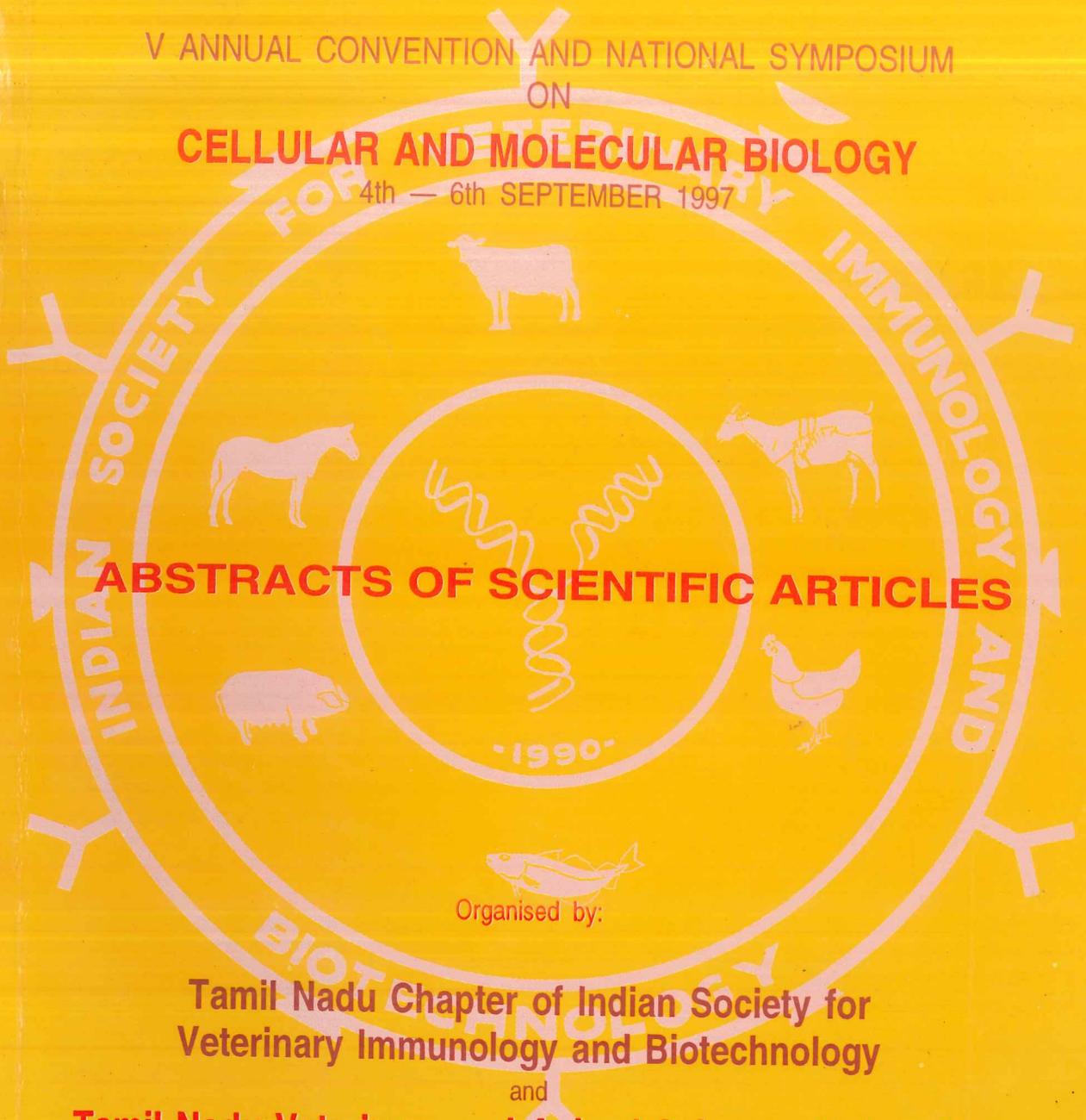


**INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY  
AND BIOTECHNOLOGY**

V ANNUAL CONVENTION AND NATIONAL SYMPOSIUM  
ON

**CELLULAR AND MOLECULAR BIOLOGY**

4th — 6th SEPTEMBER 1997



**ABSTRACTS OF SCIENTIFIC ARTICLES**

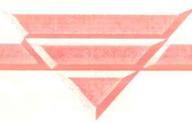
Organised by:

**Tamil Nadu Chapter of Indian Society for  
Veterinary Immunology and Biotechnology**

and

**Tamil Nadu Veterinary and Animal Sciences University**

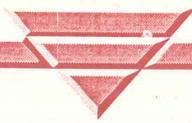
**Chennai - 600 007**



With Best Wishes  
from

Dr. SHANTHA KUMAR

Axon Drugs Pvt. Ltd.  
Chennai



no  
22.9.97

**INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY  
AND BIOTECHNOLOGY**

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Chennai - 600 007**



## Tamil Nadu Veterinary and Animal Sciences University

CHENNAI 600 007

**Dr.S. SHANMUGASUNDARAM**, Ph.D.,  
Vice Chancellor.

Dated: 28.7.97

### MESSAGE

I am happy to note that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and its Tamil Nadu Chapter is organizing its fifth annual convention and national symposium on "Cellular and Molecular Biology" from 4.9.97 to 6.9.97 at Madras Veterinary College, TANUVAS.

I hope that the Symposium on Cellular and Molecular Biology will discuss the important aspects in livestock production, protection, livestock products and Immunology and Biotechnology in the Veterinary curriculum. The discussions during the different scientific sessions and the recommendations drawn will be useful in the field of Animal Biotechnology in the Veterinary Faculty. The souvenir released during the symposium will be useful for the scientists working the field of Veterinary Immunology and Biotechnology.

I wish all the success for the fifth annual convention and National Symposium on Cellular and Molecular Biology.

S/d.  
**S. Shanmugasundaram**  
Vice Chancellor  
TANUVAS

Telex No:041-5049 MVC IN  
Fax:0091-44-581886  
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## Tamil Nadu Veterinary and Animal Sciences University

CHENNAI 600 007. INDIA

**Dr.V.D. PADMANABAN**, M.V.Sc.,Ph.D.,F.I.C.A.I.,  
REGISTRAR

Dated: 1.9.97

### MESSAGE

I am extremely delighted to note that V Annual Convention and National Symposium on 'Cellular and Molecular Biology' is being organized by the Indian Society for Veterinary Immunology & Biotechnology and its Tamil Nadu Chapter at Madras Veterinary College, during 4th to 6th September, 1997. I learn that many scientists from all over India are participating in the symposium. Different sessions including Production Biotechnology, Protection Biotechnology & Immunology, Products Biotechnology, Embryo Transfer Biotechnology and Immunology and Biotechnology in Veterinary curriculum are being conducted. This is the right time to take steps to introduce Biotechnology discipline into the Veterinary curriculum. I expect that senior scientists and academicians who are participating in the Symposium will take suitable steps and give recommendations for speedy introduction of Biotechnology into the Veterinary curriculum.

I am sure that all the scientific sessions will bring about fruitful results and recommendations for the development of Immunology and Biotechnology. I wish the Convention and National symposium a grand success.

S/d.  
**V.D. Padmanaban**  
Registrar

**Capt. Dr.D. EBENEZER**  
M.V.Sc., (ANIMAL NUTRITION) DPAD(IIM)  
DIRECTOR  
DEPARTMENT OF ANIMAL HUSBANDRY  
Chennai - 600 006.

Off:454214  
Fax:457413  
Res:618699

Date: 29.8.97

## MESSAGE

I am delighted to learn that the Fifth Annual Convention and National Symposium on "Cellular and Molecular Biology" has been organised jointly by the Indian Society for Veterinary Immunology and Biotechnology - Tamilnadu Chapter and TANUVAS from 4.9.97 to 6.9.97.

During recent years 'Biotechnology' has emerged as a very dynamic scientific faculty, extending its application on almost every aspect of life. The organisers have rightly chosen vital applications of Biotechnology in Veterinary medicine, viz., Livestock Production Biotechnology, Protection Biotechnology, and Embryo Transfer Biotechnology as the theme of this Symposium.

I am confident that the discussions, deliberations, and decisions made during this meet, meaningful application of Biotechnological techniques in the field of veterinary medicine and livestock production. I am sure the Souvenir brought out on this special occasion will be very useful to the scientific community at large.

I wish the fifth annual convention and the national symposium a very grand success.

S/d.  
**Dr.D. EBENEZER**

# INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY

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TANUVAS

# ISVIB TAMIL NADU CHAPTER

## V Annual Convention and National Symposium on Cellular and Molecular Biology

September 4-6, 1997

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# LIST OF COMMITTEES FOR THE CONDUCT OF FIFTH CONVENTION AND NATIONAL SYMPOSIUM ON CELLULAR AND MOLECULAR BIOLOGY

4-6th SEPTEMBER 1997

<b>Reception Committee</b>	Chairman : Dr.R. Jayakumar Members : Dr.K. Vijayarani Dr. Ramesh Dr.G. Thirumurugan Mr. Velmurugan
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Dr. Latha  
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**NATIONAL SYMPOSIUM ON  
"CELLULAR AND MOLECULAR BIOLOGY"  
&  
FIFTH CONVENTION OF ISVIB**

**PROGRAMME**

**THURSDAY, 4.9.1997**

Registration of Delegates	: 10 a.m. to 11 a.m.
Inaugural Session	: 11 a.m. to 1.15 p.m.
Welcome Address	: Dr.K. NACHIMUTHU Secretary - ISVIB & Professor & Head, Dept. of Animal Biotechnology.
Presidential Address	: Dr.V.D. PADMANABAN President-ISVIB & Registrar, TANUVAS
Inaugural Address	: Dr.S. RAMACHANDRAN Secretary (Retd) Dept. of Biotechnology Government of India, New Delhi.
Release of Souvenir & Special Address	: Dr.S. SHANMUGASUNDARAM Vice Chancellor, TANUVAS.
Receipt of Souvenir & Special Address	: Capt. Dr.D. EBENEZER, Director of Animal Husbandry, Government of Tamil Nadu.
Felicitation	: Dr.I. ALFRED JAYAPRASAD, Dean, Faculty of Basic Sciences, TANUVAS.
Vote of Thanks	: Dr.A. ALBERT, Organising Secretary - ISVIB V Convention and Professor & Head, Central University Laboratory, Madhavaram.
Lunch	: 1.30 to 2.30 p.m.

**Scientific Session I:  
PRODUCTION BIOTECHNOLOGY**

**2.30 - 4.00 p.m.**

- Key Note Address : 1. Mycotoxins in Feed: A universal problem and the natural, novel biotechnological solutions.  
Dr.G. Deva Gowda, Dept. Poultry Science, Bangalore.
2. Role of Environmental physiology in Animal Production.  
Dr. Khub Singh, Director, N.I.A.N. Bangalore.

**CONTRIBUTORY PAPERS**

Tea : 4.00 p.m. - 4.15 p.m.

**Scientific Session II:  
PROTECTION BIOTECHNOLOGY & IMMUNOLOGY  
4.15 - 6 p.m.**

- Key Note Address : Vaccination, Perceptions, Progress & Prospects of Rinderpest eradication from India.  
Dr.M. Rajasekar, Project Co-ordinator, AICRP on Animal Disease Monitoring & Surveillance, IAH & VB Campus, Bangalore.

**CONTRIBUTORY PAPERS**

**FRIDAY - 5.9.97**

**Scientific Session III:  
PROTECTION BIOTECHNOLOGY & IMMUNOLOGY (Contd)  
9.00 - 11.30 a.m.**

**CONTRIBUTORY PAPERS**

Tea : 11.30 - 11.45 a.m.

**Dr. Richard Masilamony Oration Award Function : 11.45 a.m - 1.00 p.m**

- Oration : Dr.P.K. Uppal, Project Director (Rtd), National Research Centre on Equines. Retrospective and Prospective Development of Vaccinology In India

**Scientific Session IV:  
EMBRYO TRANSFER BIOTECHNOLOGY**

2.00 - 3.30 p.m.

Key Note Address : Laboratory Production of IVF embryo in Farm Animals: Role of growth factors.  
Dr.S.M. Totey, Scientist IV & Head,  
Embryo Biotechnology Lab, N.I.I., New Delhi.

**CONTRIBUTORY PAPERS**

Tea : 3.30 - 3.45 p.m.

**Scientific Session V:  
PRODUCTS BIOTECHNOLOGY**

3.45 - 5.30 p.m.

Key Note Address : 1. Patenting in India  
Dr.H.M. Jagannatha, Astra  
Biochemicals India, Bangalore.  
2. Current status of Veterinary Vaccine  
Production in India.  
Dr. V.A. Srinivasan, General  
Manager, Indian Immunologicals,  
Hyderabad.

**CONTRIBUTORY PAPERS**

General Body Meeting : 5.30 p.m.

**SATURDAY, 6.9.97**

**Scientific Session VI:  
Immunology & Biotechnology in Veterinary Curriculum**

9.00 - 11.00 a.m.

Key Note Address : 1. Dr.M.V. Subba Rao, Professor &  
Head, Dept. of Microbiology,  
College of Veterinary Science,  
Tirupathi.  
2. Dr.K.S. Palanisamy,  
Professor & Head,  
Dept. of Microbiology,  
Madras Veterinary College.

Tea : 11.00 a.m. - 11.15 a.m.

**Plenary Session  
11.15 a.m. - 12.00 noon**

**Validatory Function  
12.00 - 1.00 p.m.**



**Prof. B.B. Mallick**

## FIRST PRESIDENT OF ISVIB

**Prof. B.B. Mallick**, D.Sc. (Sorbonne), FAI, FNAVS,  
Vice Chancellor  
West Bengal University of Animal &  
Fishery Sciences,  
Calcutta - 700 037.

**Prof. B.B. Mallick** — 'The founder member and first president of ISVIB (1990-94)' was born on 1st August, 1935, did his graduation from Calcutta University and subsequently graduation in Veterinary Sciences from Bengal Veterinary College, Calcutta. He did his postgraduation thesis from IVRI and went to Pasteur Institute/National Veterinary School, Maisons - Alfort, Paris and completed doctoral and post doctoral work leading to D.Sc. from Sorbonne University (Paris).

He was appointed as Professor of Bacteriology and Virology, IVRI, in the year 1969 and thereafter he held various other positions like Project Coordinator (RVP); Advisor, Andhra Pradesh Agriculture University; Head, Division of Veterinary Public Health; Division of Virology and also Station Incharge, Mukteswar - Kumaon Campus of IVRI. He was Professor of Eminence, officiated as Director, IVRI and Central Institute for Research on Goat, Makhdoom earlier. Dr. Mallick served as Joint Director (Academic) and Dean and Joint Director (Research) at IVRI.

**Prof. Mallick** has done work on Salmonella, Brucella, Clostridia, Mycoplasma, New Castle disease, IB, IBD, ILT, Rabies Virus, Sheep Pox etc. His special interest has been Viral Ecology, Infectivity and Viral Immunity. His work on immunostimulant, its polyclonal response, CMIR and immunomodulatory effect did reveal new scientific information.

In recognition of fundamental contribution he was given the following **International Awards and Prizes**:

1. **Weber Price** - by National Veterinary Academy, France
2. **Medal of Honour and Citation** - by the President, Republic of France.
3. **Prof. K.S. Nair Prize** (Twice - 1974, 1976) - by Indian Veterinary Association.
4. **Dr. P.Richard Masillamony oration award** by (1994) ISVIB.

**Prof. Mallick** has 237 Research publications-International and National Journals of repute to his credit and he has edited two books and contributed chapters in many text books. He is on the editorial board of the International Journal of Veterinary Microbiology, Immunobiology and Infectious Disease. He has served on the Editorial Board on all the important Scientific Indian Journals connected with Animal Science.

**Prof. Mallick** has served as the President, Indian College of Allergy and Applied Immunology, Vice President, Indian Association of Virologist, Vice-President, Indian Tissue Culture Society, Vice-President, Indian Association of Veterinary etc. He has elected as President of the Section of Medical and Veterinary Sciences for the 77th Session (1989-90) of the Indian Sciences Congress. He was President of the Indian Society for Veterinary Immunology and Biotechnology.

**Prof. Mallick** was invited to deliver ICAI - 1988 Oration and was awarded the Gold Medal.

**Prof. Mallick** has worked as consultant with WHO on the Ecology of Influenza viruses. He has also served in the faculty of FAO/SIDA, International Course in Sweden leading to FRVCS. He delivered a series of lectures on tropical animal diseases.

He was invited by the XXII World Veterinary Congress authorities to chair the session on Immunostimulant held at Montreal, Canada, 1988 and also visited Plum Island USA and WHO International Immunology Laboratory, Paris.

He was invited by the XIII International Congress of Allergology and Clinical Immunology, Montreux, Switzerland during 16-21 October, 1988 to present a paper and lead a group discussion and also visited WHO International Laboratory of Immunology, Paris, France.

He did visit Pakistan during November, 1989 in a delegation for assessing the scope for the collaboration work between the two countries in the field of Animal Science in general and Animal Health in particular.

He also visited France, USA (Washington DC, Maryland Univ., California, Davis, Los Angeles), Tokyo and Bangkok during September, 1990 to see the latest technologies being practiced in the leading laboratories in the field of Immuno-Biotechnology and Viral Immunology.

**Prof. Mallick** was invited to chair the session on "Current Status of Viral Vaccine" at XXIV World Veterinary Congress held in August, 1991 at Rio de Janeiro, Brazil.

**Prof. Mallick** has been nominated by the Indian National Science Academy, New Delhi for a Scientific Exchange Visit to France during 1993-94, as an outstanding Scientist of International repute.

The pinnacle of glory came to **Prof. Mallick** with the "Felicitation" given to him by the 3 National Academies of France.

The National Academy of Sciences, National Academy of Medicine and National Academy of Veterinary Sciences of France felicitated **Prof. Mallick** on his contribution in the field of Veterinary Science in general and Viral Immunity in particular.

**Prof. Mallick** was appointed as first Vice-Chancellor of the West Bengal University of Animal & Fishery Sciences in the beginning of 1995 for a tenure of four years. He has put all his endeavour to develop the infrastructure for this newly established University, which is second of its kind in this country. He has organized three faculties viz. 1. Faculty of Veterinary Sciences and Animal Husbandry, 2. Faculty of Dairy Technology and 3. Faculty of Fishery Sciences in this University. The much needed research programmes have been given priority and research projects in all sectors have been planned and started. The Extension Education programme has also got a boost so that the State of West Bengal can get the benefit of improved germplasms and required technology transfer for agrarian upliftment. The conservation of available germplasms and their suitable eco-friendly distribution has been planned.

On behalf of the ISVIB the secretary ISVIB thanks Prof. B.B. Mallick and place it in the record the yomen service rendered by him for the Association during his tenure.

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**PRODUCTION  
BIOTECHNOLOGY**

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# 1. Polymorphism of Exon 2 of the Bola-DRB3 Gene in Buffaloes Detected by PCR-RFLP

T.V. Aravindakshan & A.M. Nainar

Dept. of Animal Biotechnology, Madras Veterinary College,  
Chennai - 600 007.

Polymerase chain reaction (PCR) primers specific to exon 2 of the bovine lymphocyte antigen (BoLA)-DRB3 gene was used successfully to amplify the equivalent region in 34 Murrah and 36 Surti buffaloes selected at random. The 304 bp amplified product of the DRB3 gene was separately digested with BstYI, HaeII and RsaI enzymes. Digestion with BstYI enzyme did not reveal any polymorphism and all animals showed a single restriction pattern 'b' previously described for cattle. Digestion with HaeII resulted in five patterns of which four patterns corresponded to the HaeII patterns previously reported in cattle. A new HaeII pattern was observed in both the breeds of buffaloes studied. The fragment analysis with RsaI revealed 13 different RFLP patterns either in homozygous or heterozygous form. All these RsaI patterns corresponded to the RsaI pattern previously described for cattle. The high degree of similarity in the RFLP patterns of cattle and buffalo observed in the present study provided evidence for the strong conservation of restriction sites previously reported in cattle.

## 2. Karyological studies in Rabbits and Hares

P. Kumarasamy, P. Thangaraju & A. Mahalinga Nainar

Dept. of Animal Biotechnology, Madras Veterinary College, Tamil Nadu  
Veterinary and Animal Sciences University, Chennai - 600 007.

The leukocyte culture technique was adapted for the karyological studies in rabbits and hares. Eighteen male and ten female adult rabbits and two adult female hares were utilised for this study. Two mitogens, namely pokeweed mitogen and phytohemagglutinin and their combination were compared for their efficacy in terms of mitotic drive and mitotic index. The mean mitotic drive were  $65.14 \pm 0.24$ ,  $58.99 \pm 1.05$  and  $66.16 \pm 0.97$  per cent for pokeweed mitogen, Phytohemagglutinin and their combination respectively. The corresponding mitotic index were  $2.37 \pm 0.21$ ,  $1.46 \pm 0.18$  and  $3.25 \pm 0.24$  per cent respectively. A comparison showed that the pokeweed and its combination with phytohemagglutinin was found to be significantly superior than phytohemagglutinin in terms of mitotic drive and mitotic index.

Chromosomal spreads of male and female rabbits were prepared and it was observed that the diploid chromosome number in rabbits studied was 44 ( $2n = 44$ ). Morphological study of the rabbit chromosome revealed that 17 autosome pairs were either metacentric or submetacentric or subtelocentric chromosome and four autosome pairs were acrocentric. The X chromosome was the third largest submetacentric and Y chromosome was the smallest submetacentric chromosome. G- and C-banding were carried out for identification of the individual chromosomes of the rabbit and chromosome was numbered according to the banding pattern.

The diploid chromosome number in South Indian hare was  $48(2n = 48)$  comprising of 46 autosomes and two sex chromosomes. Among the autosomes 16 were submetacentric or acrocentric, 26 were submetacentric and four were acrocentric. The X chromosome was the one among submetacentric chromosome. The individual chromosomes were identified based on G-banding pattern.

### 3. Breed Characterization and differentiation by RAPD analysis

Dr. A Mahalinga Nainar & C. Annapoorani

Dept. of Animal Biotechnology, Madras Veterinary College, Tamil Nadu  
Veterinary and Animal Sciences University, Chennai - 600 007.

Genomic DNA was isolated from Murrah, Surti, Non-descript buffaloes and Jersey cross bred cattle. The average yield of DNA from 10 ml of blood was 381  $\mu$ g. The Genomic DNA were amplified for Random Amplified Polymorphic DNA (RAPD) analysis employing seven sets of random oligonucleotide primers (10 mer). The PCR amplified products from individual template DNA as well as the pooled DNA from each breed revealed RAPD band pattern on agarose gel electrophoresis. Two primers NM<sub>2</sub> and NM<sub>3</sub> identified breed specific bands approximately at 0.5 kb size in Murrah and 1.3 kb in Surti. The Jersey cross bred cattle produced entirely different RAPD pattern compared to buffalo breeds. The frequency of breed specific markers in Murrah, Surti and Non-descript was found to be 0.85, 0.07 and 0.21 for NM<sub>2</sub> and 0.42, 0.71 and 0.57 for NM<sub>3</sub>, respectively. Band sharing between the breeds for the primer NM<sub>2</sub>/NM<sub>3</sub> was 0.50/0/0.90 in Murrah and Surti; 0.93/0/80 in Surti and Non-descript and 0.46/0.88 in Non-descript and Murrah. These observations indicate that the RAPD analysis employing short random primers will be useful in breed characterisation and breed identification.

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**PROTECTION  
BIOTECHNOLOGY  
AND  
IMMUNOLOGY**

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#### 4. Studies on some mouse anticattle monoclonal antibody-probes for buffalo (*Bubalus bubalis*) leukocytes

Dr. Pradeep Singh & Dr. A.A. Grewal

Department of Immunology, College of Veterinary Science,  
Punjab Agricultural University, Ludhiana - 141004, INDIA.

Water buffalo (*Bubalus bubalis*) is a very important dairy animal, however the knowledge about its immune system is quite scanty. Among the diverse leukocytes, the lymphocytes are the key cellular elements that determine immunological competence and specificity, while monocytes/macrophages and granulocytes function as accessory cell types in diverse immune responses. The monoclonal antibody-probes are useful to characterise the cell surface markers on the leukocytes as well as to understand the ontogenic development pathways of the diverse leukocyte subpopulations and resolve highly complicated network of immune system. It is understood that inspite of likely unique features of buffalo immune system, some of the monoclonal antibody-probes available for cattle leukocytes may share some evolutionary conserved surface molecules and specificities with that of buffalo leukocytes. In the present study, the application of some anticattle leukocyte monoclonal antibody-probes i.e. CC15 (BoWC1), IL-A11 (BoCD4), IL-A24 (monocytes/macrophages and granulocytes), IL-A43 (BoCD2, IL-A105 (BoCD8), IL-A111 (BoCD25), J11 (bovine MHC class II) and VPM30 (B cells) for characterisation of water buffalo immune system was studied. Surface phenotype analysis of *Theileria annulata* parasitized cow calf lymphoblasts vaccine cell line developed in this laboratory was also conducted where in two additional monoclonal antibodies IL-A2 (BoIgG) and IL-A30 (BoIgM) were also used. In addition to the above mentioned monoclonal antibodies, anti bovine IgG FITC conjugate was also used as marker of B cells.

The anticattle monoclonal antibodies (CC15, IL-A11, IL-A43, IL-A111, J11, VPM30 and anti-bovine IgG FITC conjugate) stained statistically similar proportions of cells in peripheral blood mononuclear cells (PBM) of water buffalo ( $40.03 \pm 13.7$ ;  $19.58 \pm 5.72$ ;  $45.66 \pm 6.27$ ;  $3.00 \pm 2.62$ ;  $40.88 \pm 10.60$ ;  $21.24 \pm 4.1$  and  $25.61 \pm 7.42$  respectively) when compared with cattle PBM ( $31.49 \pm 8.52$ ;  $21.2 \pm 4.35$ ;  $42.94 \pm 6.12$ ;  $2.80 \pm 2.24$ ;  $40.88 \pm 10.60$ ;  $23.98 \pm 8.48$  and  $23.02 \pm 8.86$  respectively). IL-A24 positive cells were higher in water buffalo calves ( $25.44 \pm 12.31$ ) as they were found to be statistically different from cattle calves ( $13.25 \pm 8.85$ ) at 5% level of significance but not at 1% level. IL-A105 positive cells in PBM from buffalo calves were also higher than the cattle calves. These higher number of CD8 cells and monocytes may be of significance for low susceptibility of water buffalo to intracellular pathogens. The trend for CD4:CD8 ratio in water buffalo was for a value lesser than one, while reverse trend was seen in case of cattle. The *Theileria annulata* cow calf lymphoblasts vaccine cell line developed in this laboratory was found to be a heterogeneous cell line with B cell characteristics.

## 5. Effect of spontaneous fowl pox virus infection on RSV induced sarcomas in broiler chicks

Alka Tomar, A.S. Panisup & B. Singh

Immunology Section, Indian Veterinary Research Institute,  
Izatnagar: 243 122, U.P.

Two trials were conducted in Synthetic Dam Line (SDL) coloured broiler chicks procured from CARI, Izatnagar (Courtesy Dr.B.P.Singh & Dr. Dev Roy). The chicks were infected at 3 weeks of age with BS RSV (RAV-I) @  $10^3$  pock forming units/chick. Hatchmate control chicks were inoculated similarly with normal saline solution alone. The experimental chicks were fed with grower's ration *at lib* and were reared in the same environment.

Tumour formation could be observed in RSV infected chicks by 9-10 days post infection (DPI). Tumour size was measured in 3 different planes at weekly intervals with the help of Vernier calipers. Tumor volume was calculated employing the formula used for determining volume of an ellipsoid i.e.  $\frac{4}{3} \cdot \pi \cdot r_1 \cdot r_2 \cdot r_3$ , where  $r$  is the radius of tumor measured in 3 different planes.

Birds of Trial I, carried out during summer, experienced spontaneous fowl pox infection. Cutaneous pock lesions were observed around 5 weeks of age in RSV inoculated as well as in control chicks. No such infection was seen in chicks of Trial II, carried out during winter.

Tumor volume in the experimental chicks was found to be maximum during III to V weeks post RSV infection. In chicks of Trial I, tumor volume varied between 0.49 to 42.55 cu cm (Mean values  $2.25 \pm 0.77$ ,  $13.5 \pm 5.8$ ,  $7.64 \pm 3.49$  at III, IV and V weeks, respectively). In chicks of Trial II, tumor volume varied between 0.01 to 209.31 cu cm (Mean values  $8.38 \pm 4.34$ ,  $32.79 \pm 14.59$ ,  $51.11 \pm 26.79$  at III, IV and V weeks, respectively). Comparison of tumor volumes in these 2 trials revealed a significant difference ( $p < 10 - 20\%$ ). In spite of dual infection in Trial I, number of birds surviving by VII weeks post RSV infection was higher (42.86%) than in Trial II (28.57%).

The results indicated that spontaneous fowl pox infection in post RSV inoculated chicks provided partial cross protection against RSV induced sarcomas.

## 6. PCR Analysis of 16S rrn Gene Loci of *Salmonella typhimurium*

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Determination of rRNA gene restriction patterns have been successfully used in epidemiological studies for typing of *Salmonella* species using PCR 16s rrn gene loci probe generated by employing primers based on rrn b gene *E.coli*.

A total of twenty poultry isolates along with two reference strains obtained from UK were used in this study. The pathogenicity of each isolate was tested in chick and mice. DNA for PCR amplification was purified by standard methods and a crude preparation of DNA was also used. Crude preparation of DNA was prepared by boiling the bacterial pellet and centrifuging it. The PCR reaction was carried out in 100  $\mu$ l reaction mixture which contained standard PCR mix, template DNA, primers 100 (1  $\mu$ M), and Taq DNA polymerase (2 units). Temperature profile 94°C for 5 min and thereafter 30 cycles of 50°C (1.5 min), and 94°C (1 min). A final extension at 72°C for 7 min was also given. A PCR product of 550 bp was obtained from all the isolates which indicated highly conserved nature of the gene. PCR product was purified, labelled and used as probe for studying different field isolates. Results obtained will be presented and discussed.

## 7. Amplification of Plasmid Associated Virulence Gene by PCR of *Salmonella typhimurium* - Indian Poultry Isolate.

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Virulence region of *S. typhimurium*, *S. dublin*, *S. choleraesuis* and *S. enteritidis* have been found to be located in the 6.4 kb region is almost identical in all the subtypes of *Salmonella*. The standard methods used in studying the relationship between presence of a plasmid and virulence or increase in virulence involves showing the presence of plasmid by isolating it or by Southern hybridization and lethality study by host (mice/chicks). These techniques are time consuming and difficult in applying in large number of samples. On the basis of sequence analysis of *S. typhimurium* mfk A gene and *S. choleraesuis* virulence plasmid mba region a primer pair has been designed which gives PCR product of 472 bp, for detection of the presence of virulence gene by PCR. We have investigated the relationship between lethality and presence of virulence gene in *S. typhimurium* isolates. Lethality was tested by intraperitoneal inoculation and oral inoculation while presence of virulence gene by PCR. In PCR all the *S. typhimurium* isolates (20) gave an identical product of 472 bp while no such product was obtained with *E. coli* and *P. multocida*. The lethality of isolates were different. The isolate which does not show any lethality of very low mortality also generated virulent gene amplified product. Thus, we did not find any correlation between presence of virulence gene and lethality in *S. typhimurium*. In *S. gallinarum* a positive correlation was reported for the presence of 85 kb plasmid, lethality in chicks and amplification of virulence gene. It thus appears that in *S. typhimurium* virulence is governed by other factors also.

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## 8. A Biomolecule of *Str. suis* type 4 - possible candidate for subunit Vaccine

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*Str.suis* is known to cause variety of clinical conditions in swine and other heterologous hosts. Besides being a cause of piglet mortality, it is well known zoonotic agent responsible for meningitis, pneumonia and arthritis in human beings as a emerging pathogen in India. Considering its emerging trend, a preliminary study was carried out to enucleate the potential candidate for subunit vaccine for the type which is prevalent in some parts of the country. The fractionization of *Str. suis* type 4 through gel chromatography revealed seven protein fractions of molecular weights ranging from 20 KD - 88 KD.

The immune response against fractions 71 KD, 57 KD, 20 KD, and wc antigen of *Str. suis* type 4 was studied by inoculating these fractions into rabbits. On precipitation test, 57 KD fraction was found best in its antigenic nature. However, in mouse protection test, the 44 KD fraction was found both protective as well as anigenic in nature and is a suitable candidate for subunit vaccine.

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## 9. Foot and Mouth Disease in Indian Elephants

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During early 1997, two elephants aged 7 and 45 years, respectively, maintained at Children's Park, Guindy, Chennai, suffered from Foot and Mouth disease (FMD) for a fortnight. Both the elephants showed vesicular lesions in the trunk, mouth and feet. Trunk epithelial tissue collected from one of the elephants was proved to contain 'O' type of FMD virus antigen in micro complement fixation test. Epidemiology of FMD in elephants is discussed.

## 10. Usefulness of Plate ELISA for the Detection of Antibodies in Leptospirosis

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&

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Plate enzyme-linked immunosorbent assay (ELISA) has been standardized for the detection of leptospiral antibodies using pooled antigens. Human sera samples from suspected patients were analysed. The antigens for coating the plates were prepared by sonicating the leptospiral cell pellet from the serovars *autumnalis*, *australis*, *icterohaemorrhagiae* and *patoc*. The conjugate used for this study was Protein A - horseradish peroxidase conjugate. The results obtained were compared with the standard microscopic agglutination test (MAT) and usefulness of ELISA for routine antibody detection is discussed.

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## 11. Evaluation of Infectious Bursal Disease Virus Oil Emulsion Vaccine of Bursal Tissue Origin under Experimental Conditions.

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Inactivated IBDV OEV was prepared by incorporating IBDV antigen prepared from the infected bursa using field isolate. Three batches of vaccine each containing IBDV antigen  $10^3$ ,  $10^4$  or  $10^5$  CID<sub>50</sub> were evaluated under experimental conditions. IBDV OEV produced satisfactory seroconversion when vaccine was given to 3-week old seronegative chicken. Though single vaccination of IBDV OEV containing IBD antigen with  $10^5$  CID<sub>50</sub> to chicks at 21-days of age itself showed seroconversion, the method of preparation involves ultracentrifugation. In spite of observing high seroconversion when the chicks were given single IBDV OEV at 28-days of age with any of the three batches, the double vaccination of chicks with either at 14 and 21 or 14 and 28-days of age was preferred for conducting field trials in five flocks, as majority of the outbreaks were in the age group of 2 to 6-weeks with average mortality of 50% in 5 to 6-week old chicken.

## 12. Molecular Characterization of Fowl Pox Virus Isolate

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Six Fowl Pox Virus (FPV) isolates were obtained from field outbreaks and characterized by conventional techniques. The restriction profiles of the isolates were studied using Hind III and EcoRI enzymes. Complete digestion and good resolution of DNA fragments were obtained for the isolates with both the enzymes with minor variations in few isolates.

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## 13. A Rapid Slide Coagglutination Test for Detection of Egg Drop Syndrome -76 Virus Antigen

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&  
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Egg drop syndrome-76 antibody coated *Staphylococcus aureus* strain Cowan I (Containing protein A) was used in a rapid Coagglutination assay for the detection of Egg drop syndrome-76 virus antigen. The reaction was found specific and equally sensitive to the haemagglutination test in detection of the Egg drop syndrome-76 virus antigen. The test was easy to perform and rapid as it required only 15 to 30 seconds to reveal the positive result. Being a specific serological test, it may be recommended as a rapid diagnostic test for the Egg drop syndrome-76 infection.

## 14. A Rapid Protocol Incorporating Mercuric Chloride Yields Genomic DNA of *Clostridium septicum* Suitable for Cloning.

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*Clostridium septicum* is a gram positive endospore forming bacterium traditionally implicated in sheep, malignant oedema of cattle, necrotic enteritis in chicken, neonatal septicaemia in foals, (Jones *et al.*, 1993), thromboembolic meningoencephalitis in calves (Selmiya *et al.*, 1992) and gas gangrene of Man. Due to extraordinarily high production of DNase by this organism, extraction of genomic DNA of this organism is an arduous task with concomitant difficulties in cloning. A method is presented, based on the ability of mercuric chloride to inhibit DNase activity, high SDS conc. and freeze thaw cycles for extraction of genomic DNA of *C. septicum* suitable for cloning without recourse to cesium chloride, ethidium bromide density gradient centrifugation. The genomic DNA so obtained was of good integrity, yield and quality, suitable for subsequent cloning manipulations.

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## 15. Characterization of Newcastle Disease Virus Isolates from Desi Chicken

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The present study has been undertaken to isolate and characterize Newcastle disease Virus (NDV) strains from Desi chicken.

A total of eleven strains of NDV were isolated in embryonated chicken eggs and identified as NDV by hemagglutination inhibition (HI) test and Virus Neutralization test (VNT). The isolates were characterized by Mean Death Time (MDT), Intracerebral Pathogenicity Index (ICPI), Intravenous Pathogenicity Index (IVPI), stability of hemagglutination at 58°C, Agglutination of mammalian erythrocytes and adsorption of hemagglutination by chicken brain cells. The results of these tests indicated that these isolates were not lentogenic. The isolates were also characterized by Monoclonal antibody typing which placed them in groups C1, E and L. The NDV isolates were passaged in MDBK cells also for differentiating them as velogenic and lentogenic. A 21 mer oligonucleotide probe (complementary to the conserved region of the fusion protein), labelled with <sup>32</sup>P-ATP was also used to differentiate the isolates. The results of these experiments indicated that the eleven isolates studied in this study were of either velogenic or mesogenic in nature but not lentogenic.

## 16. An Improved Method for Concentration and Purification of Infectious Bursal Disease Virus Suitable for Immunoassay

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An attempt was made to develop a simple and effective method to purify the local infectious bursal disease virus (IBDV) isolates grown in the bursa of Fabricius of susceptible chicken by discontinuous CsCl gradient centrifugation using Beckman L7 - 85 Ti 60 rotor. In the first method, the virus was purified from the bursal material by discontinuous CsCl gradient centrifugation after 5 per cent polyethylene glycol treatment (PEG: MW 6000) followed by extraction with an equal volume of trichloro trifluoroethane (Freon).

In the Second method, the virus was purified by discontinuous CsCl gradient centrifugation without PEG treatment and freon extraction. The PEG precipitation followed by Freon extraction method yielded 4.5 mg/ml of purified virus which is 50 per cent higher than the second method, and the purity was further confirmed by SDS-PAGE analysis. The PEG was found to be highly effective in precipitating the particles and Freon was useful in releasing of the intact virion from the bursal homogenate.

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## 17. Use of Polymerase Chain Reaction Assay for Detection of Leptospire in Blood Samples from Human Donors

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&

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Blood samples from human blood donors are usually checked for HIV virus and hepatitis virus. Recently, there have been instances of human leptospirosis transmitted through blood transfusion. In the present study, blood samples from donors obtained from a private Blood Bank were screened by dark field microscopy (DFM) and polymerase chain reaction (PCR) assay. Of 126 blood samples tested by DFM, 38 samples (30.15%) were found to be positive for leptospire. All the 38 samples were tested by PCR assay and 32 samples were found positive with the amplification of 285 bp with the leptospira specific primers. Risk of leptospirosis through blood transfusion is discussed in the paper.

## 18. Importance of Nested PCR for the Detection and Confirmation of Canine Parvovirus

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No other disease in Veterinary medicine has created public interest like the recent outbreaks of canine parvoviral enteritis. By using primers based on the sequence of the portion of the canine parvovirus (CPV) genome, a rapid and specific assay for identification of the polymerase chain reaction (PCR) was established. A specific 1 kb PCR amplicon was obtained. For further confirmation of the PCR amplicon, nested PCR was carried out using the 1 kb PCR product and a 600 bp specific sequence was amplified. The primers used consisted of one inter primer lying within the 1 kb PCR product and an outer primer of the first set. These results suggest that nested PCR assay can not only detect the presence of CPV in dogs but also can confirm the specificity of PCR early enough to prevent secondary infection in veterinary hospitals.

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## 19. Detection of Salmonella Enterotoxigenicity by Polymerase Chain Reaction

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The Polymerase chain reaction (PCR) was evaluated for detection of *Salmonella* enterotoxin determinant *stn*. For PCR, primers which amplified 617 bp fragment of *stn*. were used. A cloned 617 bp large PCG fragment was labelled with digoxigenin and was used as gene probe for the hybridization of analysis of blotted PCR product. A total of 17 strains of *Salmonella enterica* (belonging to different serovars), 2 strains of *S. bongori* and 2 strains of *E. coli* were tested with PCR. All the strains and serovars of *S. enterica* such as serovar Typhimurium, Enteritidis, Dublin, Newport, Typhi, etc. were found to carry *Salmonella* enterotoxin determinant *stn*. However, using CHO cells for testing the enterotoxigenicity, only a limited number of *stn* positive strains (8) showed phenotypically enterotoxin effect (elongation of cells). In contrast to *S. enterica*, *S. bongori* and *E. coli* were found neither genotypically nor phenotypically enterotoxin positive.

## 20. Application of Polymerase Chain Reaction for Diagnosis of Blue Tongue Virus

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Blue tongue is an OIE List A disease affecting mainly sheep. In this study the polymerase chain reaction (PCR) was used to diagnose and confirm the presence of Blue tongue virus (BTV) in blood samples, chicken embryos and tissue culture fluid. The sheep blood samples were obtained from suspected blue tongue outbreaks in Tamil Nadu and Andhra Pradesh. The primers used for PCR were from genome segment 10 of BTV. The PCR resulted in an amplicon of size 251 base pairs. In this study, PCR detected blue tongue virus antigen in 37.5% of blood samples.

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## 21. A simple and Rapid Procedure for Extraction of High Molecular Weight DNA from *Salmonella gallinarum* DNA Fingerprinting Studies

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DNA fingerprinting studies for subtyping of bacterial species require highly intact and good quality DNA. The present study on extraction of genomic DNA from *Salmonella gallinarum* was carried out by two different procedures viz. neutral SDS - proteinase K lysis method and procedure using CTAB (Hexadecyl trimethyl ammonium bromide). The neutral SDS - Proteinase K lysis procedure could not yield highly intact genomic DNA consistently and copious amounts of exopolysaccharides were interfering the process. Apart from being laborious and time consuming, the method yielded genomic DNA at concentrations of 750-1950 µg/ml and a purity (OD260/OD280 ratio) of 1.23 to 1.50. Whereas the procedure using CTAB was found effective in removing contaminating exopolysaccharides and residual proteins, was rapid and yielded highly intact genomic DNA consistently at concentrations of 3400-5300 µg/ml with a purity of 1.7 to 1.85. The suitability of the DNA obtained by this method for DNA fingerprinting studies was confirmed by restriction digestion and Southern hybridization analysis.

## 22. Use of Recombinant Probe in Characterization of Leptospiral Isolates

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A recombinant probe derived from genomic library of serovar *australis* of *L. interrogans* was selected on the basis of hybridization, which showed clear signals with slot blot containing 21 reference serovars representing 21 serogroups using enhanced chemiluminescence kit (ECL, Amersham). This probe was used for hybridization of Hae III digested Southern blot for characterization of isolates. This method was found to be sensitive and superior to bacterial restriction endonuclease analysis. The results will be discussed.

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## 23. Identification of Newcastle Disease Virus by Slot Blot Hybridization using Digoxigenin Labelled Probe

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Newcastle disease (ND) is a contagious melody causing substantial loss to the poultry husbandry. Therefore, it was felt essential to diagnose ND by a rapid molecular biology tool like nucleic acid hybridization technique. This technique will avoid separation of RNA by electrophoresis. It was accomplished by extracting RNA from clinical materials/infected allantoic fluids/purified viruses by proteinase-K method. These extracted RNA samples were denatured and slot blotted onto nylon membrane. Then the NDV detection probe, sequence of which maps to 5' noncoding region of fusion gene of NDV 5' - ACG GGT AGA AGA TTC TTG ATC CCC GTT GGC - 3' was labelled with digoxigenin by 3' end labelling method. This probe was hybridized with RNA extracted from NDV suspected materials (Velogenic, Mesogenic and Lentogenic isolates). It was concluded that this nucleic acid hybridization technique was found to be highly sensitive, specific and rapid. It can be conveniently employed for detection of even picograms of viral RNA as compared to other conventional tests.

## 24. Rabbit Serum Total T4 and T3 Estimation by Radioimmunoassay

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Serum total T4 and T3 concentration in 29 rabbits of Saidapet Veterinary Hospital Farm, Chennai was estimated by RIA. The animals [12 Nos. - Soviet Chinchilla (2.100 to 3.125 kg) and 17 Nos. White Giant (2.200 to 3.300 kg)] are reared by hanging cage system fed with standard compounded ration, greens and carrots. Total T4 concentration of Soviet Chinchilla and White Giant ranged from 16.86 to 25.51 and 13.83 to 23.60 ng/ml, and total T3 from 0.16 to 3.25 and 0.67 to 2.94 ng/ml, respectively. Animals with fungal dermatitis showed very negligible decline in thyroid hormone values compared to apparently healthy ones. Increase in thyroid hormone values noticed in female animals than in males of both breeds. Within breeds the value was higher in Soviet Chinchilla than in White Giant. Irrespective of breed/sex the weight of the animals found positively correlated with the serum thyroid hormones in rabbits.

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## 25. Adaptation of Canine Adeno Virus - 1 (CAV-1) to MDCK Cells and Its Use in Detecting CAV-1 Antibodies

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Canine adenovirus-1 (CAV-1) is the causative agent of Rubarth's disease, also called as infectious canine hepatitis. The present paper describes the adaptation of CAV-1 to MDCK cells to study its cytopathic effects and also to use it as a source of antigen for the detection of CAV-1 antibodies. The virus was serially passaged 15 times in MDCK cells and titrated. Serum neutralisation test was standardised using the cell line adapted virus for detecting CAV-1 antibodies.

## 26. Generation of Somatic Cell Hybrids by Electrofusion

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Hybridomas are generated by fusing lymphocytes with non secreting cancer cells using polyethylene glycol (PEG), which functions like a cationic glue to adhere the negatively charged cell membrane to one another. Recently, a new technology for the production of hybridomas using precise transient electric fields to induce somatic cell hybridization, a technique referred to as electrofusion has been reported. In this, cells are first exposed to an alternating electrical (AC) field to cause them to align into a "string of pearls" formation in preparation for fusion - a process named 'dielectrophoresis'. The current is then switched to a direct electrical field (DC) to bring about dielectric break down of cell membrane to produce pores. The pores of adjacent cells form small channels between the two cells, which eventually broaden, causing the cells to fuse. When the direct electrical field is removed, the remaining pores in the membrane of the heterokaryon cell close yielding an intact hybrid.

Sixteen electrofusion experiments for the production of hybridomas have been carried out using BTX Electro Cell Manipulator (ECM 200) and the parental cell lines used were splenocytes from Balb/c mice immunized with IBDV and Sp2/0 in the ratio of 4:1. The fusion medium consisted of 0.25 M sucrose, 0.1 mM  $Mg^{2+}Cl_2$  and 0.1 mM  $Ca^{2+}Cl_2$  with pH 7.2. The cells were then treated with facilitator, 0.05% trypsin, at 37°C for 30 seconds, centrifuged and resuspended in ice cold fusion medium. An aliquot of 0.7 ml was placed in the fusion chamber under sterile condition. The cells were exposed to an alternating electric current 29V for 30  $\mu$  sec, which induced alignment of cells. A single square wave electric pulse of 640 V for 10  $\mu$  sec applied thrice which resulted in fusion of aligned cells. The cells were then transferred into culture media and incubated for 4 hours at 36°C and then distributed into 96 well micro titre plates and cultured in HAT selection medium. Hybridoma growth was observed after 14 days. The frequency of hybridoma productions were evaluated.

## **27. The Application of Indirect ELISA for the Detection of Morbillivirus Group-Specific Antigen from the Autopsy Materials of Goat.**

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In May, 1994 the State of West Bengal, first experienced a Rinderpest like disease in caprine and during February, 1995 a massive catastrophe occurred among the goats. The disease was latter confirmed to be the cases of PPR (Peste des Petits Ruminants) caused by a morbillivirus which is closely related to the rinderpest virus. The diagnosis of the disease is usually based on AGPT and CIE tests. It has been observed that to get the visible precipitation line, an optimum concentration of the antigen is required. But in most of the cases the autopsy material (suspected to be PPR) collected under field conditions do not meet the proper requirements and as a result it poses a great difficulty towards the application of the above mentioned tests. To eliminate all these possible practical difficulties efforts were made to develop an Indirect ELISA test to detect the morbillivirus group specific antigen from the autopsy material of goats (viz. spleen, tongue, gum, etc.)

The results of the Indirect ELISA revealed that the maximum concentration of the morbillivirus antigens were present in the tongue, gum and spleen. Moreover, the indirect ELISA test showed the potentiality as a rapid and sensitive technique to detect the morbillivirus antigens which were present in the tongue, gum and spleen. The indirect ELISA test also showed the potentiality to detect the morbillivirus group-specific antigens from the autopsy materials in comparison with AGPT and CIE tests.

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## **28. Antigenic Analysis of Infectious Bursal Disease Virus Isolates using Monoclonal Antibodies**

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The antigenic nature of infectious bursal disease virus (IBDV) isolates from our state was assessed using neutralizing and non-neutralizing monoclonal antibodies (MAbs) obtained from Japan, Germany and USA. Virus neutralization and combined immunoprecipitation and immunoblotting assays were performed using these MAbs. All the isolates tested belonged to the classical serotype I virus group only. No antigenic variation was seen among the IBDV isolates screened.

## 29. Cross-Reactive Cellular Immune Responses Induced by a Live Infectious Bronchitis Virus Vaccine

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Infectious bronchitis virus (IBV) strains have been classified into several serotypes based on virus neutralization tests. However, it has been observed that IBV vaccines not only protect chickens against challenge with the same serotypes but also induce varying degrees of cross-protection against strains of other serological types. The aim of the present study was to establish whether this cross-protection is due to cross-reactive cellular immune responses stimulated by the vaccine virus to serologically distinct strains of IBV.

Two weeks-old specific-pathogen-free chickens were vaccinated with the HI20 vaccine virus intra-nasally. On days 4, 7, 11 and 14 postvaccination (p.v.) spleen mononuclear cells (MNC) prepared from vaccinated and unvaccinated (control) chickens were used in virus antigen-induced lymphoproliferation assays. Supernatants from such stimulated MNCs were used for interleukin -2 (IL - 2) and interferon gamma (IFN - $\gamma$ ) assays. The virus antigens used included a homologous strain M41, a heterologous but partially protected IBV strain 7 and another heterologous but unprotected IBV strain 793/B.

In LP assays on days 11 and 14 p.v., 4/8 chickens responded to the homologous antigen (strain M41), 1/8 to an heterologous antigen (Strin 7) and none to the other heterologous antigen (strain 793/B). When the IL-2 levels in the supernatants of the virus-antigen stimulated cultures from vaccinated chickens were compared, highest levels were seen in IBV strain M41 stimulated cultures followed by IBV strain 7 and 793/B stimulated cultures. IFN- $\gamma$  production was also stimulated by the homologous and one heterologous IBV (strain 7).

These results show that cellular immune responses induced by a live IBV vaccine are cross-reactive and these could have contributed to the variable cross-protection seen in *in-vivo* studies.

### 30. The Effect of Zinc Deficiency on Immune Response in Chicks against Newcastle Disease Viral Infection

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Two feeding trials were conducted using 130 and 80 day-old broiler chicks (VEN COBB) to assess the possible immunomodulatory property of dietary zinc against Newcastle disease virus. The diets providing optimum (50 mg Zn/kg diet) and low (25 or 35 mg Zn/kg diet) levels of zinc did neither suppress nor enhance the immune response. Instead, zinc supplementation exceeding 75 mg/kg diet suppressed the humoral immunity after a lag phase of 7 days of post-vaccination. The cellular immune response of birds did not vary much ( $p < 0.1$ ) between low (25 mg), optimum (50 mg) and excess (75 and 100) zinc diets. The level of zinc in serum, liver and spleen did not vary much among the treatment groups ( $p < 0.05$ ) and failed to reflect the dietary zinc status. Eventhough, the histological changes of Bursa of Fabricius and thymus did show mild lymphoid depletion on low zinc diet birds, this was not reflected on the immune response. It may be concluded that under practical feeding conditions, wherein the diets are likely to be deficient than surplus in zinc, the breakdown in immunity against Newcastle disease virus may not be attributed to the dietary zinc.

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### 31. Efficacy of Bursal Derived Killed Infectious Bursal Disease Vaccine in Experimental Chicks

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An experimental study was conducted to compare the immune potentials of full dose and half dose of bursal derived IBD oil emulsified vaccine (OEV) and a full dose of chick embryo derived IBD OEV. The antibody response was detected by quantitative agar gel precipitation test (QAGPT). Challenge studies were also performed. The bursal derived OEV at full and half dose provided higher immune response and better protection than chick embryo derived OEV, when vaccinated at seventh day of age. A half dose of killed bursal derived OEV is recommended.

## 32. Rabies Vaccine

P. Madusudhan

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If Jenner is associated with small-pox, Louis Pasteur is, besides being the pioneer in the field of evolving a prophylactic treatment, for Rabies. The success achieved in the control of diseases, through application of vaccines, right from the time of these two fathers of vaccinology, has been spectacular. Vaccines that are in general use today are, the products of the development efforts spanning several decades and reflect the technology of times.

Advocates for both live and inactivated vaccines, have been vigorous in their beliefs. The view held by proponents of live modified vaccines, from the point of view of long lasting immunity coupled with their outright condemnation of inactivated vaccines due to adverse reaction are no longer valid in view of the application of scientific advances like cell culture, purification procedures, etc.

Though DNA was known much earlier, it was only in 1953 (Crik and Watson) that it was suggested to be double helix in structure, and once it was established that DNA is the basis of heredity, different disciplines like nucleic acid chemistry, biochemistry etc., made a united approach possible. Gene was characterised, defined and its structure was synonymous with function.

Though endonucleases and ligase made a dent in creating a chimeric molecule (recombinant) DNA, other studies like gene cloning, gene expression, gave rise to the much desired vaccine. The vector system-prokaryotic and eukaryotic have enlarged and even plants have been targeted for vaccine production. The recombinant DNA Rabies vaccine-Raboral by Merieux in France & V.R.G. in USA have revolutionised the concept of wildlife control of Rabies.

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## 33. Novel Adjuvant for Newcastle Disease Live Mesogenic Vaccine

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New Castle disease is endemic in Tamil Nadu especially in Namakkal, the poultry belt of India. Using filter paper sampling technique seroconversion following vaccinations were found not satisfactory. To improve the existing vaccine a novel adjuvant was developed. The adjuvant when mixed with RDVK and given at eighth week of age to the chickens, protective immunity of that vaccine was prolonged compared to the non-adjuvanted RDVK. Comparative efficacy of mineral oil and vegetable oil as an adjuvant was studied. The advantage of the newly developed adjuvant will be discussed.

### 34. Comparative Analysis of Efficacy of Formal Inactivated, Liquid Paraffin Adjuvanated Formalised Vaccine and Groundnut Oil Adjuvanated Formalised Vaccine against Fowl Cholera

W.M. Paul, B.P. Rajkumar, H.K. Mukhopadhyay, T.G. Prabhakar & N. Dorairaj  
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Namakkal - 637 002.

Three types of vaccines viz., formalin inactivated whole cell vaccine, liquid paraffin adjuvanated, formalised vaccine and groundnut oil adjuvanated formalised vaccine against fowl cholera were prepared using a very virulent resident isolate based on the mean death time determined by the mouse bio-assay. The efficacy of the vaccines were tested by challenge exposures. Of these three types of inactivated vaccines, oil adjuvant vaccine was found more potent and efficacious than that of whole cell inactivated aqueous vaccine which is the cheapest in cost analysis. However, groundnut oil adjuvanated vaccine is cheapest in cost analysis. However, groundnut oil adjuvanated vaccine is cheaper and as efficacious as the liquid paraffin adjuvanated vaccine.

### **35. Study on Immune Status of Commercial Layer Chicks against Infectious Bursal Disease (IBD) in Namakkal Area**

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Dept. of Microbiology, Veterinary College and Research Institute,  
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**N.D.J. Chandran**

&

**K. Shoba**

Avian Disease Laboratory Namakkal, Tamil Nadu

The present study was undertaken to assess the decay pattern of IBD maternal antibody in commercial layer by 21 days of age under field conditions. The immune responses of chicks to three different commercial IBDV line 'intermediate' and inactivated vaccines namely A, B and C administered simultaneously on 14th day of age followed by a booster vaccination with live 'intermediate' IBDV vaccine on 24th day of age were studied in six commercial poultry farms for each vaccine. Vaccine B had produced significantly ( $p < 0.01$ ) higher immune response. Analysis of immune response with the present vaccination schedule revealed that the birds remained susceptible between three to six weeks of age.

### 36. Effect of Tuftsin on Embryo Vaccination with Newcastle Disease Virus Vaccine

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Chennai - 600 007.

The effect of tuftsin on embryo vaccination with NDV-F was compared with regular post-hatch vaccination. Initial attempts with NDV-F embryo vaccination resulted in poor hatchability and embryo death. Subsequent experiments employed the use of EMS and 5-FU along with vaccine. Treatment of the vaccine with EMS also resulted in poor hatchability. However, when NDV-F vaccine was treated with 5-FU and used for embryo vaccination, the hatchability was not affected. It is evident from the result, that embryo vaccination resulted in a gradual increase in HI antibody titres from the day of hatch upto a period of 2 weeks of age, thereafter the titres declined gradually. With regard to post hatch vaccination the rise in the antibody titres was observed from second week onwards. The titre reached the peak during 4th week and started declining from the 5th week of age onwards.

When tuftsin was used along with vaccine as immunostilumator, better seroconversion was noticed in both embryo and post hatch vaccination. On challenge, 100% survivability was observed at 5 weeks in both the embryo and post-hatch vaccination birds. The hatchability of tuftsin administered embryos were more than the embryos vaccinated without tuftsin.

Although the antibody titers of both embryo and post-hatch vaccinated chicks differed in the early weeks, not much difference was observed in the later stages. Under the circumstances in the absence of maternal antibodies embryo vaccination will be of great help during the first two weeks of age which is considered as the most susceptible period for picking up NDV infection.

### 37. Antigenic Variation Among Different Isolates of *Pasteurella multocida* in Namakkal and The Efficacy of the Resident Isolate as Vaccine Strain

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Department of Microbiology  
Veterinary College and Research Institute,  
Namakkal

Out of 68 suspected cases of fowl cholera from 43 commercial poultry farms, 15 isolates of *Pasteurella multocida* were obtained comprising of 10 isolates from acute cases and five from chronic cases. The isolates were numbered from FCNI to FCN 15.

Antigenic variation among the different isolates were found out using biotyping, antibiogram, passive mouse protection test and SDS-PAGE. All the 15 isolates were categorised into group I, II, III and IV based on the result of the above tests. It was envisaged that *P. multocida* isolates FCN 7 and FCN 14 were similar in all respects and deviated from other isolates, where as FCN 9 had many characters in common with the other isolates except FCN 7 and FCN 14 and it was also found to be highly virulent than any other. Therefore, FCN 9 and FCN 14 were used for the preparation of fowl cholera vaccines and tested in different commercial farms having problems of fowl cholera.

It was observed that the mortality rate was curtailed only with the vaccine which contained the local resident isolate of the vaccine strain.

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### 38. Usefulness of Monthonys Buffer in Counter Immunoelectrophoresis in Animal Health

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The use of veronal Buffer (VB) is well documented in immunoelectrophoretic in Animal Health. Sodium diethyl barbiturate and diethyl barbituric acid used in VB come under 'Controlled Substances'. Import of these chemicals from many reputed firms is possible only with the production of 'Import permit' from the Drug controller.

This presentation deals with the use of Monthonys Buffer for counter immunoelectrophoresis in Animal Health.

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### 39. Comparison of HI and DIA for Routine Detection of Antibodies to EDS - 76 Virus

Andrew Chandramohan, R. Govindarajan & R.A. Venkatesan

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Madras Veterinary College,  
Chennai - 600 007

Serological tests like Hemagglutination Inhibition, ELISA, Serum Neutralization, Fluorescent Antibody and Agar Gel Immunodiffusion are commonly employed for detection of egg drop syndrome virus antibody. ELISA, Serum Neutralization and Fluorescent Antibody tests are expensive and need sophisticated equipments. A simple, cost effective, pen - side test will be of great advantage for quick detection of EDS-76 virus antibody. The present study was carried out to compare HI and DIA for a quick seromonitoring of EDS-76 virus infection. It is concluded that HI test can best be employed for seromonitoring of EDS-76 virus infection for all practical purposes.

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### 40. Validation of A-B ELISA for Infectious Bovine Rhinotracheitis

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ICAR Project on Animal Disease Monitoring and Surveillance, IAH & VB  
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Infectious bovine rhinotracheitis (IBR), a virus disease of cattle and buffaloes causes respiratory and genital tract infections resulting in considerable economic losses to rapidly developing dairy industry. The disease is caused by bovine herpes virus-1 (BHV-1) and is reported world-wide. The virus causes latent infection and precipitates clinical symptoms in animals under stress conditions.

The present investigation was undertaken to validate Avidin-Biotin Enzyme linked immunosorbent assay (A-B ELISA) for detection of IBR antibodies. The optimisation of A-B ELISA indicated 1:200 dilution of BHV-1 antigen, 1:100 of test control sera and 1:20,000 dilutions of biotinylated antibovine IgG and avidin-HRP conjugate were adequate. Negative/positive cut-off absorbance at 0.2, was double the absorbance of negative control sera. Absorbance of positive control sera ranged from 0.5 to 1.0 at 492 nm.

Serum samples (6,949) collected during 1995-97, were resourced from national serum bank facility of the Animal Disease Monitoring and Surveillance (ADMAS) project, Bangalore. A total number of 5,111 cattle from 22 States and 1,838 buffaloes from 11 States were screened. The overall prevalence was 34.52 per cent with 44.51 and 6.75 per cent in cattle and buffaloes, respectively. The A-B ELISA kit will be introduced in the national network of 32 State level ELISA laboratories for detailed survey.

## 41. Comparison of a Latex based Field Kit with standard Hemagglutination and Hemagglutination Inhibition Test for Newcastle Disease Virus Detection

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Veterinary and Animal Sciences University, Chennai - 600 007.

Rapid field diagnostic kit had been based on the technique of Latex immunoassay for rapid detection of New castle Disease virus (NDV) from suspected tissue sample. The developed kit has been compared with OIE approved Hemagglutination (HA) and Hemagglutination inhibition (HI) tests for NDV. A total of 258 suspected tissues from field outbreaks were tested with LAT and HA & HI. The percentage of positive samples were 69.76 and 63.85 by HA & HI and LAT respectively. Very high significant association ( $p \leq 0.01$ ) was observed between the two tests. The Latex test was found to be 97.63% sensitive and 87.74% specific for the detection of NDV antigen.

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## 42. Development of Rapid Field Diagnostic Kits for Duck Plague

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Dot-Enzyme linked immunosorbent assay (DOT-ELISA) and Latex agglutination test (LAT) have been developed for detecting Duck plague virus (DPV) antigen from field samples. These tests have been compared with conventional Duck embryo inoculation, Virus neutralization test (VNT) and Fluorescent antibody test (FAT). The sensitivity of the Dot-ELISA and FAT were found to be 93.21 per cent and 95.62 per cent, respectively in detecting DPV antigen.

### 43. Effect of Vacuum Packaging on Physico-Chemical and Organoleptic Qualities of Mutton in Different Packaging Materials

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Effect of vacuum packing on physico-chemical, microbial and organoleptic characteristics of mutton packed in low density polyethylene monolayer, low density polyethylene two layer, polyester/polyethylene and multilayer material and stored upto 120 hours at  $4 \pm 1^\circ\text{C}$  and upto 60 days at  $-10^\circ\text{C}$  were studied.

Mutton packed under vacuum in multilayer and stored upto 120 hours at  $4 \pm 1^\circ\text{C}$  recorded the lowest pH.

Highest Munsell colour-hue was noticed in mutton packed under vacuum, in low density polyethylene monolayer material and stored upto 120 hours of duration at  $4 \pm 1^\circ\text{C}$ . Mutton packed by ordinary method, in low density polyethylene monolayer material and stored upto 72 hours at  $4 \pm 1^\circ\text{C}$  recorded the lowest thiobarbituric acid number and the highest appearance, flavour, juiciness and tenderness scores.

Mutton packed under vacuum, in multilayer material and stored upto 30 days recorded the highest moisture percent. Highest Munsell colour value and chroma were observed in mutton packed under ordinary methods, in low density polyethylene monolayer and stored upto 30 days at  $-10^\circ\text{C}$ . Mutton packed under vacuum, in multilayer and stored upto 60 days at  $10^\circ\text{C}$  recorded the lowest tyrosine value and total viable count.

From this study the vacuum packaging method and the multilayer material were found to be the most suitable method and material for storing mutton for short and long periods.

#### ✓44. The Level of Cadmium in Buffalo Muscle and Organs

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The level of cadmium in the muscle and organs of Buffaloes (*Bubalus bubalis*) were compared between those slaughtered at the Department of Meat Science and Technology, Madras Veterinary College, Chennai and those obtained from the Corporation Slaughter house, Perambur, Chennai. The analyses for the toxic element - cadmium was made using Atomic Absorption Spectrometer (AAS) during the years 1991 to 1996.

The level of toxic metal, cadmium in various organs and muscle of buffaloes slaughtered at Madras Veterinary College were below the detectable level ( $< 0.5$  ppm) whereas in the market samples analysed, the heart had the least cadmium content of  $0.69 \pm 0.05$  ppm while the spleen had the highest content of  $1.25 \pm 0.12$  ppm. It was found that the spleen in buffaloes was the organ of choice. The kidney tissue also contained an equally higher concentration of  $1.04 \pm 0.06$  ppm of cadmium. However, the animals did not show any clinical symptoms for cadmium toxicity during Ante-mortem inspection nor lesions during post-mortem examination.

## ✓ 45. Toxic metal - Chromium in Muscles and Organs of Cattle

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Tamilnadu

Tannery industry is well established in Tamilnadu. It has been estimated that vast areas of cultivable land is now polluted with the discharge of tannery effluents. Animals grazing on these areas may contain chromium in their organs and muscle.

A study was made at Madras Veterinary College, Chennai during the year 1993 to 1996 to detect the level of chromium in the fresh muscle and organ samples (liver, kidney, spleen and heart) of cattle (*Bos indicus*) slaughtered at Madras Veterinary College and Market using Atomic Absorption Spectrometer (AAS).

The study revealed that the muscle of cattle slaughtered at Madras Veterinary College had the highest chromium content of  $3.47 \pm 0.33$  ppm and least in heart  $2.63 \pm 0.21$  ppm. Whereas in market samples the highest content of chromium was found in heart ( $8.06 \pm 0.21$  ppm) and least in kidney ( $7.09 \pm 0.07$  ppm). Harrison and Staples (1955) found that the maximum tissue levels for chromate poisoning was to be 30 ppm in liver. In the present study it was below this level. At present there is no standards available for fresh meat in India. The statistical analyses had revealed highly significant ( $p < 0.01$ ) difference in chromium content between the two sources but not between their organs.

## 46. Efficacy of Liquid Nitrogen, Water Ice and Freezing Mixture in Carabeef Preservation

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Tamilnadu

A number of methods for short-term preservation have been tried to retain the initial quality of meat during transport from the place of production to the quality assessment laboratory. However, high cost, non-availability or chemical alteration of the meat disqualified several of the methods.

A study on the comparative efficiency of preservation of carabeef (*longissimus dorsi*) samples in the thermocool containers (height 17.5 cm × width 18.4 cm) with liquid nitrogen, water ice and freezing mixture individually and in combination was made. The odour of the preserved portions and control samples, were tested at intervals of half an hour along with an assessment of pH, moisture, water holding capacity, tyrosine value and thiobarbituric acid number at the time of observation of spoilage by odour.

The results of the investigation showed that the combined preservation with liquid nitrogen and freezing mixture showed the longest period of safe-keeping, namely 45.78 hours, whereas preservation with freezing mixture alone showed a shelf-life of 44.42 hours, with liquid nitrogen and water ice combination keeping the carabeef safe for 43.32 hours.

Considering the availability and the cost of the preservatives explored, it is safe to recommend the wider usage of freezing mixture consisting of water ice and salt in the proportion of 3:1 at 10 kg of freezing mixture for every 1 kg. of meat.

## 47. Faecal Streptococci Organisms in Pork, Ham, Bacon and Sausages

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Tamilnadu.

Pork and pork products like Ham, Bacon and Sausages are popular choice of meat products of convenience. However, these products provide an excellent environment for the growth of microorganisms as it is contaminated during processing and packaging. The overall microbial load in the pork and pork products depend upon the number of organisms in the fresh meat, handling, machineries, hygiene in the slaughter house, types of packaging and time and temperature of storage. The present study concerns about the microbe faecal streptococci contamination in pork and pork products available in the meat shops of Chennai City. The samples were grouped into two. The Group-I comprising of samples manufactured by private companies and Group-II consisting of the samples manufactured and marketed by Madras Veterinary College, Chennai. The sample of Group I and II were further classified into fresh (on the day of purchase) stored (7 days from the day of purchase) and marketed. (15 days from the day of purchase). The samples were subjected to direct plate count method (K.F. Streptococcal agar) and most probable number method (MPN) K.F. The study revealed highest faecal streptococci ( $1.3 \times 10^4$ ) in direct plate count in stored Ham of group II, whereas it was  $1.8 \times 10^3$  in MPN method. The faecal streptococci count was slightly above the suggested level of  $10^3$  for frozen food. The bacon, sausages and pork of both the groups also had faecal streptococci organisms which were below the standard levels. The present study also revealed that samples frozen at  $-10^\circ\text{C}$  for 15 days had low faecal streptococci count in samples of both groups.

## 48. Synchronisation of Estrus in Kheri Sheep with Progesterone and Prostaglandin F2 Alpha

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Estrus synchronisation in sheep is used to improve fertility and to facilitate the use of artificial insemination and embryo transfer techniques. In this trial the efficacy of two synchronisation methods were tested on native Kheri ewes which were to be used as recipients in an embryo transfer programme. During autumn, twenty adult cycling Kheri ewes were randomly allotted to two equal groups. Group-1 ewes were treated with laboratory prepared vaginal pessaries impregnated with 0.35 g progesterone (CDH, India) and kept *in situ* for twelve days. Group - 2, ewes were given two 10 mg does of prostaglandin F2 alpha (Lutalyse, Unichem, India) ten days apart. Estrus was detected by parading aproned rams starting 24 hours after pessary withdrawal or second prostaglandin F2 alpha injection.

Progesterone (P4) treatment induced heat in less (60%) proportion of ewes as compared to prostaglandin F2 alpha (PGF) administration which induced heat in 90% of ewes ( $P < 0.01$ ) within 72 hours of end of treatment. The mean time interval between end of treatment and onset of estrus was later in PGF treated ewes ( $P < 0.05$ ) than P4 treated ewes  $52.0 \pm 3.46$  h. vs  $32.0 \pm 2.44$ h) while the duration of estrus was more ( $36.0 \pm 6.19$ h) in P4 treated ewes  $P > 0.05$ ) as compared to PGF treated ewes ( $24.0 \pm 0.33$  h). The results indicate that though more number of sheep can be synchronised by use of prostaglandin F2 alpha tight synchrony is achieved by progesterone treatment in Kheri ewes.

## 49. Progesterone Profile and Superovulatory Response of Kangeyam Cows Treated with Syncromate B and Folltropin

Joe Arosh, D. Kathiresan, P. Senthilkumar, T.G. Devanathan & K. Nachimuthu

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The objective of this study was to find out the relationship between progesterone profile and superovulatory response in Syncromate-B treated Kangeyam cows. In six Kangeyam cows the oestrus was synchronized with Syncromate-B System. On the day 6 of oestrous cycle (day 0 - behavioral oestrus), all the cows received 6 mg norgestomet ear implant and an intramuscular injection of 3 mg norgestomet and 5 mg oestradiol valerate. The day of implant insertion was considered as day 1 of implant period. The implant was removed on day 9. On the day 7 of implant period superovulatory treatment was initiated with Folltropin. A single injection of 25 mg of PGF $2\alpha$  was administered 12 hrs before implant removal. Artificial insemination was done 36, 48, 60 h after the implant removal. Embryo collection was performed non surgically on day 7 after the first insemination. The mean number of ovulations, total embryo and transferable embryos were  $4.83 \pm 0.40$ ,  $3.5 \pm 0.22$  and  $2.16 \pm 0.31$ , respectively.

The progesterone concentration on the day of SMB implantation was  $1.62 \pm 0.33$  ng/ml. It declined less than 0.5 ng/ml within 24 hours of initiation of SMB treatment and remained below 0.5 ng/ml during the entire implant period and superovulatory oestrus. Afterwards, the progesterone concentration gradually increased and reached the concentration of  $8.87 \pm 0.66$  ng/ml on the day of embryo collection. Progesterone level on the day of implant insertion and embryo collection was positively correlated with superovulatory response, where as the concentration during implant period, on the day of initiation of superovulatory treatment and superovulatory oestrus was negatively correlated. Hence it is concluded from the present study that the progesterone concentration during implant period and on the day of initiation of superovulatory treatment in SMB can not be used to predict the superovulatory response in superovulated cows.

## 50. Oestrus Response and Pattern of Estrum of Kangeyam Cows Treated with Two Synchronization Agents

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The objective of the study was to compare the two synchronization agent on estrus response, pattern of estrum and hormonal profiles in Kangeyam cows. Twelve Kangeyam cows were divided into two groups. In group-I n=6 the estrum was synchronized by administering 25 mg of PGF2  $\alpha$  on day 9 of oestrous cycle. In group-II (n=6) the estrum was synchronized with Syncromate-B system at any stage of the oestrous cycle. The implant was kept *in situ* for 10 days. A single injection of 25 mg of PGF2  $\alpha$  was administered 12 hours before implant removal. The onset and duration of estrum were 76 hrs and 20 hrs in PGF2  $\alpha$  and 48 hrs and 16 hrs in SMB group, respectively. SMB group had significantly early onset and shorter duration of estrum than PGF2  $\alpha$  group. The progesterone concentration on day of estrum was less than 0.5 ng/ml in both the groups. The LH peak concentration was  $10.7 \pm 1.13$  ng/ml in both the groups, but the onset of LH surge was earlier (52h) in SMB group than in PGF2  $\alpha$  group (68h). The oestradiol concentration was  $160 \pm 7.65$  pg/ml in SMB group and 110.47 pg/ml in PGF2  $\alpha$  group. Based on the present study it is concluded that the onset of estrum and LH surge were earlier in SMB group than PGF2  $\alpha$  group. The oestradiol concentration was higher in SMB group than PGH2  $\alpha$  group.

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**EMBRYO TRANSFER  
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## 51. Comparative Efficiency of Different Retrieval Techniques on Recovery Rate, Quality and Maturation of Sheep Oocytes *in vitro*

A. Palanisami, G. Ramathilagam, D. Kathiresan & K. Nachimuthu  
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Follicular oocytes retrieved by different techniques from ovaries obtained from slaughter houses would be the least expensive and most abundant source of oocytes for *in vitro* ovaries were randomly grouped and the oocytes recovery rate, quality and per cent maturation were recorded for each technique. Among the retrieval techniques, the slicing technique (7.45 oocytes per ovary) was found to be the best technique when compared to puncturing (4.44 oocytes per ovary), aspiration (3.28 oocytes per ovary) and post aspiration slicing (1.99 oocytes per ovary). Time taken to process single ovary for collection of oocytes in slicing technique was less than a min. Among the different grades of oocytes collected, the percentage of grade-1 oocytes were higher in slicing ( $34.7 \pm 0.12$ ) technique and grade-11 in puncturing ( $38.0 \pm 0.12$ ) when compared to other retrieval techniques. When oocytes of Grade-1 and Grade-11 were collected by slicing technique and mature *in vitro* yielded higher percentage of maturation 67.04, 2.28 and 57.10, 1.93 respectively.

## 52. Influence Of Ovine and Porcine FSH on the Quality of Embryos in Superovulated Tellicherry goats.

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Twelve healthy, cyclical parous Tellicherry goats weighing between 20 and 30 kgs were divided equally into two groups as FSH-O and FSH-P. The estrous cycle was controlled by using Syncromate-B system (3 mg SMB Implant + 0.5 ml SMB injection). The implant was kept *in situ* for 9 days. The superovulation treatment was initiated on 7th day after implant insertion with 180 mg NIH-FSH-SI in 8 divided equal doses and 180 mg NIH-FSH-PI in 8 divided step down doses in FSH-O and FSH-P group, respectively. All the goats were bred to fertile buck during superovulatory estrus. The embryo collection was performed surgically 3 days after mating and the quality of embryos were assessed and graded. The mean numbers of transferable and non transferable embryos were  $13.16 \pm 1.76$  and  $1.17 \pm 0.40$  in FSH-O group and  $7.61 \pm 1.96$  and  $0.83 \pm 0.43$  in FSH-P group, respectively. It was observed that the total number of embryos recovered, number of fertilized and transferable embryos were significantly higher in FSH-O group than FSH-P group. However the number of non transferable embryos and unfertilized oocytes showed no significant difference among two groups. It is concluded that the FSH-O treatment can be preferred for superovulation than FSH-P to produce more number of transferable embryos in Tellicherry goats.

## 53. Rabbit Peritoneal Fluid as Medium for *in vitro* Maturation and Fertilization of Caprine Oocytes

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An attempt was made to test the efficacy of rabbit peritoneal fluid as a maturation and fertilization medium for caprine oocytes under *in vitro* conditions. For this five different media, i.e., TCM-199, TCM-199 + 20% FCS, TCM-199 + FCS + hormone (FSH, HCG and estradiol 17  $\beta$ , TCM-199 + 50% rabbit peritoneal fluid (rPF) alone were included in the experiment. A total of 907 ovaries were collected and sliced. Slicing produced 3028 usable oocytes. These oocytes were incubated in the above media. The sperm capacitation and acrosomal reaction were induced by exposure of sperm cells to 20  $\mu\text{m}$   $\text{Ca}^{+2}$  ionophore for ten minutes. Matured oocytes and capacitated sperms were co-incubated for 5 hr in a 5%  $\text{CO}_2$  incubator at 38.5°C with 95% humidity in fertilization medium consisting of Fert-TALP supplemented with epinephrine and hypotaurine. After 5 hr of incubation, oocytes were transferred to a culture medium which consisted of TCM-199 + 20% FCS for a further 43 hr, under the same conditions of incubation.

A maximum ( $92.22 \pm 2.26$ ) *in vitro* maturation occurred when oocytes were incubated in rPF along while the TCM-199 (control) gave the minimum maturation rate ( $23.40 \pm 1.89$ ). The remaining three media, i.e., TCM + FCS, TCM + FCS + hormones and TCM + rPF gave maturation percentages of  $56.80 \pm 2.21$ ,  $70.22 \pm 1.95$  and  $80.11 \pm 1.85$  respectively. A total of 652 out of 1255 oocytes (52.16%) were found fertilized (2-4 cell stage) when examined at 48 hr post insemination. It was concluded that rPF can be used as a highly satisfactory medium for the IVM and IVF of caprine immature oocytes.

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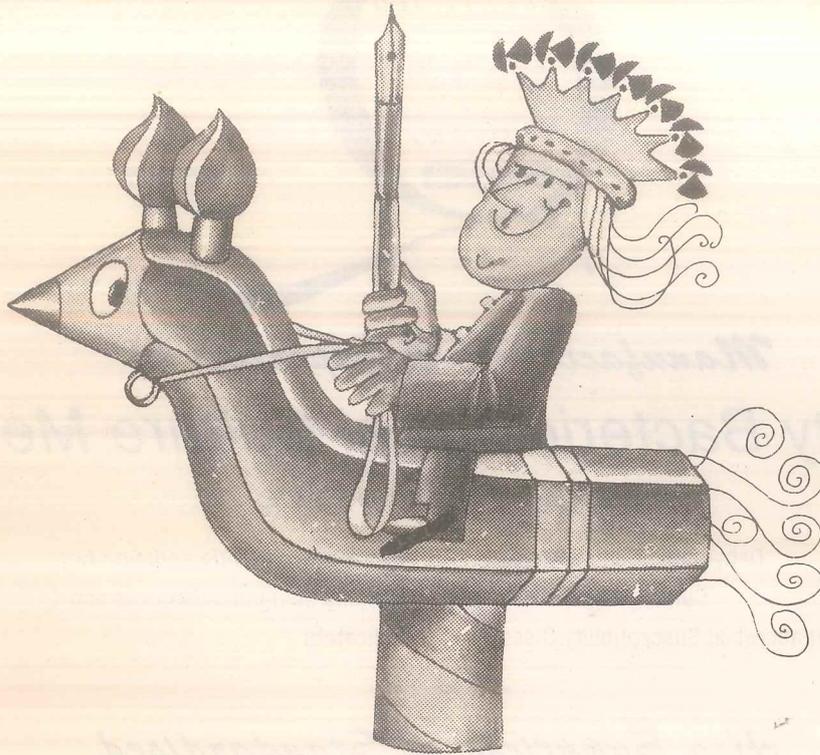
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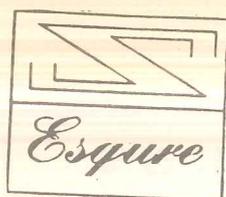
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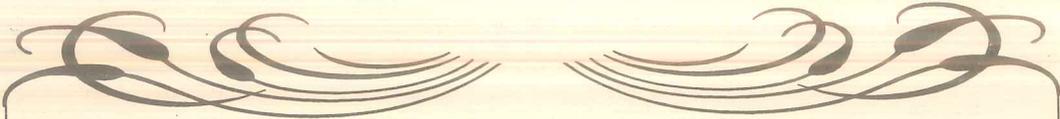
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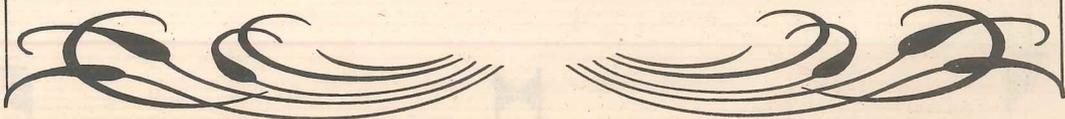
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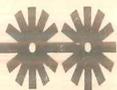
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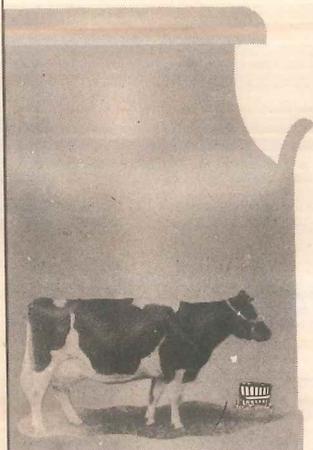
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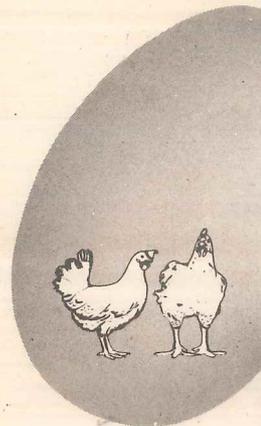
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**Other benefit-packed features include: Low stage design, low-tilt eyepiece tube, eye-level riser, four-filter linear slider, convenient filter-switching mechanism, Nomarski DIC and phase contrast, photomicrography...**

Manufactured by

**Nikon**  
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Sole Distributors in India

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With Best Compliments from



Producer of GIBCO BRL Products

**Which Gibco BRL FBS Product is Right for Your Needs?**

**Sera Descriptions and Usage**

Product	Description/Usage Guidelines	Source	Cat.No.	Size
Certified FBS	<ul style="list-style-type: none"> <li>Undergoes special biochemical/hormonal profile and bacterial tests. Guaranteed low endotoxin (&lt; 15 mg/dl).</li> <li>Use with your most sensitive, precious cells</li> </ul>	United States	16000-036	100 ml
			16000-044	500 ml
Qualified FBS	<ul style="list-style-type: none"> <li>Our most popular FBS product! High quality and exceptional value.</li> <li>Suitable for general applications, especially those that do not require defined biochemical/hormonal profiles or bacteriophage testing</li> </ul>	United States  Countries which meet USDA importation requirements	26140-087	100 ml
			26140-079	500 ml
			26140-095	1,000 ml
			10437-010	100 ml
Dialyzed FBS	<ul style="list-style-type: none"> <li>Serum is dialyzed against 0.15 M NaCl using a 12,000 to 14,000 molecular weight cut-off membrane until the level of glucose is &lt; 5.0 mg/dl as determined by glucose/peroxidase method.</li> <li>Ideal for radiolabeling assays</li> </ul>	United States  Countries which meet USDA importation requirements	10437-028	500 ml
			10437-036	1,000 ml
			26300-053	100 ml
			16300-061	500 ml
ES Cell-Qualified FBS	<ul style="list-style-type: none"> <li>Specially tested for the ability to sustain undifferentiated cell morphology of embryonic stem cells</li> <li>Crucial for the successful maintenance of embryonic stem cells</li> </ul>	United States  Countries which meet USDA importation requirements	10440-014	100 ml
			10440-22	500 ml
			16141-061	100 ml
			16141-079	500 ml
			10439-016	100 ml
			10439-024	500 ml

**GIBCO BRL CUSTOM PRIMERS**

Scale	\$/base	Setup charges \$	MODIFICATIONS														
			Purity (\$ per primer)					Modified bases (additional \$ per base)				5' Modification (additional \$ per primer)					
			Deprotected	Desalted	HPLC	PAGE	Cartridge	Mixed Bases	Inosine (deoxy)	Phosphorothiocates (S-ciligos)	Uracil (deoxy)	Phosphate	Biotin Fluorescein	Rhodamine	Primary Amine	HEX	TET or 6-FAM
50nm (> 5 OD)*	1.49	0	0	0	NA	NA	24	0	8	5	5	25	49	49	49	49	79
200 nm (> 20 OD)*	2.19	0	0	0	75	75	24	0	8	8	5	25	49	49	49	49	79
1 μmole (> 50 OD)*	5.89	0	0	0	100	129	30	0	16	13	10	56	110	110	110	110	NA

\*Minimum yield for unmodified, standard or desalted purity primers > 20 bases; yields for shorter primers or other purifications will be proportionately less.

Price Formula: (Number of total bases) × (\$/base) + (number of modified bases) × (addl.\$/base) + purification charge + 5' modification charge

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