



# XIII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology (ISVIB)



## & National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security"

15-17 February, 2007

**COMPENDIUM**

EcoRI - Apo I 4259  
 Aar II 4281  
 Cla I - BspD I 20  
 Hind III 29  
 EcoR V 185  
 Nhe I 229  
 BamHI I 375  
 Sma I 459  
 EcoS7 I 4046  
 Xba I 3901  
 Sma I 3844  
 Sma I 3787  
 Sma I 3756  
 Pvu I 3733  
 Pst I 3007  
 BsrD I 3002  
 Ase I 3537  
 Bsu I 3433  
 BsrD I 3420  
 EcoS7 I 3000  
 AluNI 2884  
 BsrV I 2682  
 Dri I 2575  
 Ayr III - Pci I 2473  
 BsrB I 2404  
 Ear I 2351  
 Ssp I 2350  
 Nde I 2306  
 BsrAP I 2291  
 Pst217 I - Acc I 2244  
 BsaA I 2225  
 EcoR V 185  
 Nhe I 229  
 BamHI I 375  
 Sma I 459  
 EcoR II 471  
 Sma I 459  
 Sty I 424  
 Sph I 562  
 EcoN I 622  
 Sal I 651  
 Hinc II 651  
 Acc I 651  
 PstAI 712  
 EcoR I 939  
 Nhe I 933  
 PstM I 1315  
 Bam I 1353  
 Sty I 1360  
 PstM I 1364  
 Ase I - SmaB I 1  
 PstM I 1438  
 Msc I 1444  
 Sty I 1447  
 PstM I 1480  
 PpuD I 1580  
 Bgl I 1650  
 BspE I 1664  
 DsaB I 1668  
 Xba I 2020  
 PstM II 2064  
 BsrB I 2122  
 Dri I 2162  
 PfuPI - Tth111 I 2217

**College of Veterinary and Animal Sciences, Pookot,  
Lakkidi P.O., Wayanad, Kerala - 673 576**

# WORLD CLASS SOLUTIONS FOR YOUR LABORATORY REQUIREMENTS



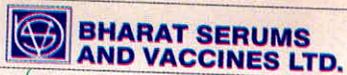
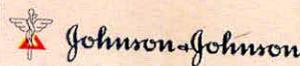
*Reliable Solutions  
Committed Support*



## Chemik Chemicals

XXV / 776/2, T.M.K. Complex,  
Mannath Lane, M.G. Road, Thrissur.

E-mail : chemctr@dataone.in  
Ph : 0487 2339011, 3292385



**XIII ANNUAL CONVENTION OF INDIAN SOCIETY FOR  
VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY  
&  
NATIONAL SYMPOSIUM ON**

**“Veterinary Biotechnology and Immunology for  
Sustainable Rural Livelihood Security”**

**15 - 17 February, 2007**

**COMPENDIUM**

**College of Veterinary and Animal Sciences, Pookot,  
Lakkidi P.O., Wayanad, Kerala, INDIA. PIN 673 576**

**Organized by**



**KERALA AGRICULTURAL UNIVERSITY**



**INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY**



**KERALA STATE VETERINARY COUNCIL**

## MESSAGE

**SHARAD PAWAR**  
MINISTER OF AGRICULTURE  
& CONSUMER AFFAIRS  
FOOD AND PUBLIC DISTRIBUTION  
GOVERNMENT OF INDIA



शरद पवार  
SHARAD PAWAR



कृषि, उपभोक्ता मामले, खाद्य और  
सार्वजनिक वितरण मंत्री  
भारत सरकार  
MINISTER OF AGRICULTURE  
& CONSUMER AFFAIRS  
FOOD AND PUBLIC DISTRIBUTION  
GOVERNMENT OF INDIA

31 January, 2007

### MESSAGE

I am happy to ~~note that~~ Indian Society of Veterinary Immunology and Biotechnology is organizing its XIII annual convention and National Seminar on "Veterinary Biotechnology and Immunology for Sustainable Rural Livestock Security at College of Veterinary and Animal Sciences, Pookot, Wayanad, Kerala from 15<sup>th</sup> to 17<sup>th</sup> February, 2007.

The Veterinary Biotechnology, immunology and the associated processes offer a great promise both in terms of advancement of biological research and generation of a wide range of biological products and services. A large number of cutting edge technologies have been developed in niche areas of Animal Sciences. These modern technologies are becoming increasingly important and finding a common place in animal science research and development today. At the heart of R&D activities under the National Agriculture Research System, lies the need for establishing the linkages for exploring fruitful partnerships and agreements for management of human resources, sharing of technologies and services for sustainable rural livelihood security. I hope that the deliberations of the seminar will result in recommendations which help planners to formulate more effective strategies for future.

I wish the Seminar all success.

(SHARAD PAWAR)

## MESSAGE

**KANTI LAL BHURIA**  
MINISTER OF STATE FOR AGRICULTURE,  
CONSUMER AFFAIRS,  
FOOD AND PUBLIC DISTRIBUTION  
GOVERNMENT OF INDIA



कांतिलाल भूरिया  
KANTI LAL BHURIA



कृषि, उपभोक्ता मामले,  
खाद्य और सार्वजनिक वितरण  
राज्य मंत्री  
भारत सरकार  
नई दिल्ली-110001  
MINISTER OF STATE FOR  
AGRICULTURE, CONSUMER AFFAIRS,  
FOOD AND PUBLIC DISTRIBUTION  
GOVERNMENT OF INDIA  
NEW DELHI-110001

31 JAN 2007

### MESSAGE

I am very happy to learn that the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) is organizing its XIII Annual Convention and a National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security" at the College of Veterinary and Animal Sciences, Pookot.

It is the need of the day that the fruits of research in biotechnology should reach the rural society where the majority of the Indian farming population lives and to sustain high economy - wide growth rate has to be achieved through Innovation. I hope that this symposium will help to evolve effective strategies in biotechnology and immunology for sustaining and development of livelihood of the rural people. I am sure that the compendium proposed to be released to commemorate the symposium will reflect the scientific vision and advances made in the area of veterinary biotechnology and immunology in this aspect.

I congratulate ISVIB and wish all the success in this endeavor.

  
(Kanti Lal Bhuria)

# MESSAGE

V.S. ACHUTHANANDHAN  
CHIEF MINISTER OF KERALA



V. S. ACHUTHANANDAN  
CHIEF MINISTER OF KERALA

PHONE { OFFICE : 2333812  
          : 2333682  
          FAX : 0471-2333489

18.01.2007

## Message

I am glad to know that the Indian Society of Veterinary Immunology and Biotechnology has decided to conduct its XIII<sup>th</sup> Annual Convention and National Seminar on "Veterinary Immunology and Biotechnology for sustainable Rural Livelihood Security" at the College of Veterinary & Animal Sciences, Pookot from February 15<sup>th</sup> - 17<sup>th</sup> 2007 and bringing out a Souvenir cum compendium in connection with the convention.

I hope that the Seminar will discuss various aspects of modern trends and developments in the field of Immunology and Biotechnology and evolve effective strategies for sustainable Rural Livelihood Security. I am sure, the Souvenir will comprise of messages, report of the society, lead papers and abstracts of papers presented by the delegates.

I wish the Convention and the Seminar a grand success.

V.S.Achuthanandan

Dr.K.Anil Kumar

College of Veterinary & Animal Sciences, Pookot

Lakkidi.P.O, Wayanad-673 576

## MESSAGE

DR. A.L. CHAUDHARY  
PRESIDENT  
VETERINARY COUNCIL OF INDIA



DR. A. L. CHAUDHARY  
PRESIDENT

Veterinary Council of India  
(Statutory body of Government of India)  
'A' Wing, 2<sup>nd</sup> Floor, August Kranti Bhavan  
Bhikaji Cama Place, New Delhi – 110 066  
Ph: 011- 26184149, 26184354  
Fax: 011-26182434  
e-mail: [vcinfo@nic.in](mailto:vcinfo@nic.in)  
website: [www.vci.nic.in](http://www.vci.nic.in)

### MESSAGE

It is heartening to note that the Indian Society of Veterinary Immunology and Biotechnology (ISVIB) is holding its XIII<sup>th</sup> Annual Convention and National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security" at College of Veterinary & Animal Sciences, Pookot, Kerala from 15<sup>th</sup> to 17<sup>th</sup> February, 2007.

The role of animal husbandry in food security and for that matter rural livelihood security is well documented. This however needs to be further augmented with the application of newer biotechnological tools in respect of animal production by way of genetic manipulation, diagnostics and disease control measures, improving nutritive values of low quality roughages for animal feeding etc.

I complement the organizers of the Symposium for choosing this topic which is relevant to the present day needs. I hope, this Symposium will provide a platform for effective interaction among the scientists, academicians, administrators and grass root workers to evolve strategies in biotechnology and immunology for sustainable rural livelihood security and the deliberations will be very useful towards the overall development in the field of Immunology and Biotechnology

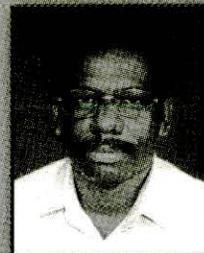
I wish the Symposium a grand success.

(Dr. A.L Chaudhary)

Dated: 6.2.2007

# MESSAGE

DR. E. NANU  
DEAN  
COLLEGE OF VETERINARY & ANIMAL SCIENCES  
MANNUTHY



## KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF VETERINARY AND ANIMAL SCIENCES  
MANNUTHY - 680 651, THRISSUR, KERALA, INDIA

Phone : (0487) 2370451, 2370344 (O)  
(0487) 2370468 (R)  
Fax : 91-0487-2370388  
E-Mail : deanvetkau@yahoo.com

Prof. (Dr.) E. NANU, Ph.D., FNAVS,  
DEAN-IN-CHARGE

Date.....8.2.2007.....

### MESSAGE

I have immense pleasure to learn that the College of Veterinary and Animal Sciences, Pookot has taken the lead to organize the National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security". In developing countries, biotechnological application relating to livestock need to be suitable for animal owners who are resource – poor small scale operators owning little or no land and only few animals. Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely dictated by commercial considerations and socio economic goals. Using technology to support livestock production is an integral part of viable agriculture in multi enterprise systems. Livestock are part of a fragile ecosystem and a rich source of animal biodiversity as local species and breeds possess genes and traits of excellence. Molecular markers are increasingly being used to identify and select particular genes that lead to these desirable traits and it is possible to select superior germplasm and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies. These technologies have been used in the genetic improvement of livestock, particularly, in cattle and buffalo, and the economic returns are significant. However, morbidity and mortality among animals produced using assisted reproductive technologies lead to high economic losses, so the principal application of animal biotechnology at present is the production of cheap and dependable diagnostic kits and vaccines. The organizers have also taken an earnest effort to bring out a souvenir cum compendium in connection with the convention.

I wish the Annual Convention of Indian Society of Veterinary Immunology and Biotechnology and the National Symposium all success.

  
Dr. E. NANU

**DR. P. P. BALAKRISHNAN**  
ASSOCIATE DEAN  
COLLEGE OF VETERINARY & ANIMAL SCIENCES  
POOKOT



## KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Pookot, Lakkidi P.O., Wayanad district, Kerala. PIN 673 576

Phone: 04936 256 380 Fax: 04936 256 390

DR. P. P. BALAKRISHNAN, M.V.Sc., Ph.D.,  
Associate Dean

Date: 2-02-2007

### FROM THE ORGANIZING SECRETARY'S DESK

Diseases pose a serious threat to animal and human health world wide. In the past few years, there has been an increase in several emerging diseases, resulting in fatal consequences among animals thereby affecting the farming community.

However, recent advances in frontier technologies in the field of biotechnology and immunology have indeed strengthened the realm of veterinary science. Genetic manipulation for better performance, drug design, disease resistance and quality assurance, besides diagnosis of diseases and their control are certain areas of thrust and focus that require our attention.

I feel extremely pleased to be associated with the conduct of the XIII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology and National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security" as the organizing secretary.

I am even more proud to say that the venue selected for the present convention for the first time in Kerala at College of Veterinary and Animal Sciences, Pookot, will for certain hold a wonderful platform for healthy discussions and deliberations, as the major population of Wayanad encompasses rural folk involved in animal/dairy farming.

This national symposium will hold five scientific sessions on different topics in which lead papers and contributory papers will be presented by eminent scientists and academicians. Let us hope that the best will evolve from these deliberations and it should provide necessary guidelines to overcome the present challenges faced by a veterinary scientist in order to help to develop a better society and better production of livestock. I wish to place on record my sincere appreciation to my colleagues for the earnest efforts taken to make this three day programme a grand success.

Dr. P. P. Balakrishnan



## ABOUT THE COLLEGE

*The College of Veterinary and Animal Sciences, Pookot, the second institution of higher learning in veterinary education was established by the Government of Kerala in 1998 in Wayanad district. Historically, Wayanad is one of the most vibrant districts of Kerala. Countless evidences about New Stone Age civilization can be seen on the hills of Wayanad especially in the world famous caves of Ampukuthimala located between Sulthan Bathery and Ambalavayal. In ancient times, this land was ruled by the Rajas of the Veda tribe and in later days, Wayanad came under the rule of the Pazhassi Rajahs of Kottayam royal dynasty. In the later years, Wayanad fell into the hands of the British who opened up the plateau for cultivation of tea and other cash crops and laid roads connecting it with Kozhikode and Thalassery.*

*This institution, located at a distance of 76 kilometers from Kozhikode is situated 700 m above mean sea level, at the crest of the Thamaraserri ghat pass, along the national highway to Mysore. The sprawling campus, located on 100 acres of land is affiliated to the Kerala Agricultural University. Nestled among some of the highest hills of Wayanad, this veritable cenotaph of higher learning offers a wonderful experience in veterinary education amidst a breathtaking view of misty mountains and enchanting valleys interspersed with tea, coffee and cardamom plantations*

*The College has 17 departments planned according to the Veterinary Council of India guidelines and equipped to meet the increasing demands of veterinary education. A central instrumentation facility is equipped with latest instruments and individual departments have been planned and equipped for taking up avant-garde research in veterinary science. The teaching veterinary complex at the gateway of the institution has facilities which meet national standards. Also situated at the entrance of the college is the sales and information counter with facilities for sale of all University produce as well as publications. Instructional farms are located at Sugandagiri, about a kilometer away from the Academic block. Separate farms for cattle and buffalo, goats, pigs, poultry and a rabbit unit are positioned at various points along the meandering road to Sugandagiri.*

*At present there are five batches of undergraduate students studying in this institution. Students are housed on campus in separate hostels for boys and girls which have been designed and furnished with all modern amenities including indoor gymnasiums, as well as well equipped recreation and reading rooms.*

*The institution strives for the upliftment of the backward district of Wayanad and Kerala state as a whole. It aims to become an institution of national repute in the years to come.* ■



**Committees for the Organisation of XIII Annual Convention of Indian Society for  
Veterinary Immunology and Biotechnology and National Symposium on  
"Veterinary Biotechnology and Immunology for Sustainable  
Rural Livelihood Security" 15 - 17 February, 2007**

**ORGANISING COMMITTEE**

Chairman : Hon. Vice Chancellor  
Secretary : Dr. P. P. Balakrishnan  
Joint Secretary : Dr. K. Anilkumar  
Chairman and conveners of all committees  
President Students Union, COVAS, Pookot

**ACCOMODATION**

Chairman : Dr. Sreekumaran T.  
Vice Chairperson : Dr. Latha C.  
Convener : Dr. C. N. Dinesh

**FOOD**

Chairman : Dr. T. V. Viswanathan  
Vice Chairman : Dr. C. T. Sathian  
Convener : Dr. K. S. Anil

**TECHNICAL SESSIONS & PUBLICATION**

Chairperson : Dr. P. T. Philomina  
Convener : Dr. Chintu Ravishankar  
Joint convener : Dr. Renuka Nayar

**PROGRAME & STAGE**

Chairperson : Dr. K. Devada  
Convener : Dr. Bindu Lakshmanan  
Joint convener : Dr. Shynu M.

**TRANSPORTATION**

Chairman : Dr. N. Gopakumar  
Convener : Dr. Rajeev T. S.  
Joint convener : Dr. Balusami

**PUBLICITY**

Chairman : Dr. Francis Xavier  
Convener : Dr. Girish Varma  
Joint conveners : Dr. Reeja George  
: Dr. Jayakumar C.

**INVITATION**

Chairman : Dr. Harshan K. R.  
Convener : Dr. Lucy Sabu  
Joint conveners : Dr. Senthil Kumar  
: Dr. Dildeep S.

**RECEPTION AND REGISTRATION**

Chairman : Dr. H. Subramanian  
Convener : Dr. Ally K.  
Joint convener : Dr. Indu V. Raj

**FINANCE**

Chairman : Dr. N. Ashok  
Convener : Dr. Reghu Ravindran  
Joint conveners : Dr. Radhika G.  
: Dr. Sanis Juliet

**ENTERTAINMENT**

Chairperson : Dr. A. P. Usha  
Convener : Dr. Ajith kumar S.  
Joint conveners : Dr. John Abraham  
: Dr. Sreeranjini A.R.

**VOLUNTEER**

Chairperson : Dr. Metilda Joseph  
Convener : Dr. Anoop S.  
Joint conveners : Dr. Beena V.  
: Dr. Priya P. M.

**DECORATION**

Chairperson : Dr. Radhamma Pillai.  
Convener : Dr. Promod K.  
Joint conveners : Dr. Nisha A.R.  
: Dr. Deepa Surendran



## INDEX

### LEAD PAPERS

1. **SUSTAINABLE RURAL LIVELIHOOD SECURITY ROLE OF LIVESTOCK**  
Dr. K. P. Agrawal
2. **EMERGING VIRAL THREATS IN LIVESTOCK AND POULTRY**  
Dr. R. K. Singh
3. **BIOTECHNOLOGY FOR RURAL LIVELIHOOD SECURITY**  
Dr. V. Ramaswamy
4. **USE OF BIOTECHNOLOGICAL METHODS FOR LIVESTOCK DISEASE DIAGNOSIS**  
Dr. P. Ramadass
5. **GLOBAL CHALLENGES IN VACCINOLOGY AND CONTROL OF ANIMAL DISEASES – STRATEGIES, APPROACH AND BLUE PRINT**  
Dr. V. Purushothaman

### ORAL PRESENTATIONS

#### SESSION 1: BIOTECHNOLOGY FOR RURAL LIVELIHOOD SECURITY

1. **THE EFFECT OF PROBIOTIC ON THE HUMORAL IMMUNE RESPONSE IN BROILER CHICKS TO NEW CASTLE DISEASE VIRUS.**  
R. Karunakaran, K. Nachimuthu, R. Kadirvel and V. Balakrishnan
2. **FMD STATUS, OCCURRENCE AND DISTRIBUTION OF FMD VIRUS TYPES IN THE STATE OF KARNATAKA- INDIA FROM 1977-2004**  
Raveendra Hegde, V. R. Girish, Giridhar. P and C. Renuka Prasad
3. **DEVELOPMENT OF PROBIOTICS FOR BROILER CHICKS TO AUGMENT "GUT HEALTH" AND GROWTH PERFORMANCE**  
R. Karunakaran, K. Nachimuthu, R. Kadirvel and V. Balakrishnan
4. **THERAPEUTIC EFFICACY OF METHANOLIC EXTRACT OF *ACHILLEA MILLEFOLIUM* LEAVES AGAINST *TRYPANOSOMA EVANSI*.**  
Shaba, P, Pandey, N.N., Sharma, O.P, Rao, J.R. and Singh, R.K
5. **EFFECT OF SUPPLEMENTATION OF INORGANIC SELENIUM SOURCES ON IMMUNE RESPONSE OF LAYERS**  
B. E. Aruna, B. S. V. Reddy, R. G. Glori doss and N. Suma
6. ***IN VITRO* ASSESSMENT OF BACTERIOSTATIC POTENCY OF EGG YOLK Ig Y AGAINST SALMONELLA.**  
Vikrama Chakravarthi.P, Shanmugasundaram.K, Aravinthan.P, Malmurugan.S and Sugumar. K
7. **IMMUNOSTIMULATORY POTENTIAL OF *EMBLICA OFFICINALIS* (AMLA) ON MURINE IMMUNE SYSTEM**  
R. S. Suja, A. M. C. Nair, S. Sujith, J. Preethy and A. K. Deepa
8. **EVALUATION OF VARIOUS ATTRIBUTES OF SPERMATOZOA IN RELATION TO ZONA PELLUCIDA PENETRATION TO ASSESS BUFFALO SEMEN FERTILITY**  
S. Selvaraju, J.P. Ravindra and J. Ghosh
9. **GENOTYPING BY *ICAA* PCR, OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM CASES OF BOVINE MASTITIS**  
Rajeev N. Kuler, Shrikrishna Isloor, V. V. S. Suryanarayana , B. M. Veeregowda , N. B. Sridhar and D. Ratnamma.



10. **IMMUNOMODULATORY EFFECT OF VITAMIN C IN BROILER CHICKEN UNDER OVERCROWDING STRESS**  
Karthiyayini.K and Philomina. P.T.
11. **IMMUNOMODULATORY EFFECT OF GARLIC POWDER AND NEM SEED CAKE FEEDING IN BROILER CHICKEN**  
Lonkar, V. D. and Jalaludeen A.
12. **FATTYACID SYNTHESIS AND DESATURATION IN PORCINE PINEAL GLAND, LIVER AND BRAIN: RELATION WITH SERUM GLUCOSE LEVELS**  
Mohan.N. H., B. C.Sarmah, M. K.Tamuli, Anubrata Das, K. M. Bujarbaruah and D. Kalita
13. **COMMERCIAL PRODUCTION OF SEX SORTED SEMEN FOR PREDICTION OF SEX AND ITS SCOPES**  
V. S. Binoy and Ani S. Das
14. **AN AYURVEDIC SUPPLEMENTATION (BR) ENHANCES HUMORAL IMMUNE RESPONSE IN HEAT STRESSED CHICKENS**  
V. Ramnath, P. S. Rekha and K. R. Mejo.

## SESSION 2: PRESENTATION FOR YOUNG SCIENTIST / MID CAREER SCIENTIST AWARD

### ISVIB Young Scientist Award

1. **MOLECULAR CHARACTERIZATION OF A NEWLY IDENTIFIED DUCK RANTES CC-CHEMOKINE HOMOLOGUE AND FUNCTIONAL STUDIES USING A CHICKEN B-CELL LINE, DT40**  
D. S. Arathy, Soja Saghar Soman, & E. Sreekumar
2. **ANALYSIS OF TISSUE mRNA EXPRESSION PROFILE AND ANTI-BACTERIAL SPECTRUM OF ANATIN-2, THE FIRST  $\hat{A}$ -DEFENSIN HOMOLOGUE IDENTIFIED IN DOMESTIC DUCK**  
Soja Saghar Soman, D. S. Arathy & E. Sreekumar
3. **CHARACTERIZATION OF A VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATE ADAPTED IN CHICK EMBRYO FIBROBLAST CULTURE**  
V. Umapathi
4. **DEVELOPMENT OF A REVERSE TRANSCRIPTION - POLYMERASE CHAIN REACTION LINKED ELISA FOR SENSITIVE DIAGNOSIS OF RABIES**  
R.P. Aravindh Babu, S.Manoharan, G.Dhinakar Raj and P. Ramadass
5. **BUFFALO POX: INVESTIGATION OF OUTBREAKS, ISOLATION OF THE VIRUS, MOLECULAR AND EM CONFIRMATION AND ATTEMPTS FOR DEVELOPMENT OF A LIVE ATTENUATED VACCINE**  
B. M. Chandranaik
6. **IMMUNOLOGICAL EVALUATION OF IMMUNOMODULATORY EFFECT OF INTERFERON-GAMMA (IFN- $\alpha$ ) IN CHICKEN VACCINATED WITH NEWCASTLE DISEASE VIRUS**  
Basavaraj S. Binjawadagi, Y. Hari Babu and E. Sreekumar
7. **ASSESSMENT OF CELL MEDIATED AND MUCOSAL IMMUNE RESPONSES IN CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINES**  
S. Varalakshmi, J. John Kirubaharan & V. Purushothaman
8. **SURVEILLANCE AND CHARACTERIZATION OF AVIAN INFLUENZA VIRUSES IN INDIAN POULTRY**  
S. Nagarajan<sup>1</sup>, V. Ramaswamy<sup>2</sup>, S. Gounalan<sup>1</sup>, K. Rajukumar<sup>1</sup>, H.V. Murugkar<sup>1</sup>, C.Tosh<sup>1</sup>, B. Pattnaik<sup>1</sup> and H.K. Pradhan<sup>1</sup>

### ISVIB Mid Career Scientist Award

1. **A FIELD STUDY OF BACTERIAL ETIOLOGY OF ABORTIONS AMONG MIGRATORY SHEEP AND GOATS IN NORTH-WEST HILL STATES OF INDIA WITH EMPHASIS ON QUICK DETECTION OF CHLAMYDIAE EMPLOYING TWO PCR TESTS**  
Mandeep Sharma



**2. IMMUNOLOGICAL COMPARISON BETWEEN INDIGENOUS AND COMMERCIAL BREEDS OF CHICKENS**

G. Dhinakar Raj\*, T.M. Chozhavel Rajanathan and P. Ramadass

**3. DEVELOPMENT OF RECOMBINANT ANTIGENS FOR DIAGNOSIS OF AVIAN MYCOPLASMOSIS.**

G. Venkatesh

**4. EMERGENCE OF BLUETONGUE VIRUS SEROTYPES FROM HOST AND VECTOR (CULICOIDES) IN INDIA WITH SPECIAL REFERENCE PROPHYLAXIS IN TAMIL NADU STATE.**

A. Wilson Aruni, M. Parthiban, N. Daniel Joy Chandran, A. Koteeswaran and K.S. Palaniswami

**F M Burnett Memorial Award**

**1. EFFECTS OF EXPOSURE TO HEAVY METAL POLLUTED WATER ON THE IMMUNE SYSTEM OF FISH.**

Madhu Prahha Saxena, Hari Mohan Saxena and Gurinder Kaur Sangha

**2. PEN-SIDE KITS FOR DIAGNOSIS OF PESTE DES PETITS RUMINANTS**

G. Dhinakar Raj, V. Thiagrajan, M. S. Shaila and R. K. Singh

**ISVIB Scientist Award**

**1. QUICK DETECTION OF CHLAMYDIALES AND CHLAMYDIACEAE EMPLOYING POLYMERASE CHAIN REACTION.**

Mandeep Sharma, R. C. Katoch, Subhash Verma and Pooja Soneja

**2. DETECTION OF *BABESIA BIGEMINA* DNA IN TICKS BY DNA HYBRIDIZATION USING A NONRADIOACTIVE PROBE GENERATED BY ARBITRARY PCR**

Reghu Ravindran, Jammi R. Rao, Ashok K. Mishra

**3. ABROGATION OF DTH RESPONSE AND MITOGENIC LECTIN-AND ALLOANTIGEN-INDUCED ACTIVATION OF LYMPHOCYTES BY CALCIUM INHIBITORS TMB-8 AND BAPTA-AM**

Hari Mohan Saxena, Madhu Dikshit

**SESSION 3: LIVESTOCK DISEASES - DIAGNOSIS AND CONTROL**

**1. COMPARISON OF PCR WITH CONVENTIONAL TECHNIQUES FOR THE DIAGNOSIS OF BRUCELLOSIS IN CATTLE**

P. Kaushik, D. K. Singh and A. K. Tiwari

**2. APPLICATION OF POLYMERASE CHAIN REACTION (PCR) TO DIAGNOSE ABORTIVE CHLAMYDIOSIS IN CATTLE, SHEEP AND GOATS OF HIMACHAL PRADESH**

Abhishek Gandhi, Mandeep Sharma, Prasenjit Dhar and V. K. Gupta

**3. NEWCASTLE DISEASE VIRUS INDUCED CYTOPATHIC EFFECT IN-VITRO IS CAUSED BY APOPTOSIS**

Ravindra P. V., A. K. Tiwari, Yoginder Singh Rajawat, Ravi Sundersan, Barkha Ratta, Uttara Chaturvedi, Sudesh Palia, Subudhi P. K. and A. Rai

**4. DEVELOPMENT OF PCR FOR DIFFERENTIAL DIAGNOSIS OF INFECTIOUS CANINE HEPATITIS AND INFECTIOUS LARYNGO TRACHIEITIS**

Uttara Chaturvedi; A. K. Tiwari; Barkha Ratta; Sudesh Kumar; Ravindra P.V.; Y. S. Rajawat; Mitesh Mittal and A. Rai

**5. PCR FOR DETECTION OF CLASSICAL SWINE FEVER IN CLINICAL SAMPLES**

Barkha Ratta, A. K. Tiwari, N. N. Barman, Sudesh Kumar, P. V. Ravindra, Y. S. Rajawat, Uttara Chaturvedi, Subudhi P. K. and A. Rai

**6. ISOLATION AND MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS***

P. Ramadass, Shrine Nagalakshmi and T. M. A. Senthil Kumar

**7. NUCLEIC ACID BASED DIFFERENTIATION OF *PASTEURELLA MULTOCIDA* SEROTYPES**

P.X. Antony, G.K. Nair, V. Jayaprakasan, M. Mini and T. V. Aravindakshan



8. **CLASSICAL SWINE FEVER IN MEGHALAYA AS CONFIRMED BY ELISA AND PCR**  
I. Shakuntala, Babita Devi, Ashok Kumar, R. H. Begum and K. M. Bujarbaruah
9. **INSITU HYBRIDISATION TECHNIQUE FOR THE DETECTION OF INFECTIOUS BURSAL DISEASE INFECTION**  
A. Wilson Aruni, M. Parthiban, Hudson Taylor and V. Purushothaman
10. **SEQUENCING OF NUCLEOPROTEIN GENE OF NEWCASTLE DISEASE VIRUS LOCAL ISOLATE D58**  
R.N. Ramani Pushpa, J. John Kirubakaran, and V. Purushothaman
11. **DIAGNOSIS OF CANINE PARVOVIRUS INFECTION IN FAECAL SAMPLES OF DOGS BY CONVENTIONAL AND MOLECULAR TECHNIQUES**  
Harmandeep Kaur, P.N.Dwivedi, Dipak Deka, Sandeepa Bhan and Ramneek
12. **IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* BY CULTURE METHOD AND POLYMERASE CHAIN REACTION (PCR) FROM CHICKEN MEAT**  
T.Arulkumar and A. Manikavasaka Dinakaran
13. **NATURAL INFECTION OF VACCINIA VIRUS IN CATTLE, BUFFALOES AND HUMANS**  
Parimal Roy, V.Purushothaman, Andrew Chandramohan and S.Panneerselvam
14. **DEVELOPMENT OF TAQMAN QPCR ASSAY FOR RAPID AND SENSITIVE DETECTION OF ORTHOPOXVIRUS**  
R. K. Singh, V. Balamurugan, M. Hosamani, V. Bhanuprakash and M. P. Yadav
15. **APPLICATION OF TAQMAN QPCR FOR SPECIFIC DETECTION OF CAPRIPOXVIRUSES**  
V. Balamurugan, V. Bhanuprakash, M. Hosamani, D. J. Kallesh and R. K. Singh
16. **PATHOGENESIS OF PPR: DETECTION OF VIRAL ANTIGEN IN ATYPICAL ORGANS FROM EXPERIMENTALLY INFECTED GOATS**  
Sen, V. Balamurugan, P. Saravanan, J. Sarkar, K. K. Rajak, V. Yadav and R. K. Singh
17. **SIMULTANEOUS DETECTION OF *BABESIA BIGEMINA* AND *THEILERIA ANNULATA* INFECTIONS IN CATTLE BY PCR-RFLP**  
Reghu Ravindran, B. C. Saravanan, J. R. Rao and A. K. Mishra
18. **USE OF NESTED PCR FOR ENHANCED DETECTION OF *PASTEURELLA MULTOCIDA* CAUSING HAEMORRHAGIC SEPTICAEMIA FROM CLINICAL SAMPLES**  
Sunitha Karunakaran, G.Krishnan Nair and M. Mini
19. **DEVELOPMENT OF PCR AS A ROUTINE DIAGNOSTIC TECHNIQUE FOR CANINE EHRlichiosis**  
Asha Rajagopal, S.Abdul Basith and S.Gomathinayagam

#### **SESSION 4: GENETIC MANIPULATIONS**

1. **EVALUATION OF COMBINED RECOMBINANT OUTER MEMBRANE PROTEINS LIPL32 AND LIPL41 FOR THE SERODIAGNOSIS OF CANINE LEPTOSPIROSIS**  
T. M. A. Senthilkumar, M. Subathra, and P. Ramadass
2. **GENOTYPING OF CANINES USING MICROSATELLITE MARKERS**  
M. Parthiban, A. Wilson Aruni, P. Sathya and P. Ramadass
3. **MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF LOCAL INFECTIOUS BURSAL DISEASE (IBD) ISOLATES TARGETING THE HYPERVARIABLE REGION OF VP2 GENE**  
A. Wilson Aruni, M. Parthiban, Hudson Taylor and V. Purushothaman
4. **MOLECULAR MODELLING OF TWENTY FIFTH BINDING DOMAIN (TBD) PROTEIN AND ITS RELATED STRUCTURES IN DEVELOPMENT OF RAS SIGNALLING INHIBITORS PREVENTING CANCER**  
A. Wilson Aruni and K. Gajendran
5. **CLONING OF CANINE INTERLEUKIN-4 GENE IN MAMMALIAN EXPRESSION VECTOR**  
S. S. Salunkhe, A. Rai, A. Saxena, P. K. Gupta, A. K. Tiwari, and M. Sandey



6. **GENETIC POLYMORPHISM IN DRB3 EXON 2 IN NILI-RAVI BREED BY POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM AND ITS ASSOCIATION WITH MASTITIS**  
Sudhir Kumar, M. L. Sangwan, Anurag Barwar and Sonika Ahlawat
7. **SEQUENCE ANALYSIS OF FOWL ADENOVIRUS - 4 (FAV-4) FIBRE GENE AND ITS SECONDARY STRUCTURE PREDICTIONS**  
Sandeepa Bhan, Ramneek, D. Deka, P. N. Dwivedi, A. Singh and M S Oberoi
8. **ISOLATION OF EMBRYONIC STEM CELLS AND THEIR CELLULAR LINEAGE DIFFERENTIATION**  
A. Mangala Gowri, A. A. Palanisamy, A. Mahalinga Nainar, C. Balachandran, G. Dhinakar Raj and P. Ramadass
9. **SEQUENCE ANALYSIS OF MORBILLIVIRUS CD150 RECEPTOR-SIGNALING LYMPHOCYTE ACTIVATION MOLECULE (SLAM) OF DIFFERENT ANIMAL SPECIES**  
J. Sarkar, V. Balamurugan, A. Sen, P. Saravanan, B. Sahay, K. K. Rajak, T. J. Rasool and R. K. Singh
10. **AMPLIFICATION, CLONING AND SEQUENCING OF ENOLASE GENE**  
Aralikatti, S. S., Suryanarayana, V. V. S., Honnappagol, S. S., Renuka Prasad, Byregowda, S. M., Boogi, V. C., Isloor, S and Rathnamma; D.

### SESSION 5: ADVANCES IN VETERINARY IMMUNOLOGY

1. **ACTIVATION OF CHICKEN B CELLS ENHANCES THE BINDING OF INFECTIOUS BURSAL DISEASE VIRUS TO THEM BY UPREGULATING THEIR EXPRESSION OF THE PUTATIVE TARGET MOLECULE**  
Hari Mohan Saxena and Daya P. Gangale
2. **PROTECTIVE ANTIGEN OF *BACILLUS ANTHRACIS* FOR DIAGNOSIS AND VACCINE DEVELOPMENT**  
V. Purushothaman, Parimal Roy, B. Balaganesan, T. Vijayanand and Sankaramoorthy. A.
3. **ISOLATION, SEROTYPING AND ANTIBIOGRAM OF *PASTEURELLA MULTOCIDA* ISOLATES ASSOCIATED WITH H. S. OUTBREAKS IN BUFFALOES OF HIMACHAL PRADESH**  
Vipin Katoch, Mandeep Sharma, Prasenjit Dhar and Rajinder Kumar
4. **EVALUATION OF INACTIVATED OIL EMULSION INFECTIOUS BURSAL DISEASE VIRUS VACCINE OF TISSUE CULTURE ORIGIN.**  
A. Thangavelu, K. S. Palaniswami and V. Purushothaman
5. **ISOLATION AND IMMUNOLOGICAL CHARACTERIZATION OF MYCOPLASMA SPECIES FROM BOVINE MASTITIS**  
Sanjeev Kumar and Y. Hari Babu
6. **SEROLOGICAL EVIDENCE OF EDS-76 IN BROILERS**  
Priya, P. M., Nair, G. K., Chintu Ravishankar and Mini, M.
7. **PROTECTIVE ROLE OF CELL MEDIATED IMMUNE RESPONSE IN CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINE**  
S. Varalakshmi, J. John Kirubakaran and V. Purushothaman
8. **CROSS PROTECTION STUDIES WITH *PASTEURELLA MULTOCIDA* VACCINES PREPARED IN NORMAL AND IRON RESTRICTED MEDIUM**  
M. Vidhya, N. Daniel Joy Chandran, W. Manohar Paul and G. Dhinakar Raj
9. **IMMUNOHISTOCHEMICAL CONFIRMATION OF GUT ASSOCIATED ANTIGENS IN *HAEMONCHUS CONTORTUS***  
P. Parvady devy, S. Gomathinayagam, Lalitha John and G. Dhinakar Raj
10. **DEVELOPMENT OF TRANSFORMED FIBROBLASTS ANTIGEN AS A DIAGNOSTIC ANTIGEN FOR AL/SV INFECTIONS IN POULTRY**  
Alka Tomar; Rajeeva; Suresh, V; Anita P; Pankaj Kumar Mandal and Vipasha.



## POSTER PRESENTATIONS

### SESSION - I

1. **DETECTION OF *CAMPYLOBACTER JEJUNI* BY POLYMERASE CHAIN REACTION**  
Jatinderpal Singh, T. S. Rai, A. K. Arora, Ramneek and S. K. Jand
2. **EFFECTS OF EXPOSURE OF FISH TO HEAVY METALS ON SERUM PROTEINS AND LYMPHOCYTES**  
Madhu Prabha Saxena, Hari Mohan Saxena and Gurinder Kaur Sangha
3. **CYTOPATHIC EFFECT OF PPR VACCINE VIRUS STRAINS IN VERO CELLS**  
Raveendra Hegde, Amitha R. Gomes, S. M. Byre Gowda, Santhosh A. K. and C. Renuka Prasad
4. **EFFECT OF DIETARY SUPPLEMENTATION OF HUMIC ACIDS ON IMMUNITY STATUS OF BROILER**  
R. Nagaraju, B. S. V. Reddy, R. G. Glori doss and B. N. Suresh
5. **EFFECT OF DIETARY ENZYMES ON LYMPHOID AND OTHERS ORGANS OF BROILERS**  
B. N. Suresh, B. S. V. Reddy, R. G. Glori doss and C. Ramesh
6. ***IN VITRO* ASSESSMENT OF BACTERIOSTATIC POTENCY OF EGG YOLK Ig Y AGAINST *ESCHERICHIA COLI*.**  
Shanmugasundaram.K, Vikrama Chakravarthi.P, Aravinthan.P, Malmurugan.S and Sugumar.K
7. **THE RAISING OF ANTI *ESCHERICHIA COLI* IMMUNOGLOBULIN Y FROM THE EGG YOLK OF IMMUNIZED CHICKEN.**  
Vikrama Chakravarthi.P, Shanmugasundaram.K, Aravinthan.P, Malmurugan.S and Sugumar.K
8. **THE RAISING OF ANTI SALMONELLA IMMUNOGLOBULIN Y FROM THE EGG YOLK OF IMMUNIZED CHICKEN.**  
Shanmugasundaram.K, Vikrama Chakravarthi.P, Aravinthan.P, Malmurugan.S and Sugumar.K
9. **PURIFICATION AND PARTIAL CHARACTERIZATION OF IgG FROM BLACK BENGAL GOAT**  
T. Siva Subramani and S. Batabyal
10. **COUNTER IMMUNOELECTROPHORESIS OF PURIFIED IgG OF BLACK BENGAL GOAT AND GAROLE SHEEP**  
T. Siva Subramani, S. Batabyal and Rajani Kumar Paul
11. **RESTRICTION ENDONUCLEASE ANALYSIS OF *PASTEURELLA MULTOCIDA* ISOLATES FROM POULTRY USING *HPAII* AND *HHAI*.**  
Mann A S, Arora A K, Jand S K and Pavitar Kaur
12. **DIAGNOSIS OF GENITAL CAMPYLOBACTERIOSIS IN BREEDING BULLS BY POLYMERASE CHAIN REACTION**  
Kairay N.K, Sharma N.S., Arora A.K., Ramneek, Pavitar Kaur and Jand S.K.
13. **EFFECT OF PROBIOTICS WITH ENZYMES ON IMMUNE RESPONSE IN BROILERS**  
J.Balachandar, P.S. Reddy and P.V.V.S.N. Reddy
14. ***IN-VITRO* ANTIVIRAL ACTIVITY OF PLANT EXTRACTS ON GOATPOX VIRUS REPLICATION**  
V. Bhanuprakash, M. Hosamani, V. Balamurugan, G. Pradeep, D. Swarup and R. K. Singh
15. **COMPARATIVE EVALUATION OF IMMUNE RESPONSE OF SHEEP AND GOATS TO PPR VACCINE STRAINS**  
A.K.Santosh, Raveendra Hegde, Shrikrishna Isloor, K. Prabhudas, C. Renukprasad, D. Ratnamma, B. M. Veeregowda and Amita Gomes



**16. PATHOLOGY OF 'ACUTE CLASSICAL SWINE FEVER'.**

Rameshkumar P., Chintu Ravishankar, Sarmadha M.K., Senthamil selvan P., Sreekumaran T., Priya P.M., Japrkasan V., Mini M., and Jayesh V.

**17. STANDARDIZATION OF LARGE SCALE PRODUCTION OF HOMOLOGOUS LIVE ATTENUATED PPR VACCINE IN INDIA**

Raveendra Hegde, Amitha R. Gomes, Paramananda Hugar, Byregowda S. M, Giridhar P. and C. Renukaprasad

**18. CLONING OF CANINE INTERFERON GAMMA GENE IN MAMMALIAN EXPRESSION VECTOR**

S. S. Salunkhe, A. Rai, A. Saxena, P. K. Gupta, A. K. Tiwari, M. Sandey and Sudarshan kumar.

**19. *IN VITRO* MATURATION OF BOVINE OOCYTES –THE ROLE OF OVUM RETRIEVAL METHODS**

Magnus Paul K, Ratheesh Babu M, Vijayakumaran. V and Sreekumaran.T

**20. ANALYSIS OF CUMULUS-OOCYTE COMPLEX YIELD AND MORPHOLOGY IN CATTLE BASED ON OVUM RETRIEVAL METHODS**

Magnus Paul K, Ratheesh Babu M, Vijayakumaran V and Sreekumaran T

**21. DETECTION OF BLUETONGUE VIRUS IN CHICKEN EMBRYOS TISSUES BY IMMUNO HISTOCHEMICAL TEST.**

Byregowda S. M, Suguna Rao, C. Renukaprasad and Ramesh

**22. POLYMORPHISM OF MICROSATELLITE MARKER S0005 IN INDIGENOUS PIG POPULATION OF KERALA**

Ariprasath K and Usha A. P.

**23. EFFECT OF GOOSEBERRY (*EMBLICA OFFICINALIS*) AND INDIAN GALLNUT (*TERMINALIA CHEBULA*) ON SPLEENIC ANTIBODY FORMING CELLS IN COCKERELS UNDER INDUCED HEAT STRESS**

Mejo. K. R., Ramnath. V. and Philomina.P.T.

**24. IMMUNOMODULATORY EFFECT OF GOOSEBERRY (*EMBLICA OFFICINALIS*) AND INDIAN GALLNUT (*TERMINALIA CHEBULA*) IN COCKERELS UNDER INDUCED HEAT STRESS**

Mejo.K.R., Ramnath.V. and Sreekumar.K.P

**25. COMPARATIVE STUDY OF ANTIGENIC PREPARATIONS FROM *SETARIA DIGITATA***

Sadish. S., Chattopadhyay U. K., Bhattacharya. D.

**26. DETECTION OF NEW CASTLE DISEASE VIRUS ANTIBODIES IN EGG YOLK OF LAYERS**

Nidhin Raj, Praseena Poulouse, Surya P.S., Lonkar V.D., Chintu Ravishankar and Priya P.M.

**27. ASSESSMENT OF NEW CASTLE DISEASE VIRUS ANTIBODIES IN BROILERS**

Surya P.S., Praseena Poulouse, Nidhin Raj, Chintu Ravishankar and Priya P.M.

**SESSION - II**

**1. ATYPICAL CASE OF RECURRENT TYMPANY IN BOVINE OF KASHMIR VALLEY**

F. U. Peer and Md. Moin Ansari

**2. A STUDY ON *PSEUDOMONAS AERUGINOSA* AFFECTIONS AND ITS ANTIBIOGRAM ASSAY IN DIFFERENT LIVESTOCK IN HIMACHAL PRADESH**

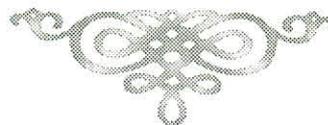
R. C. Katoch and Mandeep Sharma

**3. TUBERCULOSIS IN CATTLE IN AND AROUND PALAMPUR CITY OF HIMACHAL PRADESH**

Aneesh Thakur, Mandeep Sharma, Prasenjit Dhar and Vipin Katoch

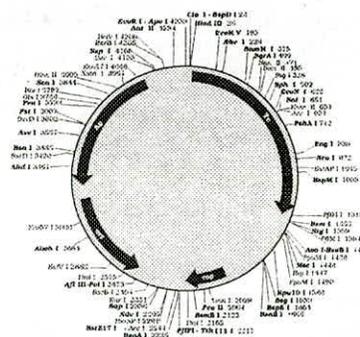


4. **STUDIES ON CLINICO-THERAPEUTIC OF MANGE IN CATTLE OF KASHMIR VALLEY**  
F.U.Peer and Md.Moin Ansari
5. **SUSTAINABLE DUCK REARING SYSTEM USING FRESH PRAWN WASTE**  
John Abraham and Reghu Ravindran
6. **COLIFORM INFECTION IN POULTRY REARED UNDER DEEP LITTER IN HILLY AREAS OF WAYANAD DISTRICT**  
Lonkar V. D., Balachandar J and Balusami C
7. **ANTIOXIDANT EFFECT OF *EMBLICA OFFICINALIS*.**  
Sajitha. I. S and C. R. Lalithakunjamma
8. **PYOGRANULOMATOUS INFECTION (BUMBLE FOOT) IN JAPANESE QUAIL REARED UNDER DEEPLITTER SYSTEM IN COLD CLIMATIC CONDITON**  
Lonkar V.D., Balusami C. and Balachandar J.
9. **ASPERGILLOSIS IN LAYER JAPANESE QUAIL REARED UNDER DEEP LITTER SYSTEM**  
Lonkar V.D., Ramesh Kumar, Balachandar J. and John Abraham
10. **SERUM TOTAL PROTEIN, ALKALINE PHOSPHATASE AND ASPARTATE AMINOTRASAMINASE CHANGES DURING TIBIAL FRACTURE HEALING IN DOGS.**  
S. Anoop and T. P. Balagopalan.
11. **EFFECT OF DIETARY NEEM (*AZADIRACHTA INDICA*) AND GARLIC (*ALLIUM SATIVUM*) ON CERTAIN BLOOD BIOCHEMICALS IN BROILER CHICKEN**  
Sreekumar, K. P., Preethymol, J., Lonkar, V. D., Sumi, C.P. and Asha, A.
12. **VISCERAL GOUT IN LAYER CHICKEN - A CASE REPORT**  
Lonkar V.D. and John Abraham
13. **EFFECT OF DIETARY SUPPLEMENTATION OF NEEM (*AZADIRACHTA INDICA*) AND GARLIC (*ALLIUM SATIVUM*) ON HAEMATOLOGY OF BROILER CHICKEN**  
Sreekumar, K.P., Lonkar, V. D., Sumi, C.P., Asha, A. and Preethymol, J.
14. **SALMONELLOSIS IN PIGS: AN EPIDEMIOLOGICAL STUDY**  
B.R. Harish, Shivaraj.B.M, B.M Chandranaik, M. D. Venkatesh and C.Renukaprasad
15. **CERTAIN BIOCHEMICAL PARAMETERS OF THE CROSS-BRED GOATS IN KERALA**  
Roymon Jacob, K. S. Sujatha and Sisilamma George
16. **INFLUENCE OF LEVEL AND DEGRADABILITY OF DIETARY PROTEIN ON THE PERFORMANCE OF EARLY LACTATING CROSSBRED DAIRY COWS. II. ONSET OF POST PARTUM HEAT**  
Ally, K., Mercy, M.D. and Viswanathan, T.V.
17. **FATTY LIVER KIDNEY SPLEEN AND HEART SYNDROME IN LAYER CHICKEN**  
Balachandar J., Lonkar V.D., Ramesh Kumar and Ajay Kumar





# Lead Papers



## SUSTAINABLE RURAL LIVELIHOOD SECURITY ROLE OF LIVESTOCK

**K. P. Agrawal**

National Coordinator, National Agricultural Innovation Project  
Indian Council of Agricultural Research  
KAB-II, Pusa Campus, New Delhi-110012

Livestock in India is characterized by very large numbers, across all the species. In 2000, it had 218.18 million cattle, 93.77 million buffaloes, 123 million goats, 57.96 million sheep, 10.50 million pigs and 402 million poultry birds. India ranks 1<sup>st</sup> in cattle and buffaloes, 2<sup>nd</sup> in goat, 3<sup>rd</sup> in sheep and 7<sup>th</sup> in poultry 70 percent of the livestock population is owned by small and marginal farmers and by landless labourers.

In spite of large livestock population, the contribution of livestock to the economy of the country does not commensurate with their enormous numbers. For instance, cattle milk yield is about half of the world average of 2070 kg/animal/annum and just 12-15 percent of the yield in USA, Canada and Israel. Productivity of species other than dairy and poultry has been stagnating at an extremely low level. Poor genetic potential, feed and fodder deficiency, poor management, adverse climatic conditions and lack of use of modern technologies are the probable reasons for low productivity of animals. Other factor responsible is lack of forces of selection as a result of which the poor yielders do not get out of the population and hence contribute off springs from generation to generation.

Our strengths are livestock number and economic production system and weaknesses are poor genetic potential of animals, deficient feed and fodder, poor management, adverse climatic condition and lack of use of modern technologies. Improvement in production depends on extent of genetic gain through use of reproductive biotechnologies (30%), feed and fodder availability (30%) and scientific management including disease control measures (40%). Use of biotechnologies to enhance livestock production and an integrated approach which are important for Sustainable Rural Livelihood Security have been discussed in the present communication.

### BIOTECHNOLOGIES TO ENHANCE LIVESTOCK PRODUCTION

Advances in Animal Biotechnology during the past few decades have been phenomenal. Biotechnological interventions can be introduced at different level of production, namely: breeding, feeding, management, disease control including conservation of indigenous livestock species breeds. The focus in this paper is cattle centric being an important livestock species of the country.

India has rich and diverse genetic resources, with some of the best breeds of dairy, draught and dual purpose cattle. Over 30 indigenous cattle breeds are essentially the result of long-term natural selection and evolution over the centuries and are fully adapted to different agro-eco-regions of the country, withstand tropical diseases and perform reasonably well even with low inputs in terms of feed and fodder. The average annual growth rate of cattle has been consistently amongst the lowest, when compared to growth rates of other species of livestock. During 1987-1992, the annual growth rate of cattle was 0.48% (5.92% in cross-bred and 0.12% in



indigenous), whereas that of buffaloes was 2.09%. Indigenous cattle breeds are declining at a faster rate for example, in Andhra Pradesh, Angola pure-bred population is decreasing and the Punganur breed has almost vanished. Similarly, Shawl population has dwindled in Haryana and Amritmahal in Karnataka. In Karla, the Vector cow is almost on the verge of extinction. The possible reasons for the decline in the population are inadequate inputs in terms of feed / fodder, shelter and management, poor reproductive management, dilution of breeds due to inter-mixing, indiscriminate infusion of exotic germplasm for cross-breeding for enhancing milk production, absence of specific strategies and programmes for conservation of indigenous breeds and loss of breeds due to geographical re-organisation. Breeding tracts and organised farms of some of the established breeds such as Sahiwal, Red Sindhi and Tharparkar have been lost to Pakistan. On account of the prospects of quick economic gains, cross-breeding has encroached into even the native tracts of superior indigenous breeds, which has led to dilution of these breeds, some of which are threatened and heading toward extinction if no action is taken to arrest this trend. Even in the cross-bred strains, genetic stabilization is yet to take place. The male calves of crossbred cattle are generally considered by the farmer as inferior as they are not useful as draught animals. These calves are neglected and starved to death, or more often than not, sold for slaughter for meat and hides, illegally in most states. These calves can not be slaughtered due to social and ethical reasons in India.

### FEATURES OF INDIGENOUS CATTLE

- 1) Cattle are the first animals domesticated by man for agricultural purpose.
- 2) Brahman breed in USA introduced in 1849 has been developed from Indian cattle breeds.
- 3) Indian cattle breeds like Gird and Angola (Nellore) exported to Brazil in 1906 are thought to be the world's oldest domestic cattle breeds and can be seen all over Brazil whereas, in their native country, India, these breeds are rarely found.
- 4) Cow was a second outcome (Rotan) of "Saundra Manthan"
- 5) A great medicine called "Panchagavya" (cow milk, cow ghee, cow curd, cow urine and cow dung) was made from indigenous cow by the sage, "Dhanwanthri".
- 6) The Indian breeds excel in draught capacity, exhibit distinct superiority in utilizing poor quality feed (grass, crop residues and crop by-products) and are adapted to adverse climate.
- 7) Cattle produce fuel, fertilizer, nutrition and generates more power than all of India's generating plants. Eight hundred million tones of manure produced every year is the biggest energy source. To replace dung with coal would cost India \$1.5 billion per year.
- 8) Cow dung if packed on the outside walls of a house keeps the house warm in winter and produces a cooling effect in summer. It also keeps flies away, and its smoke acts as a repellent for mosquitoes.
- 9) India is first in milk production. Average milk yield varies from 1800 to 3500 kg per lactation.
- 10) Before mechanization in agriculture, India depends on bullock power. Bullocks used to do two-thirds of the work on an average farm. Some fifteen million bullock carts were used to move approximately fifteen billion tones of goods across the nation. Converting Indian agriculture from bullocks to machinery has costed an estimated thirty billion dollars plus maintenance and replacement costs to the country.

### Plight of the Cow in Modern India

Late Shri Nathuram Mirdha, the then Chairman of the National Agricultural Commission, when asked by a foreign delegation as to what, according to him, was the culture of India. His reply was "Agriculture and no other culture is the culture of India" and the king-pin of agriculture in our country is the cow, other-wise known as "Kamdhenu". Now situation is fast changing. Not only productivity is poor, there has been negative growth rate in cattle population in nineties. The cow whom we call "Gau Mata" is becoming a burden on the society. Reasons are explained below.

**Blind following of Western Agriculture:** Blind following of western agriculture, particularly in respect of mechanization of agriculture without looking into the nature of our soil, size of our holdings, number of man power available, the capacity of our bullocks and ignoring completely our traditional methods of farming has created havoc to our farmers as well as to agricultural labourers. Use of chemical fertilizers and pesticides on a massive scale has not only increased the cost of production but has also poisoned our soil, water and crops and caused health hazards to the people and cattle.



**Indiscriminate Breeding:** In order to improve the productivity cows, are indiscriminately cross-bred. The exotic and cross-bred cows and bullocks are misfit to our climate and unable to tolerate the heat of summer. Exotic animals brought with them many diseases which did not exist earlier in our country. The quality of their milk is inferior to that of the indigenous breeds. Their male calves have no humps and are not capable of plunging and other agricultural operations as are our indigenous breeds. Indiscriminate breeding has led to dilution of our precious breeds indigenous and few cow breeds are at the verge of extinction.

**Illegal Slaughtering:** A recent report by the Union Ministry of Environment (MOE) claims that "the massive illegal slaughtering of animals has posed a serious threat to their existence and there has been an alarming decrease of animal - human population ratio". The report also states that the cattle human ratio came down from 430 animals per thousand human population in 1951 to 278 in 1981 which has now reached the alarming level of 200 per 1000. Central Leather Research Institute, Chennai in its recent analysis mentioned that "indiscriminate slaughter of animals to meet the internal and export demand for meat had contributed to the low growth rate of animals". Cattle population, which was 204.58 million in the year 1992 decreased to 195.58 million in 1997. For the first time since 1951, a negative growth in the cattle population of the country has been noticed which is indeed a cause of great concern for cattle wealth.

**Feed and Fodder Deficiency:** Livestock production particularly ruminants in India depends on crop residues and crop by-products. There is a wide gap in demand and supply of animal feed and fodder. The overall deficiency of feeds and fodders is estimated to be about 30 percent in respect of total digestible nutrients (TDN) and as high as 70 percent in digestible crude protein. Under feeding, protein and vitamin deficiencies and imbalance of trace elements result in various reproductive abnormalities both in male and female. Poor nutrition during early life in female retards the onset of puberty. In parous cows, it leads to anoestrus, anovulation and metabolic disorders. During early pregnancy, moderate to severe under-nutrition may lead to embryonic mortality and/or abortion. During late pregnancy it has an adverse effect on calf birth weight. A poor body condition score (BCS) at calving adversely affects milk production and fertility characterized by prolonged post-partum oestrus interval, reduced conception rate and more services per conception. The nutrient deficiencies and imbalances during initial period of lactation can lead to several disorders, which affect the post-partum reproduction. A very low protein diet can cause cessation of oestrus, if fertilization occurs, foetal death or the birth of premature and weak offsprings result. The plane of nutrition also influences the performance of breeding males. Ration containing low protein adversely affects semen volume and quality. Continued feeding of low protein ration also affects sex libido.

**Poor Reproductive Management:** Reproductive efficiency is determined by many different processes, namely: age of puberty, pattern of oestrous cycle and oestrous behaviour, length of breeding season, ovulation rate, lactational anoestrous period, inter-calving period and reproductive lifespan. These traits influence the breeding efficiency. Here ability of breeding efficiency is 0.30. Selection based on this trait is advantageous in terms of reduction in age at first calving and first service period. Ample scope exists for increasing the reproductive efficiency by modification in the traditional methods of breeding, feeding, management and disease control.

**Adverse Climatic Conditions:** Environment is one of the determining factor in the production and reproduction of farm animals all over the world. Inborn production potentialities can be severely affected by exposure to adverse climatic conditions. Season affects the breeding efficiency in animals. Season of calving affects lactation performance, lactation length and calving interval. Winter calvers produce more milk in a lactation as compared to those calve in rainy season. Those who calve in rainy season have least calving interval. Oestrogen activity of some fodders also influences seasonal activity.

**Lack of Forces of Selection:** Other factor responsible for poor productivity is lack of forces of selection as a result of which the poor yielders do not get out of the population and hence contribute off springs from generation to generation. Slaughter is being used through out the world to restrict an increase in population of undesirable livestock. The need for slaughter is also important if there is a programme of genetic transformation of the population through crossbreeding. In view of the religious taboo and legislative restrictions on the slaughter of the cows in many states of India, the poor yielders do not get out of the population, and contribute off springs from generation to generation. These low yielding cows compete with better stock in respect of feeding, housing and other managerial necessities.



**Lack of Use of Modern Breeding Tools:** Advances in animal breeding during the past few decades have been phenomenal. Farm animal breeding has entered into an era of new biotechnology revolution which includes artificial insemination, embryo transfer, cryopreservation, sexing, transgenesis, cloning etc. These technologies still in infantile stage in India, have revolutionised animal agriculture in developed countries of the world.

## BIOTECHNOLOGICAL APPROACHES

Poor productivity of indigenous cattle is the main culprit endangering cattle wealth of India. Following approaches, though do not directly help in conservation but are important have been described below.

**Scientific Approach of Animal Keeping:** There is a great potential to improve productive efficiency of farm animals. In an American study with dairy cattle, it was estimated that nearly \$300 million could be saved with only one approach, i.e. scientific approach of animal keeping. Breakup of savings is as \$135 million by reducing the average calving interval, \$ 85 million by reducing calf mortality from 10 percent to 4 percent, \$57 million by reducing the number of sterile culled cows from 5 percent to 2 percent and \$ 20 million by reducing the number of services per conception from 2 to 1.5.

**Artificial Insemination:** Artificial insemination is one of the most effective ways of bringing significant genetic improvement in farm animals. The impact of AI in our country is too meagre in comparison to developed World because of low coverage, lack of proper infrastructure including non-availability of progeny tested bull semen, improper refrigeration, lack of transport facilities and inadequate trained and motivated personnel. Nearly three-fourths of the dairy cattle in the United States are artificially inseminated as against only one-fifth of cattle and one-tenth of buffalo in India.

**Embryo Transfer:** The application of artificial insemination technique has provided considerable opportunity to improve the genetic potential of the livestock by using semen from proven males. The embryo transfer technique permits exploitation of superior female genotype, giving more off springs from the same genetic donor than would arise under normal conditions of breeding. It has an added advantage of using the genetically poor female as foster mother to carry genetically valuable embryos to full term and further, has a control on the production of her own off springs of poor genetic make up.

**Sexing :** A sex ratio favoring males is more desirable in meat breeds because of the faster growth and lean carcass and females in dairy breeds. The technology of sexing is of much relevance in India where the male calves of dairy cattle breeds can not be slaughtered due to social, ethical and legal reasons.

**Cloning :** Ability to produce genetically identical animals has far reaching benefits both in research and commercial animal production. Multiplication of superior animals, reviving endangered or extinct species / breeds and reproducing deceased animals are important advantages of cloning technology.

**Transgenesis :** Transgenic animals are of particular interest in relation to growth, production traits, reproduction and disease resistance. The production of pharmaceutical proteins and nutraceuticals (genetically engineered whole milk) promises immediate application of transgenics. The amount of milk needed to provide a national supply of these pharmaceuticals are really very reasonable. Assuming the animals produce 1 g of the protein per liter milk and one has a purification efficiency of 30% (that is, 30% of the protein is recovered in the pure sample), then a pig can produce 75 g of protein per year, a goat 100 g, a sheep 125 g, and a cow 3 kg. As the need of blood-clotting factor IX is 2 kg / yr, one transgenic cow can meet out pharmaceutical requirement (blood-clotting factor IX) of a country. For other proteins the demand is larger (for example, for tissue plasminogen activator it is 75 kg per year and for human serum albumin it is about 1,000 kg / yr), but nonetheless a limited number of animals is all one now needs to meet the national demand for pharmaceutical proteins that used to be astronomically expensive.

**Sound Breeding Policy and Strategies:** Sound Breeding Policy and Strategies should be developed State-wise / District-wise to safe guard native breeds from further dilution. Cross-breeding with exotic strains should be totally banned in the home tracts of the important cattle breeds. Import of germplasm should be allowed only in very specific cases and after taking all the precautions to prevent the ingress of diseases into the country. Religious execution of National Cattle Breeding Policy (NCBP) whose main focus is: i) improvement of nationally



important indigenous breeds (draught and dual purpose) through selective breeding in their home tracts, ii) improving low producing non-descript cattle by upgrading with other superior indigenous dairy breeds, iii) as an alternative to ii), low producing non-descript cattle can be cross-bred with exotic dairy breeds and iv) to continue intense breeding among cross-bred cattle using pedigreed / proven bulls can safeguard indigenous cattle breeds from further dilution/extinction. There is a need to develop region-specific strategies and detailed district level breeding plans, in consonance with the national breeding policy by each State Government. Strategies for implementing the breeding programme should include: 1) establishing an institutionalized monitoring mechanism from the Centre downwards, 2) drawing of region-specific and breed-specific breeding strategies in each state, 3) fixing targets in terms of actual numbers of cattleheads of the particular breed, infrastructure facilities such as sperm stations, bull farms etc. for each state, 4) organizing regular review meetings to assess the progress in terms of the physical and financial targets, and to draw corrective action plan wherever the targets are not met and 5) ensuring adequate funding from Central Government to the State Governments to implement the programmes

**Creation of Scope for Larger Use of Indigenous Cattle Breeds:** The guidelines issued by the Centre for conservation of indigenous breeds, their selective breeding for genetic improvement and use of indigenous breeds for upgrading low producing non-descript cattle in resource-poor areas should be strictly adhered to. However, introduction of cross-breeding with exotic cattle even in such areas has seriously limited the scope of use of bulls of indigenous cattle breeds outside their breeding tract. The states should be directed to specifically delineate and identify the geographical boundaries of the areas where non-descript cattle should be upgraded by crossing with bulls of indigenous breeds. Once such areas are earmarked, no cross-breeding of non-descript cattle, other than with bulls of indigenous breeds, should be permitted. There is a need to provide an incentive to the farmers in the breeding tracts to rear male stock of indigenous breeds up to the breeding age.

**Ensuring Supply of Good Quality Breeding Material in the Breeding Tracts:** Due to indiscriminate cross-breeding, there has been a general lack of emphasis on selective breeding in the breeding tracts. The supply of superior quality germless, to match the breedable females in the breeding tract should be ensured. For sourcing cross bred bulls, the military dairy farms should be used as a major source of contribution to the bull production programme.

**Promotion of Breeders' Organisations:** Presently, the conservation of indigenous breeds is mainly in the domain of the Government and semi-Government agencies. Breeders in other countries form strong proactive groups, which lobby for furthering their interests, as for example, the Brahman Breeders Association in USA. Like privatisation in various sectors, cattle and livestock breeding should also be encouraged in the private sector, with Government playing a facilitating role and providing infrastructural inputs, wherever required.

**Enhancing the Role of Voluntary Organizations:** There are large number of Goshalas, Goosanders and Pinjrapoles in the country. These organizations have quite sizeable population of excellent animals of various indigenous breeds. Efforts made by such agencies towards improvement and conservation are not based on scientific methodologies. The efforts are also diluted because of their supporting old, diseased and destitute animals, mostly of non-descript breeds. At present the terminologies of Goshalas, Gosadans and Pinjrapoles are often loosely used and are inter-changeable at will. These organizations should be provided with scientific and technical inputs and training to staff in genetic evaluation and selection of germplasm for breed improvement and upgradation programmes should also be organized.

**Data Base:** Data especially in the context of the breed improvement and conservation programmes over years is lacking. The quinquennial Livestock Census, conducted by the Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Govt. of India suffers from all kinds of qualitative and quantitative problems, apart from lack of timeliness. The problems of lack of timely and quality data are further compounded by lack of perception of the data-collectors while categorising the breeds and quantifying their performances. For example, there is a tendency to club both non-descript and good indigenous cattle breeds into a single category of indigenous cattle, resulting in distortions while recording parameters such as average productivity etc. There is a need for training the collectors and developing suitable formats for reliable data on different breeding aspects. A few points to be critically considered in data collection are: 1) A reliable data base should be developed with regard to all the details of recognized indigenous breeds, including their breeding tracts, numbers, characteristics, genetic make-up, the institutional farms where they are being preserved and /or conserved and so on. Separate data bases



should be developed with regard to non-descript and cross-bred cattle, 2) The census data should be up-dated from time to time through surveys, 3) The Livestock Census must be conducted in a timely manner and 4) A breeding network should be set up by computerising and net-working all AI outlets, sperm stations, breeding farms and Goshalas and other agencies involved in the production of breeding material and implementation of breeding programmes.

### **SUSTAINABLE RURAL LIVELIHOOD SECURITY**

Farming System Approach for the performance of Indian Agriculture in transforming India from a begging bowl status in the mid 1960s to self sufficiency during 1990s is remarkable. More ever, food self sufficiency has not reached at the household level in several parts of the country. Malnutrition in women and children is continued to remain high, farms are shrinking in size and natural resources are depleting and degrading in quality. Another notable feature of agricultural transformation is the growing regional disparity in development. Disadvantaged regions did not gain much from the development efforts. A balanced regional development in disadvantaged regions assumes special significance in the planning and development of the programmes under component 3 (Sustainable Rural Livelihood Security) of the Project.

#### **Features of Rural Livelihood Security Programmes**

The Indian agriculture has achieved phenomenal success in over coming food crisis. Plenty of research information is available, but a majority of this information does not reach the farmers and is at times unsuitable for its utilization in the fields. Under rural livelihood security programmes of NAIP, efforts are being made for integrated approach and packaging of location specific available technologies, testing in the farmers fields, training the farmers for its adoption with emphasis on efficient management of natural resources. The ultimate aim is to develop location specific models through Farming System and Participatory Approaches in agriculture and allied sectors for long-term sustainability. The main features of livelihood programmes are given below:

- The programmes in this component will be operated in consortium mode.
- To build partnership, pool competence and resources from conventional and non-conventional resources and build social capital for better ownership and sustainable model for rural development
- The Programmes are bottom-up, technology-driven with supportive institutions and policies.
- To develop synergy with Development Departments, NGOs, Civil Society Organizations etc. to achieve larger, wider and with faster impact.
- The targeted group is rural families in disadvantaged regions of the country, namely: chronically drought and flood prone rainfed, hill and mountains, dryland, coastal and tribal areas including lands which have been left behind in economic development.
- Enriched environment and natural resources, improved quality of life and human values and create opportunities of gainful self employment to ensure sustainable livelihood.
- Integrated and multidisciplinary approach through diversification, horticultural, livestock and fisheries, bee keeping, organic farming, integrated pest management, integrated plant nutrient management, natural resources management, agro processing and value addition.
- To develop and improve the marketing and export agricultural systems in order to increase demand for local products and sustainable production in rural areas.
- To address equity, drudgery reduction ,and promotion of self help groups (SHGs).
- Design and undertake training programmes for knowledge transfer by employing bottom up and participatory approach.
- Develop and publish appropriate literature in local language for the extension workers, farmers and rural poor.
- Improve and develop sustainable farming system models in the less favorable environments and regions through action research, packaging of available proven technologies, technological dissemination including knowledge empowerment.



### **Criteria for Selection of Disadvantaged Regions / Operational Areas**

150 disadvantaged districts identified by the Planning Commission for support under the "Work for Food Programme" covering 27 states have been included for developing the programmes. 150 districts have been grouped into clusters (Table 1). A cluster consisting of contiguous districts of similar socio-economic and agro-ecology is preferred for consortia formation. Selection of operational areas within the identified districts are based of subindices, namely: i) Infrastructure Status, ii) Agricultural Status, iii) Food Availability Status, iv) Nutritional Status, v) Health and Sanitation Status and vi) Economic Status.

### **Consortia Formation and Partners**

A consortium is a formal group of partners to carry out the identified task by sharing research agenda and research resources according to an agreed work plan. The partners in each consortium should be identified on the basis of problems to be tackled and their strength. The range of partners that may be engaged in forming a consortium includes; i) research institutes within and outside the ICAR System, ii) Farmer Organizations, iii) Private Enterprises, iv) State Development and Extension Departments, v) Market Establishments, vi) Financial Institutions, vi) Voluntary Organizations/ NGOs and vii) International Organizations.

### **Eligibility and Selection of Consortia**

The consortia are identified under two distinct categories: i) Competitive and ii) Sponsored, 10 in each category. The consortia under competitive category are selected through an open, wide, transparent and objective two stage competitive process. First, concept notes (CNs) on sub-project proposals from prospective consortia are called. The consortia whose concept notes pass through the first round screening are asked to submit full proposals. The full proposals are rigorously screened and finally the subprojects to be awarded are selected.

Under sponsored category, the proposals are not invited but are sponsored to the identified institutions. The sponsored programmes can also work in a limited competitive mode where, for an identified research area, a certain number of potentially capable institutions may be identified and requested to come forward with proposals and partners. The rest of the process is the same as for the competitive mode.

### **How is Farmers' Participation Planned in the Project ?**

Component 3 is applied / action / adaptive research component where models for ensuring benefits to the society through agricultural research will be available for adoption at farmer's field. Hence participation of stakeholders including the farmers, the farm laborers, the fishermen and the people engaged in on farm / off- farm livelihood activities have been ensured at every level of the project development and operation.

1. Farmers have been involved at the highest governance and executive levels by representation of 2 farmers in National Steering Committee, one in Project Management Committee and 1 or 2 in Consortia Advisory Committee.
2. The projects are developed in consortium mode in collaboration with development departments, private sector, farmer organizations, NGOs and other stakeholders.
3. Participation of stakeholders including farmers has been ensured in the Interaction Workshop before finalization of the full project proposal for each consortium.
4. The participation of stakeholders including the farmers and farm laborers at different stages of project development, adoption and dissemination of appropriate technologies have been ensured for the success of the programme.
5. A campaign strategy through the tools like organizing krishi meals, study tours, exhibitions, rural heats, radio & television broadcasts, publishing in local papers, pamphlets, brochures, etc. will be a part of each consortium plan.
6. A learning and capacity building plan for the stakeholders will also form an integral part of each consortium.
7. Benefits to farmers in terms of technology adoption, income and employment enhancement, nutritional improvement etc. have been included as important indicators of monitoring and impact assessment. Further, each consortium will have to give a detailed uptake plan so that stakeholders, farmers and fishermen are not left in the lurch after the project activities are withdrawn.



Table 1. GROUPING OF 150 DISTRICTS IN CLUSTERS

Cluster	States	Districts	No.
1	Arunachal Pradesh	Upper Subansiri	7
	Manipur	Tamenlong	
	Meghalaya	South Garo Hills	
	Mizoram	Siaha	
	Nagaland	Mon	
	Sikkim	North Sikkim	
	Tripura	Dhalal	
2	Bihar	Araria, Vaishali, Gaya, Madhubani, Muzaffarpur, Nawadah, Samastipur, Sheohar, Kathar, Jamui, Lakhisarai, Monghayr, Purnea, Supaul, Darbhanga	15
3	H.P.	Chamba	5
	J & K	Doda & Kupwara	
	Uttaranchal	Champawat & Tehri Garhwal	
4	Chhatisgarh	Bastar, Dantewada, Kanker, Korbha, Sarguja, Jaspur, Dhamtari, Raigarh, Bilaspur, Rajnandgaon	10
5	Maharashtra	Gadchiroli, Gondya, Chandrapur, Bhandara, Dhule, Nandurbar, Hingoli, Nanded, Aurangabad, Ahmednagar, Yawatmal	11
6	Karnataka	Chitradurga, Davangere, Bidar	8
	Tamil Nadu	Tiruvannamalia, South Arcoti Cuddalore, Villupuram, Nagapattinam	
	Kerala	Wayanad	
7	Jharkhand	Saraikela, Singhbhum West, Godda, Simdega, Gumla, Chatra, Garhwa, Palamau, Latehur, Lohardagga, Dumka, Jamtara, Sehebganj, Pakur	14
8	Uttar Pradesh	Sonabhadra, Mirzapur, Kushinagar, Unnao, Raebareli, Sitapur, Hardoi, Fatehpur, Lakhimpur Kheri, Barabanki, Lalitpur, Banda, Chitrakoot, Mahoba, Hamirpur	15
9	Haryana	Satyamev Puram	2
	Punjab	Hoshiarpur	
10	Orissa	Koraput, Malkangiri, Nabarangpur, Rayagada, Mayurbhanj, Sundergarh, Keonjhar, Phulbani, Boudh, Nuapada, Kalahandi, Sambalpur, Deograh, Jharsuguda, Sonepur, Bolangir, Dhenkanal, Ganjam	18
11	West Bengal	Malda, West Midnapur, Bankura, West and North Dinajpur, Murshidabad, Purulia	6
12	Madhya P.	Umaria, Shahdol, Balaghat, Mandla, Barwani, Shivpuri, Sidhi, Tikamgarh, Chattarpur, Betul, Jhabua, Khargone, Khandwa, Seopur, Dhar	15
13	Andhra P.	Adilabad, Mahbubnagar, Rangareddy, Khammam, Warangal, Nalgonda, Anantpur & Cudappah	8
14	Gujarat	Dangs, Dohad, Panch Mahals, Sabarkantha, Narmada, Banaskantha	6
15	Assam	Kokrajhar, North Cachar Hill, Karbi Anglong, Dhemaji, North Lakhimpur	5
16	Rajasthan	Banswara, Dungarpur, Udaipur, Sirohi, Karauli	5
16	27	150	150



## **LEAD PAPER**

### **EMERGING VIRAL THREATS IN LIVESTOCK AND POULTRY**

**R. K. Singh**

Station-In charge cum Head, Division of Virology  
IVRI, Mukteswar 263138, UA

#### **Abstract**

Emerging infectious diseases (EIDs) can be defined as diseases that have recently increased in incidence or geographic range, recently moved into new host populations, recently been discovered or caused by newly evolved pathogens. The line between human and animal pathogens has become finer in the past few years. There has been tremendous increase in number of zoonotic diseases that affect both humans and animals. Notable among them are Hantavirus diseases, Lyme borreliosis, Hendra, Nipah, and West Nile viral diseases, monkeypox, avian influenza, and the coronavirus severe acute respiratory syndrome (SARS). RNA viruses have high mutation rates (RNA polymerase lacks proof-reading activity) and therefore have more potential to emerge and re-emerge. Quasi-species have been reported for many RNA viruses, which allow considerable plasticity within the viral population for adaptation in a harsh new environment of a new host, selective pressure of the host immune system or antiviral drugs. The influenza epidemics or annual outbreaks of FMD in animals result due to antigenic drift resulting in appearance of a new variant. A zoonotic disease in pig workers in 1998 in Malaysia was attributed to the Nipah virus. Hendra virus is closely related to Nipah virus (approx. 78% N gene sequence homology) and is fatal to horses and humans, under experimental conditions. The sensitivity of a passive surveillance system is always a concern. In general, any system will find large outbreaks but finding smaller outbreaks or scattered cases of disease is the real challenge warranting effective disease surveillance employing highly sensitive and specific assays.



## LEAD PAPER

### **BIOTECHNOLOGY FOR RURAL LIVELIHOOD SECURITY**

**V. Ramaswamy,**

Dean,

Faculty of Basic Sciences,

Madras Veterinary College, TANUVAS, Chennai-7, TAMILNADU

#### **Introduction.**

1. Agricultural biotechnology is a collection of scientific techniques, including genetic engineering, that are used to modify and improve plants, animals and micro-organisms for human benefit. It is not a substitute for conventional plant and animal breeding but can be a powerful complement. The present paper explores what roles biotechnology may play in contributing to sustainable agriculture and rural development, particular concerns for biosafety and biodiversity.
2. Agriculture comprising crop and animal husbandry, fisheries, , agro-forestry and agro-processing are the backbone of India's national food security and rural livelihood security systems. There are about 110 million operational holdings in the country. The smaller the farm, the greater is the need for higher productivity and marketable surplus to enable a family have some cash income. The human population ping predominantly young, the youth can be attracted and retained in farming only if it is intellectually satisfying and rewarding. But that could happen only through technology upgrading. Biotechnology provides an opportunity to convert bio resources into economic wealth. There are a myriad applications of biotechnology in agriculture and these deserve to be tapped. There is a worldwide food crisis . Currently 790 million people are undernourished and around one third of the world's children go to bed hungry. Biotechnology holds the promise to double food production, ensure adequate nutrition and rid small farmers from poverty. Biotech applications are already yielding health benefits by releasing what are considered life saving and life enhancing drugs As nano biotechnonology is taking rapid strides, this new area should direct research on the basis of priorities, social, economic and equity issues. But the potential contribution of modern biotechnology to the achievement of global food security remains uncertain, at least for the present.

#### **II. Agricultural biodiversity**

1. Agricultural biodiversity encompasses the variety and variability of animals (including aquatic animals), plants, forestry and microorganisms – at the genetic species and ecosystem levels—necessary to sustain the key functions of the agro-ecosystem and its structure, as well as processes for and in support of agricultural production and food security. The biological resources contained within agricultural biodiversity are of direct and vital importance to the food security and socio-economic development of all countries.

#### **III. Potential of biotechnology for sustainable agriculture and rural development.**

2. Agriculture biotechnologies have major potential for facilitating and promoting sustainable agriculture and rural development. They could also generate environmental benefits; especially where renewable genetic inputs can be effectively used to substitute for dependency on externally provided agrochemical inputs. The fact that genes or genotypes (e.g. varieties, breeds) can constitute locally renewable resources is of profound significance to the further development of sustainable agriculture and rural development.
3. Nevertheless, the extent to which modern biotechnology will contribute to the achievement of food security for all is still an open question ! Science alone is unlikely to provide a complete solution to the problems of rural development. There are many processes, factors and socio-economic structures underlying poverty in rural areas, such as lack of access to land and other productive resources, low purchasing power, political powerlessness, fragile environment and distance from markets. Agricultural (or indeed plant



- biotechnology) research is but one factor, which could impact on rural poverty; it is not a panacea for sustainable agriculture and rural development.
4. Over the longer term, there is little doubt that some biotechnological approaches to agricultural improvement could generate social, economic and environmental benefits if specifically targeted at the specific needs of poorer groups. While a vast range of approaches for the biotechnological improvement of such agronomic traits are either under study or in early development phases, given the current lack of focused public sector support for pro-poor agricultural biotechnology it is unlikely that poorer farmers will have economic access to such improvements in the short term.
  5. Field trials of cash crops like cotton and mustard have thrown up prospects of disease-free and pest-free crops, apart from greater yields to make the seeds of these crops cost-effective. Genetic modifications of cattle could improve milk yields enormously even as India is already the world's largest milk producer. Yet the need to preserve some resilient and centuries-old Indian breeds of cow have time and again been recognized by veterinary scientists. Biotech applications could promote rapid multiplication of river and sea fish or seafood to help improve nutrition or provide the desired diet for the non-vegetarians. The transgenic approach should be considered as complimentary and resorted to when other options to achieve the desired objectives are either not available or not feasible. High priority should be accorded in the transgenic approach to the incorporation of resistance to insects and pests and diseases, including viruses and to drought and salinity. Biotechnology could contribute to the breeding of plant varieties or animal breeds tolerant to pests or pathogens that are currently controlled by agrochemicals, which could allow reductions in agrochemical use through the substitution effects of particular genes conferring tolerance. Over the longer term there is much promising agricultural biotechnological research that in theory might be harnessed for sustainable agriculture and rural development objectives, such as increasing yields and sustainable utilization of plant genetic resources for food.

#### **IV Assessing impacts of biotechnology on health and the environment.**

1. There are concerns about potential risks posed by some aspects of biotechnology. These risks fall into two basic categories; the effects on human and animal health and the environmental consequences. Caution must be exercised in order to reduce the risk of transferring toxins from one life form to another, of creating new toxins or transferring allergenic compounds from one species to another, that could result in unexpected allergic reactions. Risks to the environment include the possibility of out-crossing, leading, for example, to the development of more aggressive weeds or wild relatives with increased resistance to diseases or environmental stresses, upsetting ecosystem balance. There is also the potential loss of biodiversity, for example, resulting from the displacement traditional cultivars by a small number of genetically modified cultivars, and the potential for increased crop vulnerability resulting from the possible widespread adoption of varieties with simple, monogenetic, disease resistance mechanisms. However, in principle, these latter effects are no different from those that may result from many conventional approaches to plant breeding.
2. Genetic engineering approaches have considerably broadened the range of gene pools, which are accessible for crop improvement purposes. If countries expect to benefit from modern biotechnologies in their agriculture and food sectors, they will have to give serious consideration to the drafting of biosafety regulations that are tailored to meet their socio-economic needs.
3. There is no evidence to suggest that transgenic crops or biotechnology *per se* would either decrease or increase biodiversity in agricultural or in "natural" ecosystems. Within agricultural systems, plant biotechnology research could be applied to either increasing or decreasing genetic diversity, depending on research objectives. With modern biotechnological methods, the use of the genetic resources from wild crop relatives may actually increase. The selective advantage that a particular genetically modified organism will confer in the agro-ecological niche in which it is applied should be considered in risk assessment.
4. Many naturally occurring plant proteins and compounds can be anti-nutrients, toxic or allergenic. Indeed, a significant number of crop species are toxic if not cooked or prepared properly to reduce or inactivate



such compounds. There is currently no scientifically accepted evidence to suggest that transgenic foods *per se* are any more or less toxic or allergenic for humans than their conventionally bred counterparts. Indeed, genetic engineering approaches and other research approaches are under way to develop “functional foods” or “nutraceuticals” which would contain lower levels of allergens and toxins or higher levels of beneficial compounds than conventional foods.

#### **V. Biotechnology and the private sector**

1. The global market for agricultural biotechnology products was less than US\$ 500 million in 1996 but is projected to increase significantly. As a result, the past decade has seen a major increase in private-sector investment in agricultural biotechnology. According to FAO, Private-sector agricultural research in the OECD countries is now in excess of \$7 billion and accounts for half the world’s entire agricultural research investments. As a result of recent mergers and acquisitions, there are now fewer small agricultural biotechnology companies.
2. For commercial reasons, richer farmers are likely to be the main target for most privately funded plant biotechnology research as reflected in the crop focus of current agricultural biotechnology research, which is heavily biased towards major commercial –often export—crops, such as maize, soybean, canola, cotton, tobacco, tomato, potato, squash and papaya, rather than the food staples of poorer populations, such as millet, sorghum, cassava, sweet potato and plantains. There is a need at both the national and international levels to stimulate research and development for non-export staple crops.

#### **VI. Future for agricultural biotechnology and sustainable agriculture and rural development.**

1. Expenditures on food staples typically absorb half the income of people below the poverty line. Food staples are their main source of nutrients. There is little doubt that if plant biotechnology research were applied to well-defined social or economic objectives, such as improving the food staples of the poor, it could benefit poorer rural and urban groups.
2. There remains the valid concern that the needs of poorer farmers or nations are unlikely to be a factor, which favorably steers the research objectives of biotechnology research, which is dependent on private investment. At the government level, there are currently no policy instruments, which promote the type of biotechnological research that could contribute to food and livelihood security in resource-poor situations, especially in developing countries. Long-term public-sector investment in agricultural research will be essential to address the needs of poorer farmers and consumers, who do not constitute a significant commercial market for private-sector biotechnology research and development. Increased participation by farmers and other key actors in the overall sustainable agriculture and rural development process is of vital concern.
3. It is important to strengthen the communication among public-sector agricultural biotech research, on-farm research and farmer groups to facilitate the realization of sustainable agriculture and rural development.
4. There is a need to adopt a holistic and integrated approach in the application and evaluation of the impacts of agricultural biotechnology. Evaluation of newly engineered crops must consider biodiversity as a value; monitoring bio-indicators can help in reaching decisions about their environmental impacts. Many actions in several fields need to be developed by Governments and by international organizations to make sure that the pro-poor potentialities of agricultural biotechnologies are realized. Care should be taken that the current gap between developing and developed countries does not increase as a result of their lack of appropriate action concerning these key issues.



## **LEAD PAPER**

### **USE OF BIOTECHNOLOGICAL METHODS FOR LIVESTOCK DISEASE DIAGNOSIS**

**Dr. P. Ramadass**

Professor & Head, Department of Animal Biotechnology,  
Madras Veterinary College, Chennai  
Tamil Nadu Veterinary and Animal Sciences University

Diagnosis of disease is very essential for improvement of livestock production. Unless animals are maintained in good healthy conditions, our livestock production could not improve. So, in any good livestock management, control of diseases is of prime importance. Even though one should aim to prevent the occurrence of diseases by regular vaccination, once the disease occurs, it should be promptly diagnosed at very early stage of infection, so that, it could be effectively treated and controlled, without much loss to the health of animals.

Conventionally, diagnosis of disease is achieved by either direct demonstration of the disease causing agents and indirectly through demonstration of antibodies produced by these microbes. Direct demonstration of the disease causing agents should be given more importance, because that will give the state of active infection. Direct microscopical examination is possible in bacterial and parasitic infection or use of immunofluorescence or immunoperoxidase methods could be used for viral pathogens. However, for demonstration of microbes, under microscope, large number of pathogens should be there in the sample, which occurs only at advanced stage of infection. Demonstration of antibodies has been achieved using haemagglutination inhibition test, agglutination test, complement fixation test and neutralization test. These tests lack sensitivity and more cumbersome to conduct the test.

Advent of immunoassays have revolutionized the diagnosis. Enzyme linked immunosorbents assay (ELISA) has been used for almost all diseases for demonstration of antibodies as well as disease causing agents. Immuno assays coupled with monoclonal antibodies and recombinant proteins have increased the sensitivity and specificity of this diagnostic method. Another area, which has developed, recently is the DNA-based methods in disease diagnosis. Nucleic acid probes can be used to identify the presence of microorganisms in tissues by hybridization. Recently, polymerase chain reaction (PCR) method has been increasingly used for detection of pathogens in clinical samples. Very minute amount of nucleic acid of microbes could be amplified to million times within matters of few hours and analysed for the microbes. A modification of PCR method, random amplified polymorphic DNA fingerprinting has been used for detection and characterization of microbes directly in clinical samples. Real time PCR method has been used for quantification of the nucleic acid

#### **Enzyme-linked immunosorbent assay (ELISA)**

Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs can provide a useful measurement of antigen or antibody concentration. There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen. Recently developments in monoclonal antibodies and recombinant DNA technologies have led to the increased use of ELISA with greater sensitivity and specificity. Use of monoclonal antibodies with ELISA formats has led to the specific detection of pathogens. Monoclonal antibody-based competitive ELISA for simultaneous detection of rinderpest virus and peste des petits ruminants virus antibodies has been reported (Choi *et al.*, 2003).

Recombinant N antigen was expressed in a baculovirus and a ruminant morbillivirus (RPV and PPRV)-specific monoclonal antibody for simultaneous detection of RPV and PPRV antibodies. PPRV H gene of the vaccine strain (Sungri-96) was amplified by reverse transcription (RT)-PCR, cloned into a eukaryotic expression



vector (pTarget), and subsequently transfected and expressed in Vero cells (Balamurugan *et al.*, 2006). A stable Vero cell line was developed after 20 repeated passages by using G418 antibiotic selection pressure (400 to 600 microg/ml). The integration of PPRV H gene in the Vero cell genome and its genomic transcription were confirmed by PCR and RT-PCR assays, respectively, and the 70-kDa PPRV H protein was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. The recombinant protein reacted specifically with PPRV anti-H neutralizing monoclonal and polyclonal antibody in competitive, sandwich, and indirect ELISA, respectively, indicating that the native form of the protein was expressed. Evaluation of the protein in competitive ELISA and indirect ELISA vis a vis whole virus was done using 306 and 146 goat field serum samples, respectively; comparable results were obtained with high degrees of relative diagnostic specificity (93.53% and 100%, respectively) and sensitivity (99.04% and 79.16%, respectively). This study shows that the PPRV H protein could be a sustainable source of safe antigen in countries of non-endemicity without the need to handle infectious virus for serodiagnosis.

Wanzala, *et al.* (2002) used a monoclonal antibody-based antigen-ELISA for detecting live *Taenia saginata* cysts in bovines.

A sandwich ELISA was optimized by van den Heuvel *et al.* (2003) for detection of major internal structural protein (p24) produced by bovine leukemia virus in short term cultures of peripheral blood mononuclear cells. Envelop glycoprotein (gp51) of bovine leukemia virus was cloned in to a baculovirus and the recombinant antigen was used in ELISA for detection of BLV antibodies (De Giuseppe *et al.*, 2004).

A 287bp fragment from the middle region of the nucleocapsid protein of canine distemper virus (CDV) was amplified from the conjunctival samples of distemper-infected dogs and was cloned into pRSET B vector (Latha *et al.*, 2006). The recombinant protein was expressed as a 16-kDa-fusion protein with histidine tag in *E. coli*. Sera of distemper-infected and vaccinated dogs contained IgG antibodies against the purified recombinant protein as observed by enzyme linked immunosorbent assays (ELISA) and showed a strong correlation ( $r=0.882$ ,  $p<0.0001$  at 95% CI) and good agreement ( $\kappa=0.718$ ) with the conventional tissue culture viral antigen based ELISA. Further, the results of recombinant protein based ELISA and Western blotting with the sera from the infected and vaccinated dogs correlated well ( $\kappa=0.8226$ ). These findings recommend the use of the recombinant protein in the serodiagnosis of canine distemper virus infection in dogs.

Using ELISA with recombinant antigen has improved the detection sensitivity of the method. Bruderer *et al.* (2004) differentiated infection from vaccination in FMD infection using ELISA based on recombinant 3ABC. The test is based on the detection of antibodies against the recombinant non-structural protein 3ABC. In another study, a recombinant surface protein of *Theileria annulata* was used for the detection of circulating antibodies against tropical theileriosis (Bakheit *et al.*, 2004). This method had a sensitivity of 99.1% and specificity of 90.47%, when taking the IFAT as a reference test.

A recombinant leptospiral protein LipL32 was evaluated for use in the diagnosis of bovine leptospirosis by ELISA (Bomfim *et al.*, 2005). The MAT of 150 serum samples from cattle suspected of leptospirosis showed that 125 (83.3%) samples had positive reciprocal agglutination titres. In rLipL32 IgG ELISA, 83.3% of the samples were positive. The sensitivity and specificity of ELISA were 100%. The rLipL32 IgG ELISA can be an alternative to the MAT for diagnosis of leptospiral infection in cattle.

Monoclonal antibody-based dot-blot ELISA was evaluated for detection of *Leptospira* species in bovine urine samples (Suwimonteerabutr *et al.*, 2005). The results were compared with dark field microscopy (DFM), microbial culture and PCR assay. All urine samples with positive results when tested by use of the Mab-based dot-blot ELISA, except one sample that had positive results only for the PCR assay.

A sandwich-ELISA for the diagnosis of PPR infection in small ruminants using anti-nucleocapsid protein monoclonal antibody has been developed (Singh *et al.*, 2004). This test is specific to PPRV as it failed to detect rinderpest vaccine virus. The compared well with internationally accepted commercial Immune-capture ELISA kit, which uses biotinylated monoclonal antibody against the nucleocapsid protein. This test showed an overall diagnostic specificity of 92.8% and diagnostic sensitivity of 88.9% compared to the commercial kit.

Recently, ELISA method has been clubbed with PCR methods to increase the sensitivity of both methods. Morato *et al.* (2003) developed a PCR-ELISA method for diagnosis of brucellosis. After amplification of a 223 bp



sequence of a gene that codes for the synthesis of an immunogenetic membrane protein specific for the *Brucella* genus (BCSP31), the Digoxigenin-labeled amplified product was hybridized with a biotinylated capture probe which was complementary to the inner part of the amplicon. The hybrid was captured on streptavidin-coated microtitre plate and detected by using an antidigoxigenin Fab-peroxidase conjugate. PCR-ELISA was used for detection of *Babesia bovis* in cattle (Thammasirak *et al.*, 2003). The detection of around  $2.4 \times 10^{-80\%}$  parasitemia (equivalent to 1 infected erythrocyte per 2 ml) was achieved.

### Nucleic acid probes

Nucleic acid probe technology is increasingly used in diagnosis of infectious diseases of veterinary importance. Advances in molecular cloning techniques that made possible the preparation of gene specific probes led to the use of hybridization techniques for the detection of bacterial and viral pathogens in clinical samples. Nucleic acid hybridization can provide confirmation of diagnosis before the results of culture and biochemical tests are available. Nucleic acid probe technology has helped in the detection of virtually any organisms in food, clinical samples or environment. It is now possible to detect wide variety of viral, bacterial and protozoan pathogens in urine, stools, tissues and sputum using radio labeled or non-radio labeled assay systems.

One of the important uses of nucleic acid probes is in the detection of pathogens in clinical samples.  $^{32}\text{P}$ -labeled DNA probes had been used for the detection of leptospires in blood, urine and liver specimens Terpstra *et al.*, 1986). Probes have been prepared for the detection of *Mycobacterium tuberculosis* (Klehin and Edwards, 1987), *Campylobacter* spp. in stool samples (Gebbart *et al.*, 1989), Foot and mouth disease virus from oesophageal and pharyngeal fluids (Rossie *et al.*, 1983), babesiosis (McLaughlin *et al.*, 1986) and trypanosomiasis (Ambrosio *et al.*, 1988) in blood smears.

For rapid and specific identification of *M. tuberculosis* and other mycobacteria several DNA probes have been developed. Commercially marketed probes for *M. tuberculosis* and *M. avium* are also available. These probes are being used in several countries for rapid confirmation of the identity of mycobacterial isolates.

Other uses of nucleic acid probes include differentiation of virulent from avirulent organisms, typing of microorganisms using DNA homology studies, detection of contaminants in the culture, study of mechanisms of pathogenesis and genetic analysis using restriction fragment length polymorphisms.

Gen-Probe products are available for the culture identification of *Mycobacterium tuberculosis*, *M. avium intracellulare* complex, *Cryptococcus neoformans*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Haemophilus influenzae*, *Enterococcus* sp. and *Listeria monocytogenes*. The sensitivity and specificity of the probes are excellent and they provide species identification within one working day.

### Polymerase chain reaction (PCR)

This is a specific *in vitro* amplification of DNA with the use of a pair of primers and DNA polymerase. Amplification is carried out in a thermal cycler, which can be programmed to run cycles of 3 temperature segments, viz., denaturation of target DNA at  $94^{\circ}\text{C}$  for 2 min, annealing of primers to target DNA at  $55^{\circ}\text{C}$  for 1-2 min and synthesis of new strands at  $72^{\circ}\text{C}$  for 1-2 min. At the end of 25-30 cycles of amplification, about 3-5 million times amplification of sample is achieved. PCR assay has now generally superseded the use of specific gene probes in diagnosis, as it is more sensitive and very rapid.

PCR technique represent the ultimate in sensitivity and under optimum conditions are expected to detect 1-10 organisms. This method has shown useful for early confirmation of diagnosis in very early stages of mycobacterial diseases. PCR assay system for tuberculosis is also commercially available from ROCHE (Amplacor) and has been reported to be reproducible, sensitive as well as specific.

Similar to probe techniques, one of the main uses of PCR method is detection of pathogens. PCR method has been used for detection of FMD virus from infected tissues (Meyer *et al.*, 1991), canine parvovirus from faecal samples (Meerarani *et al.*, 1996), leptospires from blood samples (Ramadass *et al.*, 1997), *Mycoplasma pneumoniae* from throat swab samples (D'Oliveira *et al.*, 1995) and *Ehrlichia canis* from tissue samples (Iqbal and Rikibrisa, 1994). RNA viruses have been detected by reverse transcription of the total RNA followed by amplification (RT-PCR).



Bricker and Halling (1994) used a PCR assay with five oligonucleotide primers, which identified selected biovars of four species of *Brucella*. The assay identified three biovars of *Brucella abortus*, three biovars of *Br. mellitensis*, biovar 1 of *Br. suis* and all *Br. ovis* biovars. The assay exploited the polymorphisms arising from species-specific localization of the genetic element IS711 in the *Brucella* chromosome. These primers did not cross react with other bacterial species tested. Although *Brucella* spp. represent a highly homogenous group of bacteria, RFLPs of selected genes display sufficient polymorphism to distinguish *Brucella* species and biovars (Al Dahouk *et al.*, 2005). PCR-RFLP analysis shows excellent typeability, reproducibility, stability, and epidemiological concordance. Consequently, PCR-RFLP assays of specific gene loci can serve as tools for diagnostic, epidemiological, taxonomic, and evolutionary studies. Various PCR-RFLPs used for the identification of *Brucella* species and biotypes are reviewed.

Van Eys *et al.* (1989) developed PCR for detection of leptospires in urine samples of infected cattle and stated that urine sample containing as few as 10 leptospires gave positive result in their PCR assay. Gravekamp *et al.* (1993) reported two sets of primers (G1&G2 and B64 I & B64 II) derived from genomic DNA libraries of leptospiral serovar *icterohaemorrhagiae* (RGA) and *bim*, enabled the amplification of all pathogenic leptospiral species. Bal *et al.* (1994) tested urine samples from patients at different stages of leptospirosis and were able to detect leptospires in approximately 90% of urine samples. They concluded that the detection of leptospires in urine with PCR was a promising approach for early diagnosis of leptospirosis and was useful in studying long term shedding.

Tan *et al.* (1997) studied the usefulness of PCR in the detection of *My. tuberculosis* in different clinical samples. A wide range of clinical samples including sputum, bronchoalveolar lavage fluid, cerebrospinal fluid, pleural fluid, gastric aspirate, pus and tissues were tested. This method did not require routine DNA extraction before PCR. The sample was boiled for 10 min and ultrasonicated for 15 min to release the DNA. The sensitivity was 100% for all types of specimens, while the specificity ranged from 95% for sputum to 88% for bronchoalveolar lavage fluid and pleural fluid and 85% for non-pulmonary specimens.

Bonovska *et al.* (2005) used PCR for detection of *Mycobacterium tuberculosis* in experimentally infected dogs. Tissue samples from internal organs of experimentally infected dogs as well as non-infected but contact animals were investigated by direct PCR. Eighty-three (86.5%) of PCR results coincided with bacteriological finds, 82 (85.4%) with the pathological and 71 (74.0%) simultaneously with bacteriological and pathological results. The observed specific DNA products in tissue samples of infected and non-infected dogs demonstrate significantly sensitivity of PCR method. It could be assumed that the transmission of *M. tuberculosis* infection is possible by close contact between ill and healthy dogs and that the naturally infected dogs or dogs suffering from tuberculosis may serve as a permanent source of infection to humans and other animals.

A PCR-based method for tuberculosis detection in wildlife was reported by Henault *et al.* (2006). PCR seems an interesting alternative method for rapidly screening these species in epidemiological enquiries and immediate decision-making to avoid transmission to livestock. We describe here the validation process for a PCR diagnostic method compared to bacteriology in a wildlife TB survey.

Immunofluorescence assay was compared with RT-PCR and nested PCR in the diagnosis of canine distemper (Jozwik and Frymus, 2005). Blood samples of 23 dogs clinically suspected of distemper were examined by RT-PCR combined with nested PCR, and the results were compared with the detection of the CDV antigen in the smears from the mucous membranes by the direct IF test. On the 23 dogs, 12 were positive in nested PCR, six in the IF assay and only two in single RT-PCR. It is concluded that nested PCR seems to be the most sensitive method for ante-mortem diagnosis of canine distemper, especially in its subacute or chronic forms.

Other uses of PCR include studies of genetic diseases like sickle cell anaemia,  $\beta$ -thalassemia and phenylketonuria, sexing of embryos and molecular gene mapping.



### FDA-approved molecular diagnostic tests

Test	Method	Company
<i>Chlamydia trachomatis</i>	PCR	Roche
<i>Mycobacterium tuberculosis</i>	PCR TMA	Roche Gen-Probe
Group A <i>Streptococci</i>	Hybridization	Gen-Probe
HIV quantitation	PCR	Roche
<i>Neisseria gonorrhoeae</i>	LCR	Abbot
Culture confirmation for bacteria and fungi	Hybridization	Gen-Probe

TMA – transcription-mediated amplification; LCR- ligase chain reaction

### Random amplified polymorphic DNA Fingerprinting

A modification of PCR method is Random amplified polymorphic DNA (RAPD) fingerprinting, which is now frequently used for detection and characterization of pathogens. This is also known as arbitrarily primer PCR, which uses arbitrary primers, which produces reliable and easily obtainable typing schemes. We use a short oligonucleotide primer to produce random but experimentally reproducible sets of amplified DNA fragments. The multiple products resulting from RAPD analysis are then separated according to size by conventional agarose gel electrophoresis and the DNA banding patterns of different isolates can then be compared.

Tcherneva *et al.* (2000) differentiated *Brucella* species by RAPD analysis and concluded that this technique was a simple, quick and sensitive technique for the epidemiological investigation of brucellosis. AP-PCR method has been used for characterization of leptospiral serotypes. Pershina *et al.* (1999) studied 13 strains of four leptospiral species belonging to *L. interrogans*, *L. parva*, *L. illini* and *L. inadai*. Analysis of polymorphisms of amplicon length by the PCR with short sh1 and sh2 primers revealed genotypical differences at the inter- and intraspecies levels, as well as at the subserovar level.

Ramasoota *et al.* (2001) compared *Mycobacterium avium* complex (MAC) strains from pigs and humans in Sweden by RAPD method. The results indicated that pigs and humans might be infected with the same type of MAC strains, since 14 strains from human and 8 strains from pigs were essentially identical.

Zucker *et al.* (1996) differentiated *Pasteurella multocida* subspecies *multocida* (*P.m.sp.m*) isolates from the respiratory systems of pigs using PCR fingerprinting technique. Two single primers were tested for their abilities to generate individual fingerprints by using PCR. Primer 1 (core sequence of the M13 phage) grouped the 44 *P.m.sp.m* strains into five distinct fingerprinting profiles, while Primer 2 (GACA)<sub>4</sub> grouped them into seven profiles. The results suggested that PCR fingerprinting was an efficient technique to detect DNA polymorphisms in the species *P.m.sp.m*.

AP-PCR assay was developed to analyze the genomic DNA of *Salmonella enteritidis* isolates from human outbreaks and from avian sources. The AP-PCR generated 7 distinct randomly amplified polymorphic DNA patterns among the *S. enteritidis* isolates studied. Differences in the DNA patterns among isolates of *S. enteritidis* phage types 13a and 8 as well as among *S. enteritidis* phage type 14b were observed. The authors concluded that AP-PCR could be used to determine the differences among isolates within the same phage types and may be useful for tracing back the source of *S. enteritidis* outbreaks more precisely.

Restriction-site-specific PCR (RSS-PCR), a modification of AP-PCR method was used for rapid detection and characterization of enterohaemorrhagic *E. coli* O157:H7 strains in environmental samples (Kimura *et al.*, 2000). RSS-PCR involved amplification of DNA fragments using primers based on specific restriction enzyme recognition sequences, without the use of endonucleases, to generate a set of amplicons that yield "fingerprint" patterns when resolved electrophoretically on an agarose gel. The RSS-PCR method identified all 28 isolates that were shown to be *E. coli* O157:H7 by the MAb-ELISA as belonging to O157:H7 serotype. Of the remaining 26 ELISA-confirmed non-O157:H7 strains, the method classified 25 strains as non-O157:H7.



## Multiplex PCR

This is one of the recent modifications of PCR, in which more than one primer pairs are used for simultaneous detection of more than one pathogen in clinical samples. The major advantages of multiplex PCR (m-PCR) are conservation of reagents and template, and reduction in preparation and analysis time required to identify multiple target sites in one assay, as opposed to running separate analysis for each target.

A m-PCR assay was developed using two primer sets for the identification and differentiation of *Campylobacter coli* and *C. jejuni* (Harmon *et al.*, 1997). Primer set 1 amplified a 460 bp fragment present in *C. coli* and *C. jejuni*, primer set 2 amplified a 160 bp target unique to *C. jejuni*. The authors concluded that the m-PCR assay offered an alternative to traditional biochemical typing methods for the identification and differentiation of *C. coli* and *C. jejuni*, isolated from poultry and it was accurate, simple to perform and could be completed within 8 hrs.

*Clostridium perfringens*, a common inhabitants of the gastrointestinal tract of warm blooded animals, causes several forms of extensive disease, including fatal enterotoxaemia in domestic animals and human beings. The virulence of the organism is associated with the production of as many as 17 exotoxins and 4 of the these ( $\alpha$ ,  $\beta$ ,  $\bullet$  and  $\iota$ ), the so called the major toxins form the basis for division of the species into 5 toxigenic types. PCR primers were developed against genes of major toxins of *Cl. perfringens* (*cpa*,  $\alpha$  toxin; *cpb*,  $\beta$  toxin; *etx*,  $\bullet$  toxin; *iA*,  $\iota$  toxin and *cpe*, enterotoxin and used in a m-PCR assay (Meer and Songer, 1997). They concluded that the m-PCR was effective, simpler assay and may be an useful alternative to standard *in vivo* typing methods.

Fratamico *et al.* (2000) developed a m-PCR assay for rapid detection and identification of *E. coli* 0157:H7 in food and bovine faeces. They used primers targeting the *aecA*, *stx*<sub>1</sub>, *stx*<sub>2</sub>, *flic*<sub>h7</sub> and *hly*<sub>933</sub> genes of *E. coli* 0157:H7. This assay permitted the detection of *E. coli* 0157:H7 in various types of complex samples, identification of H7 serogroup and identification of the types of Shiga toxin produced in a single assay, resulting in savings in cost and time.

A m-PCR assay was developed for detection of *Brucella abortus* and/or *Mycobacterium bovis* infection in cattle (Sreevatsan *et al.*, 2000) using bovine milk and nasal secretions. Authors concluded that this method was found to be an optimal tool for both screening of herds and testing of individual animals in a disease eradication programme.

Greco *et al.* (2001) optimized a m-PCR assay for the simultaneous detection of several species of small ruminant mycoplasmas. Two sets of oligonucleotide primers specific for *Mycoplasma agalactiae* (Ma) and *Mycoplasma mycoides* cluster (Mm cluster) were used. The m-PCR was able to amplify a 375 bp fragment of Ma chromosomal DNA and a 257 bp fragment of Mm cluster chromosomal DNA. The m-PCR assay identified 35 Ma strains, 12 Mm cluster strains out of 43 positive samples. In four samples, both Ma and Mn clusters were revealed. The m-PCR assay was able to detect 1 pg of mycoplasma DNA.

Moody *et al.* (2000) developed a multiplex real-time quantitative RT-PCR for measuring IBD virus RNA in blood using ABI Prism 7000 sequence detection system coupled with TaqMan chemistry. The results demonstrated that the course of IBDV infection can be monitored by quantifying IBDV RNA extracted from blood of infected chickens using TaqMan technology.

## Real-time PCR

Real-time PCR system relies on the detection and quantitation of a fluorescent reporter, the signal of which increases in direct proportion to the amount of PCR product in a reaction. The reporter is a fluorescent probe or a double-stranded DNA-specific dye SYBR Green which upon excitation emits light. Thus, as a PCR product accumulates, fluorescence increases. There are two general methods for the quantitative detection of amplification fluorescent probes. The TaqMan probes and Molecular beacons use the fluorogenic 5' exonuclease activity of Taq polymerase to measure the amount of target sequences in cDNA samples. TaqMan probes are 20-30 bases long oligonucleotides that contains a reporter fluorescent dye usually on the 5' base and a quenching dye on the 3' base. The close proximity of the reporter and quencher prevents emission of any fluorescence while the probe is intact. During the reaction, when the polymerase replicates a template on which a TaqMan probe is bound, its 5' exonuclease activity cleaves the probe. This ends the activity of the quencher and the reporter dye starts to emit fluorescence which increases in each cycle proportional to the rate of probe cleavage. Accumulation



of PCR products is detected by monitoring the increase in fluorescence of the reporter dye. A real-time PCR was developed for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs with diarrhoea (Decaro et al., 2005). The method was demonstrated to be highly specific and sensitive, allowing a precise CPV-2 DNA quantitation. Comparison between real-time PCR, hemagglutination (HA) and conventional PCR revealed that real-time PCR to be more sensitive than HA and conventional PCR, allowing to detect low viral titres of CPV-2 in infected dogs.

The MB fluorescent probe was used to develop two Bluetongue serogroup-specific assays: a quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR) and a traditional RT-PCR (Orru et al., 2006). These were tested using BTV-RNAs extracted from the blood and organs of BT-affected animals, and from virus isolate suspensions. To evaluate the specificity and sensitivity of the MB probe, all positive samples (and negative controls) were tested using the developed quantitative real time RT-PCR and traditional RT-PCR assays. The former test had a detection limit of  $10^3$  cDNA molecules per reaction with a log-linear quantification range of up to  $10^{11}$  ( $R_2 = 0.98$ ), while the latter test was able to detect 500 cDNA-BTV molecules/PCR. The results showed that the MB fluorescent probe was both rapid and versatile for the laboratory diagnosis of Bluetongue and for quantifying levels of viraemia in BTV-affected animals. An "in silico" comparison of the primers and MB fluorescent probe used in this study showed that it was possible to detect all 24 serotypes of BTV.

## References

- Ambrosio, E.E. *et al.* 1988. *Res.Vet.Sci.* 60: 168.  
Al Dahouk, S. *et al.* 2005. *Crit Rev Microbiol.* 31:191  
Balamurugan V. *et al.* *Clin Vaccine Immunol.* 13:1367.  
Bal, A.E., *et al.* 1994. *J.Clin.Microbiol.* 32: 1894.  
Bonovska, M. *et al.* 2005. *J. Vet. Med. B. Infect. Dis. Vet. Public Health.* 52: 165.  
Bricker, B.J. and S.M.Halling. 1994. *J.Clin.Microbiol.* 32: 2660.  
Decaro, N. *et al.*, 2005. *Vet. Microbiol.* 105: 19.  
D'Oliveira, C. *et al.* 1995. *J.clin.Microbiol.* 33: 2665.  
Fratamico, P.M., *et al.* 2000. *J.Food.Protection.* 63: 1032.  
Gebbert, C.J. *et al.* 1989. *J.Clin.Microbiol.* 27: 2717.  
Gravekamp, C *et al.* 1993. *J.Gen.Microbiol.* 139: 1691.  
Greco, G., *et al.* 2001. *Mol.Cell. Probes.* 15: 21-25.  
Harmon, K.M., *et al.* 1997. *Mol.Cell.Probes.* 11: 195.  
Henault, S. *et al.*, 2006. *Dev Biol (Basel).* 126: 123.  
Iqbal,Z and Y.Rikibrisa. 1994. *Vet.Microbiol.* 42: 281.  
Jozwik, A. and T. Frymus. 2005. *Vet. Res. Commun.* 29: 347.  
Kimura, R. *et al.* 2000. *Appl.Environ.Microbiol.* 66: 2513.  
Kiehin, T.E. and F.F.Edwards. 1987. *J.Clin.Microbiol.* 25: 1551.  
Latha, D. *et al.* 2006. *Vet. Microbiol.* In press.  
McLaughlin, G.L. *et al.* 1986. *J.Protozool.* 33: 125.  
Meer, R.R. and J.G.Songer. 1997. *Amer.J.Vet.Res.* 58: 702.  
Meerarani, S. *et al.* 1996. *Ind. Vet.J.* 73: 1013.  
Meyers, R.F. *et al.* 1991. *J.Virol.Meth.* 34: 161.  
Orru G. *et al.* 2006. *J Virol Methods.* 2006. 137:34  
Pershina, M.I., *et al.* 1998. *Mol.Gen. Mickrobiol. Virasol.* 1: 29.  
Ramadass, P. *et al.* 1997. *Ind.Vet.J.* 74: 457.  
Ramasoota, P. *et al.* 2001. *Vet.Microbiol.* 78: 251.  
Rossi, M.S. *et al.* 1989. *Arch.Virol.* 99: 67.  
Singh, RP. *et al.* 2004. *Arch. Virol.* 149: 2155.  
Sreevatsan, S. *et al.* 2000. *J.Clin.Microbiol.* 38: 2602.  
Suwimonteerabutr, J. *et al.* 2005. *Am. J. Vet. Res.* 66: 762.  
Tan, M.F. *et al.* 1997. *J.Med.Microbiol.* 46: 164.  
Tcherneva, E. *et al.* 2000. *J.Appl.Microbiol.* 88: 69.  
Terpstra, W.J. *et al.* 1986. *J.Med. Microbiol.* 22: 23.  
Van Eys, G.J.J.M. *et al.* 1989. *J.Clin.Microbiol.* 22: 2258.  
Zucker, B. *et al.* 1996. *J.Vet.Med.* 43: 585.



## LEAD PAPER

### **GLOBAL CHALLENGES IN VACCINOLOGY AND CONTROL OF ANIMAL DISEASES – STRATEGIES, APPROACH AND BLUE PRINT**

**Dr. V. Purushothaman, Ph.D.,**

Professor and Head, Dept. of Veterinary Microbiology,  
Madras Veterinary College, Chennai – 600 007.

Vaccines have accomplished near miracle in the fight against infectious diseases. They have cosigned small pox in man globally and Rinderpest in animals in many countries to history. Yet these victories make enormous gaps in the demand and delivery. The situation is worrisome not only in human health but also in animal health. The vaccines have the aim of priming the immune system to destroy swiftly specific disease causing agent. Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer are also being developed).

- 1 Types of vaccines
- 2 Developing immunity
- 3 Vaccination schedule
- 4 Vaccine Controversies
- 5 Adverse effects (known and suspected)
- 6 Economics of vaccine development
- 7 Preservatives

#### **Types of vaccines**

Vaccines may be living, attenuated viruses or bacteria which intentionally give rise to inapparent-to-trivial infections. Vaccines may also be killed or inactivated organisms or purified products derived from them.

There are four types of traditional vaccines:

- Inactivated - these are previously virulent micro-organisms that have been killed with chemicals or heat. Examples are vaccines against Rabies, Foot and Mouth and Fowl Cholera. Most such vaccines may have incomplete or short-lived immune responses and are likely to require booster doses.
- Live, attenuated - these are live micro-organisms that have been cultivated under conditions which disable their virulent properties. They typically provoke more durable immunological responses and are the preferred type. Examples include PPR vaccine, Sheep Pox vaccine, Toxoids - these are inactivated toxic compounds from micro-organisms in cases where these (rather than the micro-organism itself) cause illness. Examples of toxoid-based vaccines include tetanus and enterotoxaemia.
- Subunit - rather than introducing a whole inactivated or attenuated micro-organism to an immune system, a fragment of it can create an immune response. Characteristic example is the subunit vaccine against HBV that is composed of only the surface proteins of the virus (produced in yeast)
- Conjugate - certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g. toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the Fowl Cholera vaccine. Recombinant Vector - by combining the physiology of one micro-organism and the DNA of the other, immunity can be created against diseases that have complex infection processes
- DNA vaccination - in recent years a new type of vaccine, created from an infectious agent's DNA called DNA vaccination, has been developed. It works by insertion (and expression, triggering immune system recognition) into animal cells, of viral or bacterial DNA. Some cells of the immune system that recognize the proteins expressed will mount an attack against these proteins and cells expressing them. Because



these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be attacked instantly by the immune system. One advantage of DNA vaccines is that they are very easy to produce and store. As of now, DNA vaccination is still experimental, but shows some promising results.

### **Developing immunity**

The immune system recognizes vaccine agents as foreign, destroys them, and 'remembers' them. When the virulent version of an agent comes along, the immune system is thus prepared to respond, by neutralizing the target agent before it can enter cells, and (2) by recognizing and destroying infected cells before that agent can multiply to vast numbers.

### **Efficacy of vaccines**

Vaccines do *not* guarantee complete protection from a disease. Even after a vaccination, there is still a possibility that a vaccinated animal may get the disease. Sometimes this is because the host's immune system simply doesn't respond adequately or at all. This is due to a 'low titre of antibodies'.

Adjuvants are typically used to boost immune response. The efficacy or performance of the vaccine is dependent on a number of factors:

- the disease itself (for some diseases vaccination performs better than for other diseases)
- the strain of vaccine (some vaccinations are for different strains of the disease)
- whether one kept to schedule for the vaccinations)
- some animals are 'non-responders' to certain vaccines, meaning that they do not generate antibodies even after being vaccinated correctly
- other factors such as genetic predisposition

### **Adverse effects (known and suspected)**

#### ***Economics of vaccine development***

One challenge in vaccine development is economic: many of the diseases most demanding a vaccine, although some contend pharmaceutical firms and biotech companies have little incentive to develop vaccines for these diseases, because there is little revenue potential, the number of vaccines actually administered has risen dramatically in recent decades. This increase, particularly in the number of different vaccines administered

One of the most significant drivers of value in the vaccines market has been the development and commercialization of new immunological techniques. The development and application of new technologies that have enabled the successful commercialization of new vaccine types, including gene-deleted, subunit and – most recently – 'naked' DNA products, as well as vectored vaccines. The advantages that such approaches offer over classical vaccines are also described, and examples of commercial products in each category are identified.

Other new approaches that have yet to yield commercial products – including synthetic vaccines – are also discussed, along with developments in related fields such as adjuvant technology and vaccine delivery. 'Edible' (plant-derived) vaccines could be a commercial reality before the end of the decade, and may eventually represent a further significant milestone in the transformation of the market.

#### ***Developing vaccines in the 20<sup>th</sup> century***

Existing vaccines can be divided into two broad groups: living and non living vaccines. Living vaccines induce immunity by mimicking natural infections using attenuated forms of pathogens. Non living vaccines use the whole pathogen (killed bacteria, parasites or viruses), or components of them (subunit vaccines), to induce protective immunity. Many of the vaccines developed during the last few decades are based on the subunit approach, that is, they contain one or more protective antigens a number of steps+. Briefly, the pathogen is grown in laboratory conditions and then dissected into individual components. Each component is then tested for its ability to induce immunity. This approach is time-consuming and allows the identification only of those antigens which can be purified in quantities suitable for vaccine testing. In many cases the most abundant proteins are not suitable vaccine candidates, and the genetic tools needed to identify less abundant components may be inadequate or not available at all. In many cases, the antigens expressed during infection *in vivo* are not expressed during the *in vitro*



cultivation. Therefore, the approach can take years or decades and in many cases it can fail to identify protective antigens which are relevant *in vivo* during infection. Once the suitable antigen is identified, it needs to be produced on a large scale, often by growing the pathogen itself. Cloning of the gene coding for the antigen is often necessary in order to better characterize and produce the identified antigen(s). Finally, the new molecule can enter vaccine development. While successful in many cases, this approach took a long time to provide vaccines against those pathogens for which the solution was easy and failed to provide a solution for those bacteria and parasites that did not have obvious immunodominant protective antigens]. When dealing with non-cultivable microorganisms, we had no approach to vaccine development.

#### *Developing vaccines in the year 2000 by reverse vaccinology*

The availability of whole genome sequences has entirely changed our approach to vaccine development and introduced a new way of thinking in this process. This approach, starts from the genomic sequence and, by computer analysis, predicts those antigens that are most likely to be vaccine candidates. There are a number of novel features in this approach. We do not need to cultivate the microorganism. The entire process starts *in silico*, in a clean room containing only a computer, no need of fermenters, pipettes and laboratory. The process can be equally applied to cultivable and non-cultivable microorganisms. Pathogens dangerous to handle can be studied as easily.

#### **References:**

1. Chiron S.p.A., Reverse vaccinology, a genome-based approach to vaccine development, 2001.
2. A.A. Potter and L.A. Babiuk, New Approaches for Antigen Discovery, Production and Delivery: Vaccines for Veterinary and Human Use, Current Drug Targets – Infectious Disorders, 2001, I, 249-262.



*With best compliments from*

# **VISION SCIENTIFIC**

**2<sup>nd</sup> floor, Delta Centre,  
MC Road, Angamali, Kerala - 683 572**

E-mail: [visionscientific@yahoo.com](mailto:visionscientific@yahoo.com)





## **SESSION 1: BIOTECHNOLOGY FOR RURAL LIVELIHOOD SECURITY**

### **1. THE EFFECT OF PROBIOTIC ON THE HUMORAL IMMUNE RESPONSE IN BROILER CHICKS TO NEW CASTLE DISEASE VIRUS.**

**R.Karunakaran, K.Nachimuthu, R.Kadirvel and V.Balakrishnan**

Department of Animal Nutrition, Madras Veterinary College, Chennai-7, Tamil Nadu.

The effect of probiotic, *Lactobacillus acidophilus* 0.5, *Lactobacillus Salivarius* 0.25, *Saccharomyces* sp 0.125 and *Torulopsis* sp 0.125 x 10<sup>6</sup> cfu per kg diet on the humoral immune response of broiler chicks (cobb) of 0-6 weeks of age vaccinated with new castle disease virus vaccine (Lasoto strain) was studied. In a feeding experiment, birds in treatment-1 (T1) were fed with a control diet and birds in treatment-2 (T2) were fed with the diet supplemented with probiotic. Birds in both a treatments were vaccinated with New castle disease virus on the seventh day of hatch and weekly hemagglutination inhibition (HI) titre were assayed. The mean logarithmic value of HI titre in vaccinated control birds (T1) at 1, 2, 3, 4 and 5<sup>th</sup> week of post vaccination were 0.737, 1.963, 2.27, 1.921 and 1.307, respectively, whereas in the vaccinated probiotic birds (T2), the weekly HI titre were 1.097, 2.942, 2.654 and 2.110, respectively. There was a significant increase in HI titre (P<0.01) in probiotic birds compared to control birds. It is concluded from this study that probiotic enhanced humoral immune response in broiler chicks to New castle disease virus.

### **2. FMD STATUS, OCCURRENCE AND DISTRIBUTION OF FMD VIRUS TYPES IN THE STATE OF KARNATAKA- INDIA FROM 1977-2004**

**Raveendra Hegde, V.R. Girish\*, Giridhar. P and C. Renukprasad**

Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore-24

Foot and mouth disease (FMD) status is an important determinant in international trade of livestock and their products and countries reporting FMD suffer from severe production losses and are deprived of export opportunities. In India, the disease is endemic with more than 5000 outbreaks being reported every year. Present study discusses the status of FMD in the state of Karnataka for the past 28 years from 1977 to 2004 to know the prevalence and distribution of serotypes. Of the 1949 clinical samples processed by micro complement fixation test (1977-1996) maximum of 525 samples were found to be positive for 'O' type followed by Asia-1 (321 samples), 'A' (189 samples) and 'C' type (86 samples) test. When the clinical samples (1,922 samples) were screened by sandwich ELISA (1997-2004), type 'O' was found to be the most prevalent followed by type 'A' and type Asia-1 whereas serotype 'C' was not recorded in the state since 1994. The study revealed that serotype 'O' was the most prevalent type during the last 28 years. Out of the 1683 outbreaks typed during the study, type 'O' was encountered in 962 outbreaks with Relative frequency indexes (RFI) of 57.15%, 'Asia-1' in 421 outbreaks with RFI of 25.16% followed by 'A' in 214 outbreaks (RFI-12.71%) and 'C' in 86 outbreaks (RFI-5.11%). However, data for outbreaks typed in last twelve years revealed (764 outbreaks) that the maximum were due to type 'O' (620 numbers) with a RFI of 81.15% followed by Asia-1 (RFI-11.12%) and 'A' (RFI-7.2%). The study provides information on status of the disease that can help in taking up the preventive measures for effective control of the disease. Clinical samples processed, outbreaks reported and serotypes recorded during 28 years will be discussed.

\*Intervet India Pvt. Ltd., Intervet house, 33, Pune-Nagar Road, Pune-14



### 3. DEVELOPMENT OF PROBIOTICS FOR BROILER CHICKS TO AUGMENT "GUT HEALTH" AND GROWTH PERFORMANCE

**R.Karunakaran, K.Nachimuthu, R.Kadirvel and V.Balakrishnan**

Department of Animal Nutrition, Madras Veterinary College, Chennai-7, Tamil Nadu.

A study was undertaken to isolate probiotic microflora from chicken gastrointestinal tract and to evaluate their effect on the performance of broiler chicks. *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Saccharomyces sp* and *Torulopsis sp* were isolated from the chicken gut. A probiotic was developed with *L.acidophilus* 0.5, *L.Salivarius* 0.25, *Saccharomyces* 0.125 and *Torulopsis sp* 0.125  $10^6$  cfu/g and was evaluated in a feeding trials with broiler chicks. There was a significant ( $P>0.01$ ) 12.2% increase in weight gain in probiotic fed birds (1635.5g) over the control birds (1437.3 g) at the sixth week of age. The probiotic feeding lowered the intestinal coliform count ( $5.263_{10}$  cfu/g) by 74.5% when compared to control feeding ( $10^{5.863}$  cfu /g). The total microbial population in the droppings of probiotic birds ( $10^{6.28}$  cfu /g) increased by 12% over control birds ( $10^{6.23}$  cfu / g). It is concluded that probiotic feeding improved growth rate and gut health of the broiler chicks.

### 4. THERAPEUTIC EFFICACY OF METHANOLIC EXTRACT OF *ACHILLEA MILLEFOLIUM* LEAVES AGAINST *TRYPANOSOMA EVANSI*.

**Shaba, P<sup>1</sup>. Pandey, N.N<sup>1</sup>. Sharma, O.P;<sup>2</sup> Rao, J.R<sup>3</sup> and Singh, R.K<sup>4</sup>**

Division of Medicine, Indian Veterinary Research Institute, Izatnagar -243 122, India.

Animal trypanosomosis had re-emerged in recent years. It is causing havoc in Sub-Saharan Africa and Latin America where the disease is endemic. Resistance to available trypanocides is on the increasing thereby jeopardizing chemotherapy, which is the only mean of combating the disease in infected animals. In search for trypanocidal compounds from medicinal plants, *Achillea millefolium* leaves were screened. Vero cell line maintained in Dubecco's Modified Eagle Medium (DMEM) and seeded in flat bottom ELISA plates were incubated with *Trypanosoma evansi* for more than 12 h. Powdered leaves were dissolved in DMEM and solubilized in 1% dimethylsulphoxide. Concentrations (250-1000  $\mu\text{g/ml}$ ) of methanolic extract of *Achillea millefolium* leaves was added to the Vero cell culture medium and incubated at appropriate conditions. The cytotoxicity test of it was carried out on Vero cell line at different concentrations (100-1.56  $\mu\text{g/ml}$ ) and incubated at same conditions for 72 h. After 72 h, supernatant was discarded and ELISA plates were stained with crystal violet in phosphate buffered solution and incubated in ordinary incubator for 24 h. *In vitro*, *Achillea millefolium* extract induced immobilization and killing of the parasites in concentration-time depended manner. There was significant reduction of parasites counts from concentration of 750  $\mu\text{g/ml}$  and complete killing of parasites at 1000  $\mu\text{g/ml}$  in 7h of observation, which was equivalent to 50  $\mu\text{g/ml}$  of diminazen acetate (Berenil), standard drug, where no parasite could be detected. There was significant difference between the treated group and untreated control ( $P < 0.05$ ). Both immobilized (clumped trypanosomes) and apparently killed trypanosomes were injected into two groups of mice and observed for a period of 30 days. Mice injected with immobilized trypanosomes developed parasitaemia while, the other group did not. The extract was cytotoxic to Vero cell line at concentrations of 100-6.25  $\mu\text{g/ml}$  compared to 100-12.5  $\mu\text{g/ml}$  of diminazen acetate (Berenil).

Keywords: *Trypanosoma evansi*, antitrypanosomal activity, *Achillea millefolium* leaves.

2-Regional Station, Palampur 176 061, 3-Division of Parasitology, 4-Regional Station, Mukteswar



## 5. EFFECT OF SUPPLEMENTATION OF INORGANIC SELENIUM SOURCES ON IMMUNE RESPONSE OF LAYERS

**B.E. Aruna, B.S.V. Reddy, R.G. Glori doss and N. Suma**

Department of Animal Nutrition, Veterinary College, Hebbal, Bangalore -24

A trial was conducted to study the effect of supplementation of selenium (Se) in either inorganic or organic form on immunity of layers. One hundred sixty BV-300 layers of 32-weeks of age were randomly distributed into 10 groups of 16 each. The control group (T<sub>1</sub>) was fed a basal diet formulated in conformation with BIS (1992) excepting Se. The test groups (T<sub>2</sub>, T<sub>3</sub>, T<sub>7</sub> and T<sub>9</sub>) were fed the same basal diet in which sodium selenate, an inorganic source of Se (46%) was included (0.3 ppm) and inturn with Copper (1.5ppm in T<sub>7</sub> and T<sub>9</sub>) and Vitamin E (15ppm in T<sub>3</sub> and T<sub>9</sub>). The test groups (T<sub>3</sub>, T<sub>6</sub>, T<sub>8</sub> and T<sub>10</sub>) were fed the same basal diet in which Selplex, a commercial organic source of Se (2000ppm in chealated form) was included (0.15%) and inturn with Cu (1.5ppm in T<sub>8</sub> and T<sub>10</sub>) and Vitamin E (15ppm in T<sub>6</sub> and T<sub>10</sub>) where as T<sub>4</sub> was incorporated with Selenosac EC, an another commercial organic source of Se (200ppm), Cu (1%) and VitaminE(10%). Antibody titer for Newcastle disease (ND) and infectious bursal disease (IBD) were conducted at 28-days interval during the 84 days' feeding trial. The ND antibody titre as determined by HI test and average log<sub>2</sub> HI titre were 5.50, 5.92, 8.50, 8.25, 6.75, 8.83, 6.50, 8.33, 7.75 and 8.50 in T<sub>1</sub> to T<sub>10</sub>, respectively. The average IBD antibody titre determined by ELISA method was found to be 852.0, 1264.8, 1812.1, 1478.5, 1210.0, 1724.5, 1130.1, 1494.0, 1305.6 and 1154.8 in T<sub>1</sub> to T<sub>10</sub>, respectively. Selenium unsupplemented group recorded lowest antibody titre and selenium supplementation in the organic form showed better immune response than inorganic form. Vitamin E addition further improved the antibody titre while addition of copper did not influence immune response. From the results, it may be concluded that the organic Se employed in the present study could improve the immune response of layers.

## 6. *IN VITRO* ASSESSMENT OF BACTERIOSTATIC POTENCY OF EGG YOLK IgY AGAINST SALMONELLA.

**Vikrama Chakravarthi.P<sup>1</sup>, Shanmugasundaram.K<sup>1</sup>, Aravinthan.P,  
Malmurugan.S<sup>2</sup> and Sugumar. K<sup>2</sup>**

Department of Veterinary Microbiology, VCRI, Namakkal, Tamil Nadu

Food borne disease which is due to Salmonella could prevent by using chemical preservatives and antimicrobial agents. But the development of resistance and side effects due to indiscriminate usage without proper medical intervention necessitated the need for alternate strategies. Of the available options the application of polyclonal antibodies like immunoglobulin Y could regard as a good alternative. The O antigens of food poisoning pathogen Salmonella was prepared and used to immunize commercial layer chickens in order raise immunoglobulin against Salmonella. The eggs, which contain antisalmonella IgY, were collected on 30<sup>th</sup> day of first injection of antigen and stored at 4°C. The antibacterial IgY was separated by water dilution method (10 times diluted with distilled water, pH 5-5.5, incubated at 4°C for 6 hrs) and purified by 60 percent ammonium sulphate. The recovery of IgY was in range of 57-62 per cent. The pathogens in Tryptic soya broth (approximately 6 X 10<sup>8</sup>/ml) were cultured with specific IgY @ 20 mg/ml and the inhibitory effect was measured in UV spectrophotometer at 550 nm. The resultant growth curve indicated that the application of polyclonal antibodies (IgY) on meat could be used to prevent the Salmonella food poisoning. The process enhances the safety of various food products as it's neutralize or reduce the ability of food borne pathogen like Salmonella to proliferate prior to human consumption and there by increasing the face value and safety index of the food product.

1. Teaching Assistant, COVAS, Pookot, 2. Assistant Professor, VCRI, Namakkal.



## 7. IMMUNOSTIMULATORY POTENTIAL OF *EMBLICA OFFICINALIS* (AMLA) ON MURINE IMMUNE SYSTEM

**R. S. Suja , A. M. C. Nair, S. Sujith, J. Preethy, A. K. Deepa**

Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences,  
Mannuthy, Kerala Agricultural University, Thrissur- 680651, Kerala, India.

The aqueous extract of dried *Emblica officinalis* (Amla) fruit pulp was studied for its effect on cellular and humoral immune functions in mice. The extract was administered orally at two dose levels of 100 mg/kg body weight and 200 mg/kg body weight for 19 days. Administration of *Emblica* at both dose levels significantly augmented Sheep red blood cells induced Delayed Type of Hypersensitivity reactions in mice. A marked dose related increase in Haemagglutination antibody titre was also observed indicating stimulation of humoral immune functions. Besides, it produced activation of macrophage functions, as evidenced by the increased Macrophage Migration Index in amla treated animals and a significant rise in respiratory burst activity of peritoneal macrophages, detected through NBT reduction assay. The total leukocyte count and relative lymphoid organ weight were also effectively attenuated by the administration of *Emblica officinalis*. The results thus obtained indicate the ability of aqueous extract of *Emblica* to stimulate humoral and cell mediated immunity along with macrophage phagocyte system in mice.

## 8. EVALUATION OF VARIOUS ATTRIBUTES OF SPERMATOZOA IN RELATION TO ZONA PELLUCIDA PENETRATION TO ASSESS BUFFALO SEMEN FERTILITY

**S. Selvaraju, J.P. Ravindra and J. Ghosh**

Animal Physiology Division, National Institute of Animal Nutrition and Physiology,  
Adugodi, Bangalore-560 030

The aim of this study was to evaluate various attributes of spermatozoa in relation to zona pellucida penetration rate to assess semen quality in buffalo. The percentages of (mean  $\pm$  SE) progressive forward motility, acrosome integrity, sperm nuclear morphology and mitochondrial membrane potential were  $51.67 \pm 2.69$ ,  $77.51 \pm 7.50$ ,  $84.64 \pm 0.86$  and  $19.65 \pm 3.18$ , respectively. Plasmalemma integrity was assessed by three different methods. The plasmalemma integrity assessed by vital stain ( $59.59 \pm 2.66$ ) was significantly ( $P < 0.01$ ) higher than hypoosmotic-swelling (HOS) test ( $51.25 \pm 2.32$ ). However, the plasmalemma integrity assessed by vital stain did not differ with fluorogenic staining ( $52.97 \pm 7.86$ ). The subpopulation of spermatozoa positive for plasmalemma functional membrane and acrosomal integrity measured by hypoosmotic swelling-Giemsa (HOS-G) test ( $42.87 \pm 4.56$ ) was significantly ( $P < 0.05$ ) lower than functional membrane integrity. Sperm-zona penetration differs significantly ( $P < 0.05$ ) between animals. The percentages of penetrated and polyspermic oocytes were  $71.33 \pm 8.22$  and  $77.96 \pm 1.64$ , respectively. The average number of spermatozoa penetrated zona pellucida was  $3.35 \pm 0.64$  (range 1.24 to 4.97). The sperm zona penetration had significant correlations with plasmalemma integrity (vital:  $r = 0.83$ ; fluorogenic:  $r = 0.82$ ; HOST:  $0.89$ ;  $P < 0.05$ ), plasmalemma functional membrane and acrosomal integrity (HOS-G;  $r = 0.85$ ;  $P < 0.05$ ) and mitochondrial membrane potential ( $r = 0.89$ ;  $P < 0.05$ ). However, no significant correlations was observed between the sperm zona penetration and acrosomal integrity ( $r = 0.51$ ), and sperm nuclear morphology ( $r = 0.34$ ). Stepwise regression model for buffalo sperm zona penetration ability, using results from different spermatological parameters as independent variables showed that Hyp-osmotic swelling test (functional membrane integrity;  $P < 0.019$ ), HOS-G test (sperm subpopulation having both functional membrane integrity and acrosomal integrity,  $P < 0.020$ ) and mitochondrial membrane potential ( $P < 0.034$ ) had significant influence on zona penetration. The present study suggests that sperm subpopulation positive for functional membrane integrity and acrosomal integrity and mitochondrial membrane potential are important attributes determining sperm zona penetration.



## 9. GENOTYPING BY *ICA*A PCR, OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM CASES OF BOVINE MASTITIS

**Rajeev N. Kuler**<sup>1</sup>, **Shrikrishna Isloor**<sup>2</sup>, **V V S Suryanarayana**<sup>3</sup>, **B M Veeregowda**<sup>4</sup>,  
**N B Sridhar**<sup>5</sup> and **D. Ratnamma**<sup>6</sup>

Department of Veterinary Microbiology  
Veterinary College, KVAFSU, Hebbal, Bangalore-560024

Bovine mastitis adversely affects both quantity and quality of milk. Occurrence of mastitis involves interaction of microbes, management and environmental factors. Multiple organisms that most frequently cause mastitis can be classified into two groups based on their sources as contagious and environmental pathogens. *Staphylococcus aureus* is one of the most common pathogens responsible for contagious mastitis. The ability of *S. aureus* to form biofilm *in vivo* is considered to be a major virulence factor influencing its pathogenesis in mastitis. The implication of biofilms in chronic infections has triggered an increasing interest in the characterization of genes involved in biofilm formation. Biofilm formation is considered to be a two step process in which the bacteria first adhered to a surface, mediated by a capsular antigen namely, capsular polysaccharide/adhesion (PS/A), followed by multiplication to form multilayered biofilm, which is associated with polysaccharide intercellular adhesion (PIA). The intercellular locus consisting of the genes *ica* ADB and C encodes the proteins mediating the synthesis of PIA and PS/A in *Staphylococcus* species. Among the *ica* genes, *ica* A and *ica* D have been reported to play an important role in biofilm formation in *S. aureus* and *S. epidermidis*. The aim of present study was to characterize the *S. aureus* isolates based on *ica*A specific PCR. A total of 25 *S. aureus* isolates were recovered from clinical and subclinical mastitis (6/25 from clinical mastitis and 19/25 from subclinical mastitis). These 25 isolates were subjected for *ica*A specific PCR. Twenty three of the 25 *S. aureus* possessed the *ica*A gene. This study indicated the high prevalence of the *ica*A genes among the *S. aureus* mastitis isolates. PCR based amplification of *ica*A gene not only helps us to identify the potential of *S. aureus* to produce biofilms but also its role in establishment of infection in both clinical and sub clinical mastitis.

1: PG student; 2,4,5 and 6: Assistant Professors; 3: Principal Scientist, IVRI, Bangalore

## 10. IMMUNOMODULATORY EFFECT OF VITAMIN C IN BROILER CHICKEN UNDER OVERCROWDING STRESS

**Karthiyani.K\*** and **Philomina. P.T\*\***

Department of Physiology, College of Veterinary and Animal Sciences, Mannuthy  
Kerala Agricultural University, Thrissur- 680651, Kerala, India.

The effect of vitamin C on the immune responses of broiler chicken of vencob strain under overcrowding stress (348cm<sup>2</sup>/ bird as against 696cm<sup>2</sup>/bird) was evaluated during summer and rainy seasons of Kerala. Four week old broiler chicks were divided into three groups with 12 birds in each group *viz* G-I- unstressed control group (floor space / bird as 696cm<sup>2</sup>), G-II-stressed control (floor space / bird as 348cm<sup>2</sup>), G-III-stressed (floor space / bird as 348cm<sup>2</sup>) birds supplemented with vitamin C @ 0.03 per cent in the feed and reared upto eight weeks of age. The blood samples were collected at fortnight intervals for the evaluation of the total lymphocyte count (TLC), lymphocyte proliferation response, plasma globulin concentration and albumin: globulin ratio (AGR). Overcrowding stress and dietary supplementation of vitamin C did not produce any significant effect on the value of TLC in overcrowded birds. It was seen that overcrowding (348 cm<sup>2</sup>/bird) produced a significantly (P ≤ 0.05) lower plasma globulin concentration in six and eight week old birds both in summer and rainy seasons. Vitamin C was found to be effective in rectifying the reduced globulin level in six and eight week old broiler chicks



in both the seasons. The AGR value was increased by the applied stress in eight week old birds in the summer and in six and eight weeks old birds in the rainy season. Vitamin C could not produce any significant reduction in the AGR value of eight week old birds in summer and six week old birds in the rainy season. However, dietary supplementation of vitamin C @ 0.03% level for 28 days produced a significant ( $P \leq 0.05$ ) reduction in the AGR value in eight week old birds in the rainy season. In both summer as well as rainy seasons the stressed control birds in the G-II group had a lower lymphoblastogenic response than that of birds in control G-I group. Vitamin C could improve the lymphoproliferation in response to mitogen in overcrowded broilers of GIII group at sixth and eighth week of age both in summer and rainy seasons. The results of the study suggested that vitamin C @ 0.03% level in the feed could improve the immune status of overcrowded birds in summer and rainy seasons.

\*Assistant Professor, Department of Physiology, COVAS, Mannuthy.

\*\* Professor & Head, Department of Veterinary Physiology, COVAS, Pookot.

## 11. IMMUNOMODULATORY EFFECT OF GARLIC POWDER AND NEEM SEED CAKE FEEDING IN BROILER CHICKEN

Lonkar V.D<sup>1</sup> and Jalaludeen A<sup>2</sup>

Dept. of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, Kerala Agricultural University, Thrissur- 680651, Kerala, India.

The immunomodulatory effect of the dietary inclusion of Garlic Powder (GP) and Neem Seed Cake (NSC) in broiler chicken was investigated for a period of 6 weeks. Two hundred and ten day-old straight run commercial broiler chicks were randomly distributed into seven different treatments with three replicates of ten chicks each. The experiment was carried out with two levels of GP (0.5 & 1.0 %), two levels of NSC (1.0 & 2.0 %) and two levels of GP and NSC combination (0.5 % GP + 1.0 % NSC & 1.0 % GP + 2.0 % NSC). The weight of bursa of fabricious (BF) and spleen (SP) was recorded from two birds from each replicate at the end of sixth week during slaughter. The birds supplemented with combination of 0.5 % GP and 1.0 % NSC and 1.0 % GP and 2.0 % NSC had significantly ( $P < 0.05$ ) higher BF weight than that of control birds. The weight of BF of birds supplemented with other levels was numerically higher than control group. The weight of SP of birds supplemented with different levels of GP and NSC alone and their combinations was significantly ( $P < 0.05$ ) more than the control birds. The increased weight of BF and SP might be due to induced lymphoid proliferation. Moreover, the dietary supplementation of GP, NSC and their combination had no deleterious effect on livability. It was concluded that inclusion of GP, NSC and their combination in broiler diet may improve the immunity and thereby health status of broilers.

**Key words:** Garlic, Neem, Immunomodulation, Broiler chicken

1. Teaching Assistant, Dept. of LPM, COVAS, Pookot.

2. Associate Professor & Head, Dept. of Poultry Science, COVAS, Mannuthy, Thrissur

## 12. FATTY ACID SYNTHESIS AND DESATURATION IN PORCINE PINEAL GLAND, LIVER AND BRAIN: RELATION WITH SERUM GLUCOSE LEVELS

Mohan.N.H., B.C.Sarmah<sup>1</sup>, M.K.Tamuli, Anubrata Das,  
K.M.Bujarbaruah and D.Kalita<sup>1</sup>

National Research Centre on Pig, Indian Council of Agricultural Research,  
Panjabari road, Guwahati- 781022. Assam

The pineal gland plays a major role in sexual development, hibernation, metabolism and seasonal breeding. The pineal synthesizes and secretes the hormone melatonin and pineal peptides. The pineal varies from the brain tissue in various aspects, one of which is with regards to fatty acid synthesis and composition. The present study was conducted with an objective of comparing the expression of genes concerned with fatty acid synthesis (Fatty



acid synthase, FAS; EC 2.3.1.85) and desaturation (Stearoyl CoA desaturase, SCD; EC.1.14.99.5) in pineal with that of liver and brain cortex. The porcine tissues (pineal, liver, and brain) were collected at the time of slaughter. Following isolation of RNA, the cDNA was subjected to real time polymerase chain reaction using gene specific primers. Serum Glucose concentration was also estimated in these tissues. FAS gene expression was 7.14 and 3.17 times higher respectively in liver and brain than pineal tissue. The SCD gene expression was 5.16 and 2.07 times higher respectively in liver and brain than pineal tissue. The serum glucose levels were more correlated with the FAS expression in pineal, followed by liver and brain. With respect to SCD, the correlation between gene expression and serum glucose levels were almost similar in the tissues studied.

1. College of Veterinary Science, AAU, Khanapara, Guwahati- 781022. Assam

### **13. COMMERCIAL PRODUCTION OF SEX SORTED SEMEN FOR PREDICTION OF SEX AND ITS SCOPES**

**V.S.Binoy\* & Ani.S.Das\*\***

Kerala Livestock Development Board Limited

The first mammal (the rabbit) whose sex was predetermined by the separation of X-and Y-chromosomes bearing sperm was reported upon in 1989 and this was followed in 1991 by the first pigs. This innovative work was carried out at the USDA laboratories in Maryland in the USA. Since that time there have been a series of major developments in this area that have involved personnel who are either employed by or are closely associated with XY, Inc. These include the first sex predetermined cattle, the first sheep following intrauterine insemination, the first cattle after AI with sorted sperm, the first cattle with sorted sperm which were subsequently frozen and the first sex predetermined horse. A major breakthrough in the progress towards the commercialization of sex sorted sperm was the development of the MoFlo<sup>R</sup>, a flow cytometer capable of sorting cells at very high speeds and a fruitful synergism has developed between the manufacturer, Cytomation and XY, Inc. with developments taking place both in relation to more efficient sperm sorting and in sperm treatments for insemination.

This paper is to give a glance on the procedures and its scopes for future biotechnological advancements. The procedure of sex sorted sperm production and predetermination of sex include semen collection, sperm evaluation, sperm assessment, staining sperm with HOECHST 33342, startup and alignment procedures of MoFlo<sup>R</sup> sperm sorting, pre-freeze processing of sperm, sperm freezing, sperm handling and thawing and artificial insemination. The potential scopes of sex sorted sperm production and genetic pre-selection may include breeding of farm animals for desired sex, assisted reproduction of endangered species, breeding bull production, breeding bull selection, sexed in vivo embryo production, IVF using sexed sperms, microinjection of sexed sperm for embryo production and multiplication of such sexed embryos through cloning, research applications etc. Sexing technology is a very effective research tool for the study of sex-influenced traits in animals used for food production. It is also a powerful tool for study of gender linked animal diseases as well as fertility and infertility problems.

\* Dr.V.S.Binoy, Deputy General Manager (BT), KLD Board Ltd, Mattupatti.P.O.,Munnar (via), Idukki (Dist)-685616. \*\*  
Dr.Ani.S.Das, Managing Director, KLD Board Ltd, 'Gokuom' Pattom, Trivandrum-4

### **14. AN AYURVEDIC SUPPLEMENTATION (BR) ENHANCES HUMORAL IMMUNE RESPONSE IN HEAT STRESSED CHICKENS**

**V. Ramnath, P. S. Rekha and K. R. Mejo.**

Dept. of Physiology, College of Veterinary and Animal Sciences,  
Kerala Agricultural University, Mannuthy, Thrissur - 680 651.

Study was conducted on 30 local strain male chickens (Gramapriya) to investigate the effect of heat exposure ( $40 \pm 1^\circ\text{C}$  in an environmental chamber for 4h daily for 10 days) on the humoral immune response and



also to investigate the effect of oral administration of an ayurvedic supplementation (Brahma Rasayana) @ 2g/kg bw daily for 10 days prior to and during the experiment. Heat stress brought about a low profile of humoral immune response as indicated by reduced titres of anti-SRBC haemagglutinin (HA), haemolysin (HL), IgG and IgM. Heat stress in chickens also led to rapid catabolism of preformed antibodies against a known antigen as indicated by a progressive decline in the titre of antibody. The BR supplementation in heat stressed chickens brought about a significant immunopotentiative response on all the above immune parameters. Treated chickens also exhibited more splenic cells that produce antibodies against RRBC, which were confirmed by Plaque Forming Cell assay (PFC) and Rosette Forming Cell assay (RFC). Results thus suggest that BR supplementation could improve the humoral immune status of the heat stressed chickens.

## **SESSION 2: PRESENTATION FOR YOUNG SCIENTIST / MID CAREER SCIENTIST AWARD**

### **ISVIB Young Scientist Award**

#### **1. MOLECULAR CHARACTERIZATION OF A NEWLY IDENTIFIED DUCK RANTES CC-CHEMOKINE HOMOLOGUE AND FUNCTIONAL STUDIES USING A CHICKEN B-CELL LINE, DT40**

**D. S. Arathy, Soja Saghar Soman, & E. Sreekumar**

Molecular Virology and Immunology Laboratory, Department of Molecular Microbiology,  
Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram-695014

Ducks are an important agricultural species, with more than 3 million metric tons of meat produced annually (<http://www.fao.org>), primarily in Asia. Ducks are implicated in the dispersal and evolution of influenza A viruses. Understanding immune response of ducks to this virus will be a critical step in global influenza eradication programmes. Ducks are also natural hosts of a duck hepatitis B virus (DHBV) similar to the hepatitis B virus and are an important model for hepadna virus replication. RANTES (Regulated on Activation Normal T cell Expressed and Secreted) is a pro inflammatory cytokine typically inducible and participate in the generation of both innate and adaptive immune response. The cDNA clone encoding duck RANTES with 81% homology to reported chicken RANTES has been described (Sreekumar *et al*; 2004) from our laboratory. In the present study the full length gene of duck RANTES was cloned and exon –intron organization was studied. Exon –intron organization revealed presence of three exons and two introns in the gene. Promoter sequence by genome walking could identify major transcription factor binding sites in the immediate upstream sequences of the gene. The prokaryotic expression and purification of recombinant RANTES was standardized. The purified protein was used for hyper immunization in rabbit. An indirect ELISA was standardised to find out the antibody titre. A Chemotactic assay of eukaryotically expressed protein was standardized with a modified Boyden chamber assay. A chicken B cell line DT40 cells were used for the assay. A known chemotactic peptide fMLP was included as positive control. The recombinant protein was found to be functionally active. It is the first report on the production and characterization of recombinant avian RANTES.

#### **2. ANALYSIS OF TISSUE mRNA EXPRESSION PROFILE AND ANTI-BACTERIAL SPECTRUM OF ANATIN-2, THE FIRST $\beta$ -DEFENSIN HOMOLOGUE IDENTIFIED IN DOMESTIC DUCK**

**Soja Saghar Soman, D. S. Arathy & E. Sreekumar**

Molecular Virology and Immunology Laboratory, Department of Molecular Microbiology,  
Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram-695014

Beta-defensins are a group of peptides with broad spectrum microbicidal activity and providing a link between innate and adaptive immune response. A novel beta defensin, *Anatin-2* was identified from domestic



duck (*Anas platyrhynchos*) by screening of an enriched cDNA library constructed from mitogen-stimulated splenic mononuclear cells by subtraction hybridization. Molecular analysis of *Anatin-2* showed, the mature protein is having 42 amino acids, it is cysteine rich and cationic. The mature protein with the signal peptide has 64 amino acids. In Phylogenetic analysis, the protein showed close relation to chicken heterophil peptide (Gallinacin-2) and Turkey heterophil peptide (THP-1). The tissue expression profile of *Anatin-2* was done by RT-PCR. The high expression level of *Anatin-2* mRNA in bone marrow and spleen indicates it is a myeloid defensin. Basal level expression of *Anatin-2* was noted in bursa of Fabricius, kidney, Lung, Brain, Ovary, Small intestine, Gall bladder and Oesophagus. His-tagged *Anatin-2* cDNA was cloned to PET32a expression vector and expressed in BL21DE3 pLys S cells. The molecular weight of expressed *Anatin-2* was around 6kDa as detected by Western blot, using anti-His antibody. The expressed protein was purified by Ni-NTA affinity chromatography and 90% purity was obtained. Antimicrobial assay of the purified protein was standardized with standard plate count assay and bacter-titer-glo assay (Promega) against *Escherichia coli* NCIM 2685, *Staphylococcus epidermidis* NCIM 2493, *Bacillus cereus* NCIM 2322 and *Salmonella typhimurium* NCIM 2501. The protein at a concentration of 61 g/ml showed more than 50% reduction in the growth of these microbes. This is the first report of the identification and characterization of beta-defensin of Duck origin.

### 3. CHARACTERIZATION OF A VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS ISOLATE ADAPTED IN CHICK EMBRYO FIBROBLAST CULTURE

**V. Umapathi**

Jr. Research Officer

Department of Veterinary Biochemistry, College of Veterinary & Animal Sciences,  
G.B. Pant University of Agriculture & Technology, Pantnagar- 263 145, UTTARANCHAL.

Infectious bursal disease virus (IBDV) has been classified into different pathotypes viz. classical virulent, attenuated, antigenic variant and very virulent strains. Out of these, the very virulent pathotype, isolated in various parts of the country, continued to cause economic impact on Indian poultry industry. In contrast to the common observation that very virulent isolates of IBDV are refractory to adaptation to chick embryo fibroblast (CEF) culture, a field virus isolated from Uttaranchal has been successfully adapted to CEF culture. The isolate showed very virulent nature when studied for pathological and molecular characteristics. The isolate caused 40% mortality in four weeks old specific antibody negative (SAN) white Leghorn chicks. Reverse transcription-Polymerase chain reaction followed by restriction fragment length polymorphism (RT-PCR/RFLP) and nucleic acid sequencing of hyper-variable region of the vp2 gene of the virus also indicated the very virulent nature of the virus. Then the virus isolate was adapted to CEF culture and characterized again for the same parameters at 10<sup>th</sup> passage. No mortality was observed in SAN chicks and molecular studies were also indicative of attenuated nature of the virus. As the cross neutralization experiment showed a close relationship of this CEF adapted field isolate with other field viruses and considerable distant relationship with the existing vaccine strain (Georgia, an intermediate vaccine strain), this isolate may be considered as one of the potential candidates for vaccine development to control the IBD in India.

### 4. DEVELOPMENT OF A REVERSE TRANSCRIPTION - POLYMERASE CHAIN REACTION LINKED ELISA FOR SENSITIVE DIAGNOSIS OF RABIES

**R.P. Aravindh Babu, S.Manoharan\*, G.Dhinakar Raj and P. Ramadass**

Department of Animal Biotechnology, Madras Veterinary College, Chennai – 600007

Rapid, readily available and accurate diagnosis of rabies is the keystone of prevention. A sensitive and specific RT-PCR coupled with an ELISA detection system for detecting rabies virus genome in brain tissues was optimized. A set of primers for the use in RT-PCR-ELISA was chosen from three sets of primers analyzed, based on the highest sensitivity of the primers in detecting rabies virus genome in terms of FFD<sub>50</sub> (Focus Forming Dose)



of the virus and the ability to amplify viral genome from tissue samples of different species of animals. RT-PCR was optimized using the chosen set of primer JW12-JW6dpl labeled with digoxigenin and biotin respectively at the 5' end. To facilitate the detection of PCR product, an ELISA detection method was then standardized. This RT-PCR-ELISA had an analytical sensitivity of 0.001 FFD<sub>50</sub> of the virus, which was 10 times higher, compared to the conventional RT-PCR followed by agarose gel electrophoresis detection. The RT-PCR-ELISA was compared with Fluorescent antibody test (FAT), the gold standard test for rabies diagnosis and could detect 90.38% samples while FAT could detect only 81.25%. Also RT-PCR-ELISA could detect viral genome in 4 samples, which are unable to be processed by FAT. The R<sup>2</sup> values between RT-PCR-ELISA OD values and titre of CVS, PV and Street rabies virus were 0.966, 0.946 and 0.996 respectively. Thus RT-PCR-ELISA was found to be a more sensitive technique than the conventional RT-PCR followed by agarose gel electrophoresis detection. This test could be a useful tool in detection of rabies virus not only in the post mortem samples but also in the ante mortem samples like saliva and cerebrospinal fluid due to its higher sensitivity.

**Keywords:** rabies ; RT-PCR ; RT-PCR-ELISA; diagnosis

## **5. BUFFALO POX: INVESTIGATION OF OUTBREAKS, ISOLATION OF THE VIRUS, MOLECULAR AND EM CONFIRMATION AND ATTEMPTS FOR DEVELOPMENT OF A LIVE ATTENUATED VACCINE**

**B. M. Chandranaik**

Scientist-1, Diagnostic Virology, Southern Regional Disease Diagnostic Laboratory,  
IAH&VB, Hebbal, Bangalore-560 024

Buffalo pox is a highly contagious orthopox viral infection of cattle and buffaloes, which virtually terminates their productivity by inducing acute mastitis and fibrosis of udder. Present paper describes the disease investigations undertaken during massive outbreaks in several districts of Tamilnadu and Andrapradesh. During our study, Pustular pox lesions were observed on teats and mammary parenchyma of cattle and buffaloes apart from taking toll of many calves. In the areas of outbreaks, disease was of significant zoonotic importance since similar lesions were produced on the hands, legs and face of people in close contact with the affected animals. Scab materials in viral transport media (VTM), pustular fluid in VTM and blood were collected from affected animals and human beings.

The materials collected were processed in the laboratory for virus isolation as per the procedure outlined in OIE manual for pox virus isolation. The processed, filter sterilized materials were inoculated to 9-11 day old chicken embryos by CAM route and Primary calf kidney cells. The characteristic pock lesions were noticed after seven blind passages on the CAM and the virus produced cytopathic effect (CPE) after five passages in cell culture system. The virus was confirmed by Haematoxylin and Eosin staining of cover slip cultures inoculated with the virus. The staining revealed characteristic eosinophilic, intracytoplasmic, cow dry Type-A inclusions. Further the virus was confirmed by a sensitive and rapid PCR using the primers that amplify "A type inclusion" gene with an amplicon size of 552 bp.

The pock lesions on the CAM were subjected for electron microscopy by negative contrast electron microscopy (At TANVASU) which revealed pox like virions of a complex symmetry measuring about 220nm in diameter. Molecular sequencing of the virus was carried out at IVRI, Muktheshwar, with an acceptable homology.

Even though the outbreaks were brought under control by adopting stringent hygienic practices and antibiotic therapy, by the time the animals recovered they had lost their productivity by more than 50 % due to acute/chronic mastitis. Hence, considering the urgent need of a vaccine the virus isolated has been adopted to vero cell line and has been given 24 serial passages for attenuation. More details will be discussed during the presentation.



## 6. IMMUNOLOGICAL EVALUATION OF IMMUNOMODULATORY EFFECT OF INTERFERON-GAMMA (IFN- $\alpha$ ) IN CHICKEN VACCINATED WITH NEWCASTLE DISEASE VIRUS

**Basavaraj S. Binjawadagi<sup>1</sup>, Y. Hari Babu<sup>2</sup> and E. Sreekumar<sup>3</sup>**

Dept. of Veterinary Microbiology, Veterinary College,  
KVAFSU, Bidar, Karnataka, India.

A total of 180 day old layer chicks (B. V 300) were divided into test groups I and II (64 chicks each) and control group (52 chicks). All the groups were vaccinated with lentogenic LaSota strain of Newcastle disease virus (NDV) on seventh day by intraocular route and with mesogenic R2B strain of NDV during 9th week by subcutaneous route. Test group I was administered with rchIFN- $\alpha$  @ 5  $\mu$ gm/ chick by subcutaneous route along with each vaccination, test group II was administered with same dose and route of rchIFN- $\alpha$  six hours after each vaccination, whereas the other group remained as vaccinated control group. Blood samples were collected from the birds on '0' day, 14th, 21st, and 28th day post immunization. The results of haemagglutination inhibition (HI) test showed higher titres ( $2.86 \pm 0.09$ ) in test group II followed by test group I ( $2.38 \pm 0.09$ ) and control group ( $2.00 \pm 0.09$ ). Results of leucocyte migration inhibition test (LMIT) showed highest mean migration inhibition (MI) values in test group II ( $45.50 \pm 2.29\%$ ), followed by test group I ( $39.33 \pm 1.52\%$ ) and control group ( $24.33 \pm 1.40\%$ ). Further, mean phagocytic index (PI) values assessed by nitro blue tetrazolium (NBT) reduction assay also showed higher values in test group II ( $79.17 \pm 2.89\%$ ) compared to test group I ( $70.17 \pm 1.19\%$ ), whereas, control group ( $50.33 \pm 2.47\%$ ) again showed results lower compared to any of the test groups. All the above results indicated that, chicken administered with rchIFN- $\alpha$  showed higher protective levels of humoral, cell mediated and non-specific immune responses. Further, interferons given after six hours after vaccination had better immunostimulating effect than when given along with vaccines. Thus, the results advocated that rchIFN- $\alpha$  may be used as an immunomodulator for enhancing the immunity in chicken vaccinated against Newcastle disease virus.

1. Veterinary Officer, Dept. of AH & VS, Govt. of Karnataka.
2. Prof. & Head, Dept. of Vety. Microbiology, Veterinary College, Bidar, Karnataka
3. Scientist-B, Rajiv Gandhi Center for Biotechnology, Thiruvananthapuram, Kerala

## 7. ASSESSMENT OF CELL MEDIATED AND MUCOSAL IMMUNE RESPONSES IN CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINES

**S. Varalakshmi, J. John Kirubaharan & V. Purushothaman**

Department of Veterinary Microbiology,  
Madras Veterinary College, Chennai

The data generated was statistically analysed by independent *t* test, one way ANOVA and correlation (Pearson - *r* value). One way ANOVA was carried out to find immune response between different time points and independent *t* test was performed to find out the significance between vaccinated different groups. Correlation - coefficient (Pearson "*r*" value) was calculated to find out the relationship between different immune responses

In the present study attempts were made to find out the protective role of CMI response in chickens against Newcastle disease. The protective role was assessed by specifically suppressing the B cell response using Cyclophosphamide. The drug Cyclophosphamide suppressed the bursa of Fabricius to 95%, producing a near total suppression of humoral immune response. The suppression of humoral immune response was evidenced by undetectable HI titres and low IgG ELISA titres at the time of challenge with virulent virus in ablated birds that have been vaccinated, Despite these birds having high CMI response, they were not protected in challenge. This clearly established the fact that neutralizing antibodies in serum are essential for protection and protection role of



CMI response alone against virulent virus challenge is questionable for NDV. This finding was further substantiated, when birds were vaccinated with SDS treated protein of NDV, SDS damages the conformational epitopes of NDV. In the absence of conformational epitopes though CMI response was produced, neutralizing antibodies in circulation were either in low level or in undetectable range necessary to protect birds from virulent virus challenge. Further good mucosal immune response was produced in chickens that have been vaccinated locally as evidenced by high IgA and IgG titres. Even in this case birds were not protected against virulent virus challenge in absence of circulating antibodies. In non-ablated birds that have been vaccinated all the three immune responses were found and these birds withstood local and parantrol virulent virus challenge. Statistically significant positive correlation existed between all three immune responses.

Hence it could be concluded that,

- 1) Circulating neutralizing antibodies are essential for protection against virulent Newcastle disease viruses
- 2) The CMI response alone is not protective since NDV replicate quickly and come out of the cell, before CMI response identified virus infected cell
- 3) Mucosal immune response is produced in high level in birds that have been vaccinated.
- 4) Positive correlation exist between all the three immune responses and all these three responses are essential for protection of birds against virulent virus challenge.

## 8. SURVEILLANCE AND CHARACTERIZATION OF AVIAN INFLUENZA VIRUSES IN INDIAN POULTRY

**S. Nagarajan<sup>1</sup>, V. Ramaswamy<sup>2</sup>, S. Gounalan<sup>1</sup>, K. Rajukumar<sup>1</sup>, H.V. Murugkar<sup>1</sup>,  
C.Tosh<sup>1</sup>, B. Pattnaik<sup>1</sup> and H.K. Pradhan<sup>1</sup>**

1. High Security Animal Disease Laboratory, Indian Veterinary Research Institute,  
Anand Nagar, Bhopal 462021, Madhya Pradesh

2. Dean, Faculty of Basic Science, Madras Veterinary College, TANUVAS, Chennai - 600 007

Recent outbreaks of H5N1 worldwide among poultry and human beings highlighted the zoonotic potential and the economic devastation of the avian influenza viruses. In this paper, we report the results of first ever surveillance and isolation of avian influenza viruses (AIV) in India. A total of 13,175 serum and 8,442 clinical samples (tissue, dead birds, swab and fecal) collected or received from 26 States and 6 Union territories of India were tested.

The seroprevalence of AIV was found to be 5.35% of which 0.03% was positive for H5 antibodies and the remaining samples were positive for H9 antibodies. Samples from 18 States were serologically positive for H9 antibodies. The Maharashtra state was positive for H5 and H9 AIV antibodies. A total of 23 H5N1 isolates were isolated from three states and six H9N2 isolates were isolated from four states during this study.

The sequencing and phylogenetic analysis of the HA gene of six H9N2 isolates revealed that they are closely related to each other (99.3 - 99.7%) and to other Asian H9N2 isolates (90.9 - 95.1%). The amino acid analysis indicated that Indian H9N2 isolates have cleavage site sequence of ARSSR\*GLF, leucine at position 238 and serine at position 240 which indicated that these isolates are low pathogenic and recognize  $\alpha(2, 6)$  sialic acid receptors of humans and hence may not replicate efficiently in chickens. These results were corroborated by intravenous pathogenicity (IVP) index of 0.0/3.0 for both the H9N2 viruses tested.

The phylogenetic analysis of four H5N1 HA gene, revealed that the isolates were closely related to isolates of Italy, Iran, Turkey, Russia, Nigeria and China (98 - 99.1%). The sequence of cleavage site PQGERRRKKR\*GLF revealed the presence of multiple basic amino acids which indicate that the isolates are highly pathogenic. This result concurs with the IVP index of 2.63 to 2.83/3.00 for the five H5N1 isolates tested.

Analysis of the HA gene amino acid sequence of 115N1 isolates revealed that they are of avian origin and the disease was brought to India by migratory birds although entry of the disease through smuggling of birds cannot be ruled out.



The NA gene of a H9N2 and a H5N1 isolate were sequenced. Amino acid analysis of the H9N2 virus revealed that there is no deletion in the stalk region and is phylogenetically related to other Asian isolates of H9N2. On the other hand, the NA gene of H5N1 had a deletion of 20 amino acids and is related to Vietnam, Thailand and Malaysian isolates. The amino acid analysis also showed that the H5N1 viruses are susceptible to TamiFlu (Oseltamivir). The sequence analysis of the NA gene of H5N1 virus isolated from Navapur, Maharashtra revealed that the NA gene is derived from VTM lineage.

Antigenic analysis with homologous serum raised against an Indian H9N2 isolate showed that it could be used as vaccine to control the disease in case of severe outbreaks of H9N2 virus. Antigenic analysis with serum raised against a commercially available H5N2-inactivated vaccine indicated that such a heterologous vaccine could be used to control H5N1 outbreaks. This finding could be used to develop indigenous DIVA strategy for control of H5N1 outbreaks in case of reoccurrence of the disease in India.

### ISVIB Mid Career Scientist Award

## **1. A FIELD STUDY OF BACTERIAL ETIOLOGY OF ABORTIONS AMONG MIGRATORY SHEEP AND GOATS IN NORTH-WEST HILL STATES OF INDIA WITH EMPHASIS ON QUICK DETECTION OF CHLAMYDIAE EMPLOYING TWO PCR TESTS**

**Mandeep Sharma**

Department of Veterinary Microbiology,  
Dr. G.C. Negi College of Veterinary and Animal Sciences,  
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, H.P.

Reproductive diseases and the consequential economic losses are a major problem for the livestock industry. These losses are due to abortions, repeat breeding, cost of treatment, and transmission of infectious agents among the herd. It has been estimated that up to 90% of these losses are due to infectious agents. Most of the studies on the causes have been done with cattle; however, similar problems are seen in sheep and goats and wide arrays of microbial agents have been incriminated. But, information on these diseases in rangeland or migratory flocks is limited.

A field study on the bacterial etiology of abortions among sheep and goats having migratory practices in the northwest hilly states of India was carried out. A total of 203 flocks were investigated. Abortion outbreaks occurred in 51 flocks and sporadic abortions were observed in 114 flocks. Vaginal swabs from aborted sheep and goats were collected and processed for isolation of bacterial agents from 37 flocks with history of abortion outbreaks and 56 flocks with sporadic abortions. Bacteria known to cause abortions were identified in 30 flocks with abortion outbreaks including *Chlamydiae* (17 flocks), *Brucella melitensis* (4 flocks), *Listeria monocytogenes* (8 flocks), and one *Salmonella dublin*. *L. ivonovii* was also isolated from sheep in one of the flocks with *L. monocytogenes*. No specific causes of abortion were identified in flocks having sporadic abortions. The results also revealed that chlamydial abortions were more likely to occur in goats than in sheep and that *Brucella* abortions occurred more often in sheep.

The study further focused on two simple and rapid PCR techniques employed on vaginal swabs for fast detection of different members of *Chlamydiales* and *Chlamydiaceae*. The molecular diagnosis of chlamydial abortions assumes significance as chlamydiae are intracellular bacteria and their isolation still confronts. Overall, 260 deep vaginal swabs (0-15 days post abortion) comprising of 112 samples from sheep and 148 samples from goats were processed for detection of chlamydiae through isolation, DNA extraction and later employing PCR tests. Undoubtedly, it was observed that these two PCR tests provided excellent sensitivity as well as easy recognition to chlamydial infections as compared to labour intensive and cumbersome isolation procedures and indirect micro-immunofluorescence test.



## 2. IMMUNOLOGICAL COMPARISON BETWEEN INDIGENOUS AND COMMERCIAL BREEDS OF CHICKENS

**G. Dhinakar Raj\***, T.M. Chozhavel Rajanathan and P. Ramadass

Department Animal Biotechnology, Madras Veterinary College  
Tamil Nadu Veterinary and Animal Sciences University Chennai - 600 007

The commercial and desi breeds of chickens were compared for their immunological parameters such as lymphocyte subset numbers, cytokine profiles following mitogen stimulation and nitrite assays using flow cytometry, reverse transcription polymerase chain reaction (RT-PCR) and Griess reagent respectively. The percentages of immune cells in the spleen did not appear to be different between the two breeds of chickens. The cytokine interferon gamma showed a consistent expression in RIR chickens (2/3) as compared to Giriraja birds (0/3). The cytokine IL 8 showed a consistent expression in RIR chickens (3/4) as compared to Giriraja birds (1/3). The nitrite levels were found to be higher in Giriraja chickens at 24 hrs post LPS stimulation than RIR chickens. The reverse was true at 72 hrs post LPS stimulation.

Expression profiles of chicken toll-like receptors (TLRs) were analyzed in the heterophils and selected tissues of 12- and 20-weeks old white leghorn and indigenous chickens using reverse transcription polymerase chain reaction (RT-PCR). Although the expression profiles of TLRs were mostly similar between WLH and indigenous 12-weeks old chickens, more number of chickens tested had positive RT-PCR amplifications in the indigenous breed. In contrast, the 20-weeks old indigenous birds expressed a greater repertoire of TLRs. TLR2 type1, TLR2 type 2, TLR3, TLR4, TLR5 and TLR 7 were all expressed in these birds while only TLR4 and TLR7 were expressed in the corresponding WLH chickens and that too, in only 37% of the birds. In case of Aseel, the TLR repertoire was broad with 100% of the birds expressing all the TLRs tested. In the case of tissues, the differences between these two breeds were not very prominent. Of the TLRs studied, only TLR5 and TLR7 were strongly positive in 12- and 20-weeks old birds. In general, the TLR expression profiles were broader in the 20-weeks old indigenous chickens compared 10 WLH birds.

## 3. DEVELOPMENT OF RECOMBINANT ANTIGENS FOR DIAGNOSIS OF AVIAN MYCOPLASMOSIS.

**G.Venkatesh**

Scientist (SS), HSADL - IVRI, Bhopal- 21.

Avian Mycoplasmosis is wide spread and is one of the most common respiratory diseases of poultry causing significant economic losses in poultry industry. The disease occurs when birds infected with *Mycoplasma gallisepticum* (MG) or *Mycoplasma synoviae* (MS) are stressed. An attempt has been made in this study to express the antigenic proteins of MG and MS in *E.coli* and use them in diagnostic assay.

Primers were designed with built in restriction enzyme sites for amplification of p75 and p29 (TM-I) genes of MG PG31, PCR amplification of the genes was done, cloned and sequenced. Both the genes were cloned into expression vector pPROEx HTb and expressed in *E. coli* DH5 $\alpha$ . The full length *vihA* gene of local field isolate MS427 was amplified using published primers and the 5' end corresponding to MSPB protein sequences were sequenced. Based on the sequence primers were synthesized, amplified, a smaller part of MSPB and a larger part of MSPB (MSPB) genes were cloned in the expression vector pPROEx HTb and expressed in *E. coli* DH5 $\alpha$ . The recombinant proteins were purified using Ni-NTA affinity chromatography.

The four recombinant proteins obtained in this study were characterized and evaluated as diagnostic antigen. The recombinant P29 of MG and recombinant MSPB protein of MS showed a potential to be used as diagnostic antigens. A highly sensitive indirect ELISA, a rapid simple easy to perform latex agglutination test and flow through enzyme assay were developed to diagnose MG and MS separately using the respective recombinant



proteins. The MG and MS indirect ELISA had perfect agreement with commercial kit LLISA used in this study. The latex agglutination tests and flow through enzyme assays had substantial agreement with commercial kit.

**Key words :** Avian Mycoplasmosis - *Mycoplasma gallisepticum* - *Mycoplasma synoviae* - Cloning - recombinant expression - ELISA - Latex agglutination test - Flow through enzyme assay.

#### 4. EMERGENCE OF BLUETONGUE VIRUS SEROTYPES FROM HOST AND VECTOR (CULICOIDES) IN INDIA WITH SPECIAL REFERENCE PROPHYLAXIS IN TAMIL NADU STATE .

**A. Wilson Aruni , M. Parthiban, N. Daniel Joy Chandran, A. Koteeswaran and K.S. Palaniswami**

Department of Veterinary Microbiology, Madras Veterinary College, Chennai

Bluetongue an arthropod borne orbiviral infection was found to be transmitted by *Culicoides* the biting midges. The wide spread of this devastating disease is attributed to the vector in particular. The emergence of Bluetongue virus serotypes in Tamil Nadu has become common during the past years due to uncommon climatic conditions. The existence of various serotypes not reported so far in Tamil Nadu is attributed to the same reason. Out of the 904 blood samples and 50 catches of culicoides screened 154 samples were found positive for BT and out of them a total of four isolates were obtained in BHK21 cells. Isolation of Bluetongue virus from the *Culicoides* yielded one isolate Out of the 24 states in India only 11 state were reported to have been infected with BT.

The infection of BT was found to be more pronounced in the southern and south western states of India and were found to be less pronounced in the north eastern states. Isolates were initially adapted to embryonating chicken eggs and these isolates were confirmed by coverslip cultures of BHK21 cells using IFAT which showed characteristic green fluorescence. These isolates were further confirmed by RT-PCR which yielded the expected amplicon size of 495 bp.

BlastN nucleotide homology studies revealed that the sequences of two local BTV isolates viz., BTV-S (Gen Bank Accession number: AY 359960) and BTV-T (Gen Bank Accession number : AY 350061) possessed 100 % homology against BTV-1 of Australian isolate and 18 of South African isolate respectively. Isolates that obtained from other three places of Tamil Nadu were grouped as BTV-18 based on sequencing datas. The isolates were confirmed by Institute of Animal Health Pirbright .The isolate BTV-1 was used in production of a Bivalent vaccine against Bluetongue. A patent has been filed for the same.

The isolates were further confirmed by serotyping from Arboviral laboratory, Institute of Animal Health Pirbright, UK and found as BTV-1, BTV-18 .BTV-1 also The serotypes 1 and 18 were prevalent in eight states of India and BTV-18 and BTV-4 to be prevalent in six states followed by BTV-12,16 and 17 in five states.BTV-21, BTV-22 and BTV-24 were not reported so far from India. A total of 16 serotypes of BT were reported from Tamil Nadu and 21 serotypes of BTV were reported from India.

**Key words :** Bluetongue, serotype prevalence, culicoides vector, RT-PCR, sequencing.

#### ***F M Burnett Memorial Award***

#### 1. EFFECTS OF EXPOSURE TO HEAVY METAL POLLUTED WATER ON THE IMMUNE SYSTEM OF FISH.

**Madhu Prahha Saxena<sup>±</sup>, Hari Mohan Saxena<sup>#</sup> and Gurinder Kaur Sangha\***

Departments of <sup>±</sup>Livestock Production & Management and <sup>#</sup>Veterinary Microbiology, Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana, and

\*Department of Zoology, Punjab Agricultural University (PAU), Ludhiana – 141 004.

Water sample from a polluted brook of Ludhiana called Buddha Nallah showed high concentrations (in mg/L) of all the 8 heavy metals investigated - Iron (35.86), Zinc (2.75), Manganese (0.166), Nickel (0.090),



Chromium (0.065), Copper (0.045), Lead (0.044) and Cadmium (0.025). Common Carp fish were reared in a water tank containing polluted water from the Nallah. Serum protein profiles of 5 normal and 5 pollutant – exposed fish by SDS PAGE revealed that proteins p63 and p25 present in the normal fish were absent in the sera of pollutant - exposed fish. On the other hand, p56 and p22 absent in the normal fish were detected in the sera of pollutant - exposed fish. The total protein concentration in the plasma was lower in the pollutant - exposed fish compared to the normal fish. However, the difference was not significant. The response of lymphocytes to mitogen Con-A was reduced significantly in fish exposed to polluted water. Exposure of fish to the polluted water (3%) for 2 weeks led to sudden mortality among 60% of the fish. The dead fish showed generalized congestion and cyanosis on the outer surface of the body and congestion and hemorrhage in the intestines. Histopathology of lymphoid tissues of the dead fish revealed congestion, haemorrhage, lymphocytic infiltration and degenerative changes in kidney, spleen, gut and liver.

<sup>#</sup>Corresponding author.

## 2. PEN-SIDE KITS FOR DIAGNOSIS OF PESTE DES PETITS RUMINANTS

**G. Dhinakar Raj\***, **V. Thiagrajan\***, **M. S. Shaila\*\*** and **R. K. Singh\*\*\***

\* Department of Animal Biotechnology, Madras Veterinary College, Chennai- 600007

\*\* Department of Cell Biology and Microbiology, Indian Institute of Sciences, Bangalore 560012

\*\*\* Head, Division of Virology, IVRI , Muktheshwar, Uttranchal

Peste des petits ruminants (PPR) is one of the most economically important diseases affecting sheep and goats in India. An immuno filtration-based test has been developed using either mono specific serum / monoclonal antibodies (Mab) against a recombinant truncated nucleoprotein of PPR virus. Ocular swab eluate from suspected animals is coated on to the membrane housed inside the flow through module. Then it is incubated at 37°C for about one hour. Following washing, addition of Mab / mono specific serum and conjugate, a pick dot appears if PPR virus antigen is present in the swab sample. Comparison of the immuno filtration test with the lab-based antigen-competition ELISA yielded a sensitivity of 76.9% and specificity of 100%. The results of immuno filtration test for PPR virus antigen detection on field samples using either PPRV Mab or mono specific serum showed a sensitivity of 80.0% and specificity of 100%.

For qualitative PPR virus-specific antibody detection, a strip test has been developed and validated. The recombinant PPR virus antigen and anti-goat antibody coated NC strips are allowed to dry under vacuum over night at 37°C. Then the absorbent pad and sample pad are then affixed on the NC laminate overlapping the NC membrane on the top and bottom respectively. Then strips of 4-5 mm width are cut and packaged in to aluminum pouches containing silica gel. At the time of use these strips are taken out and kept in a small tube containing two drops of buffer provided, two drops of serum to be tested and one drop of the gold conjugate provided. The mixture migrates upwards due to capillary action and if antibodies are present in the serum sample being tested, two lines would appear. If no antibodies were present only one line would appear. If no lines appear the test is invalid. The present test is so user-friendly, stable, needs no gadgets, requires only 5-10 minutes to obtain results although the results are qualitative. The sensitivity and specificity of the developed strip test, in comparison with the competitive ELISA was determined to be 88.6% and 80.8% respectively. This kit was validated successfully at IVRI, Muktheshwar.



**ISVIB Scientist Award**

**1. QUICK DETECTION OF CHLAMYDIALES AND CHLAMYDIACEAE  
EMPLOYING POLYMERASE CHAIN REACTION.**

Mandeep Sharma, R. C. Katoch, Subhash Verma and Pooja Soneja  
*Indian Journal of Comparative Microbiology Immunology and Infectious Diseases*  
Vol. 25, No. 2 (July – Dec), 2004:104-108

**2. DETECTION OF *BABESIA BIGEMINA* DNA IN TICKS BY DNA  
HYBRIDIZATION USING A NONRADIOACTIVE PROBE  
GENERATED BY ARBITRARY PCR**

Reghu Ravindran, Jammi R. Rao, Ashok K. Mishra  
*Veterinary Parasitology* 141 (2006) 181-185

**3. ABROGATION OF DTH RESPONSE AND MITOGENIC LECTIN-AND  
ALLOANTIGEN-INDUCED ACTIVATION OF LYMPHOCYTES  
BY CALCIUM INHIBITORS TMB-8 AND BAPTA-AM**

Hari Mohan Saxena, Madhu Dikshit  
*Immunology Letters* 101(2005) 60-64

**SESSION 3: LIVESTOCK DISEASES - DIAGNOSIS AND CONTROL**

**1. COMPARISON OF PCR WITH CONVENTIONAL TECHNIQUES FOR THE DIAGNOSIS OF  
BRUCellosIS IN CATTLE**

**P. Kaushik, D. K. Singh\*\* and A. K. Tiwari<sup>1</sup>**

Division of Veterinary Public Health  
Indian Veterinary Research Institute Izatnagar 243122 (U.P.), India

Brucellosis is prevalent in India and is a leading cause of zoonosis world wide. Therefore, accurate diagnosis of brucellosis is sought despite of equivocal serological tests. The cross reactivity of serological test and the fastidious nature of the organism hurdles in the diagnosis of brucellosis and sought the development of a reliable and sensitive method like polymerase chain reaction (PCR). A total of 45 bovine samples (15 blood samples, 15 vaginal swabs and 15 milk samples) from 15 animals suspected for brucellosis were examined by RBPT, SAT, PCR and bacteriological isolation. Out of 15 blood samples 13 (86%) were positive in RBPT and of these positive samples 6 (46.1%) were positive in SAT with a cut off titre of  $e^{80}$ . The PCR was positive in 2 blood samples of animals positive in SAT. The vaginal swabs from these animals were also positive by PCR but the milk samples were negative by PCR while the *Brucella* could not be isolated from any of the samples. This shows the poor sensitivity of isolation method compared to PCR, to be used as a diagnostic tool.

\*\*Correspondence: E. [mail-dks@ivri.up.nic.in](mailto:mail-dks@ivri.up.nic.in)

<sup>1</sup>Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar, India



## 2. APPLICATION OF POLYMERASE CHAIN REACTION (PCR) TO DIAGNOSE ABORTIVE CHLAMYDIOSIS IN CATTLE, SHEEP AND GOATS OF HIMACHAL PRADESH

Abhishek Gandhi, Mandeep Sharma, Prasenjit Dhar and V.K. Gupta

Department of Veterinary Microbiology, Dr. G. C. Negi College of Veterinary Science,  
CSK HPKV, PALAMPUR - 176 062 (H.P.)

Chlamydial abortions and infertility is a major cause of economic losses in intensively managed sheep flocks and is regarded as a serious problem in many livestock rearing areas in the world. Clinical diagnosis is often difficult as the clinical signs and pathological lesions are not specific for *Chlamydomphila abortus* infection, in fact they can also be observed as a result of infections with other abortifacient agents. So, laboratory examination of various clinical samples is must for the diagnosis of chlamydial infections. In the present study, simple and multiplex PCR techniques on fresh field specimens of infertility comprising of vaginal swabs, uterine discharges, aborted materials and fetal stomach contents from cattle, buffaloes, sheep and goats from different parts of Himachal Pradesh were employed for quick detection of chlamydiae. Overall, 292 samples comprising of 166 cattle, 33 buffaloes, 46 sheep and 47 goat samples were processed through DNA extraction and later on employing two PCR tests. DNA was extracted from all the 292 samples using Qiagen Amp DNA minikit (Qiagen) and then simple PCR (test-1) and multiplex PCR (test-2) were conducted to recognise different members of order *Chlamydiales* and family *Chlamydiaceae*, respectively. Using simple PCR, 9 samples (3.08 per cent) were found positive for order *Chlamydiales*, whereas, out of these 9 samples on which multiplex PCR was employed, only 6 samples (2.05 per cent) were detected positive for family *Chlamydiaceae*. It may be concluded that simple PCR followed by multiplex PCR on positive samples is a sensitive and valuable method for detecting *Chlamydia* spp.

## 3. NEWCASTLE DISEASE VIRUS INDUCED CYTOPATHIC EFFECT IN-VITRO IS CAUSED BY APOPTOSIS

Ravindra PV\*, A.K Tiwari, Yoginder Singh Rajawat, Ravi Sundersan, Barkha Ratta, Uttara Chaturvedi, Sudesh Palia, Subudhi P K and A Rai

Division of Veterinary Biotechnology,  
Indian Veterinary Research Institute, Izatnagar-243122, India

Newcastle disease virus (NDV), a *paramyxovirus*, causes natural infection in birds of many species. *In-vitro* cytopathic effect caused by NDV, in chicken embryo fibroblasts (CEF) cells is associated with induction of apoptosis. We investigated the mechanism of apoptosis induction in CEF cells employing specific assays of apoptosis. CEF cells infected with multiplicity of infection 0.1 underwent apoptosis characterized by condensation of chromatin, increased cytoplasmic vacuolation as identified in electron microscopy. DNA fragmentation was documented by fragmentation assay. Flowcytometric analysis revealed the increase in percentage of annexin-V positive cells with concomitant decrease in DiOC<sub>6</sub> positive cells as compared to mock infected cells. The mean fluorescent intensity of infected cells was higher than the mock infected cells up on staining with 2' 7'-Dichlorofluorescein dye. The caspase 3 expression in NDV infected CEF cells was increased when compared to mock infected cells. Real-time PCR analysis demonstrated increased expression of caspase 3, 8, and 9. Further screening for the possible involvement of HN protein in induction of apoptosis, our results suggests that HN protein induces apoptotic changes in CEF cells transiently expressing the HN protein. In conclusion our findings imply, NDV infection *in-vitro* induces caspase dependent cell death involving both mitochondrial and death receptor pathways.



#### **4. DEVELOPMENT OF PCR FOR DIFFERENTIAL DIAGNOSIS OF INFECTIOUS CANINE HEPATITIS AND INFECTIOUS LARYNGO TRACHEITIS**

**Uttara Chaturvedi; A.K. Tiwari; Barkha Ratta; Sudesh Kumar; Ravindra P.V;  
Y.S. Rajawat; Mitesh Mittal and A. Rai**

Division of Veterinary Biotechnology  
Indian Veterinary Research Institute, Izatnagar-243 122, UP

Infectious canine hepatitis (ICH) and infectious canine laryngotracheitis (ICLT) are caused by canine adenovirus 1 (CAV-1) and canine adenovirus 2 (CAV-2), respectively. CAV-1 infection (ICH) is characterized by vomiting, diarrhea and jaundice whereas CAV-2 causes mild or inapparent respiratory tract infection. Although the clinical symptoms, gross and histopathological findings of these two diseases are characteristics, but confirmation of CAV-1 and CAV-2 is necessary for accurate diagnosis. In the present study we have developed PCR for differential diagnosis of CAV-1 and CAV-2 infection using single set of primers designed from E3 gene sequence which resulted in amplification of 508 bp fragment in CAV-1 and 1030 bp fragment in CAV-2 differentiating both the viruses.

Sensitivity study using cell culture adapted CAV-1 virus indicated that by PCR it is possible to detect as low as 0.01 TCID<sub>50</sub>/ml virus. Further this test was optimized to detect CAV-1 from faeces and urine of dogs which detected up to 1.6 TCID<sub>50</sub> / ml virus in both. Later this test was applied to screen the faecal samples received from CMVL. Out of 45 clinical samples processed 14 samples were found positive for CAV-1.

#### **5. PCR FOR DETECTION OF CLASSICAL SWINE FEVER IN CLINICAL SAMPLES**

**Barkha Ratta, A.K. Tiwari, N.N. Barman, Sudesh Kumar, P.V. Ravindra, Y.S. Rajawat,  
Uttara Chaturvedi, Subudhi P.K and A. Rai**

Division of Veterinary Biotechnology  
Indian Veterinary Research Institute, Izatnagar-243 122, UP

Classical Swine Fever (CSF) is an economically important Office International des Epizooties list A disease of pigs which causes an acute course of disease characterized by high fever and multiple haemorrhages, as well as clinically or chronic inapparent infection. A rapid method of reverse transcription-polymerase chain reaction (RT-PCR), for detection of classical swine fever virus in infected tissues, was developed using E2 and NTR sequences specific primers. Further, the nested PCR was standardized using primary amplicon as template to enhance the sensitivity of PCR. The nested PCR resulted in a band of 271 bp with both E2 and NTR specific primers. The sensitivity of PCR was checked using cell culture adapted CSF virus which detected up to 10<sup>-4</sup> dilution. The authenticity of PCR was confirmed by electrophoresis in 1.5% agarose gel and sequencing nested PCR product. Later, the test was applied to detect CSFV in suspected clinical materials, which included spleen, mesenteric lymph nodes, tonsils, part of intestine etc. More than 145 samples collected from different states of country including Assam, Mizoram, Bihar, Haryana, Punjab and Uttar Pradesh, Mandai, Pune. The sample also included PK-15 cell line adapted virulent field isolates. Out of total 145 samples, 48 samples were found positive by RT-PCR.

#### **6. ISOLATION AND MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS***

**P. Ramadass, Shrine Nagalakshmi and T.M.A. Senthil Kumar**

Department of Animal Biotechnology  
Madras Veterinary College, Chennai 600 007, Tamil Nadu.

Dung samples (640 samples) from cattle, sheep and goats were collected from Madras Veterinary College Hospital, Sheep farm, Mannavanur, and different Livestock farms from the State, and stained by acid fast staining.



One hundred and six samples were positive by acid fast staining, which were subjected to PCR using specific primers for *IS 900* gene of *Mycobacterium avium* subsp. *paratuberculosis*. Nineteen samples out of 38 bovine samples; 22 samples out of 45 goat samples and 19 samples out of 23 sheep samples were positive by PCR. JD vaccine from Iceland was used as positive control. Samples positive by PCR were decontaminated with hexadecyl pyridinium chloride and antibiotic solutions and inoculated into Herrold's Egg Yolk slants and Middle Brooks liquid medium and incubated at 37°C. The inoculated slants and liquid media were checked once in every two weeks. Colonies were tested by acid fast staining and PCR with specific primers. Three cultures were found positive, one each from sheep, goat and cattle. PCR amplicons were purified and sequenced. Twenty samples (PCR products) were sequenced and sequences were compared using BLAST programme. JD vaccine showed 97 to 100% homology against various isolates of MAP *IS 900* genes, while 84% homology against *Mycobacterium avium* (MAT) subsp. *avium*. Amplicons from white cattle showed 91-96% homology against different MAPs, while 84% homology against MAA. Goat isolates showed 100% homology against other MAP isolates. Sheep amplicons showed 98% homology against other MAPs.

## 7. NUCLEIC ACID BASED DIFFERENTIATION OF *PASTEURELLA MULTOCIDA* SEROTYPES

**P.X.Antony<sup>1</sup>, G.K.Nair<sup>2</sup>, V.Jayaprakasan<sup>2</sup>, M. Mini<sup>2</sup> and T.V.Aravindakshan<sup>2</sup>**

Department of Microbiology,  
College of Veterinary and Animal Sciences, Mannuthy, Kerala. PIN 680 651

*Pasteurella multocida* has been recognized as an important Veterinary pathogen for over a century. The organism can occur as a commensal in the naso-pharyngeal region of apparently healthy animals and it can be either a primary or secondary pathogen in the disease processes of a variety of domestic and feral mammals and birds. Knowledge of the serotype of *Pasteurella multocida* (*P. multocida*) involved in an outbreak is essential in order to constitute effective control measures. Antigenic characterization of *P. multocida* is accomplished by capsular serogrouping and somatic serotyping. Typing of *P. multocida* is principally accomplished via passive haemagglutination and gel diffusion precipitin tests. Isolates are classified into five groups according to their capsular antigens and into 16 serotypes on the basis of their lipopolysaccharide antigens. These typing techniques are carried out by reference laboratories only. This often leads to delay in knowing the serotype of the isolate. DNA based techniques for differentiation of serotypes can provide an alternative to conventional serotyping. A PCR-REA technique was standardized to distinguish *P. multocida* serotypes A:1, A:3 and B:2. When ~1000bp PCR product obtained by amplification of *ompH* gene was digested with restriction enzyme *Dra I* it generated profiles unique to the three serotypes used in the study. If unique patterns for all serotypes could be determined in a similar manner then we can have a serotyping technique that is simple, rapid and straight forward and can be performed in any laboratory that has the capacity to perform PCR.

<sup>1</sup> Rajiv Gandhi College of Veterinary and Animal Sciences, Pondicherry.

<sup>2</sup> College of Veterinary and Animal Sciences, Mannuthy, Kerala.

## 8. CLASSICAL SWINE FEVER IN MEGHALAYA AS CONFIRMED BY ELISA AND PCR

**I. Shakuntala, Babita Devi, Ashok Kumar, R. H. Begum and K. M. Bujarbaruah\***

Division of Animal Health, ICAR Research complex for NEH Region,  
Umiam, Meghalaya

Classical swine fever (CSF) is a highly contagious and often fatal viral disease of swine affecting domestic and wild pig populations. *Classical swine fever virus* (CSFV), the causative agent of CSF, is a member of the genus *Pestivirus* under the family *Flaviviridae*. During February to May 2006, death of pigs was reported in both organized and unorganized pig farms of Meghalaya and was investigated for CSF. The morbidity and mortality rates were 13.01% and 4.98%. The animals with history of sudden death were subjected for post mortem examination. Rashes on skin and hemorrhages in internal organs were observed. The lymph nodes and spleen



were collected for the detection of virus by Reverse transcription- polymerase chain reaction (RT-PCR). RNA was extracted from the collected samples using QIAGEN QIAamp viral mini spin protocol. RT-PCR was done using Hog cholera virus (HCV) group specific primer pair (HCV-1 and HCV-2) resulting in amplicon size of 478 bp. The samples were found to be positive for HCV. Serum samples were also collected from the affected farm and were screened for the presence of Swine fever virus antibodies employing CSFV Ab ELISA kit obtained from Chisso Corporation, Japan. Out of the 47 serum samples screened, 21 were found to be positive (44.68%).

Detection of swine fever specific antibodies in serum samples and presence of HCV by RT-PCR in the morbid samples confirmed the outbreaks of swine fever in the State. Regular occurrence of swine fever, in spite of the availability of vaccine and vaccination practice in the region, is the concern of professionals and farmers as it results in heavy economic loss to the pig farmers. The more effective strategies have to be devised and implement for prevention and control of this dreaded disease.

\* Director, ICAR Research complex for NEH Region, Umiam, Meghalaya

## 9. *INSITU* HYBRIDISATION TECHNIQUE FOR THE DETECTION OF INFECTIOUS BURSAL DISEASE INFECTION

A. Wilson Aruni, M. Parthiban, Hudson Taylor and V. Purushothaman

Department of Veterinary Microbiology  
Madras Veterinary College, Chennai-7

Amplified PCR product was used as a probe to detect the IBDV genomic RNA. Peroxidase labeled probe using enhanced chemiluminescence hybridization assay (ECL) was used as a simple, fast and reliable diagnostic method for detecting IBD infection. The peroxidase labeled amplicon hybridized to all the IBD viral RNA samples. The specificity of the probe was tested using nucleic acid from Chicken embryo fibroblast cells and IBD. Dot blot hybridization assay, Tissue imprint hybridization assay were compared with the *insitu* hybridization assay and found that the results of the *insitu* hybridization assay in the infected bursal section using cDNA DIG labeled probe was found to be impressive. Several cells in the lymphoid follicles were stained. Thus the assay has the potential to be very sensitive because a single infected cell can be identified. The use of DIG labeled probe afforded an early specific detection of IBDV viral mRNA in bursal and non bursal lymphoid tissues more specifically within individual cells of histologic sections as early as 24 HPI.

## 10. SEQUENCING OF NUCLEOPROTEIN GENE OF NEWCASTLE DISEASE VIRUS LOCAL ISOLATE D58

R.N. Ramani Pushpa, J. John Kirubaharan, and V. Purushothaman

Department of Veterinary Microbiology,  
Madras Veterinary College, Chennai- 600 007

Newcastle Disease (ND) is a highly contagious disease of many avian species that lead to substantial losses in the poultry industry worldwide. The etiological agent of the disease, Newcastle Disease virus (NDV), which has been designated avian paramyxovirus 1, produces a disease of varying severity in poultry, depending on virus strains and host species infected. Newcastle Disease virus isolate (D58) obtained from apparently normal village chicken with no history of vaccination was used in the present study. The ICPI value for the isolate D58 was 0.14, which was specific for less pathogenic strains. The data regarding nucleoprotein gene of local isolate D58 was not available and hence a study has been under taken to generate data regarding the NP gene of local isolate D58 with possible exploitation for use as a marker vaccine to design an eradication programme for ND. In other countries work on marker vaccine are based on the sequence of this gene because tampering of other genes affect the properties of the virus. The virus was plaque purified in chicken embryofibroblasts (CEF). The plaque purified virus was pasaged in embryonated chicken eggs. After clarification of the allantoic fluid, NDV was sedimented by ultra centrifugation and the viral RNA was isolated by using TRIZOL LS reagent. cDNA to genomic RNA was generated with gene specific primers. The low pathogenicity of the isolate was also confirmed



by sequencing the fusion protein cleavage site (FPCS) of F gene. The deduced amino acid motif in the FPCS region of the F gene was  $^{112}G-R-Q-G-R-L^{117}$  which was specific to low pathogenic type. RT-PCR of nucleoprotein (NP) gene of the isolate was done by using NP gene specific primers including both ORF and non-expressible regions. The NP gene was sequenced by primer walking. The NP gene sequence was analyzed with the available sequences in the gen bank and found to be matching. The details of the NP gene sequence along with epitopes identified for marker vaccine studies will be discussed.

## 11. DIAGNOSIS OF CANINE PARVOVIRUS INFECTION IN FAECAL SAMPLES OF DOGS BY CONVENTIONAL AND MOLECULAR TECHNIQUES

**Harmandeep Kaur, P.N.Dwivedi, Dipak Deka, Sandeepa Bhan and Ramneek**

Department of Veterinary Microbiology, College of Veterinary Science  
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab.

Faecal samples from 25 pups aging between 3–6 months with the symptoms of haemorrhagic diarrhoea were collected for diagnosis of CPV. Faecal samples were tested for CPV antigen by using HA and HI tests, virus isolation assay and PCR/Nested PCR. The HA/HI and virus isolation assay could detect CPV antigen in 15 and 11 samples, respectively. PCR/nested PCR applied directly on faecal samples could detect CPV antigen in 22 samples. In comparison to PCR/Nested PCR the sensitivity and specificity of HA/HI were 68.18% and 100%, respectively whereas sensitivity and specificity of VI was 50% and 100% respectively. A marked difference was observed between the proportion of PCR positive and HA/HI and VI negative samples suggesting Nested PCR to be more sensitive method for detection of CPV.

## 12. IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* BY CULTURE METHOD AND POLYMERASE CHAIN REACTION (PCR) FROM CHICKEN MEAT

**T.Arulkumar<sup>1</sup> and A. Manikavasaka Dinakaran<sup>2</sup>**

Department of Veterinary Epidemiology and Preventive Medicine  
Veterinary College and Research Institute, Namakkal – 637 001

The present study was undertaken with the objective of assessing *Campylobacter jejuni* in 210 chicken meat samples collected from different retail poultry meat processing plants in and around Namakkal. All the chicken meat samples were subjected to culturing, staining, motility and biochemical tests like oxidase test, catalase test, growth in the presence of 1 per cent glycine, growth in presence of 1.5 per cent NaCl and sugar fermentation tests. Twenty eight samples were found to be positive and they were again confirmed by molecular technique Polymerase Chain Reaction (PCR) by the amplification at 402 bp fragment. The percentage of *Campylobacter jejuni* positive samples were 13.33. The analysed samples showed the  $\log_{10}$  value of *Campylobacter jejuni* as  $1.15 \pm 0.51$  cfu/g. It was within the permissible level. So poultry meat in that area can be used for food consumption.

1. Teaching Assistant, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary & Animal Sciences, Pookot.

2. Professor and Head, Department of Veterinary Epidemiology And Preventive Medicine, VC&RI, Namakkal.

## 13. NATURAL INFECTION OF VACCINIA VIRUS IN CATTLE, BUFFALOES AND HUMANS

**Parimal Roy<sup>1</sup>, V.Purushothaman<sup>1</sup>, Andrew Chandramohan<sup>1</sup> and S. Panneerselvam**

Centre for Animal Health Studies,

<sup>1</sup> Dept. of Microbiology, Madras Veterinary College,  
Tamilnadu Veterinary and Animal Sciences University, Chennai

Generalized pock lesions were observed among cows and buffaloes in several villages of Tamilnadu. Clinical signs included pyraexia, anorexia, watery nasal discharge, congestion of conjunctiva and corneal



opacity in some cases. Generalized skin lesions and spontaneous recovery were noticed in about 3-4 weeks time. Milk men were also contracted and develop nodules in hand. Diagnosis of the disease was done by electron microscopy and polymerase chain reaction with specific primers. It was found that vaccinia virus which is different from cow pox virus was involved for the disease outbreak.

#### 14. DEVELOPMENT OF TAQMAN QPCR ASSAY FOR RAPID AND SENSITIVE DETECTION OF ORTHOPOXVIRUS

**R K Singh, V Balamurugan, M Hosamani, V Bhanuprakash and M P Yadav**

Division of Virology, IVRI, Mukteswar, Nainital (Distt.)  
Uttaranchal, 263 138, India

Buffalopox is a contagious, emerging and reemerging viral zoonosis affecting buffaloes (*Bubalus bubalis*), rarely cows and human beings, with 80% morbidity in affected buffalo herd. The disease is enzootic in India with frequent occurrence of outbreaks has been reported. A quantitative PCR for rapid detection and quantitation of poxvirus is optimized using viral DNA extracted from partially purified cell culture propagated *orthopoxviruses* (buffalopox and camelpox viruses), *orthopoxvirus* DNA polymerase gene specific primers and TaqMan Probe. The sigmoid curve obtained using TaqMan probe confirmed the presence of specific product at an annealing temperature of 64°C with an efficiency of 98.4% { $Y=3.362 \log(x)+28.63$ }. The reaction mixture contained 5pmol of TaqMan probe (FAM fluorescent dye labeled) and 10 pmol of each primer, 10 mM dNTPs and 2.5 Units of *Taq* DNA polymerase. The standardized assay was successfully employed for the differentiation of orthopoxvirus from *capripox* and *parapoxviruses* and found specific for *orthopoxviruses* only. The assay was also well adopted for direct detection of buffalopox virus in clinical field materials.

#### 15. APPLICATION OF TAQMAN QPCR FOR SPECIFIC DETECTION OF CAPRIPOXVIRUSES

**V Balamurugan, V Bhanuprakash, M Hosamani, D J Kallesh and R K Singh**

Division of Virology, Indian Veterinary Research Institute  
Mukteswar, Nainital (Distt.), Uttaranchal, PIN: 263 138, India

Sheep pox and goat pox are the important diseases of sheep and goats. Both the diseases are enzootic in the country. Infections often associated with morbidity of 100% and mortality may go up to 75% in adults and 100% in young, immunologically naïve lambs and kids. A QPCR has been optimized for rapid and specific detection and quantitation of capripox viruses utilizing viral DNA extracted from cell culture propagated sheeppox and goatpox viruses, *capripox virus* DNA polymerase gene specific primers and TaqMan Probe. The sigmoid curve obtained using TaqMan confirmed the presence of specific product/s at an annealing temperature 63°C. The reaction mixture containing 5pmol of TaqMan probe (Cy-5 fluorescent dye labeled) and 10 pmol of each primer, 10 mM dNTPs and 2.5 Units of *Taq* DNA polymerase was employed. The assay, optimized so, was successfully used for differentiation of *capripox* viruses from *orthopox* and *parapox* viruses. Further, TaqMan QPCR was also well espoused for direct detection of goatpox or sheeppox viruses from suspected clinical scab material. QPCR was found relatively more rapid and sensitive than the conventional PCR assay.

#### 16. PATHOGENESIS OF PPR: DETECTION OF VIRAL ANTIGEN IN ATYPICAL ORGANS FROM EXPERIMENTALLY INFECTED GOATS

**A.Sen, V.Balamurugan, P.Saravanan, J. Sarkar, K.K. Rajak, V.Yadav and R. K. Singh**

National Morbillivirus Referral Laboratory, Division of Virology,  
Indian Veterinary Research Institute, Mukteswar-263 138,  
Nainital District, Uttaranchal, India

Pathogenesis of morbilliviral infections is paradoxical in the sense that morbillivirus are responsible for both immunosuppression and a life long protection. We are trying to determine the role that the virus has in terms



of its replication rate in different tissues from diseased animals. The differential usage of CD150 and CD46 receptors by this virus as reported previously along with experimental observations have led us to hypothesise that PPR virus has epitheliotropic as well as lymphotropic lineage. We believe that the epitheliotropic lineage is responsible for the spread whereas the lymphotropic lineage is responsible for the virulence seen in PPR infections. Both lineages coexist in varying proportions during the course of the disease. There are no differences either in nucleotide or amino acid sequences, as of date, that could help us in delineating these lineages. The basis of this hypothesis is the nature and behavior of the virulent PPR virus on needle passage in experimental animals and the relative quantification of virus from organs collected after mortality. We recently, detected PPR viral antigen in some organs/tissues like thymus, heart, kidney, gall bladder and bile samples from virulent PPR virus infected goats, in which there are no earlier reports of virus detection. This is a preliminary report of a seemingly diverse characteristic of this group of viruses viz, morbilliviruses and could very well have an impact in contributing to a better understanding of the emerging area of viral pathogenesis and the species jumping attributes of morbilliviruses.

#### **17. SIMULTANEOUS DETECTION OF *BABESIA BIGEMINA* AND *THEILERIA ANNULATA* INFECTIONS IN CATTLE BY PCR- RFLP**

**Reghu Ravindran\*, B. C. Saravanan, J. R. Rao and A. K. Mishra**

Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, -243122, India

*Theileria spp.* and *Babesia spp.* (Apicomplexa, Piroplasmidae) are responsible for tick-borne protozoan diseases in domestic and wild animals. In India *Theileria annulata* and *Babesia bigemina* constitute the predominant species responsible for widespread morbidity and mortality in cattle and buffaloes. Mixed infections are common and if the animals recover from these infections, a long lasting carrier status occurs with low grade parasitaemia in the affected population. A polymerase chain reaction (PCR) for the amplification of partial 18S small subunit ribosomal DNA fragment from the genomes of *Babesia bigemina* and *Theileria annulata* was standardized. PCR products from *B. bigemina* and *T. annulata* were sequenced and analyzed for detection of the differential restriction sites. The enzyme *Cfr*131 produced a pattern, which could clearly differentiate the two species.

\*Present address: Assistant Professor, College of Veterinary and Animal Sciences, Pookot, Lakkidi, P.O., Wayanad, Kerala-673576.

#### **18. USE OF NESTED PCR FOR ENHANCED DETECTION OF *PASTEURELLA MULTOCIDA* CAUSING HAEMORRHAGIC SEPTICAEMIA FROM CLINICAL SAMPLES**

**Sunitha Karunakaran<sup>1</sup>, G. Krishnan Nair<sup>2</sup> and M. Mini<sup>3</sup>**

Department of Microbiology, College of Veterinary and Animal Sciences,  
Mannuthy, Thrissur.

Haemorrhagic septicaemia (HS) is a highly fatal, acute, septicaemic disease, principally affecting cattle and buffaloes. There are reports about significant association of *P. multocida* in the nasopharynx of apparently normal bovines and outbreak of disease. Hence detection of *P. multocida* in apparently healthy domestic ruminants can be considered as an indicator of the susceptibility of the herd for HS. But attempts to isolate *P. multocida* from a contaminated site such as nasopharynx of healthy ruminants can be frustrating. These difficulties led to the development of Polymerase Chain Reaction (PCR) methodology for detection of *P. multocida*.

The PCR band amplified directly from nasal swabs and throat swabs is often faint, which could be due to small numbers of *P. multocida* recovered from such samples. Thus some carrier animals could be missed when screening nasal swabs by conventional PCR methodology. Application of nested PCR assay could circumvent these problems since in comparison to conventional PCR; nested PCR has been shown to be more sensitive. Based on the reports of improved sensitivity of nested PCR by several workers, this study was undertaken to develop a nested PCR assay from a previously described conventional PCR method, for enhanced detection of *P. multocida*.



A total of 309 samples comprising nasal swabs, pharyngeal swabs, lung samples, blood samples and blood smears were processed for detection of *P. multocida* DNA by using *Pasteurella multocida* species specific (PM-PCR) and nested PCR primers, which is designed using primer 3 software. PM-PCR could detect *P. multocida* DNA in only 14 clinical samples as indicated by amplification of 460 bp fragment. Negative clinical samples were selected at random and subjected to nested PCR for enhanced detection of *P. multocida*. Out of the seventy-one negative clinical samples tested, nested PCR primer could detect *P. multocida* DNA in a high proportion (53 out of 71) of samples as indicated by amplification of 214 bp fragment.

Thus in the present study, nested PCR was found to be more sensitive in detection of *P. multocida* DNA from clinical samples. But a high proportion of clinical samples previously found negative by PM-PCR gave positive results in nested PCR. This high sensitivity obtained need further investigation by trying this PCR assay for detection of *P. multocida* DNA in large number of negative clinical samples. From the present study; nested PCR assay could be recommended as a promising diagnostic tool for confirming the presence of *P. multocida* DNA while screening nasal and pharyngeal swabs, lung samples, blood samples and blood smears, where conventional PCR failed to detect *P. multocida*.

1. Veterinary Surgeon, Clinical Lab, District Veterinary Centre, Palakkad.
2. Associate Professor & Head, Dept. of Microbiology, College of Veterinary & Animal Sciences, Mannuthy.
3. Associate Professor, Department of Microbiology, College of Veterinary & Animal Sciences, Pookot.

## 19. DEVELOPMENT OF PCR AS A ROUTINE DIAGNOSTIC TECHNIQUE FOR CANINE EHRLICHIOSIS

**Asha Rajagopal\*, S.Abdul Basith\*\*, S.Gomathinayagam\*\*\***

Dept of Parasitology , Madras Veterinary College

Canine ehrlichiosis is a potentially fatal, tick borne rickettsial disease of dogs caused by *Ehrlichia canis*. Rapid and accurate diagnosis of the disease is important for ensuring proper treatment and favorable prognosis. Microscopic examination of peripheral blood smears for demonstration of the typical ehrlichial inclusions is the commonly used diagnostic technique for the routine diagnosis of the disease. This technique, though simple, is less sensitive and can give false negative results especially in the early and subclinical phases of the disease characterized by extremely low ehrlichiaemia. Recently, PCR has been developed as a sensitive technique for the diagnosis of the disease. In this study three PCR protocols, nested PCR, one tube nested PCR and direct PCR were attempted for amplifying the 16SrRNA gene fragment of *E canis*. In nested PCR, amplification was done in two rounds, the first using *Ehrlichia* genus specific primers, ECC and ECB and the amplicons of the first round was used as the template for the second round using species specific primers, ECAN5 and HE3. The protocol of one tube nested PCR was standardized in which the amplification was performed in a single round by adding the genus and the species specific primers together in the same reaction mix. In direct or one step PCR amplification was done directly using the *E canis* species specific primers ECAN5 and HE3 in a single step. Nested PCR and one tube nested PCR yielded a 387 bp product in positive control and positive samples. On comparison of the two protocols, one tube nested PCR had the advantage of reduction in procedure time by about three hours. Further, this protocol was simpler and required lower quantities of reagents. More significantly, when compared with the conventional nested PCR, this protocol could reduce the chance of cross contamination between samples as the amplification was carried out in a single tube and there was no need to handle the primary PCR product for the nested reaction. Thus considering the technical ease and the time saving procedure, one tube nested PCR was more suitable as a routine diagnostic technique for the disease. Direct PCR failed to give results in any of the samples tested and all these samples tested positive by nested PCR. The failure of direct PCR to yield positive results can be attributed to its limitation in detecting low levels of *E canis* DNA in blood. In this study, three simpler and fast methods viz the lysis buffer method, dehaemoglobinization method, and boiling method were used for the extraction of DNA from whole blood. Though these alternative methods yielded DNA of less purity when compared with the commercial kit method, the PCR results were not significantly affected. These alternative methods could be used



for DNA isolation in routine PCR diagnosis thus making PCR simpler, less expensive and rapid diagnostic technique suitable for the routine diagnosis of the disease.

\* Research Assistant, IAH & VB, Palode, \*\* Professor and Head, Dept of Parasitology, Madras Veterinary College, \*\*\* Assoc. Professor, Dept of Parasitology, Madras Veterinary College

## **SESSION 4: GENETIC MANIPULATIONS**

### **1. EVALUATION OF COMBINED RECOMBINANT OUTER MEMBRANE PROTEINS LIPL32 AND LIPL41 FOR THE SERODIAGNOSIS OF CANINE LEPTOSPIROSIS**

**T.M.A. Senthikumar, M. Subathra, and P. Ramadass**

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

Leptospirosis is an important global veterinary health problem caused by spirochetes belonging to the genus *Leptospira*. Leptospire are ubiquitous in nature, reflecting their ability to adapt to both the ambient environment and the renal tubules of chronically infected reservoir hosts. Microscopic agglutination test (MAT) remains specialized test, which is not generally performed in routine diagnostic laboratories. In the efforts to develop new diagnostic tests to achieve high sensitivity, the focus is mainly on the outer membrane proteins. LipL32 and LipL41 are among the immunogenic outer membrane proteins that are highly conserved among pathogenic species of *Leptospira*. The recombinant proteins were expressed in *E. coli* system and purified as fusion proteins. The combined recombinant outer membrane proteins LipL32 and LipL41 were evaluated for use in the diagnosis of canine leptospirosis by Enzyme-Linked immunosorbent assay (IgG-ELISA) and Latex agglutination test (LAT). The sensitivity and specificity of the developed assays were analyzed with the microscopic agglutination test (MAT) using 250 canine serum samples. The assays were proved to be as sensitive, specific and accurate as compared to the standard microscopic agglutination test (MAT).

### **2. GENOTYPING OF CANINES USING MICROSATELLITE MARKERS**

**M. Parthiban, A.Wilson Aruni, P. Sathya and P. Ramadass**

Department of Animal Biotechnology, Madras Veterinary College, Chennai- 600 007

Canine genotyping was done using microsatellite marker to find out the parentage. The microsatellite markers are polymorphic DNA loci consisting of a repeat nucleotide sequence. The repeat sequence can be of 2 to 7 base pairs long. The number of repeat units vary in a population thereby creating multiple alleles for a microsatellite locus. DNA was extracted from sire, dam and progeny of the dog. The 10 canine specific microsatellite loci were amplified by PCR using fluorescent labeled forward and unlabelled reverse primer. The microsatellite markers using fluorescent dye labeled primers recommended by the American Kennel Club (AKC) was used. The PCR amplicons were separated by using electrophoresis then the dye labeled products were identified by fluorescence detection in Genetic Analyzer (ABI 3130). Genotype software – Gene mapper supplied by Applied Biosystem was used to size and genotype the alleles. Parentage of the progeny was assessed by identifying the either of the allele from dam or sire.

### **3. MOLECULAR CHARACTERISATION AND PHYLOGENETIC ANALYSIS OF LOCAL INFECTIOUS BURSAL DISEASE (IBD) ISOLATES TARGETING THE HYPERVARIABLE REGION OF VP2 GENE**

**A.Wilson Aruni, M.Parthiban, Hudson Taylor and V. Purushothaman**

Department of Veterinary Microbiology, Madras Veterinary College, Chennai-7

Infectious bursal disease suspected samples were collected from five different geographical areas in the poultry belt of Tamil Nadu for development of molecular diagnostics for IBD. Five AGPT positive IBD samples



were subjected to isolation in 5 week old chicken. IBDV RNA was extracted using guanidium acid phenol-chloroform method and 90% DMSO was used as denaturing agent for dsRNA. RT-PCR was carried out for all the five isolates and vaccine strain with one set of primers targeting the hypervariable region of VP2 gene which yielded an amplified product with the size of 474bp. The amplified PCR products were purified with QIA quick gel extraction kit. Automated nucleotide sequencing of the purified VP2 PCR amplicons of IBDV isolates yielded nucleotide size of 415bp, 410 bp, 440 bp, 418 bp and 415 bp for the five isolates respectively. After alignment, a 391 bp sequence encompassing of the variable VP2 gene from base 791 to 1182 was obtained. The nucleotide sequence was aligned and compared with the published VP2 gene sequence from classical IBDV strains, Very virulent IBDV strains, variant strains. All the viruses shared at least 94% identity and the divergence among the isolates were between 0.2% to 4.3%. Twelve very virulent specific nucleotide exchange were found to be maintained in the local IBDV isolates.

#### **4. MOLECULAR MODELLING OF TWENTY FIFTH BINDING DOMAIN (TBD) PROTEIN AND ITS RELATED STRUCTURES IN DEVELOPMENT OF RAS SIGNALLING INHIBITORS PREVENTING CANCER**

**A. Wilson Aruni and K. Gajendran**

Department of Bioinformatics & ARIS cell, Madras Veterinary College, Chennai-7

*Ras genes* encoding a family of protein are very important molecular switches for a wide variety of signal pathways that control the process of cell proliferation, cell adhesion, apoptosis and cell migration. Mutation of *Ras* family of proto-oncogenes are very common. This paper aims at molecular modeling with a view to arrive at a similar structured molecule which could reintroduce regulation of Ras system or kill cells during uncontrolled pathways. The TBD and *Ira2 Ras GAP* structures were predicted and their interaction with *Tfs1p* were studied. Where *Tfs1p* is a PEBP family of protein which can interact with Ras thereby down regulate signal transduction pathway. The docking study revealed that the amino acids involved in the interaction could be helpful in drug designing against the catalytic sites of *Tfs1p*. So the interactions could be blocked and in turn inhibition of Ras GAP activity could be prevented. Since *Ira2* resembles human NF1 (neurofibromatosis type -1) and the 3-D structure of TBD is similar to human *Ras GAP*. The interactions between TBD, *Ras GAP* and *Tfs1p* were done using Hex software. There is no 3-D structure available for the TBD. The structural similarity of yeast GAP with the human *Ras GAP* will pave its way for further exploration.

#### **5. CLONING OF CANINE INTERLEUKIN-4 GENE IN MAMMALIAN EXPRESSION VECTOR**

**S. S. Salunkhe, A. Rai, A. Saxena, P. K. Gupta, A. K. Tiwari, and M. Sandey**

Division of Veterinary Biotechnology,  
Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India.

The canine interleukin-4 gene was amplified by reverse transcriptase polymerase chain reaction with M-MuLV reverse transcriptase enzyme using oligo-dT primers from the total RNA of canine peripheral blood lymphocytes and amplification by Taq DNA polymerase enzyme with gene specific primers. The 'A' tailed product produced by Taq DNA polymerase was cloned in TA cloning vector pTargetT and transformed in *E. coli* DH5 $\alpha$  cells. The recombinant plasmid containing the canine interleukin-4 gene insert in right orientation was selected after characterization using restriction enzyme analysis, and nucleotide sequencing. The nucleotide sequence was submitted to GENBANK and accession number assigned was EF095771. The homology sequence analysis by DNASTAR revealed 99.8% at nucleotide level with the original sequence of IL-4 accession no. NM\_001003159.



## 6. GENETIC POLYMORPHISM IN DRB3 EXON 2 IN NILI-RAVI BREED BY POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM AND ITS ASSOCIATION WITH MASTITIS

Sudhir Kumar, M. L. Sangwan, Anurag Barwar and Sonika Ahlawat

Department of Animal Biotechnology

CCS Haryana Agricultural University, Hisar 125004

The genetic polymorphism in DRB3 exon 2 in Nili-Ravi (n=25) breed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and its association with mastitis was studied. The gDNA was isolated from blood by Sambrook and Russel (2001) method. The primers described by Sigurdardottir *et al.* (1991) for exon 2 of BoLA-DRB3 gene were used for amplification. Eleven genotypes viz. *b/b*, *c/c*, *f/f*, *o/o*, *s/s*, *f/o*, *b/f*, *b/o*, *o/s*, *b/l* and *l/s* with frequencies range 0.04 - 0.16 and 6 alleles viz. *b*, *c*, *f*, *l*, *o* and *s* with frequencies range 0.08 - 0.26 were found with *RsaI*. However, *HaeIII* detected 6 genotypes viz. *a/a*, *e/e*, *d/d*, *a/b*, *b/d* and *b/e* with frequencies range 0.04-0.28 and four alleles viz. *a*, *b*, *d* and *e* with frequencies range 0.08-0.6. While with *PstI*, 5 genotypes viz. *y/y*, *z/z*, *x/y*, *x/z*, *s/z* and *y/z* with frequencies range 0.08-0.32 and four alleles *s*, *x*, *y*, and *z* with frequencies range 0.04-0.42 were observed. These results revealed that breed studied was in homozygous as well as heterozygous condition. Results of this study showed that the DRB3.2 gene was found highly polymorphic in the Nili-Ravi buffalo breed. Genotypes *e/e*, *o/o* and *s/z* alleles *b* and *l* were observed in healthy animals while genotype *y/z* was observed in mastitis cases.

## 7. SEQUENCE ANALYSIS OF FOWL ADENOVIRUS - 4 (FAV-4) FIBRE GENE AND ITS SECONDARY STRUCTURE PREDICTIONS

Sandeepa Bhan, Ramneek, D. Deka, P. N. Dwivedi, A. Singh and M S Oberoi

Department of Veterinary Microbiology, COVS,

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab.

Fowl adenovirus (FAV) isolates were propagated in chicken embryo liver (CEL) cell culture and further characterized on the basis of PCR and RFLP using the primers H1/H2, H3/H4 and restriction enzymes *HaeII* and *HpaII*, respectively. Out of these two isolates of FAV-4 were processed for sequence analysis of the fibre gene by amplifying this gene using FAV-4 fibre gene specific primers and then cloning this PCR product in PGEM<sup>®</sup> Easy vector. A sequence of 1386 bps coding for 461 amino acids was derived. Phylogenetic analysis revealed almost 100 percent homology with sequences of FAV-4 fibre gene already available in the database. Secondary structure predictions were then made on the basis of derived amino acid sequence and these predictions revealed a very close relationship of FAV-4 with FAV-10. FAV-4 had two unique antigenic sites which may be involved in classification of these viruses as two different serotypes.

## 8. ISOLATION OF EMBRYONIC STEM CELLS AND THEIR CELLULAR LINEAGE DIFFERENTIATION

A. Mangala Gowri, A. A. Palanisamy, A. Mahalinga Nainar, C. Balachandran,  
G. Dhinakar raj and P. Ramadass

Department of Animal Biotechnology, Madras Veterinary College, Chennai-600 007

Stem cells are specialized undifferentiated cells in the body capable to form different tissue derivatives. Their unique characteristics make them very promising for supplying cells to tissue engineering in replacement therapies and to treat debilitating diseases (Borue *et al.*, 2004). The stem cells from embryos of mouse C57BL/6J were isolated using immuno-surgical destruction of trophectoderm cells and by selective isolation of inner cell



mass (ICM). ICM cells were cultured in ES cell culture medium and small nest of embryonic stem cell colonies were obtained. These undifferentiated stem cells were characterized for their stemness by the expression of undifferentiated stem cell specific gene OCT-4 by RT-PCR. Transplanting of stem cell colony into the testicular interstitium of mice assessed the ectopic differentiation potential of stem cells. The transplanted animals were checked for teratoma formation. Analysis for the tissue differentiation revealed a variety of differentiated cell types comprising of all three germ layers viz., ectoderm, mesoderm and endoderm

## 9. SEQUENCE ANALYSIS OF MORBILLIVIRUS CD150 RECEPTOR-SIGNALING LYMPHOCYTE ACTIVATION MOLECULE (SLAM) OF DIFFERENT ANIMAL SPECIES

**J. Sarkar, V.Balamurugan, A.Sen, P.Saravanan, B.Sahay, K.K. Rajak,  
T. J. R asool and R. K. Singh**

National Morbillivirus Referral Laboratory, Division of Virology, Indian Veterinary Research Institute,  
Mukteswar-263 138, Nainital District, Uttaranchal, India

We determined the complete nucleotide sequence of the gene coding for the morbillivirus receptor, Signalling Lymphocyte Activation Molecule (SLAM)-CD150 from four species viz., goat (*Capra hircus*), sheep (*Ovis aries*), Indian cattle (*Bos indicus*), and buffalo (*Bubalus bubalis*.) The sequences were analysed for homology with corresponding gene sequences of other species available in NCBI GenBank. SLAM or CD150 molecule has been reported as a putative receptor for most Morbilliviruses for their respective host species. The nucleotide (nt) ORF sequence of SLAM gene in all the four species studied was 1017 nucleotides in length encoding a polypeptide of 339 amino acids (aa), similar to *Bos taurus*, but different from canine, human, marmoset, and mouse SLAM, which were 1029, 1008, 1011 and 1032 nts in length, coding for 343, 336, 337 and 344 aa, respectively. Sequence analysis revealed 96.3 to 98.5% and 92.9 to 96.8% identities among the SLAM sequences of four species analyzed in the study at the nt and aa level, respectively. Sequence diversity at aa level between various species revealed that the critical functional region of SLAM protein among different species is relatively conserved; thereby facilitating this molecule to act as a receptor for morbillivirus. Phylogenetic relationship based on the aa sequences of SLAM protein of different species revealed that caprine, ovine, cattle and buffalo fall under a defined cluster but caprine SLAM is more closely related to ovine, followed by bovine.

## 10. AMPLIFICATION, CLONING AND SEQUENCING OF ENOLASE GENE

**Aralikatti, S.S., Suryanarayana, V.V.S., Honnappagol, S.S., Renuka Prasad .,  
Byregowda, S.M., Boogi, V.C., Isloor, S and Rathnamma, D.**

Veterinary Collage and IVRI Bangalore-24.

Enolase is mainly a glycolytic enzyme catalyzing conversion of 2-phosphoglycerate to phosphoenolpyruvate, a penultimate step in glycolysis. Enolase is composed of 3 isozyme subunits, alpha, beta and gamma. Alpha enolase is found in cytoplasm and cell surface, beta is striated muscle specific and gamma neuron specific.

Alpha enolase also performs several other functions in addition to glycolytic activity- like binding to plasminogen, salivary mucin, laminin of connective tissue and DNA. Enolase is also a heat shock protein and a constructing component of turtle cellular lenses and plays role in autoimmunity disorders. Enolase is found to be surface-localized or secreted in several prokaryotic and eukaryotic organisms.

Since enolase acts as an adapter molecule for binding to mucosal surface, enolase gene can be used for delivering vaccines via mucosal route. The work aims at amplification of the gene for alpha enolase protein, cloning and sequencing. Enolase gene of 1300bp was amplified at optimum annealing temperature of 52°C using specific primers designed based on database. The amplicon was cloned into KS + vector at *Bam HI* site. The clones were screened by colony lysis and positive recombinant clones were confirmed by Restriction digestion with *Bam HI* to release the insert. One of recombinant clone was sequenced. The sequence matched 80- 98 per cent with alpha enolase gene from various prokaryotic and eukaryotic organisms when blast search was done.



## **SESSION 5: ADVANCES IN VETERINARY IMMUNOLOGY**

### **1. ACTIVATION OF CHICKEN B CELLS ENHANCES THE BINDING OF INFECTIOUS BURSAL DISEASE VIRUS TO THEM BY UPREGULATING THEIR EXPRESSION OF THE PUTATIVE TARGET MOLECULE**

**Hari Mohan Saxena and Daya P. Gangale**

Department of Veterinary Microbiology, College of Veterinary Science,  
Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

Infectious Bursal Disease (IBD) is a viral disease of young chickens with high morbidity and low mortality affecting immature bursal B cells. The molecular target of IBD Virus (IBDV) on B cells is not known. We investigated whether IBDV binding is affected by activation of mature B cells with lymphokines or Lipopolysaccharide (LPS). Activation with lymphokines increased the IBDV - specific immunoperoxidase staining on B cells. The difference in spectrophotometric absorbance between the control ( $0.109 \pm 0.014$ ) and the treated cells ( $0.302 \pm 0.054$ ) was very significant ( $p < 0.01$ ,  $n = 7$ ). Activation with LPS tremendously increased the binding of IBDV. The difference between the control ( $0.109 \pm 0.014$ ) and the treated cells ( $0.173 \pm 0.017$ ) was very highly significant ( $p < 0.0001$ ,  $n = 10$ ). The increase in IBDV binding to mature B cells after activation, shown for the first time in this study, indicates that the target of IBDV may be a marker present on both, the immature and the mature B cells and is upregulated by activation of mature B cells.

### **2. PROTECTIVE ANTIGEN OF *BACILLUS ANTHRACIS* FOR DIAGNOSIS AND VACCINE DEVELOPMENT**

**V.Purushothaman, Parimal Roy, B.Balaganesan, T.Vijayanand and Sankaramoorthy. A.**

Department of Veterinary Microbiology, Madras Veterinary College, Chennai - 7.

The disease anthrax still remains on an endemic proportion in many parts of the country. Though the Sterne's strain remains to be the vaccine for long time to control this disease. The development of diagnostic assay and improved vaccine are the need of the hours as well. With the protective antigen of *Bacillus anthracis*, purified and used as an antigen for development of diagnostic assays including AGID. The strategies for development of improved vaccine for animals are also discussed.

### **3. ISOLATION, SEROTYPING AND ANTIBIOGRAM OF *PASTEURELLA MULTOCIDA* ISOLATES ASSOCIATED WITH H. S. OUTBREAKS IN BUFFALOES OF HIMACHAL PRADESH**

**Vipin Katoch, Mandeep Sharma, Prasenjit Dhar and Rajinder Kumar**

Department of Veterinary Microbiology, Dr. G. C. Negi College of Veterinary Science,  
CSK HPKV, PALAMPUR - 176 062 (H.P.)

Himachal Pradesh is a hilly state primarily focussing on agriculture and livestock rearing. However, geographical and climatic conditions of the state let the animals susceptible to various bacterial diseases resulting in disease outbreaks. The Haemorrhagic Septicaemia (H.S.) is one of the most important bacterial diseases of cattle and buffaloes, usually occurring in the form of outbreaks, and is caused by several serotypes of *Pasteurella multocida*, especially B:2 or 6:B. The present study describes the bacteriological findings of *Pasteurella multocida* type B:2 from disease outbreaks among buffaloes in Himachal Pradesh from 2004 upto 2006. A total of 8 H.S. outbreaks in buffaloes were attended and samples including visceral organs like lungs, liver, trachea,



heart, kidneys, spleen, heart blood, exudates and swabs were collected and inoculated aseptically on 5 per cent sheep blood agar (BA) and MacConkey's lactose agar (MLA). The isolates were identified as *P. multocida* on the basis of cultural, morphological and biochemical analysis and further confirmation was done by PCR-based typing of the isolates. Buffaloes from 75 per cent (6/8) outbreaks were positive for *P. multocida* type B:2 based on PM-PCR performed in IVRI, Izatnagar (U.P.). *In-vitro*, drug sensitivity pattern of the isolates showed variable results in different outbreaks, however, most of the isolates were highly sensitive to antibiotics like ofloxacin, enrofloxacin, ampicillin, amoxicillin, gentamicin and tetracycline, whereas, penicillin, streptomycin and co-trimoxazole were mostly resistant.

#### 4. EVALUATION OF INACTIVATED OIL EMULSION INFECTIOUS BURSAL DISEASE VIRUS VACCINE OF TISSUE CULTURE ORIGIN.

**A. Thangavelu, K.S. Palaniswami and V. Purushothaman**

Dept. of Veterinary Microbiology, Madras Veterinary College, Chennai – 600 007.

Inactivated oil emulsion Infectious bursal disease virus (IBDV) vaccine was prepared with a chicken embryo fibroblast (CEF) adapted IBDV. Binary ethylene imine (BEI) inactivated IBDV was emulsified with Montanide ISA 25. Field trials were conducted in broiler farms situated around Erode and Tirupur in Tamil Nadu. A total of 11,000 doses were tested. Unprimed broiler birds were vaccinated at 10 days of age. Post vaccinal seroconversion was assessed on day 21 post vaccination and at the time of disposal by sale on day 32 post vaccination. The post vaccinal neutralization titres were above  $10^3$  and  $10^5$  respectively on day 21 and 32 post vaccination. The post vaccinal quantitative Agar gel immunodiffusion (QAGID) titres ranged between  $2^1 - 2^5$  and  $2^3 - 2^6$  respectively on day 21 and 32 post vaccination. The seroconversion observed in the field condition showed that single vaccination at 10 days of age with inactivated vaccine of tissue culture origin is adequate to protect broiler birds against field IBD.

#### 5. ISOLATION AND IMMUNOLOGICAL CHARACTERIZATION OF MYCOPLASMA SPECIES FROM BOVINE MASTITIS\*

**Sanjeev Kumar <sup>1</sup> and Y.Hari Babu <sup>2</sup>**

Dept of Veterinary Microbiology, Veterinary College Bidar

Mastitis of mycoplasmal etiology is an highly complex condition which involves the interaction of infectious agent, management and environment. The main source of mastitis spread is infected udder, milker's hands, as well as milking machines which act as an important vehicle for transmitting the infection in large herds. As mastitis can not be eradicated, prevention and control by early detection holds much promise in combating the losses. Therefore isolation and identification of the causative organism is essential for achieving the ultimate aim of treating and preventing mycoplasmal mastitis. An attempt is made in the present study to isolate and immunologically characterize the mycoplasma spp of organisms from bovine mastitis milk samples. A total of 87 mastitic animals were subjected for the isolation of mycoplasma which comprised of 223 quarter milk samples and 14 bulk tank milk samples. Milk samples were aseptically collected from mastitic animals in sterilized vials containing transport medium, out of 223 samples screened four samples were positive for mycoplasma and these isolates were considered for characterization under present study. Morphologically all the four isolates exhibited a similar type of colony features. Immunological studies were conducted on the isolates with growth inhibition test against known *Mycoplasma agalactiae* antiserum. Test antiserum was raised in New Zealand white rabbit against isolate No.2 and various



immunological studies were carried out. All the four isolates exhibited positive reactions on all the immunological tests which confirmed isolates as *Mycoplasma agalactiae*.

\* Part of M.V.Sc (Veterinary Microbiology) thesis submitted to KVAFSU, Bidar  
1. Scientist, IAH&VB, RADDL, Bidar 2. Professor and Head

## 6. SEROLOGICAL EVIDENCE OF EDS-76 IN BROILERS

**Priya, P. M.\***, **Nair, G. K.\*1**, **Chintu Ravishankar\*** and **Mini, M.\*2**  
Department of Microbiology, College of Veterinary and Animal Sciences,  
Mannuthy, Thrissur, Kerala, PIN 680 651

Of 230 sera samples collected randomly from broilers of 7-week-old from various parts of Kerala, 14 (6 %) were positive for EDS-76 viral antibodies. The HI titres were ranged from 2 to  $7 \log_2$ . The mode of infection of broilers with EDS-76 virus is unknown. There was no correlation found between the infection with this virus and the onset of a particular disease. Though the results of this study reveals low positive percentage, the importance of contaminated broilers as a source of infection for laying chickens should not be ignored.

\* Assistant Professor, Dept. of Veterinary Microbiology, COVAS, Pookot  
\*1 Associate Professor & Head, Dept. of Microbiology, COVAS, Mannuthy  
\*2 Associate Professor, Dept of Veterinary Microbiology, COVAS, Pookot.

## 7. PROTECTIVE ROLE OF CELL MEDIATED IMMUNE RESPONSE IN CHICKENS VACCINATED WITH NEWCASTLE DISEASE VACCINE

**S.Varalakshmi, J. John Kirubaharan and V.Purushothaman**  
Department of Veterinary Microbiology, Madras Veterinary College

An attempt was made to assess the protective role of cell mediated immune response in the absence of humoral immune response. Suppression of humoral immune response is carried out using cyclophosphamide. The suppression was attributed 95 percent depletion of bursal follicles and lymphocytes as evidenced by histopathological examination. Apart from suppressing humoral immune response by ablating the bursa of Fabricius, an attempt was also made to vaccinate birds with SDS treated NDV proteins. Treatment of NDV with SDS was done with an idea to damage the HN and F epitopes.

Four different trials were conducted to achieve the objectives. In trial 1, live Newcastle disease vaccine (LNDV) was administered through intranasal and intraocular route. In trial 2, oral pellet Newcastle disease virus was administered as feed is given. In trial 3, live Newcastle disease vaccine (LNDV) was administered through intranasal and intraocular route followed with a booster dose on 14<sup>th</sup> day post-vaccination. All these trials were conducted in B cell ablated and non-ablated broiler chickens at 10<sup>th</sup> day of their age. In fourth trial, SDS treated NDV proteins were given along with an adjuvant on 10<sup>th</sup> day of their life followed by booster dose on 14<sup>th</sup> day post-vaccination. The fourth trial was conducted only in non-ablated birds, since SDS damages the conformational epitopes and hence cannot stimulate neutralizing antibodies.

The CMI response was assessed by two assays viz., Colorimetric blastogenesis assay (CBA) and Under agarose leukocyte migration inhibition technique (LMIT). To confirm the protective roles, birds were challenged with virulent virus by local and parenteral routes.

The data generated was statistically analysed by independent *t* test and one-way ANOVA. One-way ANOVA was carried out to find immune response between different time points and independent *t* test was performed to find out the significance between vaccinated different groups.



From the data generated in this study it is made clear that in the absence of humoral immune response, CMI response could not protect the chicks from virulent virus challenge. In all trials CMI response was detected as early as 3<sup>rd</sup> day postvaccination and remain consistent upto the point of challenge. The probable reason for CMI not protecting birds could be due to the rapid multiplication of NDV inside cells that enables release of large amount of progeny virions quickly. In such a condition, CMI response was found to unlikely to be sufficiently rapid to kill the virus infected cells and the neutralizing antibodies in circulation alone were found to neutralize the viruses.

## 8. CROSS PROTECTION STUDIES WITH *PASTEURELLA MULTOCIDA* VACCINES PREPARED IN NORMAL AND IRON RESTRICTED MEDIUM

**M.Vidhya<sup>\*</sup>, N. Daniel Joy Chandran<sup>1</sup>, W. Manohar Paul<sup>2</sup> and G. Dhinakar Raj<sup>3</sup>**

Dept of Veterinary Microbiology, Madras Veterinary College, Chennai – 7.

Pasteurellosis caused by *Pasteurella multocida* is an important respiratory pathogen of sheep in tropical climate causing high mortality and morbidity. Vaccination using standard strain of P<sub>52</sub> was considered as a means to reduce losses. Variation between the isolates at the field level necessitated the development of vaccine against *Pasteurella multocida* using locally isolated strains. Further the vaccines prepared using the culture of *Pasteurella multocida* in iron-restricted medium were shown to increase the antibody production compared to antigens obtained from strains grown under normal conditions.

*Pasteurella multocida* strains of serotype A:3 and B:2 of ovine origin were cultured in BHI broth and in brain heart infusion broth (BHI) containing iron chelator dipyrityl. Additional outer membrane proteins were expressed by both strains of *Pasteurella multocida* when grown in iron restricted medium but not in BHI broth. Four inactivated aluminium hydroxide gel vaccines were prepared with each serotype cultured in BHI broth and in brain heart infusion broth (BHI) containing iron chelator dipyrityl.

All the four vaccines were found to be potent in mice and induced 3.08, 3.12, 4.86 and 4.58 log protection against the respective homologous challenge strains. The A:3 and B:2 serotype vaccine cultured in iron restricted medium, induced significant heterologous protection in addition to the homologous protection against the respective challenge strains with 4.66 and 4.59 logs protection respectively. The heterologous protection observed in A:3 and B:2 serotype vaccines may be due to the additional outer membrane proteins produced by these strains in iron restricted medium compared to iron rich medium. Indirect ELISA carried out to assess the immune status in mice and sheep showed significant antibody response in 21<sup>st</sup> day serum compared to 0 day.

The vaccines prepared under iron restricted conditions induced significant heterologous protection in mice and the antibody response was also significantly high compared to the vaccines prepared under normal conditions.

<sup>\*</sup>PG scholar, Dept of Veterinary Microbiology, Madras Veterinary College, Chennai – 7.

<sup>1</sup>Professor and Head, Central University Laboratory, TANUVAS, Madhavaram Milk Colony, Chennai -51.

<sup>2</sup>Retd professor, Dept of Veterinary Microbiology, Madras Veterinary College, Chennai – 7.

<sup>3</sup>Associate professor, Dept of Animal Biotechnology, Madras Veterinary College, Chennai – 7.



## 9. IMMUNOHISTOCHEMICAL CONFIRMATION OF GUT ASSOCIATED ANTIGENS IN *HAEMONCHUS CONTORTUS*

**P. Parvady devy<sup>1</sup>, S.Gomathinayagam<sup>2</sup>, Lalitha John<sup>3</sup> and G.Dhinakar Raj<sup>4</sup>**  
Madras Veterinary College, Chennai-7

*Haemonchus contortus* is the most pathogenic gastrointestinal nematode species affecting sheep and goats. *Haemonchus contortus* is a voracious blood sucker which feeds mainly on blood and mucosal tissue of host causing 'Haemonchosis'. The current approach for the control of haemonchosis is the use of concealed or hidden gut proteins which make them suitable as candidate immunogen for vaccine production. The present paper confirms the immunolocalisation of the antigen in the gut of the parasite.

*Haemonchus contortus* midgut antigen was prepared and partially purified using Sephadex G-200 column. Rabbits were immunized with the partially purified midgut antigen and booster injections were given on 14th and 28th days. Serum collected on 35th day blood sample was used for the immunohistochemical study. Cryostat sections of the *Haemonchus contortus* worms were subjected to Immunoperoxidase test (IPT) and Indirect Fluorescent Antibody Technique (IFAT). The antibody raised against the gut antigens bind to the gut membranes confirming the localization of antigens in the gut of parasite.

**Keywords:** *Haemonchus contortus*, gut antigen, IPT, IFAT

1 Teaching assistant, COVAS, Pookot, Kerala.

2 Associate Professor, Dept of Veterinary Parasitology, Madras Veterinary College, Chennai.

3 Dean, Madras Veterinary College, Chennai.

4 Associate Professor, Dept of Animal Biotechnology, Madras Veterinary College, Chennai.

## 10. DEVELOPMENT OF TRANSFORMED FIBROBLASTS ANTIGEN AS A DIAGNOSTIC ANTIGEN FOR AL / SV INFECTIONS IN POULTRY

**Alka Tomar; Rajeeva; Suresh, V; Anita P; Pankaj Kumar Mandal and Vipasha.**

Tumor Immunology Laboratory, Immunology Section  
Indian Veterinary Research Institute, Izatnagar, 243 122, UP. India.

Avian leukosis / sarcoma virus ( AL / SV ) infections; caused by a group of avian retroviruses under Genus Alpharetrovirus, [ type species avian leukosis virus ( ALV ) ], in the family Retroviridae, constitute various conditions in poultry, viz., neoplastic conditions, non- neoplastic conditions, subclinical infections leading to significant production losses and nonspecific mortalities; responsible for millions of dollars of losses each year throughout the world. Recent studies have also indicated significant importance of relationship of avian tumor viruses and human health, thus, its public health significance, as well.

The virus exists in nature in two forms : infectious virus particles, termed exogenous viruses and genetically integrated avian retrovirus like elements, termed endogenous viruses ( EVs ). Exogenous viruses are transmitted vertically ( congenitally ) from infected hen to progeny through egg and horizontally from bird to bird by direct or indirect contact; and endogenous viruses are transmitted in Mendelian inheritance fashion from the parent to their progeny.

Under natural conditions, all the chickens are subject to exogenous AL / SV infections and most chickens become infected by close contact with congenitally infected birds. Chickens, infected congenitally or even upto 2 weeks of age after hatching become immunologically tolerant to the virus and develop a persistent viremia shedding viruses constantly in their surroundings. Almost all normal chickens carry either complete or defective ev loci, on an average 5 ev loci in each chicken genome.

Currently, detection of major group specific antigen ( gsAg or GSA , mainly p27 ) present in the viral core forms basis for establishing presence of exogenous AL/SV infection in commercial poultry. But, the immunological



assays or indirect biological assays are of limited value in distinguishing between ALV GSA of endogenous and exogenous origin. Additionally, gsAg or GSA based assays can not differentiate between vertical and horizontal transmission among exogenous AL/SV infection. Though, molecular techniques such as RT-PCR assays and other indirect biological assays can differentiate both exogenous and endogenous ALV, but these techniques can be applied only in well equipped sophisticated laboratories or are highly cumbersome.

Therefore, there is an urgent need for development of an alternate antigen for convenient, precise and early diagnosis of AL/SV infections in chickens under field conditions. This will help to determine prevalence of ALV infection in poultry flocks and isolation of ALV subgroup A from chickens in India with objectives to reduce incidence of ALV infection in the country, as has been achieved considerably in the developed countries. This would also help country in saving an annual loss of 3 billion eggs, besides specific and nonspecific losses occurring on account of ALV infections. These losses might become even higher if other losses such as decreased fertility, hatchability, reduced feed efficiency and delayed sexual maturity are also considered.

In this endeavour, experiments for developing a transformed fibroblasts antigen (TfAg) at the Tumor Immunology Laboratory have yielded encouraging results, which will be presented



**WE SERVE**

Research in Biochemistry Molecular Biology, Microbiology, Cytogenetics, Biotechnology, Immunology, Horticulture, Tissue Culture and Related Sciences

**WE OFFER**

Amino Acids, Carbohydrates, Enzymes, Modifying Enzymes, Lipids, Fatty Acids, Nucleotides, Electrophoresis and Molecular Biology Reagents, Agarose, HPLC Solvents, Organic and Inorganic Compounds, Microbiological Ingredients and Dehydrated Culture Media.

**WE AIM**

High Quality, Competitive Prices, Prompt Service

**An ISO 9001: 2000 Company**

**SRL Sisco Research Laboratories Pvt. Ltd.**  
2-F, Satam Industrial Estate, 'C' Wing, Chakala,  
Andheri (E), Mumbai - 400 099. Tel: 022-2820 3882 / 2821 4005  
Fax: 28380990 Email : srl@bcm3@vsnl.net.in / srlmarketing@dishnetdsl.net

Visit Online : [www.srlchem.com](http://www.srlchem.com)





## SESSION - I

### 1. DETECTION OF *CAMPYLOBACTER JEJUNI* BY POLYMERASE CHAIN REACTION

**Jatinderpal Singh, T. S. Rai, A. K. Arora, Ramneek and S. K. Jand**

Department of Veterinary Microbiology, College of Veterinary Science,  
Guru Angad Dev Veterinary and Animal Sciences University (GADVASU)  
Ludhiana (Punjab)-141004

*Campylobacter jejuni* has emerged as an important cause of diarrhoea or gastroenteritis world-wide. Poultry is considered to be the main reservoir of *C. jejuni* infections in humans. Since, most of the campylobacters in poultry have similar morphological and biochemical characters so a highly specific molecular test is needed to confirm *C. jejuni*. In the present study, caecal swabs (n=40) were collected from poultry farms in and around Ludhiana for screening of *C. jejuni*. These swabs were processed for the isolation of *Campylobacter jejuni* under microaerophilic (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>) conditions as per OIE guidelines. Out of 40 samples, 6 isolates were found conforming to *Campylobacter* species as per their preliminary morphological (Gram-negative curved rods, seagull shape or S-shape), cultural and biochemical (catalase and oxidase positive and motile by typical cork screw or darting motility) characters. These isolates were subjected to polymerase chain reaction (PCR) employing primers directed against *mapA* gene, specific for *C. jejuni*. All the six isolates yielded an amplicon of 589 bp. PCR employing *mapA* gene was found to be highly specific and rapid test in confirming *C. jejuni* isolates.

### 2. EFFECTS OF EXPOSURE OF FISH TO HEAVY METALS ON SERUM PROTEINS AND LYMPHOCYTES

**Madhu Prabha Saxena<sup>+</sup>, Hari Mohan Saxena<sup>#</sup> and Gurinder Kaur Sangha<sup>\*</sup>**

Departments of <sup>+</sup>Livestock Production & Management and <sup>#</sup>Veterinary Microbiology,  
Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana, and

<sup>\*</sup>Department of Zoology, Punjab Agricultural University (PAU), Ludhiana - 141004.

Water sample from a polluted brook of Ludhiana called Buddha Nallah showed high concentrations (in mg/L) of all the 8 heavy metals investigated - Iron (35.86), Zinc (2.75), Manganese (0.166), Nickel (0.090), Chromium (0.065), Copper (0.045), Lead (0.044) and Cadmium (0.025). Common Carp fish were reared in a water tank containing polluted water from the Nallah. Serum protein profiles of 5 normal and 5 pollutant - exposed fish by SDS PAGE revealed that proteins p63 and p25 present in the normal fish were absent in the sera of pollutant - exposed fish. On the other hand, p56 and p22 absent in the normal fish were detected in the sera of pollutant - exposed fish. The total protein concentration in the plasma was lower in the pollutant - exposed fish compared to the normal fish. However, the difference was not significant. The response of lymphocytes to mitogen Con-A was reduced significantly in fish exposed to polluted water.

### 3. CYTOPATHIC EFFECT OF PPR VACCINE VIRUS STRAINS IN VERO CELLS

**Raveendra Hegde, Amitha R. Gomes, S. M. Byre Gowda, Santhosh A. K and  
C. Renuka Prasad**

Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore-24

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants and is a emerging disease in India after the eradication of Rinderpest from the country. Successful live attenuated homologous vaccines have



been developed against the disease for the effective prophylaxis by IVRI, Mukteswar and TANUVAS, Chennai, using Sungri and Arasur isolates respectively. The present study describes the cytopathic effect (CPE) of these two different PPR vaccine virus strains, in vero cells. Vero cells grown in Minimum Essential Medium were infected with vaccine virus at a multiplicity of infection of 0.01 per cell at a cell density of  $1 \times 10^5$  cells/ml of the medium by co-cultivation method. As per the microscopic observations and the coverslip culture studies, the cytopathic effect was visible from 96 hr post infection in Sungri vaccine virus strain where as Arasur vaccine virus strain showed CPE, 36-48 hr post infection. With both vaccine virus strains the CPE in vero cells showed initial cell rounding, aggregation and syncytial development. The generalized CPE was noticed by 144 hr in Sungri and by 96 hr post infection in Arasur strain. However complete detachment of the cell monolayer was observed in Arasur strain by 120 hr, post infection. Infected coverslip cultures stained with H & E and May & Grunwald's Giemsa showed extensive cell vaculation, cytoplasmic extension and syncytia comprising of five to six nuclei characteristic to paramyxoviruses. Acidophilic intracytoplasmic and intranuclear inclusion bodies were also observed. The details of virus propagation in cell cultures and events in cytopathic effects will be discussed.

#### **4. EFFECT OF DIETARY SUPPLEMENTATION OF HUMIC ACIDS ON IMMUNITY STATUS OF BROILER**

**R. Nagaraju, B.S.V. Reddy, R.G. Glori doss and B.N.Suresh**

Department of Animal Nutrition,  
Veterinary College, Hebbal, Bangalore -24

A study was conducted to evaluate the humic acid (HA) based product as a substitute for antibiotic in broilers. Two basal diets viz., moderate nutrient density diet as per the BIS (1992) specified ME and CP levels and low nutrient density diet containing 3.5% lesser ME and CP values than the moderate nutrient density diet were prepared each for starter (0-14days), grower (15-28days) and finisher (29-42days) phases. Low and moderate nutrient density basal diets without added antibiotic were negative controls ( $T_1$  and  $T_6$ ) and with added antibiotic became positive controls ( $T_2$  and  $T_7$ ). Further,  $T_1$  and  $T_6$  diets were supplemented with either 0.5, 0.75 or 1g HA based product per kg of feed to result in test diets viz.,  $T_3$ ,  $T_4$  or  $T_5$  and  $T_8$ ,  $T_9$  or  $T_{10}$ , respectively. Each of such diets was offered to duplicate groups of 14 straight run commercial chicks each.

An antibody assays against ND and IBD were conducted on 10<sup>th</sup> day post vaccination (16<sup>th</sup> and 22<sup>nd</sup> day, respectively). The results revealed no significant differences ( $P \geq 0.05$ ) in antibody titre against both ND and IBD. The organometry study at the end of the experiment (42<sup>nd</sup> day) also indicated no significant ( $P \geq 0.05$ ) differences among relative weights of liver, spleen and bursa weight. It is concluded that the addition of HA upto 0.1% particularly in the antibiotic free diets can improve the health status broilers.

#### **5. EFFECT OF DIETARY ENZYMES ON LYMPHOID AND OTHERS ORGANS OF BROILERS**

**B.N.Suresh, B.S.V. Reddy, R.G. Glori doss and C. Ramesh**

Department of Animal Nutrition,  
Veterinary College, Hebbal, Bangalore -24

An experiment was conducted to study effect of supplementation of biotechnological derived enzyme preparations on immune and other organs of broilers. A basal diet ( $T_1$ ) was prepared as per the BIS (1992) specification using maize and soybean meal as the main feed ingredients. Further, the basal diet was supplemented with either NSPases (xylanase – 1000,  $\alpha$ -glucanase - 400, cellulase- 200, pectinase – 100 units/kg feed –  $T_2$ ) or lipid utilizers (lipase – 100 units/kg diet + lecithin – 20 g/kg diet –  $T_3$ ) or its combination ( $T_4$ ). Each diet was offered to three replicate of 10 chicks (Hubbard strain, unsexed) each. The trial lasted for 42 days.

Two birds from each replicate were slaughtered on the terminal day and weight of the different organs viz., liver, spleen, bursa, heart, gizzard and proventriculus as well as abdominal fat pad. The results revealed no



significant differences ( $P \geq 0.05$ ) in absolute as well as relative (% body weight) organs weight. It can be concluded that the addition of enzymes have less influence on development of vital organs of the birds.

#### **6. IN VITRO ASSESSMENT OF BACTERIOSTATIC POTENCY OF EGG YOLK Ig Y AGAINST *ESCHERICHIA COLI*.**

**Shanmugasundaram.K, Vikrama Chakravarthi.P, Aravinthan.P,  
Malmurugan.S and Sugumar.K**

Department of Veterinary Microbiology, VCRI, Namakkal, Tamil Nadu

Food borne disease which is due to *Escherichia coli* could prevent by using chemical preservatives and antimicrobial agents. But the development of resistance and side effects due to indiscriminate usage without proper medical intervention necessitated the need for alternate strategies. Of the available options the application of polyclonal antibodies like immunoglobulin Y could regard as a good alternative. The O antigens of food poisoning pathogen *Escherichia coli* was prepared and used to immunize commercial layer chickens in order to raise immunoglobulin against *Escherichia coli*. The eggs, which contain anti *Escherichia coli* IgY, was collected on 30<sup>th</sup> day of first injection of antigen and stored at 4°C. The antibacterial IgY was separated by water dilution method (10 times diluted with distilled water, pH 5-5.5, incubated at 4°C for 6 hrs) and purified by 60 percent ammonium sulphate. The recovery of IgY was in the range of 57-62 per cent. The pathogens in Tryptic soya broth (approximately  $6 \times 10^8$ /ml) were cultured with specific IgY @ 20 mg/ml and the inhibitory effect was measured in UV spectrophotometer at 550 nm. The resultant growth curve indicated that the application of polyclonal antibodies (IgY) on meat could be used to prevent the *Escherichia coli* food poisoning. This process enhances the safety of various food products as it's neutralize or reduce the ability of food borne pathogen like *Escherichia coli* to proliferate prior to human consumption and there by increasing the face value and safety index of the food product.

#### **7. THE RAISING OF ANTI *ESCHERICHIA COLI* IMMUNOGLOBULIN Y FROM THE EGG YOLK OF IMMUNIZED CHICKEN.**

**Vikrama Chakravarthi.P, Shanmugasundaram.K, Aravinthan.P,  
Malmurugan.S and Sugumar.K**

Department of Veterinary Microbiology, VCRI, Namakkal, Tamil Nadu

The Immunoglobulin from immunized chickens is useful source of polyclonal antibodies. The Immunoglobulin Y can effectively bind to antigens such as bacteria, viruses, carcinogens and toxins and can neutralize the toxic effects of the antigen. Based on the literature a study was undertaken to isolate the IgY and analyze its action against food poisoning pathogen. The O antigens of food poisoning pathogen from *Escherichia coli* was prepared and used to immunize commercial layer chickens in order raise immunoglobulin against *Escherichia coli*. The eggs, which contain IgY, were collected on 30<sup>th</sup> day of first injection of antigen and stored at 4°C. The antibacterial IgY was separated by water dilution method. The egg yolk separated from the egg albumin and 10 times diluted with distilled water and the pH adjusted to 5-5.5, followed by incubation at 4°C for 6 hrs. Then after centrifugation the supernatant (water soluble fragment (WSF) containing IgY) was estimated for total protein content and further purified by salt precipitation method using 60 % ammonium sulphate as a reagent. The globulin content estimated by Biuret method. The recovery of IgY was in range of 57-62 per cent. The purity of the immunoglobulin Y was checked by Slide agglutination method with *Escherichia coli* antigens. The serum containing *Escherichia coli* and saline were used as positive and negative control respectively. The results revealed the bacteriostatic effect of IgY against the food poisoning pathogen *Escherichia coli*. From the present study it concluded that the IgY can be used as an anti bacterial agent against the infections caused by *E. Coli*. Further *in vivo* analysis needed to authenticate the finding.



## 8. THE RAISING OF ANTI SALMONELLA IMMUNOGLOBULIN Y FROM THE EGG YOLK OF IMMUNIZED CHICKEN.

**Shanmugasundaram.K, Vikrama Chakravarthi.P, Aravinthan.P  
Malmurugan.S and Sugumar.K**

Department of Veterinary Microbiology, VCRI, Namakkal, Tamil Nadu

The immunoglobulin from immunized chickens is useful source of polyclonal antibodies. The immunoglobulin Y can effectively bind to antigens such as bacteria, viruses, carcinogens and toxins and can neutralize the toxic effects of the antigen. Based on the literature a study was undertaken to isolate the IgY and analyze its action against food poisoning pathogen. The O antigens of food poisoning pathogen from Salmonella was prepared and used to immunize commercial layer chickens in order raise immunoglobulin against Salmonella. The eggs, which contain IgY, were collected on 30<sup>th</sup> day of first injection and stored at 4°C. The antibacterial IgY was separated by water dilution method. The egg yolk separated from the egg albumin and 10 times diluted with distilled water and the pH adjusted to 5-5.5, followed by incubation at 4°C for 6 hrs. Then after centrifugation the supernatant (water soluble fragment (WSF) containing IgY) was estimated for total protein content and further purified by salt precipitation method using 60 % ammonium sulphate as a reagent. The globulin content estimated by Biuret method. The recovery of IgY was in range of 57-62 per cent. The purity of the immunoglobulin Y was checked by Slide agglutination method with Salmonella antigens. The serum containing Salmonella and saline were used as positive and negative control respectively. The results revealed the bacteriostatic effect of IgY against the food poisoning pathogen Salmonella. From the present study it concluded that the Ig Y can be used as an anti bacterial agent against the infections caused by Salmonella. Further *in vivo* analysis needed to authenticate the finding.

## 9. PURIFICATION AND PARTIAL CHARACTERIZATION OF IgG FROM BLACK BENGAL GOAT

**T. Siva Subramani <sup>1</sup> & S. Batabyal <sup>2</sup>**

Department of Veterinary Biochemistry, WBUAFS, Kolkata

This study was carried out to isolate relatively purified form of IgG from Black Bengal Goat which may aid for immuno-biochemical assays to monitor diseases, thereby helping in the conservation of the species. Healthy animals maintained at University farm were used for collection of sera samples. A combination of 50% double ammonium sulphate precipitation and gel filtration chromatography was used to isolate IgG in relatively pure form. Initially, crude IgG was obtained by 50% double ammonium sulphate precipitation which was dialyzed against several changes of the phosphate buffer saline of pH 7.2. The protein concentration of the crude IgG was 12.1 mg/ml. Dialyzed crude IgG was then purified on Sephacryl S-200 in which the proteins eluted into one small peak and one major peak. The fractions showing the peak were pooled into four, and their protein concentration were found to be 0.56 mg/ml for the minor peak, 1.73 mg/ml, 1.42 mg/ml and 0.29 mg/ml for the three fractions of the major peak. On SDS-PAGE, the pooled fraction of the major peak (purified IgG) revealed two major protein bands, 50.1 kDa and 25.7 kDa which were suggestive of heavy chains and light chains respectively.

**Key words:** Black Bengal goat, Ammonium sulphate precipitation, Sephacryl S-200, IgG

<sup>1</sup> Teaching Assistant, Department of Veterinary Biochemistry, COVAS, Pookot, Kerala

<sup>2</sup> Head, Department of Veterinary Biochemistry, WBUAFS, Kolkata.



## 10. COUNTER IMMUNOELECTROPHORESIS OF PURIFIED IgG OF BLACK BENGAL GOAT AND GAROLE SHEEP

**T. Siva Subramani<sup>1</sup>, S. Batabyal<sup>2</sup> and Rajani Kumar Paul<sup>3</sup>**

Department of Veterinary Biochemistry, WBUAFS, Kolkata

This study was carried out to check the purity of purified IgG, obtained by combination of double ammonium sulphate precipitation and gel filtration chromatography from Black Bengal Goat and Garole sheep against anti-goat IgG. Counter immunoelectrophoresis was carried out using crude IgG and purified IgG of both the species against the anti-goat IgG and stained with amido black. The crude IgG obtained by salt precipitation showed single line of precipitate for both the species against the anti-goat IgG. The purified IgG revealed double line of precipitate which may be due to the IgG subclass IgG1 & IgG2 which differ in their physico-chemical and biological properties. The purified sheep IgG also showed cross reactivity with the anti-goat IgG evidenced by double line of precipitate.

Keywords: Counter immunoelectrophoresis, IgG, anti-goat IgG, Black Bengal Goat and Garole sheep

1 Teaching Assistant, Department of Veterinary Biochemistry, COVAS, Pookot, Kerala

2 Head, Department of Veterinary Biochemistry, WBUAFS, Kolkata.

3 Teaching Associate, COVAS, Faizabad

## 11. RESTRICTION ENDONUCLEASE ANALYSIS OF *PASTEURELLA MULTOCIDA* ISOLATES FROM POULTRY USING HPAII AND HHAII.

**Mann A S, Arora A K, Jand S K and Pavitar Kaur**

Department of Veterinary Microbiology,

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab.

The present study was carried out for the molecular characterization of *Pasteurella multocida* from healthy and diseased poultry birds by using restriction endonuclease analysis. In all 69 samples from 43 different poultry farms were processed. Five poultry farms were found positive for *P. multocida* organisms. All the five *P. multocida* isolates were oxidase, catalase positive, non-haemolytic and showed no growth on McConkey's lactose agar. Biochemical study of these isolates stated that these all isolates belonged to *P. multocida* subsp. *multocida*. All the isolates were sensitive to enrofloxacin, ciprofloxacin, pefloxacin and chloramphenicol. Restriction enzymes Hpa II and HhaI were applied to find the difference among these isolates. Two different profiles were observed with both the enzymes thereby indicating the occurrence of two different strains. The results indicated that HpaII and HhaI could be used to differentiate strains of *Pasteurella multocida* from fowl cholera outbreaks.

## 12. DIAGNOSIS OF GENITAL CAMPYLOBACTERIOSIS IN BREEDING BULLS BY POLYMERASE CHAIN REACTION

**Kairay N.K, Sharma N.S., Arora A.K., Ramneek, Pavitar Kaur and Jand S.K.**

Department of Veterinary Microbiology, College of Veterinary Science

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India

Samples (62) of preputial washings from breeding bulls were processed for detection of *C. fetus* by polymerase chain reaction (PCR). Out of sixty-two preputial washings, four (6.45%) were found positive by PCR. Five isolates of *C. fetus* were confirmed by PCR and out of these 5 isolates, 3 were *C. fetus* subsp *fetus* and 2 were *C. fetus* subsp *venerealis* on the basis of biochemical tests. Whereas all 5 were confirmed as *C. fetus* subsp *fetus* by PCR. Hence, sub-species level identification of *C. fetus* by PCR and isolation method did not corroborate. PCR was found to be specific method for rapid diagnosis of genital campylobacteriosis.



### 13. EFFECT OF PROBIOTICS WITH ENZYMES ON IMMUNE RESPONSE IN BROILERS

**J.Balachandar<sup>1</sup>, P.S. Reddy<sup>2</sup> and P.V.V.S.N. Reddy<sup>3</sup>**

Department of Poultry Science, College of Veterinary Science, Tirupati

Two hundred and forty day-old Cobb broiler chicks were distributed randomly into twenty four groups of ten each. Each dietary treatments, Probiotics I @0.5g/kg, Probiotics II @ 0.5g/kg, Probiotics III @ 0.1g/kg and Enzyme mixture @ 0.5g/kg diets either singly or in all possible combinations at respective dose levels were offered to triplicate groups of chicks up to 42 days of age and their immune response to New Castle disease Vaccination was evaluated. Blood samples were collected from one bird from each replicate ten days after booster dose of New Castle disease Vaccine. The serum samples were assessed for immune response by conducting Haemagglutination inhibition test. The immunity (HI titre values) to New Castle disease vaccination was above protective levels in all the dietary treatments. However, there was no significant difference among the treatments. It was inferred that immune response to ND Vaccine was found to be good in all treatments. Probiotics and Enzymes either alone or in combination at recommended levels can be supplemented in broiler diets for better performance and health.

<sup>1</sup> Teaching Assistant, Dept of Livestock Production and Management, COVAS, Pookot, Kerala

<sup>2</sup> Associate Professor, Dept of Poultry Science, College of Veterinary Science, Tirupati, Andhra Pradesh <sup>3</sup> Professor & University Head, Dept of Poultry Science, College of Veterinary Science, Tirupati, Andhra Pradesh

### 14. IN-VITRO ANTIVIRAL ACTIVITY OF PLANT EXTRACTS ON GOATPOX VIRUS REPLICATION

**V Bhanuprakash, M Hosamani, V Balamurugan, G Pradeep, D Swarup and R K Singh**

Division of Virology, IVRI, Mukteswar-263138,  
Nainital (Distt.), Uttaranchal

Goat pox is a notifiable disease of OIE, a highly contagious viral disease of goats accompanied by fever, ocular, nasal discharges and pox lesions on skin, respiratory and alimentary track. There is no homologous prophylactic available commercially to combat the goat pox infection. The disease is enzootic in the country and occurrence of outbreak is associated with 100% morbidity and mortality up to 75%. Ample number of reports is available indicating the use of herbal preparations for veterinary use in India. In this work, four plants having known medicinal importance were screened for goatpox virus (GTPV) inhibition *invitro*. Of the four plant extracts tested, extract from *Acacia arabica* (Babul) and *Eugenia jambolana* (Jamun) leaves had inhibitions of 99.70% and 99.92% at their maximum non toxic concentrations, 99.93±0.38 and 1999.73±0.50 µg/ml, respectively in all the cytopathic effect inhibition (CPE) assays. The inhibition of GTPV virus replication was further confirmed using PCR and real time-SYBR GREEN based QPCR (P32 gene) assays specific for GTPV. The current results indicate that the extract from *Acacia arabica* and *Eugenia jambolana* leaves inhibit the goat pox virus replication *invitro*.

### 15. COMPARATIVE EVALUATION OF IMMUNE RESPONSE OF SHEEP AND GOATS TO PPR VACCINE STRAINS

**A.K.Santosh<sup>1</sup>, Raveendra Hegde<sup>2</sup>, Shrikrishna Isloor<sup>3</sup>, K. Prabhudas<sup>4</sup>, C. Renukprasad<sup>5</sup>, D. Ratnamma<sup>6</sup>, B.M. Veeregowda<sup>7</sup> and Amita Gomes<sup>8</sup>.**

Dept. of Veterinary Microbiology  
Veterinary College, KVAFSU, Hebbal, Bangalore-560024

This study was conducted to compare immune response of sheep and goats to Sungri and Arasur strains of PPR vaccine viruses. Animals from two organized farms and one unorganized sector (village) were screened for PPRV antibodies. The animals which were having per cent inhibition (PI) value < 40 by c-ELISA were selected for vaccination studies. Animals were grouped into 3 groups viz., group A - Control animals, group



B -vaccinated with Arasur strain and group C- vaccinated with Sungri strain. Sera samples were collected on 21st, 60th and 90th day post vaccination and were evaluated for immune response by c-ELISA and SNT. In Farm I, 80% (12/15) of sheep vaccinated with Arasur strain and 81.25% (13/16) of sheep vaccinated with Sungri strain showed seroconversion in c-ELISA (PI >40) after 21st, 60th and 90th day. Similarly, SNT titres of these samples were protective (e" 1:8). In farm II, 75 (6/8) and 77 (7/9) per cent sheep showed seroconversion (PI >40) after vaccinating with Arasur and Sungri strains respectively. Further, 66.6 per cent (6/9) and 71.4 per cent (5/7) of goats showed seroconversion (PI >40) after vaccinating with Arasur and Sungri strains respectively. SNT of these samples showed neutralization titer of >1:8. In village, 71.4 per cent (5/7) of goats vaccinated with Arasur strain and 83.33 per cent (5/6) of goats vaccinated with Sungri strain showed seroconversion (PI >40). SNT of these samples showed neutralization titer of > 1:8.

Statistical analysis of PI value, using one way ANOVA for c-ELISA showed significant difference between control and vaccinated animals (< 0.05-0.001). However there was no significant difference between groups B and C vaccinated with Arasur and Sungri strains respectively. SNT titers were analyzed by Chi-square test ( $\chi^2$ ). No significant difference was found between two vaccinated groups but significant difference was found between controls and vaccinated groups (B and C) animals. Both the strains of vaccine viruses exhibited good seroconversion in the field. It was concluded that either of the strains of vaccines can be used under field condition for effective control of PPR disease in both sheep and goats.

1: PG student; 2; Deputy Director, IAH and VB, Bangalore; 3, 6 and 7: Assistant Professors  
4: Project Director, PD\_ADMAS, Bangalore; 5: Director, IAH and VB, Bangalore; 8: Scientist, IAH and VB, Bangalore

## 16. PATHOLOGY OF 'ACUTE CLASSICAL SWINE FEVER'.

**Rameshkumar P., Chintu Ravishankar<sup>1</sup>., Sarmadha M.K., Senthamil selvan<sup>2</sup> P., Sreekumaran T.,  
Priya<sup>1</sup> P.M Japrakasan V., Mini M., and Jayesh V<sup>3</sup>.**

Department of Veterinary Pathology, College of Veterinary and Animal Sciences,  
Pookot, Lakkidi P.O., Wayanad, Kerala. PIN 673 576

Pigs more than 20 in number under the age group of 3-4 months died in an organized farm, in Wayanad, Kerala with the clinical history of circling, shivering, disorders in locomotion, grinding of teeth, convulsion, and recumbency. Young piglets died suddenly without any clinical signs. Carcasses were brought to COVAS, Pookot for Post Mortem examination. Necropsy was conducted and the samples were collected for histopathologic and molecular studies. Gross lesions include erythematous patches in the ear, snout, lower abdomen, inner aspect of thighs, which were dark reddish in colour and some were cyanotic. Tonsil, thymus and pharyngeal lymph nodes were severely congested. In kidney pin point petechial haemorrhages were observed all over the cortex beneath the capsule. The entire surface of the kidney revealed a typical 'turkey egg' appearance. Irregular shaped infarcts in the margin of spleen were also observed. Mesenteric lymph nodes, small intestine and large intestine were moderately congested. Histopathologically, the kidney revealed moderate to severe engorged sinuses and haemorrhage. The haemorrhages were restricted to cortex and within the glomeruli. 'Perivascular cuffing' characterized by accumulation and infiltration of lymphocytes, mononuclear cells and few plasma cells were observed in brain. As clinical signs and gross, histopathological lesions were suggestive of Classical Swine Fever, samples were sent to IVRI for confirmation of the disease. The Classical swine fever virus nucleic acid in tissue samples was detected by Real Time RT-PCR and the disease was confirmed as Classical Swine Fever.

**Key words:** Swine fever, Perivascular cuffing, turkey egg, Real Time RT-PCR, CSF

<sup>1</sup> Department of Microbiology <sup>2</sup> Department of Anatomy <sup>3</sup> Dept. of AH, Govt of Kerala



## 17. STANDARDIZATION OF LARGE SCALE PRODUCTION OF HOMOLOGOUS LIVE ATTENUATED PPR VACCINE IN INDIA

**Raveendra Hegde, Amitha R. Gomes, Paramananda Hugar, Byregowda S. M,  
Giridhar P and C. Renukprasad**

Institute of Animal Health and Veterinary Biologicals, Hebbal,  
Bangalore, Karnataka 560 024

Live attenuated homologous vaccine against Peste des petits ruminants of sheep and goats was produced on a large scale basis using roller culture bottles using seed virus developed at Indian Veterinary Research Institute, Muktheshwar, India. Vero cells between 130-150 passages with six percent foetal calf serum were used for the production of vaccine. The cells were infected with 0.01 multiplicity of infection and harvested when the cytopathic effect was 80 %. The vaccine was freeze dried in order to maintain the stability of the vaccine. Identity test and titration was performed and the vaccine titer was monitored to be minimum of  $10^5/100$  doses. In-house sterility tests and quality control tests using experimental animals and small ruminants were performed. The Vacuum and moisture content of the vaccine were also regulated to be within the normal limits. Maintenance and infection of cells with virus, bulk production and Lyophilization and in-house quality control tests will be discussed in detail

## 18. CLONING OF CANINE INTERFERON GAMMA GENE IN MAMMALIAN EXPRESSION VECTOR

**S. S. Salunkhe, A. Rai, A. Saxena, P. K. Gupta, A. K. Tiwari,  
M. Sandey and Sudarshan kumar.**

Division of Veterinary Biotechnology,  
Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India.

The total RNA of canine peripheral blood lymphocytes was isolated and reverse transcribed into cDNA using M-MuLV reverse transcriptase. The canine interferon gamma gene was amplified using Taq DNA polymerase enzyme was used to amplify the gene as it adds 'A' overhang in the PCR product. This 'A' tailed product was cloned in TA cloning vector pTarget and transformed in *E. coli* DH5 $\alpha$  cells. The recombinant plasmid containing the canine interleukin-4 gene insert in right orientation was selected after characterization using restriction enzyme analysis, and nucleotide sequencing. The nucleotide sequence was submitted to GENBANK and accession number assigned was EF095772. The homology sequence analysis by DNASTAR revealed 99.6% at nucleotide level with the original sequence accession no. NM\_001003174.

## 19. *IN VITRO* MATURATION OF BOVINE OOCYTES –THE ROLE OF OVUM RETRIEVAL METHODS

**Magnus Paul.K<sup>1</sup>, Ratheesh Babu M<sup>2</sup>, Vijayakumaran.V<sup>3</sup>, Sreekumaran.T<sup>4</sup>**

Department of Animal Reproduction,  
College of Veterinary and Animal Sciences, Mannuthy-680 651.

The following experiment was carried out to evaluate the effect of different retrieval methods like aspiration, slicing and puncture on *in vitro* maturation potential of bovine oocytes. Slaughterhouse derived bovine ovaries from South Indian breeds like Kangayam, Khillari, Hallikar and a few crossbred cattle of Kerala were subjected to three retrieval methods to yield different quality grades of oocytes. In this study, a total of one hundred and fifty ovaries were processed. Fifty ovaries were processed under each method of retrieval. Ovaries were dissected out from animals within 30-60 minutes of slaughter and transported to the laboratory within 2-4 h in freshly prepared normal saline fortified with 100 IU/ml Benzyl penicillin and 100  $\mu$ g/ml Streptomycin sulphate maintained



at 36-38°C. TL-HEPES medium was used for oocyte retrieval and classification. Based on number of layers of cumulus cells and ooplasm character, the oocytes were graded into four classes. Each quality grade of oocyte obtained through three retrieval method were subjected to maturation for 24 h in TCM-199 medium supplemented with LH, FSH, Estrogen, Pyruvate and Foetal calf serum. Culture environment was set as 38.5°C temperature, 5% carbon dioxide tension and maximum humidity in standard CO<sub>2</sub> incubator. Maturation changes were assessed by cumulus expansion, formation of M II plates and polar body extrusion. Cumulus expansion rate of A class oocytes were 83.08 per cent, 69.70 per cent and 70.37 per cent in aspiration, slicing and puncture method respectively. B class oocytes exhibited cumulus expansion rate of 68.29 per cent, 53.0 per cent and 62.07 per cent in aspiration, slicing and puncture method respectively. Retrieval method was found to have no significant effect on cumulus expansion potential of bovine COCs. Nuclear maturation rate of A class oocytes in aspiration, slicing and puncture was 81.08, 78.84 and 80.0% respectively, whereas in B class oocytes under same methods the percentage of nuclear maturation was 57.10, 41.67 and 52.63% respectively. D class oocytes failed to mature in all methods of retrieval except one oocyte in aspiration method. Polar body extrusion rate of A class oocytes in aspiration, slicing and puncture method was 45.5 per cent, 42.1 per cent and 44.0 per cent respectively. B class oocytes exhibited polar body extrusion rates of 28.6 per cent, 25.0 per cent and 26.3 per cent respectively in aspiration, slicing and puncture method respectively. Oocytes with more than three layers of cumulus cells exhibited better polar body extrusion rate irrespective of their retrieval method. Staining of 18 oocytes with fluorescent dye Hoechst 33342 as a method of cross checking, revealed 66.67 per cent Metaphase II rate. Aspiration, slicing and puncture did not differ significantly in the maturation percentage of oocytes.

1) M.V.Sc Student, Dept. of Animal Reproduction. 2) M.V.Sc Student, Dept. of Animal Reproduction. 3) Associate Professor, Dept. of Animal Reproduction 4) Professor and Head, Department of Animal Reproduction

## 20. ANALYSIS OF CUMULUS-OOCYTE COMPLEX YIELD AND MORPHOLOGY IN CATTLE BASED ON OVUM RETRIEVAL METHODS

Magnus Paul.K<sup>1</sup>, Ratheesh Babu M<sup>2</sup>, Vijayakumaran.V<sup>3</sup>, Sreekumaran.T<sup>4</sup>

Department of Animal Reproduction,  
College of Veterinary and Animal Sciences, Mannuthy-680 651

This study was designed to evaluate the effect of different retrieval methods like aspiration, slicing and puncture on yield of different grades of oocytes and their morphology. Slaughterhouse derived bovine ovaries from South Indian breeds like Kangayam, Khillari, Hallikar and a few crossbred cattle of Kerala were subjected to three retrieval methods to yield different quality grades of oocytes. In this study, a total of one hundred and fifty ovaries were processed. Fifty ovaries were processed under each method of retrieval. Ovaries were dissected out from animals within 30-60 minutes of slaughter and transported to the laboratory within 2-4 h in freshly prepared normal saline fortified with 100 IU/ml Benzyl penicillin and 100 µg/ml Streptomycin sulphate maintained at 36-38°C. TL-HEPES medium was used for oocyte retrieval and classification. Based on number of layers of cumulus cells and ooplasm character, the oocytes were graded into four classes. Class A, More than 5 complete layers of cumulus cells and uniform granulation of ooplasm. Class B, 3-5 complete layers of cumulus cells and uniform granulation of ooplasm. Class C, 1-2 complete layers of cumulus cells and uniform granulation of ooplasm. Class D, Denuded oocytes with uniform granulation of ooplasm. Total yield of COCs in slicing was  $5.42 \pm 0.56$ , which was significantly higher than aspiration and puncture which yielded  $3.7 \pm 0.43$  and  $3.66 \pm 0.43$  COCs respectively. This could be due to recovery of oocytes from a heterogeneous population of follicles in slicing method. Aspiration, slicing and puncture yielded A class oocytes at the rate of 44.86%, 37.2% and 43.17% respectively, wherein mean yield per ovary in same methods were  $1.66 \pm 0.25$ ,  $2.02 \pm 0.25$  and  $1.58 \pm 0.25$  respectively. Yield of B class oocytes by aspiration, slicing and puncture were 32.97%, 29.89% and 31.69% respectively. Mean yield of B class oocytes per ovary was  $1.22 \pm 0.12$ ,  $1.62 \pm 0.20$  and  $1.16 \pm 0.16$  respectively. Proportionate yield of D class oocytes by aspiration, slicing and puncture was 3.7%, 12.10% and 6.01% respectively.



Aspiration, slicing and puncture did not differ significantly in yield of A, B and C class oocytes. But D class oocytes were proportionately more in slicing method. This finding could be attributed to the fact that the slicing recovered oocytes from a heterogeneous population of follicles, which included all kinds of growing follicles. When percentage yield of different classes of oocytes in same retrieval method was compared aspiration yielded A and B class oocytes in significantly higher proportion than C and D class oocytes. The same trend was observed in slicing and puncturing. In all the methods, the yield of D class oocytes was least.

1) M.V.Sc Student, Dept. of Animal Reproduction, 2) M.V.Sc Student, Dept. of Animal Reproduction, 3) Associate Professor, Dept. of Animal Reproduction, 4) Professor and Head, Department of Animal Reproduction

## **21. DETECTION OF BLUETONGUE VIRUS IN CHICKEN EMBRYOS TISSUES BY IMMUNO HISTOCHEMICAL TEST**

**Byregowda S.M , Suguna Rao, C. Renukprasad, Ramesh,**  
IAH&VB, Bangalore

Bluetongue is an important disease of small ruminants particularly of sheep. The disease of global importance and twenty four serotypes have been identified world over. In India reports suggest involvement of more than twenty serotypes based mostly on serology. Isolation of virus is necessary for confirmation of the serotype and to take timely control measures.

Isolation of virus is mainly by passing the suspected blood samples in embryonated chicken eggs. Confirmation of the virus in the embryo tissues at different passage levels will be useful for further passing and improves the chances of virus recovery from the blood samples. In the present study immuno peroxidase test (IPT) was performed on tissue sections of the embryos inoculated with the laboratory adopted virus for standardization of the technique. Sections of different tissues were taken and IPT was carried out using specific hyperimmune sera raised in rabbits. The bound antibodies were detected using antirabbit HRPO conjugate and OPD as chromogen. The sections were counter stained with regular H&E.

The virus could be detected specifically in tissues where virus multiplied inside the cells. The reaction was more in liver, CAM followed by lungs and heart muscles. The technique found to be simple, easy and can be performed in any laboratory without the need for sophisticated equipments. The methodology of the test, suitability of the test for detection of virus from field suspected samples, merits and demerits will be discussed.

## **22. POLYMORPHISM OF MICROSATELLITE MARKER S0005 IN INDIGENOUS PIG POPULATION OF KERALA**

**Aripasath K and Usha A.P\***

Centre for Advanced Studies in Animal Genetics and Breeding,  
College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala – 680 651

Microsatellite markers are found to be one of the best DNA markers for molecular characterisation. Genotyping of indigenous pigs of Kerala was performed by polymerase chain reaction amplification using S0005 microsatellite marker. For use in the PCR the forward primer was polynucleotide kinase labelled at its 5' end with  $\alpha^{32}$  P ATP. The amplified products were resolved by denaturing polyacrylamide gel electrophoresis followed by autoradiography. The polymorphism of the marker was assessed with allele frequency, heterozygosity and polymorphic information content (PIC). Eight alleles were observed in the study and the observed allele size range was 202 – 240 bp. The mean heterozygosity and PIC values for the marker were 0.8475 and 0.8291 respectively. The marker S0005 is found very polymorphic in the indigenous pig population of Kerala and can be recommended for the characterisation studies of native pigs of south India.

\*Associate Professor, Dept. of Animal Breeding and Genetics, COVAS, Pookot.



### 23. EFFECT OF GOOSEBERRY (*EMBLICA OFFICINALIS*) AND INDIAN GALLNUT (*TERMINALIA CHEBULA*) ON SPLEENIC ANTIBODY FORMING CELLS IN COCKERELS UNDER INDUCED HEAT STRESS

Mejo. K. R.<sup>1</sup>, Ramnath. V.<sup>2</sup> and Philomina.P.T.<sup>3</sup>

Department of Veterinary Physiology, COVAS, Mannuthy

A total of 40 egg type Gramapriya cockerels reared under standard conditions, with 1kg body weight (3-4 months age) were used for the study. Gooseberry (*Embllica officinalis*) and Indian gallnut (*Terminalia chebula*) (3:1 @ 2.0 g/kg) was given with 30g feed. Heat stress was induced by intermittent heating for 60 min with 15 min interval, over a period of 8 consecutive hours in controlled environmental chamber (floor space 875 cm<sup>2</sup>/bird) with temperature 40 ± 1°C and relative humidity 60-70 % for 10 days. The cockerels were divided into 4 groups (G-I: Untreated, non-heat stressed (NHST), G-II: drug treated, NHST, G-III: Untreated, heat stressed (HST), G-IV: drug treated, HST). G-II to IV were immunized with 1 ml i.v of 7 % rat RBC and next day, G-III & IV were subjected to heat. Two birds from each group (I to IV) were sacrificed by cervical dislocation on 3,5,7,9, and 11<sup>th</sup> day after immunization. The spleen was excised under sterile conditions for Jerne's plaque (PFC) and Rosette-forming cell (RFC) immunocytoadherence assays to determine the antibody forming cells. Though all the birds showed peak values on 7<sup>th</sup> day, G-II exhibited the maximum PFC and RFC/7.5x10<sup>6</sup> spleenocytes. Thereafter both responses were declined in all groups. The result demonstrated that anti-RRBC PFCs and RFCs in G-II were significantly (P>0.05) higher than G-I controls; similarly G-IV showed more response compared to G-I & III cockerels. The over all improved response showed by drug treated compared to untreated cockerels could be attributed to the stimulative effect of the drug on splenic cells to produce antibodies against RRBC. The findings thus revealed that the drug treatment was beneficial in antibody formation during heat stress.

1. Teaching Assistant, Dept.of Veterinary Physiology, COVAS, Pookot.
2. Assistant Professor (SS), Dept. of Veterinary Physiology, COVAS, Mannuthy.
3. Professor & Head, Dept.of Veterinary Physiology, COVAS, Pookot.

### 24. IMMUNOMODULATORY EFFECT OF GOOSEBERRY (*EMBLICA OFFICINALIS*) AND INDIAN GALLNUT (*TERMINALIA CHEBULA*) IN COCKERELS UNDER INDUCED HEAT STRESS

Mejo.K.R.<sup>1</sup>, Ramnath.V.<sup>2</sup> and Sreekumar.K.P.<sup>3</sup>

Department of Veterinary Physiology, COVAS, Mannuthy

The study was conducted with an objective of finding immunological variations during heat stress in cockerels and the role of Gooseberry (GB, *Embllica officinalis*) and Indian gallnut (IGN, *Terminalia chebula*) as an immunomodulator. A total of 48 Gramapriya cockerels of 1kg bw (3-4 months age) were subjected to intermittent heat (40 ± 1°C & relative humidity 60-70 %) for 30 min with 30 min interval for 4h/day in controlled environmental chamber (floor space 875 cm<sup>2</sup>/bird), for 10 days. GB +IGN (3:1 @ 2.0 g/kg) was given with 30g feed prior to and during heat stress. The cockerels were divided into 6 groups (G-I: Untreated, non-heat stressed (NHST), G-II: Drug treated, NHST, G-III: Untreated, 5 days HST, G-IV: Drug treated, 5 days HST, G-V: Untreated, 10 days HST, G-VI: drug treated, 10 days HST). All birds were immunized with 1 ml i.v of 7% Sheep RBC, 7 days ahead of the heat exposures. 2.0 ml blood was collected on alternate days from 5<sup>th</sup> day after immunization and pooled serum representatives of each group was utilized for estimating circulating antibody titres viz., hemagglutinin (HA), haemolysin (HL), Mercaptoethanol sensitive (IgM) and resistant antibody (IgG) titres. The results showed peak log<sub>2</sub> HA, HI, IgM & IgG titre values in G-II compared to G-I cockerels. A similar response was noticed when G-II was compared with all the other HST groups. Drug treated cockerels showed greater tendency to attain and maintain the titre values than its untreated counterparts. The results indicated that GB+IGN supplementation



in poultry not only maintained the preformed antibody titre but also improved the humoral immune response against a challenged antigen during the period of heat stress.

1. Teaching Assistant, Dept. of Veterinary Physiology, COVAS, Pookot.
2. Assistant Professor (SS), Dept. of Veterinary Physiology, COVAS, Mannuthy.
3. Associate Professor & Head, Dept. of Veterinary Physiology, COVAS, Mannuthy.

## 25. COMPARATIVE STUDY OF ANTIGENIC PREPARATIONS FROM *SETARIA DIGITATA* Sadish.S<sup>1</sup>, Chattopadhyay U.K<sup>2</sup> and Bhattacharya. D<sup>3</sup>

The study was undertaken to evaluate the proteins obtained from *Setaria digitata* (bovine filarial worm) as a source of antigen for the diagnosis of human filariasis as the nematode shares the antigenic similarity with human filarial worm *Wuchereria bancrofti*. Adult worms (*Setaria*) were collected from the peritoneal cavity of freshly slaughtered buffaloes and different techniques were employed for the preparation of antigens. Four antigenic preparations were made viz., Whole Worm Antigen (WWA), Whole Cuticular Antigen (WCA), Surface antigens (SAP), Excretory/Secretory Antigen (E/S). These antigens were characterized by SDS-PAGE which revealed their protein profile. The antigens exhibited distinct differences in their protein pattern. WWA showed 23 peptides and WCA 17 peptides. Surface antigenic preparations exhibited 5-8 peptides within the range of 20-64kDa. E/S antigen showed four major peptides within the range of 29-66kDa. In all the antigenic preparations 29kDa peptide was consistent which was found to be of diagnostic value in patients having bancroftian filariasis by DOT-ELISA.

**Keywords:** *Setaria digitata*, *Wuchereria bancrofti*, antigen, SDS-PAGE, kDa

<sup>1</sup> Teaching Assistant, Department of Veterinary Public Health, College of Veterinary & Animal Sciences, Pookot, Kerala

<sup>2</sup> Professor, Department of Microbiology, All India Institute of Hygiene & Public Health, Kolkata

<sup>3</sup> Senior Scientist, Eastern Regional Station, IVRI, Kolkata

## 26. DETECTION OF NEW CASTLE DISEASE VIRUS ANTIBODIES IN EGG YOLK OF LAYERS

**Nidhin Raj<sup>1</sup>, Praseena Poullose<sup>1</sup>, Surya P.S.<sup>1</sup>, Lonkar V.D.<sup>2</sup>,  
Chintu Ravishankar<sup>3</sup> and Priya P.M.<sup>3</sup>**

Department of Veterinary Microbiology, College of Veterinary and Animal Sciences,  
Pookot, Lakkidi P.O., Wayanad, Kerala – 673 576

A study was conducted to assess the titre of antibodies against Newcastle disease virus (NDV) in yolk of layer hens in an organized farm. Twenty eggs were collected randomly from a shed of layers and the antibody titre was assessed using haemagglutination inhibition (HI) test. Antibodies against NDV were detected in all the eggs tested. The HI titre values was obtained ranged from 4 to 32. Forty per cent of samples had a HI titre of 4, 35% of the samples had a titre of 8, 15% of the samples had titre of 16 and 10% of the samples showed titre of 32.

1. IV B. V. Sc. & A. H. student, 2. Teaching Assistant, Dept. of LPM 3. Assistant Professor

## 27. ASSESSMENT OF NEW CASTLE DISEASE VIRUS ANTIBODIES IN BROILERS

**Surya P.S.<sup>1</sup>, Praseena Poullose<sup>1</sup>, Nidhin Raj<sup>1</sup>,  
Chintu Ravishankar<sup>2</sup> and Priya P.M.<sup>2</sup>**

Department of Veterinary Microbiology, College of Veterinary and Animal Sciences,  
Pookot, Lakkidi P.O., Wayanad, Kerala – 673 576

A study was conducted to detect the presence of antibodies against Newcastle disease virus in broiler birds (42 days old). Seventy five samples were taken randomly from a group of broiler birds at time of slaughter. The HI titre values ranged from 4 to 128. Of the samples 1.3% of the samples showed HI titre of 128, 5.3% of



the samples had titre of 64, 22.6% of the samples showed titre value of 32, 28% of the samples shown titre value of 16, 20% of the samples showed titre value of 8 and 22.6% of the samples shown titre value 4. Of the 75 samples tested, 22 (29.33%) samples had a protective titre (titre of 32 or higher).

1. IV B. V. Sc. & A. H. student, 2. Assistant Professor

## **SESSION – II**

### **1. ATYPICAL CASE OF RECURRENT TYMPANY IN BOVINE OF KASHMIR VALLEY**

**F. U. Peer and Md. Moin Ansari**

Teaching Veterinary Clinical Service Complex,  
Faculty of Veterinary Sciences and Animal Husbandry,  
Shuhama, Alustaing, Srinagar-190006, J&K, India.

A five years old crossbred Jersey cow presented to the Clinical complex, Srinagar with the history of recurrent bloat, complete inappetance and sudden drop in milk yield for the last six days was the subject of study. The gaseous distension of left paralumbar fossa increasing constantly especially after feeding with resonant sound of percussion was noticed. The rumen appeared tense on palpation with sluggish rumen motility. No pain in the xiphoid region and abdomen was observed. Temperature (103.1° F) and heart rates (82/min.) were slightly elevated. The animal responded to the conservative treatment temporarily with recurrence of bloat of severe nature was suspected for foreign body syndrome. On exploratory puncture a non-penetrating wandering sewing needle in reticulum was removed and animal recovered uneventful after the treatment with no further recurrence of bloat.

### **2. A STUDY ON *PSEUDOMONAS AERUGINOSA* AFFECTIONS AND ITS ANTIBIOGRAM ASSAY IN DIFFERENT LIVESTOCK IN HIMACHAL PRADESH**

**R. C. Katoch and Mandeep Sharma**

Department of Veterinary Microbiology, Dr. G. C. Negi College of Veterinary Science,  
CSK HPKV, PALAMPUR - 176 062 (H.P.)

*Pseudomonas aeruginosa* is a frequent pathogen and persists in various clinical conditions in humans and animals. The present study was performed to isolate *Pseudomonas* spp. from different clinical conditions of domestic animals. Further, the culture sensitivity tests were performed to know the antibiotic susceptibility of the isolates. Over a period of two years, 275 clinical samples from 197 cattle, 26 buffaloes, 12 sheep, 15 goats and 25 dogs, suffering from different disease conditions were examined for bacterial isolations. Overall, 42 (15.27 per cent) isolations of *Pseudomonas aeruginosa*, 18 (9.13 per cent) from cattle, 11 (42.30 per cent) from buffaloes, 3 (25 per cent) from sheep, 2 (13.33 per cent) from goats and 8 (32 per cent) from dogs were recovered. From cattle, 11 isolations were made from cases of mastitis, whereas, 7 isolates were detected from uterine discharges of cattle suffering from endometritis and repeat breeding. Similarly, 8 isolations were made from pus swabs of buffaloes with otitis externa, whereas, 3 *Pseudomonas aeruginosa* isolates were detected from mastitic buffalo milk. From sheep and goats, 3 and 2 isolations of *P. aeruginosa* were made from ear pus swabs, respectively, while, 5 cases of otitis externa and 3 cases of dermatitis in dogs were positive for *P. aeruginosa* infection. Culture sensitivity testing of all the isolates revealed ciprofloxacin and gentamicin to be affective in most of the isolates, whereas, ofloxacin and tetracycline showed moderate sensitivity. The other antibiotics, like ampicillin, amikacin, amoxycillin, doxycycline and erythromycin were completely resistant to most of the isolates. In conclusion, *P. aeruginosa* may be associated with different systemic and localized disease conditions in different species of animals and *in-vitro*, ciprofloxacin is highly sensitive to this bacterium.



### **3. TUBERCULOSIS IN CATTLE IN AND AROUND PALAMPUR CITY OF HIMACHAL PRADESH**

**Aneesh Thakur, Mandeep Sharma, Prasenjit Dhar and Vipin Katoch**

Department of Veterinary Microbiology, Dr. G. C. Negi College of Veterinary Science,  
CSK HPKV, PALAMPUR - 176 062 (H.P.)

Bovine tuberculosis is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In a large number of countries, bovine tuberculosis is a major infectious disease among cattle, other domesticated animals, and among certain wildlife populations. Transmission to humans constitutes a public health problem. *M. bovis* belongs to the *Mycobacterium tuberculosis* complex group of mycobacteria, which also includes *M. tuberculosis*, *M. africanum*, and *M. microti*. Bovine tuberculosis infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions. After death, it is diagnosed by post-mortem examination and histopathological and bacteriological techniques. Molecular techniques like DNA probes and PCR are advanced methods of diagnosis.

Present study was conducted on bovines in and nearby areas of Palampur city of Himachal Pradesh with the purview of determining the prevalence of tuberculosis based on tuberculin testing. A total of 241 animals were screened for tuberculosis based on single intradermal tuberculin test with the bovine PPD. Out of 241 animals, 63 (26.14 per cent) were found to be positive for bovine PPD, while, 39 were doubtful. Comparative intradermal tuberculin testing was done with bovine and avian PPD to differentiate between animals infected with *M. bovis* and those sensitised to bovine tuberculin as a result of exposure to other mycobacteria. Out of 63 animals, only 15 (6.22 per cent) were found positive on comparative tuberculin testing. Samples of lungs and lymph nodes were collected from four animals died during the study. Acid fast bacilli were demonstrated from all the samples. However, mycobacterium isolates were recovered only from two of the samples. Thus, this study revealed the overall prevalence of tuberculosis as 6.22 per cent in animals surrounding Palampur city.

### **4. STUDIES ON CLINICO-THERAPEUTIC OF MANGE IN CATTLE OF KASHMIR VALLEY**

**F.U.Peer and Md. Moin Ansari**

Teaching Veterinary Clinical Service complex  
Faculty of Veterinary Sciences and Animal Husbandry  
SKUAST-Kashmir, Shuhama, Alustaing, Srinagar-190006, India, J&K

Eighteen crossbred Jersey and Zebu cattle, aged 1 month to 2 years were presented to Teaching Veterinary Clinical Complex, Srinagar with the history of alopecia, intense itching, erythema, scabby and pustular lesions on the inner aspect of the thigh, truck, brisket and head. In addition animals were emaciated with pale mucous membrane. Temperature, pulse and respiration was normal. A combination of therapy comprising of Ivermectin, Chlorpheniramine maleate, Ampicillin-cloxacillin and Lorexane spray were instituted to the affected animals, to which they responded favourably.

### **5. SUSTAINABLE DUCK REARING SYSTEM USING FRESH PRAWN WASTE**

**John Abraham\* and Reghu Ravindran\***

College of Veterinary and Animal Sciences, Pookot Wayanad, Kerala.

A study was undertaken at Aroor region of the Alleppy district in Kerala state where a unique semi-intensive system of duck rearing is followed. The peculiarity of this system is that the birds are fed on inexpensive fresh prawn waste, which is freely available waste product of prawn peeling industry spread throughout this area. The study was carried out using structured questionnaire designed to collect data on socio-economic and



husbandry practice affecting production. The Study revealed that fresh prawn waste, which is the main unconventional protein source, meets the complete protein requirement enhancing egg production and quality. This in turn, has also solved the crisis of water and air pollution prevalent in these areas since a long time due to disposal of prawn waste into the water bodies. This system turned out to be the most economical enterprise compared to any other animal husbandry enterprises in this region. The fresh prawn waste not only incorporates a salmon red colour to the yolk but also improves considerably the egg weight, albumen index, yolk index and shell quality. This system demonstrate how a waste product, which was causing lot of environment pollution could be effectively utilized to produce high quality balanced food, the eggs for human consumption economically.

\*Assistant Professor, College of Veterinary and Animal Sciences Pookot, Wayanad, Kerala.

## 6. COLIFORM INFECTION IN POULTRY REARED UNDER DEEP LITTER IN HILLY AREAS OF WAYANAD DISTRICT

**Lonkar V.D<sup>1</sup>., Balachandar J<sup>2</sup> and Balusami C<sup>3</sup>**

Department of Livestock Production and Management  
College of Veterinary and Animal Sciences, Pookot

Different Chicken breeds were reared under deep litter system in hilly areas of Wayanad district in Kerala for a period of six months (July to December). The mean Maximum & Minimum temperature recorded during July to December was 27.03 and 20.74 respectively, while, the maximum and minimum Relative Humidity recorded was 86.38 and 64.19 per cent respectively for the same period. The mortality was reported due to different causes during this period. The per cent mortality was worked out based on postmortem reports and found that mortality per cent of 47.27, 18.18 and 7.27 were due to *E.coli*, Catarrhal Enteritis and Ascariasis respectively. The above illustration indicates that mortality due to *E.coli* was more than other causes. This suggests that the occurrence of *E.coli* infections in Poultry reared in hilly areas of Wayanad district which might be due to presence of *E.coli* in water source. Moreover cold climatic conditions might favor the growth of organisms. Further investigation has to be carried out for screening of water samples from different areas of Wayanad district for detection of *E.coli*.

**Key words:** Chicken, *E.coli*,

1 & 2 Teaching Assistants, Dept of LPM, COVAS, Pookot,

3 Assistant Professor, Dept of LPM, COVAS, Pookot

## 7. ANTIOXIDANT EFFECT OF *EMBLICA OFFICINALIS*.

**Sajitha.I.S and C.R.Lalithakunjamma\***

Department of Pathology, College of Veterinary and Animal Sciences,  
Mannuthy, Thrissur

Free radicals are produced within the body during normal biochemical reactions, and they include reactive oxygen species and nitrogen species. This is enhanced in pathophysiological conditions creating oxidative stress. Reactive species are also generated during phagocytosis, a manifestation of innate immunity. These free radicals are effectively neutralized by enhancing the cellular defenses, in the form of antioxidants. Intrinsic antioxidants include enzymes such as catalase, superoxide dismutase and glutathione peroxidase. When there is oxidative stress, these may become insufficient and require exogenous antioxidants, to reduce oxidative damage.

Aflatoxin is a mycotoxin produced by *Aspergillus flavus* or *Aspergillus parasiticus*, which has immunosuppressive and hepatotoxic effect when present in the feed of livestock in excess of the permissible levels. *Embllica officinalis*, commonly known as amla or Indian gooseberry is used extensively in Indian System



of Medicine against a variety of diseases and also as a general health tonic. Amla is found to have protective effect against toxic injury inflicted by different toxins. A study was designed to see the protective effect of amla against aflatoxin in broiler chicken. Day old broiler birds were divided into three groups (A,B,C). The birds were provided with commercial feed. Amla powder was added at 1% and 2% level to groups B and C respectively. Body weight gain, hematobiochemical parameters and liver histology were assessed. The birds in the amla fed groups showed an improved weight gain in a dose dependant manner. Mean body weight at 8<sup>th</sup> week were 2250, 2446 and 2782 g respectively for A,B and C groups respectively. Hematological profile at eighth week showed an improved picture for the amla fed birds in a dose dependant manner which indicated the protective effect of emblica against aflatoxin induced suppression of bonemarrow and decreased protein synthesis imputed to hepatic damage. All the parameters except ESR showed an increase in value with the amount of amla added. The mean ESR values ranged from 1.7 to 2.4 mm/hr, Hemoglobin 9.1 to 11.8g%, PCV 26.6 to 31.1% and the Total Leucocyte Count(TLC) 18.13 to 22.75x10<sup>3</sup>. Differential leucocyte count showed significant differences between the three groups for heterophil, lymphocyte and monocyte count.

These results were confirmed by histopathology-vascular, degenerative, necrotic, fibrotic and regenerative changes were noticed in group A birds which were reduced in the amla fed groups in a dose dependant manner.

Commercial feed available in Kerala is mostly contaminated with aflatoxin and aflatoxicosis is not merely a storage problem. Aflatoxin produce decreased weight gain, cause immunosuppression and increase susceptibility to diseases, and these affect the returns from broiler farming and the livelihood of the people in this field especially the rural farmers. Amla has got significant protective effect against aflatoxin in this study. More work is needed to estimate the dose of amla required after analyzing the protective effect at various dose rates and the economics involved, for incorporating as a feed additive in livestock feed.

\*Associate Professor and Head, Department of Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur

## 8. PYOGRANULOMATOUS INFECTION (BUMBLE FOOT) IN JAPANESE QUAIL REARED UNDER DEEP LITTER SYSTEM IN COLD CLIMATIC CONDITON

Lonkar V.D<sup>1</sup>, Balusami C<sup>2</sup> and Balachandar J<sup>3</sup>

Department of Livestock Production and Management  
College of Veterinary and Animal Sciences, Pookot

Pyogranulomatous infection (Bumble foot) is commonly observed in the heavy breeds of chicken, but its evidence in Japanese quail has not been reported. This condition is reported in Layer Japanese quail at 32 weeks of age affecting the sub dermal part of the foot. There was unilateral as well as bilateral involvement of legs. The symptoms reported such as limping of leg, reluctant to move which finally led to deprivation of feed and water. Grossly there was swelling of sub dermal part of under surface of foot which was hot and painful with abscess formation. In later stage there was caseation and necrotic tissue formation. Similar condition was found in chicken reared along with quails. The cultural isolation on agar plate showed round, smooth, glistening opaque and convex golden yellow colonies which revealed the presence of *Staphylococcus* spp. Similarly the direct smear from lesion observed under microscope revealed presence of organism. The cold stress might cause the immunosuppression in bird which led to multiplication of *Staphylococcus* spp.

**Key words:** Bumble foot, Japanese quail

1 Teaching Assistant, Dept of LPM, COVAS, Pookot, 2 Assistant Professor, Dept of LPM, COVAS, Pookot, 3 Teaching Assistant, Dept of Pathology, COVAS, Pookot



## 9. ASPERGILLOSIS IN LAYER JAPANESE QUAIL REARED UNDER DEEP LITTER SYSTEM

Lonkar V.D<sup>1</sup>., Ramesh Kumar<sup>2</sup>, Balachandar J<sup>3</sup>. and John Abraham<sup>4</sup>

College of Veterinary and Animal Sciences, Pookot

Aspergillosis is respiratory disease caused by the fungus *Aspergillus fumigatus*. Though the respiratory aspergillosis is common in commercial poultry, there is paucity of evidence in Japanese Quails. The disease was reported in the Layer Quail at 32 weeks of age. The symptoms were accelerated breathing, loss of appetite, reluctant to move, respiratory distress, emaciation and drooping of feathers. The postmortem lesions revealed that, air sac contained miliary caseous pin head like nodules on thickened air sac membrane. The nodule has typical "Saucer Shaped" appearance. Lesions in the air filled cavities showed greenish to black coloration might be due to development of pigmented conidiophores. It was found that, there was absence of nodular lesions in the lung like other poultry species. Observation of crushed nodule under microscope revealed that, it contains long septate hyphae called conidiophore which ends in conidiophore vesicle bearing conidiospores which is suggestive of *Aspergillus* spp. Cold stress, Ammonia in poultry house and dusty environment might favor the incidence of aspergillosis in Japanese Quail.

**Key words:** Aspergillosis, Japanese Quail.

1 Teaching Assistants, Dept of LPM, COVAS, Pookot, 2 Teaching Assistant, Dept of Pathology, COVAS, Pookot, 3 Teaching Assistants, Dept of LPM, COVAS, Pookot, 4 Assistant Professor, Dept of LPM, COVAS, Pookot

## 10. SERUM TOTAL PROTEIN, ALKALINE PHOSPHATASE AND ASPARTATE AMINOTRASAMINASE CHANGES DURING TIBIAL FRACTURE HEALING IN DOGS.

S.Anoop and T.P.Balagopalan.

Department of Surgery and Radiology

College of Veterinary and Animal Sciences, Mannuthy, Trichur, Kerala

The present study was undertaken with the objective of assessing the changes in the serum total protein, alkaline phosphatase and aspartate aminotransaminase, during tibial fracture healing under two different methods of external coaptation techniques, in dogs. All the animals were observed for a period of six weeks and serum was separated on the day of presentation, after first week, second week, third week and sixth week, post fracture reduction. Serum total protein values on the day of presentation were  $6.01 \pm .54$  and  $6.5 \pm .58$  respectively in group 1 and group 2 animals. It showed a decrease till second week followed by an increase thereafter in both the groups. Serum alkaline phosphatase values on the day of presentation were  $351.00 \pm 64.50$  and  $318.33 \pm 60.2$  respectively in group 1 and 2. The Values showed an increase till second week, followed by a decrease there after. Serum aspartate values were  $24.66 \pm 1.02$  and  $26.16 \pm 5.13$  respectively in group 1 and 2 on the day of presentation. The values remained at a lower level through out the period of observation in both the groups.

## 11. EFFECT OF DIETARY NEEM (*AZADIRACHTA INDICA*) AND GARLIC (*ALLIUM SATIVUM*) ON CERTAIN BLOOD BIOCHEMICALS IN BROILER CHICKEN

Sreekumar, K.P<sup>1</sup>., Preethymol, J<sup>2</sup>., Lonkar, V. D<sup>2</sup>., Sumi, C.P.<sup>3</sup> & Asha, A<sup>3</sup>.

Dept of Veterinary Physiology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India

The dietary inclusion of neem and garlic on serum glucose, total lipids, triglycerides, uric acid and creatinine in broilers were studied on 56 broiler chicken. Serum glucose level was significantly ( $p < 0.05$ ) reduced by both 1% and 2% neem seed cake (NSC), 1% garlic powder (GP) and combinations of 0.5% GP and 1% NSC and 1% GP and 2% NSC. The highest reduction of serum glucose was seen when GP and NSC were used together at 1% and



2% levels respectively. Total lipid and triglycerides were significantly ( $p < 0.05$ ) reduced by feeding 1% GP and by combination of 1% GP and 2% NSC, Neem seed cake seems to have little hypolipidemic effect in birds. Both GP and NSC increased the utilization of glucose indicating their hypoglycemic effect but only garlic powder had hypolipidemic property. Neither uric acid nor creatinine was affected by the GP and/or NSC in the diet.

**Keywords:** neem, garlic, blood biochemical, broiler chicken

<sup>1</sup>Associate Professor, Dept of Veterinary Physiology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India, <sup>2</sup>Research scholar, Dept of Poultry Science, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India, <sup>3</sup>Teaching Assistant, Dept of Veterinary Physiology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India

## 12. VISCERAL GOUT IN LAYER CHICKEN - A CASE REPORT

**Lonkar V.D<sup>1</sup> and John Abraham<sup>2</sup>**

College of Veterinary and Animal Sciences, Pookot

Visceral Gout is a disorder of uric acid metabolism characterised by deposition of urates of sodium and calcium in tissue. The disease was reported in the heavy laying hen at 30 weeks of age. There was a white pasting of preloocal feathers. The gross lesions were atrophy of the kidney, dilated ureters. The dilated ureters from atrophied lobes of kidney were full of white irregular concretions of sodium and calcium urates. In addition to kidney similar deposits were found in different parts like heart, lungs, liver, proventriculus and spleen. Histologically kidney showed feathery crystals and basophilic spherical masses of urate. Uric acid crystals surrounded by histocytes and lymphocytes with hemorrhages. Necrosis, fibrosis, hemorrhages with urate deposit and significant reduction in number of glomeruli were noticed. A sudden change in ration especially in heavy laying hens with high protein diet may precipitate the onset of disease. Similarly Vitamin A deficiency which may cause the occlusion of urethral duct by desquamated metaplastic squamous stratified epithelium prevents the excretion of uric acid and so facilitating its accumulation.

<sup>1</sup> Teaching Assistant, Dept of LPM, COVAS, Pookot <sup>2</sup> Assistant Professor, Dept of LPM, COVAS, Pookot

## 13. EFFECT OF DIETARY SUPPLEMENTATION OF NEEM (*AZADIRACHTA INDICA*) AND GARLIC (*ALLIUM SATIVUM*) ON HAEMATOLOGY OF BROILER CHICKEN

**Sreekumar, K.P<sup>1\*</sup>, Lonkar, V. D<sup>2</sup>, Sumi, C.P.<sup>3</sup> & Asha, A<sup>4</sup>. Preethymol, J<sup>5</sup>**

Dept of Veterinary Physiology, College of Veterinary & Animal Sciences,  
Mannuthy, Thrissur 680 651, India

Haematological changes in chicken due to supplementation of Garlic powder (GP), Neem Seed Cake (NSC) and their combination were investigated. There was a significant ( $P < 0.05$ ) increase in heterophil count in birds supplemented with 0.5% GP, while other haematological parameters were not significant. Supplementation of 1% GP had no influence on haematological parameters. Hb, PCV, MCV, MCH, MCHC values did not differ significantly while significant ( $P < 0.05$ ) increase in lymphocyte and decrease in monocyte, eosinophil and H/L ratio were observed in birds supplemented with 1 and 2% NSC. The TLC increased significantly ( $P < 0.05$ ) in birds supplemented with 1% NSC and not in birds supplemented with 2% NSC. Birds supplemented with combination of 0.5% GP and 1% NSC had non significant change in Hb, PCV, MCV, MCHC, heterophil, basophil values, but a significant ( $P < 0.05$ ) increase in TLC, MCH, lymphocyte and decrease in TEC, monocyte, eosinophil and H/L ratio was observed. No significant change in Hb, PCV, MCV, MCH, MCHC, TLC, TEC, monocyte, basophil values, while significant ( $P < 0.05$ ) increase in lymphocyte count and decrease in heterophil and eosinophil count was observed in birds supplemented with a combination of 1% GP and 2% NSC. Decreased H/L ratio indicated



that neem seed cake alleviate the stress. Low-level dietary supplementation of mixture of GP and NSC improves the immune status of broilers.

**Key words:** garlic powder, neem seed cake, haematological changes and broiler

<sup>1</sup>Associate Professor, Dept of Veterinary Physiology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India, <sup>2,5</sup>Research scholar, Dept of Poultry Science, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India, <sup>3,4</sup>Teaching Assistant, Dept of Veterinary Physiology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur 680 651, India

#### 14. SALMONELLOSIS IN PIGS: AN EPIDEMIOLOGICAL STUDY

**B.R. Harish, Shivaraj.B.M, B.M Chandranaik,, M. D. Venkatesh and , C.Renukprasad**

Southern Regional Disease Diagnostic Laboratory (SRDDL)

Institute of Animal Health and Veterinary Biologicals (IAH&VB)

Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU)

Hebbal, Bangalore-560024

There are several infectious and contagious diseases, which affect all ages of pigs causing major impact on swine industry in terms of high mortality and morbidity (OIE, 2004). Salmonellosis is one of the principal causes of mortality in pigs. The disease has worldwide distribution apart from involving other domestic and wild animals. The incidence has increased with the intensification of livestock production and globalised market policies. The present study attempts to make a detailed epidemiological study on the outbreaks of salmonellosis in pig farms around Bangalore during July- August 2005. The study includes investigation of field outbreaks, isolation of bacteria, clinico pathological studies and factors contributing in these outbreaks.

Out of the 735 pigs in three farms under study, 165 (38 adults, 32 female and 65 piglets) succumbed to the disease in spite of routine treatment. The affected piglets were unable to suckle the milk from mother, and succumbed to generalized septicemia with in 24-48 hours. The ailing adult and piglets showed dark red to purple discoloration of the skin, especially at the ears and ventral aspect of the abdomen. The adult pigs had infection after 2-3 days and showed high fever (105<sup>o</sup>-107<sup>o</sup>F) followed by persistent blood mixed watery diarrhea with putrid odour. The affected pigs were anorectic, dehydrated and some animals showed abdominal discomfort. The clinical materials viz. cleaned intestinal mucosa, liver from dead animals and fecal samples from ailing animals were collected for laboratory diagnosis.

The collected materials when inoculated on selective media of Mc konkey agar, yielded growth of salmonella species in 48 hours which was further confirmed by the specific biochemical tests. This was further supported by histopathological studies. The farm management and hygiene was very poor and there was no scientific approach to run the farm. This may be the major predisposing factor for outbreak of the disease. All the farms were completely depending on hotel and poultry wastage for their regular feeding. The detailed studies will be presented at symposium.

#### 15. CERTAIN BIOCHEMICAL PARAMETERS OF THE CROSS-BRED GOATS IN KERALA

**Roymon Jacob, K. S. Sujatha and Sisilamma George**

College of Veterinary and Animal Sciences

Mannuthy, Kerala

Cellular and biochemical parameters of blood reflect the health and physiological status of an individual. A study was conducted in the cross-breed goats reared in the University Goat and Sheep Farm, College of Veterinary & Animal Sciences, Mannuthy to determine the plasma concentrations of the metabolites viz, glucose, albumin, total proteins, total bilirubin, the enzymes Alanine amino transferase (ALT), and Aspartate amino transferase (AST), and the minerals sodium and potassium. The animals were classified into four different groups based on their breed and age, each consisting of 15 animals as follows: Group I. Sannen x Malabari (age 3.5 – 4 yrs), Group



II. Alpine x Malabari (age 2.5 – 3 yrs), Group III. Boer x Malabari (age 1 – 1.5 yrs), Group IV. Boer x Malabari (age 3 - 4 yrs). Among the various metabolites, no significant difference was observed in the concentration of glucose and total bilirubin between various groups. In the levels of albumin and total protein, significant differences were noticed between various groups. Among the minerals estimated, only sodium exhibited significant difference between Group II and other three groups. Other than the minerals estimated, the data presented can be used as reference values to assess the liver function of cross bred goats in Kerala.

## 16. INFLUENCE OF LEVEL AND DEGRADABILITY OF DIETARY PROTEIN ON THE PERFORMANCE OF EARLY LACTATING CROSSBRED DAIRY COWS. II. ONSET OF POST PARTUM HEAT

**Ally, K<sup>1</sup>, Mercy, M.D<sup>2</sup> and Viswanathan, T.V<sup>3</sup>.**  
Department of Animal Nutrition, CV&AS, Mannuthy

A study was conducted in early lactating crossbred cows to assess the influence of level and degradability of dietary protein on the performance. The experiment was conducted in two phases. In the first phase eighteen early lactating cows within 10 days of their lactation were divided into three groups and fed with three dietary rations (concentrate: roughage as 70:30), varying only in their crude protein (20, 15.4 and 13.2 per cent) levels. The animals were fed with the rations at the rate of 3.5 percent of their body weight, which was revised every fortnightly according to body weight. The feeding trial was done for 100 days and at the end of the trial all the animals in the 15.4 CP group and one each in the other two groups were conceived. There was no difference between the groups in the average number of days from calving to first AI, which was 122, 79.4 and 80, respectively in the three groups. In the second phase of the experiment, fourteen early lactating dairy cows within 10 days of their lactation were selected and grouped into two uniformly and fed with 13 per cent CP ration at two levels of protein degradability, 26.8 and 42.9 per cent of CP as undegradable protein (UDP). The experiment was conducted in a similar manner as in the first phase. At the end of the 100 day feeding trial, three animals in the 26.8 per cent UDP group and two animals in the 42.9 group were conceived. The average number of days from calving to first AI was 90.7 and 74.8 in the two groups respectively, which was non significant. Even though there was no significant influence either by the level or degradability of dietary protein on the onset of post partum heat in animals of the different dietary groups, it could be observed from the results that as the level of CP and degradable protein increases in the ration, the days to first observed post partum heat also tend to increase.

1 & 3- Associate Professor and Professor, Department of Animal Nutrition, CV&AS, Pookot. 2. Associate Professor, Department of Animal Nutrition, CV&AS, Mannuthy

## 17. FATTY LIVER KIDNEY SPLEEN AND HEART SYNDROME IN LAYER CHICKEN

**Balachandar J.<sup>1</sup>, Lonkar V.D.<sup>2</sup>, Ramesh Kumar<sup>3</sup> Ajay Kumar<sup>4</sup>**  
College of Veterinary and Animal Sciences, Pookot

Fatty Liver Kidney Spleen and Heart Syndrome (FLKSHS) is a syndrome reported in 32 weeks age old layer chicken. The evidence regarding the FLKSHS is scanty in layer chicken. FLKSHS arise from failure of hepatic gluconeogenesis and results in extensive fatty infiltration of body tissues with enlarged liver, kidney, spleen and heart. The affected bird was lethargic, aphagic and recumbent. The post mortem findings were enlarged pale liver, kidney, spleen and heart but inflammatory or degenerative changes were not reported. The paler of the liver and kidney may due to presence of excessive amount of fat (2-5 times than normal). FLKSHS condition may involve an interaction of nutritional (biotin, choline, fat and protein), environmental (stress), starvation, and metabolic factors.

**Key words :** FLKSHS, Layer Chicken.

1&2 Teaching Assistants, Dept. of L.P.M., COVAS, Pookot, 3 Teaching Assistant, Dept of Pathology, COVAS, Pookot, 4 Fourth year, B.V. Sc, COVAS, Pookot



# Best for less! Small, fast and flexible

## Eppendorf PCR package



Application & Technical Support



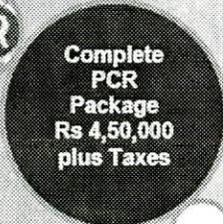
Mastercycler<sup>®</sup> Personal



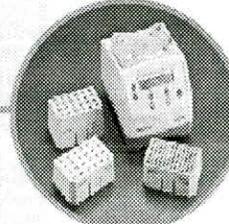
epTIPS



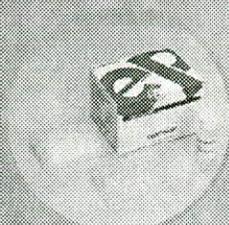
Research<sup>®</sup> Pipette



Complete PCR Package  
Rs 4,50,000 plus Taxes



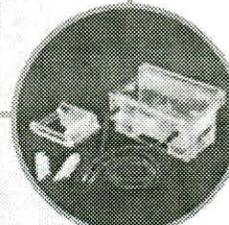
Mixmate With Adapters



Reagents



UV Transilluminator



Electrophoresis Kit



Eppendorf India Ltd. • Doshi Towers, 4<sup>th</sup> Floor • 156, Poonamallee High Road • Kilpauk • Chennai • 600 010  
Tel. 044 42111314 • Fax 044 42187405 • E-Mail: info@eppendorf.co.in • Homepage: www.eppendorf.co.in



## WITH BEST COMPLIMENTS

From



LABMATE (ASIA) PVT. LTD

YOUR TRUSTED PARTNER  
FOR

ALL LIFE SCIENCES RESEARCH INSTRUMENTS & CONSUMABLES

Instruments Division	Drug Discovery & Frontier Tech Division	Biologicals Division
<ul style="list-style-type: none"><li>❖ Luminometers</li><li>❖ Electroporation / Fusion &amp; Embryo Manipulation</li><li>❖ Gel Documentation</li><li>❖ Automated Liquid Handling System</li><li>❖ Laboratory Microscopes</li><li>❖ Freeze Dryer</li><li>❖ Cell Disruption System</li><li>❖ Automated Media Preparation &amp; Cell Culture</li><li>❖ Measurement of Dissolved CO<sub>2</sub> / O<sub>2</sub></li><li>❖ Autoclaves, Centrifuges, Fermentors, UV, Spectrometers, HPLC &amp; PCR</li></ul>	<ul style="list-style-type: none"><li>❖ Cell Morphology Bio Sensor</li><li>❖ In-Vivo &amp; Molecular Imaging</li><li>❖ MALDI-TOF, MALDI TOF/TOF, LC-MS/MS, ESI-TOF, FT-MS</li><li>❖ High Content Screening</li><li>❖ Flow Cytometers</li><li>❖ Ion Channel Analyzer</li><li>❖ Microarray &amp; Colony Picking</li><li>❖ Capillary Electrophoresis</li><li>❖ Surface Plasmon Resonance Analyzer</li><li>❖ SNP / Mutation Detection System (dHPLC)</li></ul>	<ul style="list-style-type: none"><li>❖ Molecular Biology Enzymes</li><li>❖ Antibodies against intermediates for cell signaling pathways</li><li>❖ Polymerase Enzymes, dNTPs</li><li>❖ Magnetically activated Cell Sorters</li><li>❖ Protein Manual Microarray, Blotting Membranes &amp; Devices for DNA, RNA &amp; Proteins, Electroporation Cuvettes</li></ul>

No.183 Mount Road, Chennai -600 015

Ph : 044 2220 0066 / 2220 0166

Fax : 044 2220 0056 / 2220 0156

e-mail : [labmate@vsnl.com](mailto:labmate@vsnl.com)

• Bangalore • Chandigarh • Hyderabad • Kolkata • Lucknow • Mumbai • New Delhi

web : [www.labmateasia.com](http://www.labmateasia.com)



## *Best Compliments from*

# SCIENTIFIC ENTERPRISES

Scientific Enterprises is a multi product specialist operating with emphasis on higher education and research institutions. We have growing from strength to strength over the years to be largest in this segment in Kerala.

Starting from manufacturing and stretching up to long term after sales service support, Scientific as a group has earned a name of being the most reliable support to all the educational and research institutions. More and more National & Internationals brands are looking upon us to take their products to the ultimate customers which consequently will bring in more quality and cost effective products to this market. We are in the process of ISO 9001- 2000 certification.

After sales service support is the forte of our organization and continuous effort are on to upgrade this activity.

We represent a host of manufactures, some name are as given below.

- |                     |   |
|---------------------|---|
| 1. Eutech Singapore | - Analytical Instruments like pH meters, D.O meters etc.    |
| 2. Millipore India  | - Water Purification Systems                                |
| 3. Barnstead USA    | - Deep Freezers, Incubator Shakers, High temp furnaces etc. |
| 4. Cole Parmer USA  | - Flurometers, Weather stations, Circulating baths etc      |
| 5. Sartorius        | - Electronic balances, Fermentors etc.                      |
| 6. Rudolph USA      | - Polarimeters  |
| 7. Atago Japan      | - Polarimeters  |
| 8. Olympus          | - Microscopes   |
| 9. Remi             | - Centrifuges   |
| 10. Scientech       | - CRO's, Function, Generators, Communication trainers etc   |
| 11. Meco            | - Portable Meters, Multimeters, Wattmeters etc.             |
| 12. Labline         | - Incubators, Ovens, Water baths, shakers etc.              |
| 13. Scientific Tech | - Power supplies, IC trainers , uP kits etc.                |
| 14. Mechatronics    | - VLSI Trainers, Programmers etc                            |
| 15. ADM             | - Logic Analysers, In circuit Emulators etc.                |
| 16. Transasia       | - Semi auto analysers, Micropipettes etc.                   |

## SCIENTIFIC ENTERPRISES

P.B. NO: 1951, VYTTILLA JUNCTION, NH BYEPASS, COCHIN – 682 019

PH. NO: 0484 – 2306461, FAX NO: 0484-2306954

E MAIL: [secochin@dataone.in](mailto:secochin@dataone.in), [seservice@asianetindia.com](mailto:seservice@asianetindia.com),

[seorder@airtelbroadband.in](mailto:seorder@airtelbroadband.in)



**Klenzaid's**

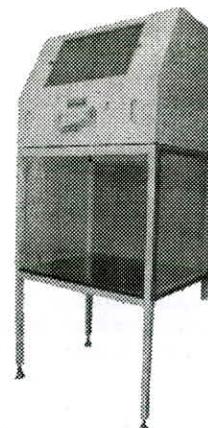
## ASEPTIC PROCESSING ZONES, MACHINERY & DEVICES

### DEVICES FOR ASEPTICS PROCESSING ZONES

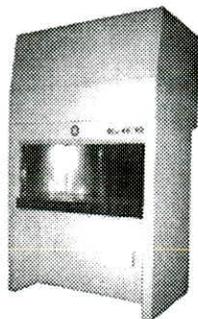
Clean air work stations, evaluative in configuration, and embodying a multitude of User-Oriented design elements and concepts at unrivalled cost-benefit equation. Made possible by use of rationalized production techniques and unremitting attention to detail. Stringent pre-despatch and post-installation qualification backed by out decades of experience in the field.



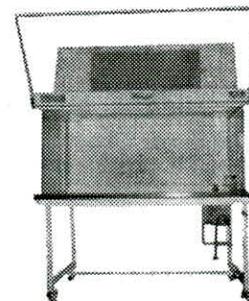
Out of consideration for several possible applications, each of a highly specialized nature, we have numerous optional features available that can be added or built into your systems at the time of manufacture or retrofitted at site at a later date, so that you may evolve or upgrade the configuration to meet your needs.



- Horizontal / Vertical - Laminar Flow Clean Air Work Stations
- Biological Safety Cabinets Type II / Type III
- Open Front Containment Stations



- Filtration Modules and Room Pressurization Systems
- Air Showers
- Pass Boxes - Static and Dynamic
- Chemical Work Stations,
- Fume Exhaust Hoods
- Garments Storage Cubicles, Hand Dryers
- Re-Circulatory Modules
- HEPA Filters, ULPA Filters.



### ***Klenzaid's Contamination Controls Pvt. Ltd.***

A-21 MIDC Industrial Area Street 3      New #13, Old E-159A VII Avenue,  
Andheri (E) Mumbai- 400 093      Besant Nagar, Chennai-600 090.  
Voice : 8218921 (10 Lines), 8323113      Voice: 91 44 244467863 / 64  
Fax : 8377927      TeleFax: 91 44 24467863  
email: klenzbio@mtnl.net.in      email: klenzaidschennai@yahoo.com  
www.klenzaid's.com

with ***Klenzaid's*** you are safe



## INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY

### PRESIDENTIAL ADDRESS

**Dr. K.S. PALANISWAMI, MVSc, Ph.D**

Respected Chief Guest Sri. S.K. Bandhopadhyay, Animal Husbandry Commissioner, Government of India, New Delhi, revered Director of Animal Husbandry Government of Kerala, Respected Colleagues, Dr. M. Balakrishnan, Associate Dean of this prestigious institution and organizing secretary of this convention, Dr. T.J. Rasool, Assistant Director General, Animal production and Breeding, ICAR, dignitaries, honorable guests, scientists, excelling scientist, P.G. students, Ladies and Gentlemen.

I extend warm greetings to all of you on behalf of Indian Society for veterinary immunology and biotechnology. This society is more than 16 years old and was founded by illustrious persons like late professor – P. Richard Masillamony, Professor B.B. Mallik, Dr.M.C. Goel, Professor B.S. Kesavamoorthy, Dr.V. Subbarao, during a meeting at Thirupathi, 1990.

The main objective of the society is to motivate all the specialists in the field of Immunology and Biotechnology to discuss, analyze and formulate suitable strategies for safeguarding animal health and thus to ensure improved animal production and make biotechnology as a research tool in the advancement science in general. To achieve this, the society convenes annual conventions and during these conventions, National Symposia are also organized to critically analyze and update our knowledge and understanding on issues concerning with the immunology and gene based technologies in animal health and production.

The first annual convention of the society was held during 1993 at Bangalore and till now 12 conventions have been held with concurrent National Symposia and one international conference at Chennai with sponsorship from veterinary Institutions in that part of the country.

The decision to hold the 13<sup>th</sup> Annual Convention and National symposium at College of veterinary and animal sciences, Pookot, Wayanad, Kerala was taken in the G.B. Meeting held during the 12<sup>th</sup> convention held at Punjab Agricultural University, Ludhiana, in November 2005. This budding institution has acquired its infrastructure in the shortest possible time due to the energetic work of Dr.P.P. Balakrishnan and his team at this scenic location. The organizing committee under the energetic stewardship of Dr.P.P. Balakrishnan made us to be in the God's own country today. The dynamism of the team with Dr. K. Anil Kumar as joint organizing Secretary, has done splendid work in organizing this convention though challenges were many.

The society has 700 life members, and to initiate, encourage and promote research in veterinary immunology and biotechnology, this society has set up several awards to the deserving members. Dr. Richard Masillamony oration is awarded to honor, the excelling service. This is the highest honor bestowed by the society followed by conferment of Fellowship of Indian Society of veterinary Immunology and Biotechnology to a maximum of three members of the society on annual basis.

The society also sent recommendation after the annual convention to the respective state and National bodies for adoption as proactive approach. The important recommendation includes research and development of cost effective and field oriented specific diagnostic kits, novel vaccines are the thrust areas for new research programmes. It is my desire that this society should co-ordinate with National bodies for identification of laboratories, create a scoring system for the validation for the Diagnostic kits and vaccines and offer its services for periodical accreditation of the laboratories in gene based technologies in animal production, health and safe animal food.

At this day of importance to the society I propose to advice the galaxy of scientist gathered here to carry forward their ambitions taking into account of technology developed, technology to be managed and technology for application.

In respect of development of technology the role of scientist is to see the sky always. Their effect is endless. New laboratory tools, methods, conjecture of different thoughts, rapidity, reproducibility, dependability, cost effectiveness, transoundary technologies and their international acceptance are their lookout. Developed technologies should say by their effective applicability, retainment of the process or products have overall include efficiency is challenge. Molecular methods like reverse genetics in vaccinology, phage display in detecting food borne pathogens should overcome the traditional methods, which is time consuming and cost expensive.

In the Management of Technology, better management system in process, product, marketing and service technology are the requirement of the day, as there are abundance in technology developments. If technology can be managed by technology forecasting, its absorption, quality assessment, its diffusion and final application, several organizations have to work together as a team by focusing on either one or several of this aspects. Different tools in this aspect could be used to see the end or abandon the out dated technology and to provide in science into possible industry adoption and customer validation of the new technology. Effective technology management results in reducing the cost policy changes and improved efficiency. Information technology to process, record and display collated date with power of digital computer is the prime driving force.

In technology application the immunologist play a major role in disease prophylaxis, their concentration is a new vaccine development and their application to create immunity or ability of the animal to fight the diseases. The process of immunization is also governed by several factors in the host and its environment in the best and its environment like nutritio, biosecurity, management, immuno suppressive parasites, mycotoxins and the stress. Hence a biotechnologist or immunologist is expected to collate inter disciplinary data to evaluate his inventions before field trails.

The theme of the conference is biotechnology and immunology for sustainable, rural livelihood security. For this purpose your technology should to simpler to adopt at rural level. The substance in animal production for example would be in concentration in areas of human biotechnology. This is to enhance growth and improved yield with low feed cost. The feed convention ratio in ruminants is not exactly calculated as in poultry sector, which is an industry. Hence rural application should be focused to dairy cattle for milk production, sheep and goats for improved growth. In the area of animal health, the threatening issue is FMD in ruminants. The early diagnosis of specific and NM specific disease in diary cattle and sheep and goat and their prophylaxis system should be based on novel biotechnology.

These are not simple but a challenging job. There may be failed attempts, it should be viewed as stepping-stones and success is inevitable for champions. They know let the best way to forecast the future is to create it. I wish you all to be champions. There will be competition to be a best champion but the competition may be a healthy one and team spirit.

Thanking you,

**Dr. K.S. PALANISWAMI**

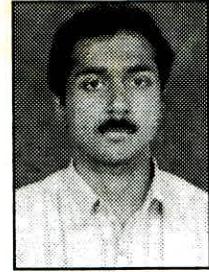


### **ISVIB- OFFICE BEARERS – OUTGOING (2004)**

- President : Dr. K.S. Palaniswamy, Director of Research, i/c., Retd. TANUVAS
- Vice-President : Dr. Alka Tomar, Senior Scientist, IVRI, Izat Nagar, U.P. 243 122.  
Dr. Probooth Borah, Associate Professor, Dept. of Microbiology,  
College of Vet. Sci, Guwahati –781 022, Assam
- Secretary : Dr. V. Ramaswamy, Professor Dept, of Animal Biotechnology, MVC. Chennai-600 007.
- Joint Secretaries (2) : Dr. B.R. Shome, Senior Scientist, ICAR Research, Complex, Meghalaya – 703 103  
Dr. M.D. Venkatesha, Scientist 3 (Research) IAH & VB, Hebbal, Bangalore-24 , Karnataka
- Treasurer : Dr.G. Dhinakar Raj, Associate Professor, Dept of Animal Biotechnology, MVC. Chennai-7
- Executive Committee  
Members (6) : Dr. Shakil Ahmad Wani, Division of Microbiology and Immunology, SKUAS, Shudhama,  
Srinagar I 90 006, J & K  
Dr. A. Palanisamy, Associate Professor, Dept of Animal Biotechnology, MVC, Chennai-7.  
Dr. T.G. Prabhakar, Associate Professor, Dept of Microbiology, MVC, Chennai-7.  
Dr. A. Thangavelu, Associate Professor, Dept. of Microbiology, MVC, Chennai-7.  
Dr. A. Raja, Assistant Professor, Dept of Animal Biotechnology, MVC, Chennai-7.  
Dr. M.Magesh, Assistant Professor, Dept of Microbiology, MVC, Chennai-7.

### **ISVIB- OFFICER BEARERS – NEW (2006)**

- President : Dr. M.V. Subba Rao, (Ex-Dean), B/2, Sr. Staff Quarters, ANGRAU, Campus, Rajendra Nagar,  
Hyderabad- 500 030 (A.P.)
- Vice-President : Dr. V. Ramawamy, Dean, Faculty of Basic Sciences, Madras Veterinary College, Chennai-600 007.  
Dr. Raj Kumar Singh, Head Division of Virology, IVRI, Mukteswar, U.P.
- Secretary : Dr. K.S. Palaniswamy , Ex Director of Research, TANUVAS, W-145, Anna Nagar, West Etn.  
Chennai-600 101.
- Joint Secretary : Dr. V. Purushothaman, Professor and Head, Dept of Microbiology, Madras Veterinary college  
Chennai-600 007  
Dr. P.N. Dwivedi, Associate Professor, Dept of Microbiology, COVS  
GADVASU, Ludhiana, Punjab.
- Treasurer : Dr. A. Thangavelu, Associate Professor, Dept. of Microbiology, MVC, Chennai-600 007.
- Executive Committee  
Members : Dr. Mandeep Sharma, Head , Dept. of Microbiology, COVAS, CSKIIPKV, Palampur, Himachala  
Pradesh.  
Dr. Ashok K. Tiwari, Division of Veterinary, Biotechnology, IVRI, Izat Nagar, Uttar Pradesh.  
Dr. Narinder Singh Sharma, Dept of Microbiology, COVAS, CADVASU, Ludhiana, Punjab.  
Dr. G. Dhinakar Raj, Associate Professor, Dept of Animal Biotechnology, MVC, Chennai-600 007.  
Dr. Parimal Roy, Associate Professor, Dept of Animal Biotechnology, MVC, Chennai.600 007.  
Dr. S. Manoharan, Assistant Professor, Dept of Animal Biotechnology, Madras Veterinary College,  
Chennai-600 007.



## *Expression of gratitude*

*It is a unique opportunity to be associated with the conduct of the XIII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology and National Symposium on "Veterinary Biotechnology and Immunology for Sustainable Rural Livelihood Security" at College of Veterinary and Animal sciences, Pookot and a proud privilege to bring out a compendium on the occasion. Efforts have been taken to prepare the compendium in a befitting manner. I am indebted to Dr. P. T. Philomina, the Chairperson and Dr. Renuka Nayar, Joint Convener of the Technical Sessions and Publication committee for their timely help and cooperation. I also sincerely thank all the other committee members and colleagues for helping me in the preparation of the compendium. I also extend my special thanks to*

- 1) Dr. P.P. Balakrishnan for his encouragement
- 2) Dr. K. Anilkumar for his support
- 3) Dr. Devada for her valuable suggestions
- 4) Dr. Reghu Ravindran for his advice
- 5) Dr. G. Radhika & Dr. Shynu for their help
- 6) Dr. Sanis Juliet for arranging for the advertisements
- 7) Dr. Reeja George for her contribution for the compendium
- 8) All scientists who have sent papers/abstracts for the symposium
- 9) All firms who have given advertisements in the compendium
- 10) Mr. Shivan, Cyber Net, Kalpetta, for setting the layout of the compendium
- 11) M/s Chinnu's Graphics, Kozhikode for their timely printing of the compendium.

Pookot  
12-2-2007

*Dr. Chintu Ravishankar*

Editor of Compendium &  
Convener,

Technical Sessions & Publication Committee.





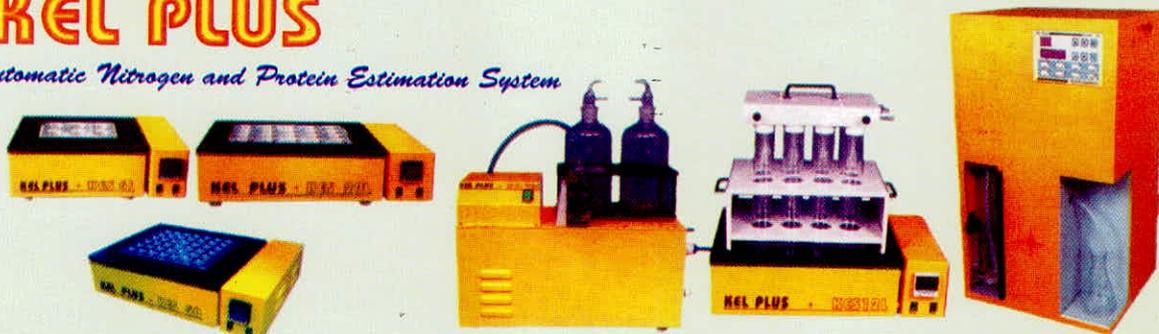
# PELICAN EQUIPMENTS



## Equipments For Agricultural Research

### KEL PLUS

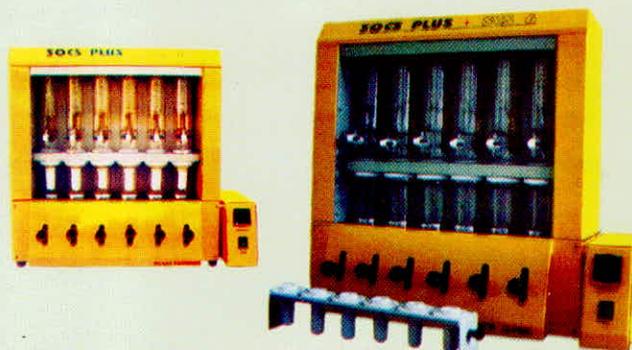
*Automatic Nitrogen and Protein Estimation System*



- KEL PLUS The Brand You Cannot Ignore.
- KEL PLUS The Technology that travels with time.

### SOCS PLUS

*Automatic Solvent Extraction System*

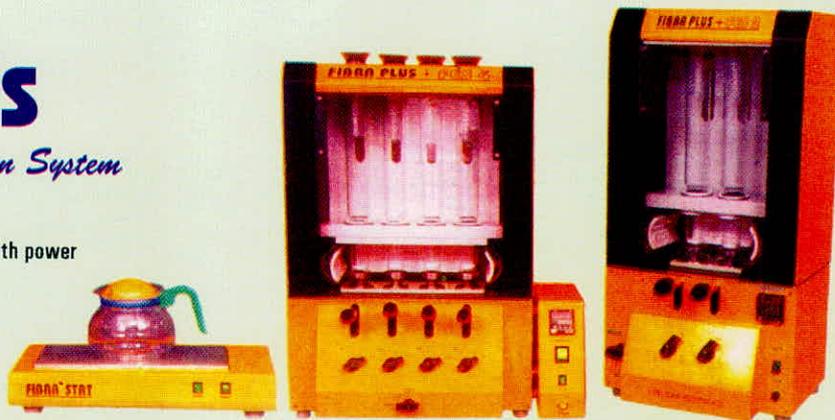


- SOCS PLUS Truly Indian. Designed for India.
- SOCS PLUS Expression of Creative Genius.

### FIBRA PLUS

*Automatic Fibre Estimation System*

- FIBRA PLUS world class Standards with power For lasting performance.



### Why PELICAN ?

- ☞ Zeal for Quality & Perfection
- ☞ Technical & Application Expertise
- ☞ Untiring Support to valued customers
- ☞ Dedicated team of Engineers and Analytical Chemists
- ☞ Excellence in After Sales Service
- ☞ Customer Satisfaction measurement & monitoring
- ☞ Quest for continuous In-house training & skill development
- ☞ ISO 9001:2000 Certified product.
- ☞ On going Research & Development Programmes to keep pace with latest technology.

No.38, II Floor, Burkit Road, T. Nagar, Chennai - 600 017. Phone : 91 - 44 - 24341830 / 24347554 Telefax: 91 - 44 - 24313738 / 26532050  
 E-mail : pelican equip@hotmail.com, pelicanequipments@vsnl.net

WITH BEST COMPLIMENTS FROM



**WORLD LEADER**

**THE MOST TRUSTED NAME IN  
ARTIFICIAL INSEMINATION & EMBRYO  
TRANSFER INSTRUMENTS,  
CONSUMABLES AND TECHNIQUES**



IN INDIA SINCE 1993

*Please contact for more information:*

**IMV INDIA PRIVATE LIMITED**

(A fully-owned subsidiary of IMV Technologies, S.A. France)

**Plot No. 747, Phase-V,  
Udyog Vihar, Gurgaon - 122 016  
Haryana (India)**

**Ph.: 0124-4004430 to 31 Fax : 0124-4002172**

e-mail : [contact@imvindia.com](mailto:contact@imvindia.com)

Web Site: [www.imv-technologies.com](http://www.imv-technologies.com)