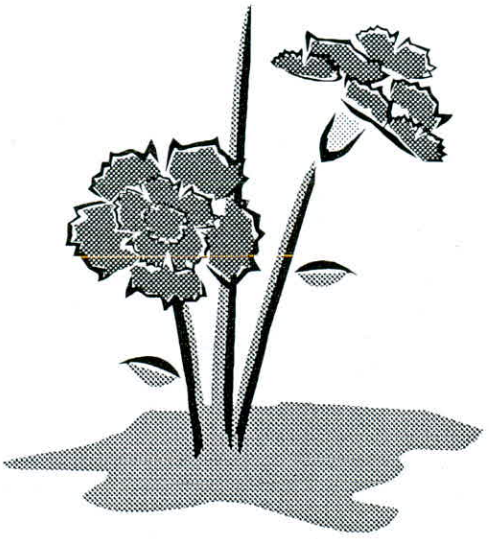

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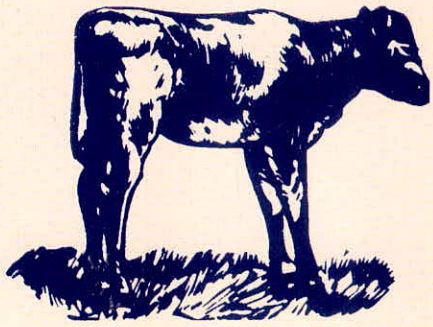

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