



**XXIV<sup>th</sup> Annual Convention and National Conference of Indian Society of Veterinary Immunology and Biotechnology & One Day Satellite Seminar for Veterinary Officers**

**On**

**Recent Trends in Veterinary Immunology and Biotechnology for Doubling Farmers' Income through Livestock Health and Production**

**5<sup>th</sup> to 7<sup>th</sup> December 2017**



**पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी**



**Organized by**

**College of Veterinary and Animal Sciences**

Maharashtra Animal and Fishery Sciences University, Nagpur

**Parbhani- 431 402 (M.S.) INDIA**

[www.mafsu.in](http://www.mafsu.in), [www.covaspbni.in](http://www.covaspbni.in)

**Compendium cum Souvenir**

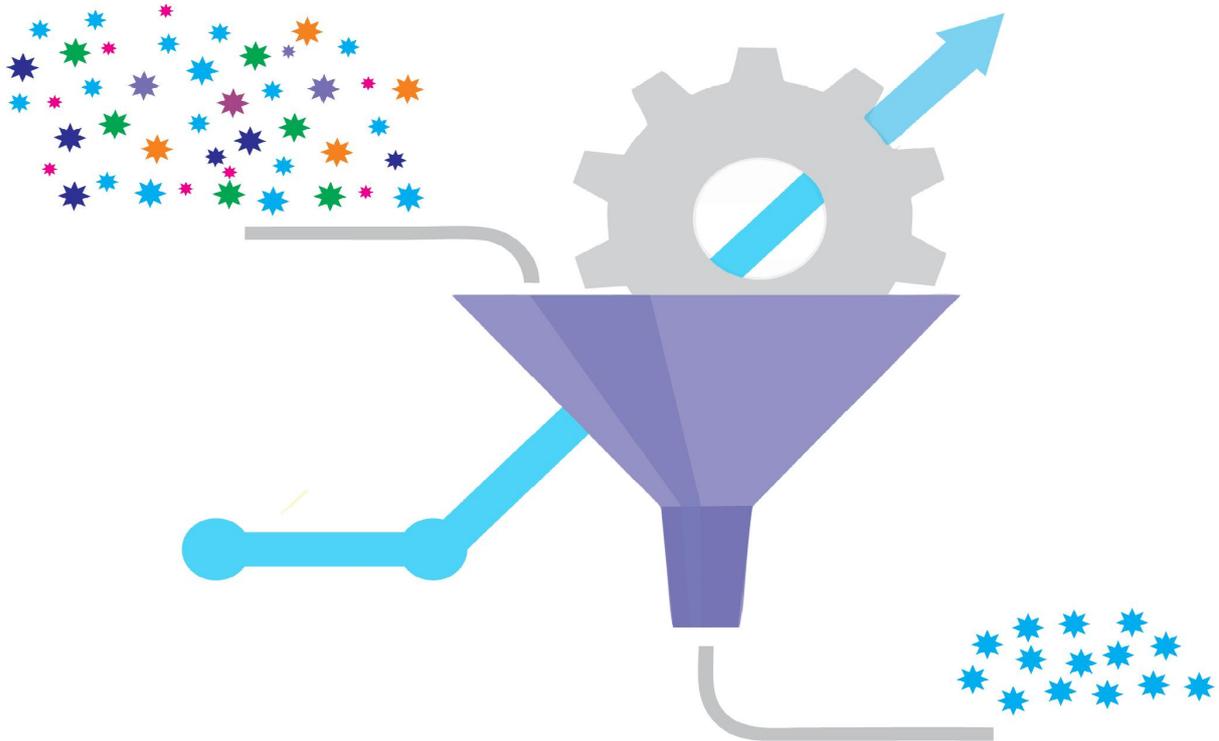
**VIBCON - 2017**

# MASTER CLONE

(Newcastle Disease Vaccine, Live)



**Protection Redefined**



-  **Uniformity in titers (Virus and Antibody response)**
-  **Less vaccinal reaction**
-  **Better immunogenicity**

**VENTRI BIOLOGICALS**

(Vaccine Division of VHPL)

'Venkateshwara House', S. No. 114/A/2, Pune Sinhad Road, Pune 411030. Tel.: +91 9021151180 Fax: +91-20-24251060 / 24251077.



Email: [ventri.biologicals@venkys.com](mailto:ventri.biologicals@venkys.com)

[www.venkys.com](http://www.venkys.com)



INVEST IN HEALTH, REAP REWARDS FOR LIFE!

# ENHANCING HEALTH OF NEW LIVES!



We have been serving Animal Health Industry since years with vaccines for poultry to enrich the lives of chicks and secure them from life threatening diseases. Now, with the same passion, we are expanding our health boundary to livestock and aqua segments. The innovation driven research allows us to deliver world class vaccines and animal health products.

# COMFORT

A complete Bedding Solution for Poultry



Poultry Industry especially Broiler Farming is facing the issues of increased respiratory disorders along with Gut health issues leading to immuno suppression and heavy losses due to N.D., VVND, Air saculitis, ILT, CRD, Coryza etc.

From various managemental techniques, antibiotics, herbal preparations, Phytogetic compounds have been tried with little success. The problem continuos with heavy losses with uncertainty in the recovery.

Temperature and humidity variation, increased oxygen demand due to heavy stocking, vaccination stress are few of the culprits for this disorder.

Increased ammonia levels in the farm adds to all these problems and / or the birds grown in Ammonia free farms have noticed very less problems as enlisted above.

### Ammonia Facts

Level	Effect
10 ppm	Trachea Irritation starts
20 ppm	Increases possibility of ND, VVND and vaccines failures
25 ppm	Air sac Inflammation, mortality, occupational hazzards
30 ppm	Air quality - less oxygen - ascitis - heart attacks

### Birds Welfareness

Food PAD lesions - hock joint injuries - Infiltrates Microbia / bacteria leading to lameness.

At VRK we have successfully reduced Ammonia level by using special Bedding Material "COMFORT".

"COMFORT" is a corn cob bedding material treated with organic acids to reduce the PH of the bedding material.

Normally uric acid in fecal matter of birds converts into ammonia at PH 7. We have decreased the PH level of this bedding material to less than 5 which can be easily tested by PH paper.

"COMFORT" stops production of ammonia.

The rice husk is normally used by farmer has very less water holding and water absorption capacity, normally it absorbs equal amount of water. However "COMFORT" can hold / absorb water 4 times of its weight keeping moisture level very very low. Beside this the density of "COMFORT" is about 0.4 as against 0.5 of rice husk and it depends upon how much sand / ash it has.

Pleasant odor, low density, less moisture and PH less than 5 in "COMFORT" stops ammonia production.

Decreased possibility of microbial / bacterial growth helps birds to be comfort and convenient to grow faster. Food pad lesions and hock joint injuries are very very negligible helping in reducing gait score. Precise targeting by reducing ammonia level in the shed not only enhances performance but also helps in keeping animal activists (bird welfareness) away from you.



COMFORT Pellet



COMFORT Mash

### Rice Husk



1st Week

### COMFORT



1st Week

### Hock joint injuries



COMFORT

Rice husk



6th Week



6thWeek



COMFORT

Rice husk



**VRK Nutritional Solutions**

ISO 9001:2008 Certified Company

Correspondence Address : 202,Ganga Collidium Phase - 1, Gangadham Phase - I , Bibwewadi - Kondhwa Road, Pune- 411 037. Maharashtra, India.

Tel/Fax : 020-24241169 Mobile : 98814 76116 E-mail : vrkgroup2009@gmail.com

G-1, Trimurti Archade, Opp. Zilla Parishad, Sangli - 416 416 Maharashtra

Web : [www.vrknutritionalsolutions.com](http://www.vrknutritionalsolutions.com)

**CH. Vidyasagar Rao**  
**GOVERNOR OF MAHARASHTRA**



**RAJ BHAVAN**  
**Malbar Hill**  
**Mumbai 400 035**  
Tel. 022-2363 2660  
Fax. 022-2368 0505

**13 November 2017**

## MESSAGE

I am pleased to know that the College of Veterinary and Animal Sciences, Parbhani is organizing the XXIV National Symposium of the Indian Society for Veterinary Immunology and Biotechnology on the theme "Recent Trends in Veterinary Immunology and Biotechnology for doubling farmers' income through Livestock health and Production during December 5-7, 2017. I am further pleased to know that a One Day Satellite Seminar for Veterinary Officers is being organised on 7<sup>th</sup> December 2017.

The role of biotechnological approaches in providing cost effective strategies for the improvement in animal health needs to be explored. It is hoped that this symposium will provide the necessary platform for scientists to deliberate on the challenges faced by the farmers to improve the production performance of the animals.

I congratulate the College of Veterinary and Animal Sciences, Parbhani for its initiative in hosting the Symposium and Satellite Seminar and convey my best wishes for the success of the events.

**(CH.Vidyasagar Rao)**



**MINISTER  
ANIMAL HUSBANDRY  
DAIRY DEVELOPMENT AND  
FISHERIES DEPARTMENT**

MAHARASHTRA STATE  
Mantralaya, Mumbai 400 032  
[www.maharashtra.gov.in](http://www.maharashtra.gov.in)

## **MESSAGE**

It gives me immense pleasure to know that College of Veterinary and Animal Science, Parbhani is organizing XXV Convention and National conference of Indian Society of Veterinary Immunology and Biotechnology and one day satellite Seminar for Veterinary Officers from 5<sup>th</sup> to 7<sup>th</sup> December, 2017.

The theme of this conference 'Biotechnology for doubling the farmers income through livestock health and production' is very much pertinent to the present need of the livestock owners and will prove highly beneficial in the coming years. Livestock, Poultry and Dairy are the promising and proven sectors of strengthening national economy. To keep these sectors progressing many farmers oriented schemes are sponsored by State and Central Government. Maharashtra Animal and Fishery Sciences University can play crucial role in development of excellent human resource, rapid disease diagnostic facilities and development of field based cost effective technologies. I am sure the deliberation in symposium will come out with fruitful conclusions and recommendation related to improvement of animal health and productivity.

The one day satellite seminar for field veterinarians will surely be helpful to them for upgradation of their knowledge and to the scientists for identifying the region based livestock disease which needs to be addressed through biotechnological research.

I extend my best wishes to the organizers of VIBCON, 2017

**(Mahadev Jankar)**



सत्यमेव जयते

**Dr. Suresh S. Honnappagol**  
Animal Husbandry Commissioner



भारत सरकार  
कृषि एवं किसान कल्याण मंत्रालय  
पशुपालन, डेयरी और मत्स्यपालन विभाग  
कृषि भवन, नई दिल्ली-११० ००१

GOVERNMENT OF INDIA  
MINISTRU OF AGRICULTURE & FARMERS WELFARE  
Department of Animal Husbandry, Dairying & Fisheries  
Krishi Bhawan, New Delhi – 110 001

## MESSAGE

I am delighted to learn that College of Veterinary and Animal Sciences, Parbhani of MAFSU is organizing XXIV Annual Convention and national conference of Indian Society of Veterinary Immunology and Biotechnology and one day satellite Seminar for Veterinary Officers.

Indian economy is basically an agrarian based and growth in agriculture will commensurate with growth of country's economy. As India happens to be the fastest growing economy in the world, Animal husbandry is expected to play an important role in achieving the desired goal. Animal husbandry has proved beyond doubt to be an effective tool in doubling farmer's income. India is among the top twelve biotechnology destinations in the world and ranks third in the Asia Pacific. Indian biotech industry shall touch USD 100 billion by 2025.

In view of the above facts, the deliberations at this annual convention will be of immense importance. I call all the participating scientists to work towards easy and rapid diagnostic tools development which will help in preventing animal diseases in near future.

I wish the VIBCON-2017 a grand success and compliment the organizers.

  
(Suresh S. Honnappagol)



# महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ

फुटाळा तलाव मार्ग, नागपूर - ४४०००१

**MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY**

Futala Lake Road, Nagpur - 440001, Maharashtra (India)

Phone: 91 712 2511088, Fax: 91 712 251182 Email: vcmafsu@gmail.com, Web: www.mafsu.in

**Shri. Anoop Kumar, I.A.S.**

Vice Chancellor

Divisional Commissioner

Nagpur Division



## MESSAGE

It gives me immense pleasure to know that College of Veterinary and Animal Sciences, Parbhani is organizing XXIV Annual Convention and National Conference of Indian Society of Veterinary Immunology and Biotechnology and One Day Satellite Seminar for Veterinary Officers from 5<sup>th</sup> to 7<sup>th</sup> December, 2017.

Biotechnology is one of the rapidly progressing sciences and is likely to be applicable to every sphere of animal and human health. Diseases of animals pose a serious threat to their health and productivity thereby leading to heavy economic losses. Biotechnology offers an effective tool in rapid and accurate diagnosis which ultimately will be highly beneficial to livestock owners. I appreciate the organization of satellite seminar for interaction with field veterinarians. This discussion will surely help the scientists in identifying regional issues which needs to be addressed through research. Maharashtra Animal and Fishery Sciences University has been striving hard for establishing liaison with field veterinarians through continuing education programme and trainings, the organization of this seminar will be a forward step in this direction.

I am sure that this symposium will provide a window for exchange of views and ideas for early disease diagnosis, newer approaches for their control and prevention and thereby increase in animal productivity resulting in doubling of farmer's income. It is learnt that scientists across the country will be participating in this august scientific gathering. I am confident that scientific deliberations of VIBCON 2017 will come up with fruitful recommendations for long term benefits.

I wish the VIBCON 2017 a grand success.

*Anoop Kumar*  
(Anoop Kumar)



# आईसीएआर-भारतीय पशु चिकित्सा अनुसंधान संस्थान (सम-विश्वविद्यालय)

इज्जतनगर - २४३ १२२ (उ.प्र.) भारत

**ICAR-INDIAN VETERINARY RESEARCH INSTITUTE**  
(Deemed University)

Izatnagar - 243 122 (U.P.) INDIA



डॉ. आर. के. सिंह  
**Dr. R.K. Singh**  
निदेशक एवं कुलपति  
**Director & Vice-Chancellor**



## MESSAGE

The Golden dream of our Hon'ble Minister to double the income of farmers by the year 2022 encompasses a holistic view to strengthen the Indian farming as an integrated enter[r]ise. Livestock farming, one of the key components of Indian farming with a significant contribution of 25.6 % of the value of agriculture output holds the key to achieve this feat in realistic terms. In this context, biotechnology is offering unprecedented opportunities for increasing animal productivity and health. The rapid development of modern biotechnology has introduced a new dimension to animal disease diagnostics, veterinary medicine and animal breeding. The innovative approaches and techniques of modern biotechnology, inter alia, gene(s) cloning and therapy; genetic manipulation of animals, embryos and rumen microbes; value addition to low quality animal feeds for improved nutritive value and, genetically engineered immunodiagnostic and immunoprophylactic agents as well as innovative and thermally stable veterinary vaccines are finding their ways into research and development programmes of developing countries.

It gives me immense pleasure to learn that the College of Veterinary and Animal Sciences, Parbhani repute is organizing XXIV National Symposium of the Indian Society for Veterinary Immunology and Biotechnology (ISVIB) on a very pertinent and contemporary theme – "Recent Trends in Veterinary Immunology and Biotechnology for doubling farmers' income through Livestock Health and Production" from Dec. 5-7, 2017 and One day Satellite Seminar for the benefit of Veterinary Officers on 7<sup>th</sup> December, 2017. I am quite hopeful that the endeavours of this nature will not only go a long way to benefit the veterinary professionals, extension education experts and other stakeholders in animal sciences by imparting the knowledge and skills in the fast evolving domains of biotechnology techniques and applications related to animal husbandry and livestock health, but also open up new prospects for addressing important regional and national issues pertaining to farmers' welfare.

I congratulate the dedicated organizers for taking this initiative as part of our collective professional commitment and also convey my best wish for the successful organization of this important event.

  
(R.K. Singh)



संचालक (शिक्षण) व अधिष्ठाता (पशुवैद्यक व पशुविज्ञान विद्याशाखा) यांचे कार्यालय  
DIRECTORATE (INSTRUCTIONS) & OFFICE OF THE DEAN, FACULTY OF VETERINARY & ANIMAL SCIENCES

# महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ

फुटाळा तलाव मार्ग, नागपूर - ४४०००१

**MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY**

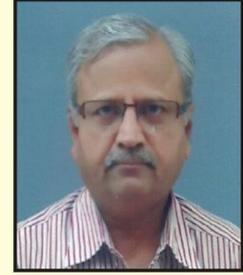
Futala Lake Road, Nagpur - 440001, Maharashtra (India)

Ph (O) : 91712-2053707, Web: www.mafsu.in email : didvmafsu@gmail.com

डॉ. अ. स. बन्नाळीकर, एम. व्ही. एस. सी. पी. एच. डी.  
संचालक (शिक्षण) व अधिष्ठाता (पशुवैद्यक व पशुविज्ञान विद्याशाखा)

**Dr. A.S. Bannaliker**, M.V.Sc., Ph.D.

Director (Instruction) & Dean (Faculty of Veterinary & Animal Sciences)



## MESSAGE

It is matter of pride and privilege that the College of Veterinary and Animal Sciences, Parbhani is organizing XXIV Annual Convention and National Conference of Indian Society of Veterinary Immunology and Biotechnology and one day satellite Seminar for Veterinary Officers from 5<sup>th</sup> to 7<sup>th</sup> December, 2017.

The Biotechnology has emerged as one of the frontier areas scientific development in the world today. Advances in the field of Biotechnology have catered to the wide range of needs of mankind including Agriculture, Animal Sciences, Environmental Science, Food Science, Medicine etc. The theme chosen by the organizers for the conference on "Recent trends in Veterinary Immunology and Biotechnology for doubling farmer's income through livestock health and production" is extremely relevant since the biotechnological applications of the recent years have proved to be powerful tool in improving the productivity of the livestock. The conference would provide a wonderful platform to the researchers for sharing their ideas and innovations in Biotechnology. The Maharashtra Animal and Fishery Sciences University since its inception has been promoting education and research in the field of Animal Biotechnology. I am sure that the deliberations at the National Convention and Seminar will come out with fruitful recommendations for the benefit of livestock farmers.

I wish the VIBCON-2017 grand success.

(A.S. Bannaliker)

Office of the Directorate, Extension Education & Training  
**Maharashtra Animal and Fishery Sciences University**  
Futala Lake Road, Nagpur



**Dr. A.P. Somkuwar**

Director  
Extension Education & Training

Ph.No. (O) : 0712-2040019  
Fax No. : 0712-2511282/2511283  
E-mail : mafsudet@yahoo.co.in  
Website : www.mafsu.in



## MESSAGE

College of Veterinary and Animal Sciences, Parbhani is organizing XXIV Annual Convention and National Conference of Indian Society of Veterinary Immunology and Biotechnology and one day satellite Seminar for Veterinary Officers from 5th to 7th December, 2017.

The theme for the convention is "Recent trends in Veterinary Immunology and Biotechnology for doubling farmers' income through livestock health and production". The convention will be attended by the scientists and field veterinarians.

Maharashtra Animal and Fishery Sciences University is working to address farmers' issues through effective extension since its inception. I believe that bringing major stakeholders on same platform will bring out unique solutions to problems of farmers and industry.

I am confident that, scientists in the area of Veterinary Science will focus their research on developing user friendly tools for disease diagnosis. I believe that the deliberations in the conference will be of immense useful for all the stakeholders.

I wish the VIBCON-2017 a grand success.

**A.P. Somkuwar**

**Government of Maharashtra**

**Dr. D.M. Chavan**  
**Additional Commissioner**  
**Commissionerate of Animal Husbandry,**  
**Pune**



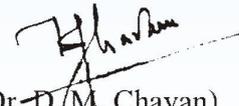
**MESSAGE**

I am pleased to know that College of Veterinary and Animal Sciences, Parbhani is organizing XXIV Annual Convention and national conference of Indian Society of Veterinary Immunology and Biotechnology and one day satellite Seminar for Veterinary Officers .

It is now a well known fact that much needed support to farmers can only be achieved by scientific practices in animal husbandry. In recent years livestock owners have adopted for highly productive breeds of cattle, sheep and goats. Further the livestock owners in the state of Maharashtra are enthusiastic in adopting to the modern day practices. The Animal Husbandry department is striving hard to make the technology available at doorsteps of the livestock owners. The modern trends in management and the high cost of animals demands for a strong diagnostic support and user friendly preventive tools such as vaccines and vaccination procedures. Application of biotechnology is need of the hour and is expected to develop simpler diagnostic tools and advancement in vaccine production.

I am happy to note that a satellite seminar for interaction with field veterinarians is also being organized. This seminar, I am sure will provide a platform to be shared by scientists and field veterinarians, which will be of mutual benefit. I am sure that scientific deliberations of VIBCON 2017 will come up with conclusions which will provide long lasting solutions for field vets and farmers.

I wish the VIBCON-2017 grand success.



(Dr. D.M. Chavan)  
Additional Commissioner

Commissionerate of Animal Husbandry, Pune

Place : Pune  
Date : 28/11/2017



# College of Veterinary and Animal Sciences

Maharashtra Animal and Fishery Sciences University

PARBHANI - 431 402 (MAHARASHTRA)



Ph. (02452) 233375 (02452) 22817'6, Fax (02452) 226188, Email - vetcp@rediffmail.com

**Dr. (Mrs.) S.B. Majee**

## Foreword

**Associate Dean  
and  
Organizing Secretary  
VIBCON-2017**



I feel privileged to be associated once again with ISVIB after a long gap of more than thirty years when in 1990 the society was first established with Dr. B.B. Mallick, former Director, IVRI, Dr. Subbarao, Dr. V.D. Padmanaban as its founder members and I was invited as rapporteur for its first conference at the Veterinary College, Tirupati. I am very fortunate to be associated with such stalwarts who helped in many ways to shape my career. It is with a sense of pride that I am bringing forth this compendium that is a mark of gratitude to them. The papers and abstracts in this compendium are a result of intellectual inputs of the faculty and students from esteemed institutes and Veterinary colleges from all over India. It is a matter of great honour that over 30 scientists have contributed their valuable time and shared their experiences with us in the form of invited and lead papers. It is the years of dedication and hard work that brings about the best and it will be my sincere endeavour to see that we keep up the standards and set the goals to be achieved. Learning is a continuous process and I believe that we learn from each other as we share our thoughts and ideas on a common platform. I hope that this compendium will serve as a reference to all, as it imbibes newer concepts and technologies that are required to update our knowledge in the vast domain of Animal Sciences, Genetics and Health related issues of livestock. Additionally, articles on recent advances in animal genetics, pathology, parasitology, immunology, biotechnology, poultry science, reproduction and clinics are included in the satellite seminar as a part of the effort in implementing Continuing Veterinary Education programmes for field veterinarians. Such an activity not only upgrades the knowledge and skills of veterinary officers but is also a positive step in improving the quality of veterinary services in our state. The deliberations and the recommendations from the sessions will highlight the role of immunology and biotechnology in alleviating the problems of the farmers and suggest ways to help them double their income using scientific means of animal husbandry under the guidance of veterinary officers on the field.

On behalf of Maharashtra Animal and Fishery Sciences University, Nagpur and College of Veterinary and Animal Sciences, Parbhani, I take this opportunity to thank Dr. R.K. Singh, President and Dr. K.S. Palaniswami, Vice President of the Indian Society of Veterinary Immunology and Biotechnology for their support and wholehearted co-operation in holding the conference at Parbhani. I thank Shri K. Umap, Commissioner of Animal Husbandry, Government of Maharashtra for deputing the officers for the satellite seminar. I place on record my deepest gratitude to Dr. B. Venkateswarlu, Honorable Vice Chancellor, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani for his wholehearted support to all our college activities and valuable guidance from time to time. I thank our Honorable Vice Chancellor, Shri Anoop Kumar, IAS for his guidance and consent for conducting the conference. I also thank Dr. A.S. Bannaliker, Director of Instructions and Dean, Faculty of Veterinary Science, Maharashtra Animal and Fishery Sciences University for deputing the faculty from the constituent colleges of this University for this conference. My sincere thanks are due to all the University authorities, Directors, Deans, technical officers, staff and Associate Deans of the constituent colleges who have supported me in my endeavours. I thank all the sponsors for their benevolence and for providing the funds, without which it would be impossible to hold this conference. Their names have been acknowledged in the compendium. I thank the faculty and supporting staff of the college for their co-operation and timely suggestions from time to time. I place on record my appreciation for the painstaking efforts of the various committees and timely execution of the work, especially to the compendium and technical committee for the completing herculean task of compilation of the proceedings within the constraints of budget and time. The committees have made all out efforts in contacting the institutes and life members personally, by post or by email, however, any lapses may please be pardoned.

Lastly, I welcome the delegates once again and thank them for the overwhelming response and for their wholehearted participation in this national event. I wish them a comfortable stay at Parbhani and look forward to exciting, vibrant and interactive sessions at the conference.

**Dr.(Mrs) Sharmila B. Majee**



## **About the College of Veterinary and Animal Sciences, Parbhani**

### **History :**

The College of Veterinary and Animal Sciences, Parbhani was established in the year 1972 as a constituent college of Marathwada Agricultural University, Parbhani in the University campus with the aim to provide the need of Human Resource Development in the Animal Husbandry sector of Marathwada region. Later on the college became a constituent college of Maharashtra Animal and Fishery Sciences University, Nagpur in the year 2000. The undergraduate teaching programme was initiated in 1972 followed by postgraduate teaching in the year 1976-77 in four disciplines and subsequently extended to another 12 departments since 1981. The latest being in Animal Biotechnology that started in 27th Oct. 2007.

### **Academic programmes :**

At present there are 18 full fledged departments involved in teaching, research and extension activities. The intake capacity of under graduates is 63, PG 39 and Ph.D. 6 students. The college has completed 42 years of eventful, glorious existence by imparting quality education to students. Most of the infrastructure requirement is fulfilled in terms of lecture halls, seminar halls library etc apart from the basic requirement. The college has a separate library building, Girls Hostel, Boys hostel, TVCC, Gymnasium and Instructional farm. The college has 3 important mandates namely to impart quality education, conduct research as per demands of public and private sector and to establish linkage between national livestock priority and livestock resources for sustainable livestock production.

It is a matter of pride that the graduates have earned laurels for the college bringing a good reputation not only within the state but also in the country. Graduates have excelled in competitive examinations such as ICAR, MPSC, Banking and other services. Over the years has provided dedicated teachers, administrators, researchers, extension workers and field veterinarians to the nation serving at their best to enrich production potential of animals thereby working for the economic up-liftment of masses. Some of Alumni are pursuing higher studies in the University and abroad and some are very successful entrepreneurs and are a source of inspiration tool for future generations. The meritorious teachers and students of college have won several prestigious awards at National and International level and brought laurels to the college. TVCC is well

equipped providing round-the-clock services to livestock owners in terms of diagnosis and treatment of the animals and also providing ample clinical and practical training to students.

The college for its future intends to perform with the following vision-

- » To impart the quality advanced education by adopting updated technologies.
- » To emphasize need based research to improve productivity and reproductive efficiency of indigenous breeds.
- » To conduct research in order to produce quality feed and fodder and quick, reliable disease diagnostic techniques.

**Projects undertaken at COVAS, Parbhani:**

1. Poultry Science/Demonstration and training programme in commercial quail production for rural women empowerment in Parbhani district of Maharashtra state/ DBT, New Delhi/ 2 years/ 12.41
2. Poultry Science/Sustainable income generating goat rearing for rural women empowerment in Parbhani district of Maharashtra/ DBT, New Delhi /2 years 22.03
3. Poultry Science/To set up infrastructure facility for experiential learning under poultry rearing./ ICAR, New Delhi /1 year / 1.00
4. Parasitology/Development of Integrated Pest Management module against important dipteran pests of livestock with reference to Culicoides Midges Diptera : Ceratopogonidae/DBT, New Delhi/3 years/ 30.95
5. Microbiology/ICAR All India networking program on Blue Tongue (ICAR adhoc research scheme) / ICAR New Delhi /15 years/ 96.00
6. Animal Genetics & Breeding/ Survey of Osmanabadi goats in Osmanabad district/ Sheep and Goat Development Corporation /3 years/ 15.50
7. Livestock Production & Management /Grassland Development scheme /RKVY /3 Years/ 10.00
8. Livestock Production & Management /Buffalo development through community based approach for enhancement of milk production in Parbhani district./RKVY /2 Years/ 42.29
9. Animal Nutrition/“Evaluation of Locally available feeds and fodders, Improve quality and formulation of complete economic rations for sugarcane based production system./ NATP /ICAR /3 Years/ 32.36
10. Physiology & Biochemistry/Assessment of mineral profile relationship of animals, soil, feeds and fodders in Maharashtra for improvement of livestock production potential /Govt. of Maharashtra/ 5 Years /13.00.

**Ongoing projects:**

1. Veterinary Biochemistry/ Effect of promoter mutation of candidate gene on growth performance and pathogenicity of Salmonella typhimurium/ SERB, Department of Science and Technology/2 years/ 50.00
2. TVCC/ Strengthening and Modernization of Teaching Veterinary Clinical Complex, Diagnostic Laboratory and Establishment of training Centre at College of Veterinary and Animal Sciences, Parbhani/ RKVY/2 years/4.59 crores.
3. Strengthening of Life Science and Biotechnology Education and Training at undergraduate level under Star College Scheme"/DBT/2 years/58.00

**Salient Accomplishments**

- » The institute participated in a project involving region-wise mapping of minerals in animals, soil and fodders that came out with area-specific mineral mixtures
- » Developed 10 acres of grass land with different fodder crops Yeshwant, Jaywant, Marvel, DHN-6, Stylo and Himata.
- » Under the Buffalo development programme the college distributed Murrah breeding bulls inclusive of free vaccination and hormonal therapy.
- » Under the ICAR Sponsored All India Network Project (AINP) on Blue Tongue, the virus strains were isolated and sent to viral repository at IVRI, Mukteshwar. » Under both the DBT projects Self help groups were formed and Japanese quails and goats were distributed to these groups.

**Extension education:**

As per the mandate of the Maharashtra Animal & Fishery Sciences University Nagpur, all faculty members of the college have to actively participate in extension programmes at college as well as in adopted villages. Faculty members of various disciplines viz. Livestock Production and Management, Poultry Science, Medicine, Pathology, Public Health, Animal Genetics and Breeding, Nutrition, Extension, Animal Reproduction and TVCC routinely visit the adopted villages and carry out extension work. Student's involvement in extension programme is through NSS camp, Organization of Animal health camps/Farmers rally, Extension surveys, organization and celebrations of World Veterinary Day, World Rabies Day, World Zoonosis Day, DNA day, World Biodiversity Day. Student's participation in the college stalls for disseminating information to farmers during the national, state and district levels exhibitions that are held regularly every year. Apart from these, continuing veterinary education programmes are held in the college. The college has entered into a Memorandum of Understanding with Mahila Arthik Vikas Mahamandal for training of women SHGs from all parts of Maharashtra as a part of women empowerment in the livestock sector for making animal husbandry and dairying a profitable enterprise at village level. The success of this programme has given the college a brand name in extension education in Parbhani and surrounding districts.



## **ABOUT ISVIB**

The Indian society of veterinary immunology and biotechnology, popularly called as “ISVIB” is a conglomerate organization of primarily veterinary scientists from microbiology, biotechnology, preventive medicine, parasitology, animal reproduction and fisheries disciplines with the objective of fostering the growth of veterinary immunology and biotechnology in the era of biological technology. The organization was started in the year 1990 by collective visionary zeal of Drs.P.Richard Masillamony, B.B.Mallick, and B.S.Keshavamoorthy, during an interactive session at Tirupathi. This registered society motivates the members to discuss, analyze and formulate suitable strategies for safeguarding animal health and thus to ensure improved animal production. To achieve this annual national conventions and symposiums are organized to critically analyse and update our knowledge and understand issues concerning with gene based technologies in animal production and health. Since then the organization has grown in strength with a current membership of over 1000 scientists both from India and abroad. The first green revolution of the country was possible by visionaries and classical genetic and microbiological technologies, with the second undoubtedly biotechnology based. This forum will maintain the tempo amongst its members and the profession by highlighting our role in the coming years for not only a hunger free world but also a world of balanced nutrition and freedom from zoonotic diseases using biotechnology in veterinary and fisheries sphere of agriculture.

## CONTENT

No.	Title	Page No.
	<b>Key Note Address</b>	
	<b>Merits and de-merits of avian influenza vaccination in poultry</b> Diwakar D. Kulkarni	1
<b>T1-01</b>	<b>Antimicrobial resistance: Current Status on its Containment Strategies in India</b> S.D. Hirpurkar <sup>1</sup> and Fateh Singh <sup>2</sup>	5
<b>T1-02</b>	<b>Antimicrobial Resistance : One Health Approach to combat</b> Sandeep Chaudhari <sup>1</sup> and Shilpa Moon <sup>2</sup>	14
<b>T1-03</b>	<b>ANTIMICROBIAL RESISTANCE: POTENTIAL RISK TO HUMAN AND LIVESTOCK HEALTH</b> V. C. Ingle, S. P. Awandkar and P.A. Tembhurne	20
<b>T1-04</b>	<b>ANTI-MICROBIAL RESISTANCE: ITS CURRENT INDIAN STATUS AND CONTROL STRATEGIES</b> <i>R.J. Zende*</i> and D.P. Kshirsagar	26
<b>T1-05</b>	<b>Antimicrobial Drug Resistance : Prospective Alternatives and Emerging Approaches</b> Dr. Ashok V Bhosle	33
<b>AB-01</b>	Application of lytic bacteriophages in the treatment of <i>Pseudomonas aeruginosa</i> mastitis. Lakshmi kavitha K <sup>1</sup> , Sambasiva Rao K <sup>2</sup> and Srivani M <sup>3</sup> .	40
<b>AB-02</b>	<b>Emergence of Virulent and Antibiotic Resistant <i>Pasteurella multocida</i> and <i>Mannheimia hemolytica</i> in Sheep and Goats of Western Maharashtra, India</b> Daphal S.H., Mhase P.P., Pawade M.M., Shelke P.R and Sangle J.D	40
<b>AB-03</b>	<b>Antimicrobial susceptibility of bacteria associated with mastitis in bovines around Western Maharashtra, India.</b> Pawade M.M., Mhase P.P., Muglikar D.M., Shelke P.R and Sangle J.D	41
<b>AB-04</b>	<b>Isolation and Identification of <i>Pseudomonas</i> spp from mastitis cases along with antibiogram pattern</b> Pawade M.M., Mhase P.P., Muglikar D.M., Shelke P.R and Sangle J.D	42

<b>AB-05</b>	<b>Phenotypic and Genotypic Antibiotic Resistance of Salmonella serovar Newport isolated from Poultry</b>	<b>43</b>
	R.N. Waghamare*, A. M. Paturkar, R. J. Zende, V. M. Vaidya, Z. B Dubal and R. S. Khilari	
<b>AB-06</b>	<b>QUORUM QUENCHING: A NOVEL THERAPEUTIC TOOL AND ANTIBIOTIC REPLACER</b>	<b>44</b>
	D.P.PATIL <sup>1</sup> , USHARAN <sup>2</sup> , A.GOPALA REDDY <sup>3</sup> B. ANILKUMAR <sup>4</sup> and S.V. LONDHE <sup>5</sup>	
<b>T2-01</b>	<b>Role of Biotechnology in Livestock Development and Sustainability of Farmers</b>	<b>45</b>
	Dr. Satish Kumar	
<b>T2-02</b>	<b>GENETIC STUDIES ON CAPRINE FECUNDITY</b>	<b>46</b>
	Aakash Y. Doiphode, Priyanka T. Wagh, Kalpesh A. Zunjarrao and Ritesh S. Vairagade	
<b>AB-07</b>	<b>Pan-genome codon usage pattern in Brucella</b>	<b>56</b>
	Gaikwad S S, Usharani J., Suryavanshi P R, Deshpande A R, Majee S B	
<b>AB-08</b>	<b>EFFECT OF DIFFERENT TROPICAL STRESS CONDITIONS ON HSP-70 PROTEIN EXPRESSION IN VARIOUS BROILER STRAINS</b>	<b>56</b>
	S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 & Suhas Amrutkar 5	
<b>AB-09</b>	<b>Development of Assisted Reproductive Biotechnologies for Species Conservation and Livestock Production: from microRNAs to ex situ approaches</b>	<b>57</b>
	Sadanand D. Sontakke	
<b>AB-10</b>	<b>EFFECT OF THERMAL CONDITIONING DURING EMBRYOGENESIS ON EPIGENETIC CHANGES IN BDNF GENE IN BROILER CHICKS</b>	<b>58</b>
	S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 and Suhas Amrutkar 5	
<b>AB-11</b>	<b>INFLUENCE OF DIFFERENT TROPICAL STRESS CONDITIONS ON PRODUCTION TRAITS IN VARIOUS BROILER STRAINS</b>	<b>58</b>
	S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 & Suhas Amrutkar 5	
<b>T3-01</b>	<b>EX-VIVO DERIVATION OF CAPRINE FETAL ADNEXA MESENCHYMAL STEM CELLS: A COMPARATIVE IMMUNOMODULATORY ANALYSIS</b>	<b>60</b>
	G.Taru Sharma	

<b>T3-02</b>	<b>Stem Cells : As Regenerative Medicine</b>	<b>61</b>
	C. H. Pawshe, S. A. Ingle, V.S. Dhaygude and R. R. Shelar	
<b>AB-12</b>	<b>ROLE OF EMBRYONIC STEM CELLS FOR OSTEOGENESIS IN THE REPAIR OF BONE DEFECTS IN RABBIT MODEL</b>	<b>67</b>
	B.K. Bhadane*, S.K.Maiti, A.K.Sharma, Naveen Kumar, Amarpal, Sadan Bag and G. K. Das	
<b>T4-01</b>	<b>Testes-mediated gene transfer (TMGT) - a potential tool for producing transgenic livestock</b>	<b>68</b>
	Abhijit Mitra <sup>1</sup> and Pramod Kumar R <sup>2</sup>	
<b>T4-02</b>	<b>ADVANCE DIAGNOSTIC TECHNIQUES IN ANIMAL REPRODUCTION</b>	<b>72</b>
	Dr. Sarita U. Gulavane- M.VSc. Ph. D.	
<b>T4-03</b>	<b>Pioneering innovations in Reproductive Biotechnology for Improved livestock Productivity</b>	<b>78</b>
	Dr Vikas D Dighe	
<b>AB-13</b>	<b>Fertility response in Indian Zebu cattle (Bos indicus) at synchronized ovulation by Ovsynch protocol and timed AI</b>	<b>86</b>
	B. L. Kumawat, N. M. Markandeya and A. B. Mali	
<b>AB-14</b>	<b>COMPARATIVE STUDIES OF BOVINE PRE-IMPLANTATION EMBRYO SEXING USING LAMP AND PCR BASED METHODS</b>	<b>86</b>
	P. Raja*, K. Vijayarani, T.M.A. Senthilkumar and K. Kumanan	
<b>T5-01</b>	<b>Herbal Nutraceuticals: Livestock Dairy Products Application</b>	<b>88</b>
	S.P. Changade, P. D. Sawale & V.K.Lule	
<b>T5-02</b>	<b>Nutraceuticals: A New Vista for Health and Medicine</b>	<b>97</b>
	Manish Kumar Chatli*, Kumbhar Vishaland Sandeep Rindhe	
<b>T5-03</b>	<b>Magnitude and current status of biotechnology in food processing</b>	<b>113</b>
	Dr. Vikas S. Waskar	
<b>T5-04</b>	<b>Milk protein as carrier of micronutrient (vitamin A) for milk fortification</b>	<b>119</b>
	Chitra Gupta <sup>1</sup> , Sumit Arora <sup>2</sup> , Sneha Shende <sup>1</sup> , Madhav Patil <sup>1</sup> and Prasad Patil <sup>1</sup>	
<b>AB-15</b>	<b>Efficacy of Herbal Liver Tonic (Superliv.Conc.)in Improving Growth Performance, Nutrient Utilization and Carcass Traits in Broiler Chicken</b>	<b>124</b>
	M.V. Dhumal*, M G Nikam <sup>1</sup> and P V Nandedkar <sup>2</sup>	
<b>AB-16</b>	<b>HOLISTIC APPROACH TO RESOLVE CURRENT ISSUES AND FUTURE THREATS IN POULTRY INDUSTRY THROUGH BIOTECHNOLOGY</b>	<b>125</b>
	V R Kulkarni	

<b>AB-17</b>	<b>Optimisation of analytical conditions for estimation of unbound vitamin A in milk-protein Vitamin A complexes prepared for milk fortification</b>	<b>126</b>
	Chitra Gupta 1 , Sumit Arora 2 & Madhav Patil 1	
<b>AB-18</b>	<b>Zinc enriched Lactic Acid Bacteria: A Novel Approach for Zinc Supplementation</b>	<b>126</b>
	V. K. Lule*, S. P. Changade, P. D. Sawale and A. R. Sarode	
<b>AB-19</b>	<b>Effect of supplementing chelated Zn, Cu and Mn on growth performance of Murrah buffalo calves</b>	<b>127</b>
	Vishal Mudgal*, N. Saxena, K. Kumar, S. S. Dahiya, B. S. Punia and M. L. Sharma	
<b>T6-01</b>	<b>Recent Advances in Nano-technology Based Novel Drug Delivery Veterinary Drugs</b>	<b>129</b>
	Dr. Abdul Samad*, Isha Naik**, Kaveri Kalola** and Dr. Mala D. Menon**	
<b>T6-02</b>	<b>Nano Particles : Green synthesis, characterisation and its use in Drug delivery system : A Review</b>	<b>134</b>
	Sudhir R Rajurkar	
<b>T6-03</b>	<b>EVALUATION OF VETERINARY PHARMACEUTICAL PRODUCT DEVELOPMENT BY GAMMA SCINTIGRAPHY</b>	<b>140</b>
	Dr. RAJIV V.GAIKWAD	
<b>AB-20</b>	<b>Antimicrobial and immunological studies of curcumin against <i>E. coli</i> induced infection in broilers</b>	<b>143</b>
	Godbole PV, Hajare SW, Poonam Bhosale, Madhuri Hedau, Ingole RS and Ingawale MV	
<b>T7-01</b>	<b>Subviral particle based infectious bursal disease vaccine: a way forward towards translation from lab to land</b>	<b>144</b>
	<u>Sohini Dey</u> <sup>#</sup> , C. Madhan Mohan, R. Saravanan, Dinesh C. Pathak, Ashis Debnath, Rahul, R. Narayan, M. Asok Kumar, A. Gulhane* and B. Piruthiviraj Kumar*	
<b>T7-02</b>	<b>Changing epidemiological patterns of Japanese encephalitis infection and impact on animal health in India</b>	<b>147</b>
	Baldev R. Gulati	
<b>T7-03</b>	<b>JOHNE'S DISEASE IN ANIMALS AND ITS CONTROL</b>	<b>151</b>
	S. Manoharan, Dr.B.Samuel Ronald Masillomony* and K.Kumanan	

<b>AB-21</b>	<b>Identification of subclinical mastitis pathogens in milk by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and antibiogram study</b>	<b>153</b>
	Ashok V Bhosle, Mahesh B Kulkarni, Avinash G Karpe, Shivaji Mehatre*, Shaunak Deshmukh	
<b>AB-22</b>	<b>Diagnosis of <i>Mycobacterium bovis</i> based on Fluorescence polarization Assay.</b>	<b>154</b>
	<sup>1</sup> Rabyia javed, <sup>1</sup> Deepti Narang, <sup>1</sup> Mudit Chandra, <sup>2</sup> S.T.Singh, <sup>3</sup> G.Filia.	
<b>AB-23</b>	<b>Expression and characterization of A4L gene of capripoxvirus for its diagnostic potential in ELISA</b>	<b>154</b>
	Aparna Madhavan, Gnanavel Venkatesan, Amit Kumar, Sargam Arya and A.B. Pandey	
<b>AB-24</b>	<b>Isolation and molecular characterization of Pasteurella multocida type B causing Hemorrhagic Septicemia in Buffaloes</b>	<b>155</b>
	K. Manimaran*, R. Bharathi, K. Karthik, S. Arunkumar, K. Shoba and K. Kumanan	
<b>AB-25</b>	<b>Expression of recombinant chimeric BP26-BLS protein in <i>E.coli</i> system and assessing its potential use as diagnostic antigen for bovine brucellosis</b>	<b>156</b>
	M. Nagalingam <sup>1</sup> , Thaslim J. Basheer <sup>1</sup> , V. Balamurugan <sup>1</sup> , Rajeswari Shome <sup>1</sup> , S. Sowjanya Kumari <sup>1</sup> , G. B. Manjunatha Reddy <sup>1</sup> , B. R. Shome <sup>1</sup> , H. Rahman <sup>2</sup> , Parimal Roy <sup>1</sup> and RK Gandham <sup>3</sup>	
<b>AB-26</b>	<b>Comparative evaluation of serodiagnostic efficacy of recombinant LLO and PI-PLC-based ELISAs for detection of listeriosis in animals and field Veterinarians</b>	<b>157</b>
	Rahul D. Suryawanshi*, S.V.S. Malik, Bhushan Jayarao, S. P. Chaudhari, Emily Savage, Nitin V. Kurkure, S. B. Barbuddhe, D. B. Rawool	
<b>AB-27</b>	<b>Pro-inflammatory cytokine and apoptotic gene mRNA levels against lentogenic and velogenic Newcastle disease virus pathotypes in <i>in-vivo</i> and <i>in-vitro</i> biological systems</b>	<b>158</b>
	Ranjani Rajasekaran, J. John Kirubaharan, P. Shilpa, M. Vidhya, N. Daniel Joy Chandran	
<b>AB-28</b>	<b>EVALUATING THE DIAGNOSTIC POTENTIALS OF RECOMBINANT IMMUNODOMINANT PROTEIN LIPL32 OF PATHOGENIC LEPTOSPIRA IN BOVINES</b>	<b>159</b>
	T.V.Meenambigai, Sworna Kumari, Gautami Sadekar, G. Balakrishnan, and K. Kumanan	

<b>AB-29</b>	<b>Application of Recent Diagnostic Techniques for Detection of Cysticercosis in Pig and Taeniasis in Human Population</b>	<b>159</b>
	Vilas M. Vaidya*, Ravindra J. Zende, Ashish M. Paturkar, Rupesh N. Waghmare and Sujata S. Bhave	
<b>T8-01</b>	<b>Nano-based diagnostic approaches for viral infections</b>	<b>161</b>
	Prasad Minakshi <sup>1*</sup> , Upendera P Lambe <sup>1</sup> , Basanti Brar <sup>1</sup> , Ikbali <sup>1</sup> , Koushlesh Ranjan <sup>2</sup> and Jyoti Misri <sup>3</sup>	
<b>T8-02</b>	<b>Role of National Animal Disease Referral Expert System (NADRES) as animal health support system in controlling livestock diseases in India</b>	<b>166</b>
	Dr. Parimal Roy	
<b>T8-03</b>	<b>Immunobiotechnological contributions to our understanding of epidemiology and evolution of animal origin enteric viral pathogens</b>	<b>169</b>
	Yashpal S. Malik	
<b>T8-04</b>	<b>An overview of Avian Mycoplasmosis</b>	<b>171</b>
	K. Manimaran	
<b>T8-05</b>	<b>Antibody and cellular response in Brucellosis affected vaccinated and unvaccinated cattle</b>	<b>180</b>
	Hari Mohan Saxena and Sugandha Raj	
<b>T8-06</b>	<b>Recent Trends in Molecular Epidemiology : Techniques and Applications</b>	<b>192</b>
	Nitin V Kurkure	
<b>T8-07</b>	<b>Development of diagnostic systems and molecular epidemiology studies of bluetongue virus in India</b>	<b>199</b>
	Sushila Maan	
<b>T8-08</b>	<b>Chronic Respiratory Disease in Poultry: Current scenario of the disease in India and advances in diagnosis</b>	<b>202</b>
	Dr. (Mrs.) Rajashree Gandge Patil	
<b>AB-30</b>	<b>Sequence and phylogenetic analysis of sheeppox and goatpox viruses by A12L gene</b>	<b>209</b>
	<u>Aparna Madhavan</u> , Gnanavel Venkatesan, Sargam Arya, Amit Kumar and A.B. Pandey	
<b>AB-31</b>	<b>Isolation and identification of <i>Microsporium canis</i> from dog</b>	<b>209</b>
	C. Sannat*, N. Rawat, R. Hazari and K. K. Kushwaha and S.D. Hirpurkar	
<b>AB-32</b>	<b>Swiss Modelling of cAMP Receptor protein</b>	<b>210</b>
	Ghorpade Prabhakar B <sup>1</sup> , S.D. Deshpande <sup>2</sup> and Bhaskar Sharma <sup>3</sup>	

<b>AB-33</b>	<b>Genetic characterization of orf virus isolate from black buck by VEGF and VLTF-4 genes</b>	<b>211</b>
	Gnanavel Venkatesan <sup>1</sup> , Sargam Arya <sup>1</sup> , Amit Kumar <sup>1</sup> Karikalan Mathesh <sup>2</sup> and A B Pandey	
<b>AB-34</b>	<b>EVOLUTIONARY ANALYSIS OF RABIES VIRUS IN MUMBAI REGION OF INDIA</b>	<b>212</b>
	R. R. Pharande, S. B. Majee, S S.Gaikwad**, A. S. Bannalika, R. S. Gandge, D.G. Dighe, S. S. Moregoankar, A. Y. Doiphode, S.A.Ingle and S. Mukherjee*	
<b>AB-35</b>	<b>Isolation and Molecular Characterization of <i>Listeria monocytogenes</i> from diarrheal cases in young animals and human infants</b>	<b>212</b>
	<u>Shubhangi Warke</u> , Tumlam U.M., V.C.Ingle and Sumedha Bobade	
<b>AB-36</b>	<b>Detection of <i>Coxiella burnetii</i> in bovine bulk milk samples by nested PCR in Nagpur region</b>	<b>213</b>
	<u>Shubhangi Warke</u> , V.C. Ingle and Sumedha Bobade	
<b>AB-37</b>	<b>Isolation, characterization and antibacterial activity of <i>Mallasezia pachydermatis</i> from dogs</b>	<b>214</b>
	V. Dilliwar, S.D.Hirpurkar, C.Sannat*, N. Rawat, R. Hazari and K. K.Kushwaha	
<b>T9-01</b>	<b>Doubling Indian Dairy Farmers' Income through Dairying</b>	<b>215</b>
	P. G. Wasnik <sup>1</sup> and S. S. Chopde <sup>2</sup>	
<b>T9-02</b>	<b>ENTOMOPATHOGENIC FUNGUSES AS BIOPESTICIDES: EMERGING CONCEPT FOR TICK CONTROL IN INDIA</b>	<b>222</b>
	GreeshmaRao U B and <u>B W Narladkar</u>	
<b>T9-03</b>	<b>Upliftment of livelihood of tribal farmers by doubling their annual income through implementing the scientific goat farming practices in Thane district (Maharashtra).</b>	<b>225</b>
	Dr. H.Y. Palampalle and Dr. S. D. Jagadale	
<b>T9-04</b>	<b>Applications of Radiomunoassay (Rai) to Improve Animal Health and Production</b>	<b>231</b>
	Dr. Chandrakant N. Galdhar	
<b>T9-05</b>	<b>Advances in Animal Husbandry &amp; Animal health Biotechnology.</b>	<b>233</b>
	Dr. S.D.Deshpande	
<b>T9-06</b>	<b>Platelet-rich plasma (PRP): A new vision for tissue repair under regenerative therapy</b>	<b>246</b>
	Dr. V. D. Aher	

<b>AB-38</b>	<b>Socio-economic profile of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>251</b>
	Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.	
<b>AB-39</b>	<b>Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>251</b>
	Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.	
<b>AB-40</b>	<b>Advance biotechnological tools for increasing fish production and biodiversity conservation</b>	<b>252</b>
	Swapnil S. Ghatge* and Milind M. Girkar*	
<b>AB-41</b>	<b>Culture of <i>Pangasius sutchi</i> in polythene lined farm ponds in Vidarbha and Marathwada region of Maharashtra</b>	<b>252</b>
	Umesh suryawanshi <sup>1</sup> , Shamkant Shelke <sup>2</sup> and Somnath Yadav <sup>3</sup>	
<b>PO-01</b>	<b>Identification of Methicillin resistant <i>Staphylococcus pseudintermedius</i> in dogs of Chennai with skin infection</b>	<b>253</b>
	Hridya Susan Varughese <sup>1*</sup> , M. AnandaChitra <sup>1</sup> , S.Rajalakshmi <sup>1</sup> , G.Dhinakar Raj <sup>2</sup> and J. John Kirubaharan <sup>1</sup>	
<b>PO-02</b>	<b>A systematic review and Meta-analysis on prevalence of Methicillin-Resistant <i>Staphylococcus aureus</i> in Animals</b>	<b>253</b>
	Hamsapriya S, Rashmi R. Kurli, Krishnamoorthy P Suresh K.P and Parimal Roy	
<b>PO-03</b>	<b>Phenotypic Characterization and 16S rRNA sequence Analysis of Equine Isolates of <i>Streptococcus pluranimalium</i></b>	<b>254</b>
	R. Javed, A. K. Taku, M.A.Bhat, R. K. Sharma and G.A.Badroo	
<b>PO-04</b>	<b>Efficacy of a Polyherbal Drug against Indigestion in Bovine</b>	<b>255</b>
	A.M.Syed, V.D.Aher, P.M.Mane and G.R.Gangane	
<b>PO-05</b>	<b>Efficacy of Polyherbal Drug against Tympany in Bovine</b>	<b>256</b>
	A.M.Syed, V.D.Aher, P.M.Mane and G.R.Gangane	
<b>PO-06</b>	<b>Therapeutic Efficacy of Herbal Drug Against Post-parturient Reproductive Disorders in Bovine</b>	<b>256</b>
	P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade	
<b>PO-07</b>	<b>Induction of Oestrus in Anoestrus Bovine by Using Herbal and Mineral Preparations</b>	<b>257</b>
	P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade	
<b>PO-08</b>	<b>Comparative Studies on Haemato- Biochemical Constituents in Anoestrus Bovine</b>	<b>257</b>
	P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade	

<b>PO-09</b>	<b>EFFECT OF <i>MORINDA CITRIFOLIA</i> JUICE ON DIABETIC RATS</b>	<b>258</b>
	Dr. N. D. Jadhav, Dr. S. R. Rajurkar and Abhinna Keshari Behera,	
<b>PO-10</b>	<b>Development and characterization of nanoparticle antimicrobial agent loaded SPRAYON type polymeric in-situ gelling bandage system for wound healing in animals.</b>	<b>259</b>
	Authors: Isha V. Naik, Kaveri R. Kalola, Dr. Mala D. Menon	
<b>PO-11</b>	<b>Effect of lower doses of the cloprostenol on oestrus synchronization, physio-chemical properties of cervico-vaginal mucus and fertility in repeat breeder cross bred cows</b>	<b>260</b>
	M.B. Amle, K.P.Khillare, M.N.Rangnekar and A.B Korade	
<b>PO-12</b>	<b>Studies on improvement of conception rate in normal cyclic cows and buffaloes with two different doses of GnRH.</b>	<b>261</b>
	S.R. Chirfare, N.M. Markandeya, B.L. Kumawat and A. Wankar	
<b>PO-13</b>	<b>Studies on improvement of conception rate in non infectious repeat breeder cows and buffaloes with two different doses of GnRH.</b>	<b>262</b>
	S.R. Chirfare, N.M. Markandeya, A.B. Mali and A.G. Sawale	
<b>PO-14</b>	<b>Efficacy of Neem extract and <i>E.coli</i> LPS for treatment of endometritis in cows and buffaloes</b>	<b>262</b>
	G. A. Thombre, M. N. Markandeya, B. L. Kumawat and V. B. Thombre	
<b>PO-15</b>	<b>Does efficacy of <i>E.coli</i> LPS improves with immunostimulant in the treatment of endometritis in dairy animals?</b>	<b>263</b>
	<u>Puro , N. A.</u> , N. M.Markandeya, A. B. Mali, and S. G. Swami,	
<b>PO-16</b>	<b>Influence of different levels of Xylanase enzyme on performance, litter quality and economics of broiler chicken</b>	<b>264</b>
	D S Gade <sup>1</sup> ,M.V.Dhumal <sup>2#</sup> , <a href="mailto:dhumalmv@gmail.com">dhumalmv@gmail.com</a> , and M G Nikam <sup>3</sup>	
<b>PO-17</b>	<b>Isolation of <i>proteus</i> from poultry faecal sample</b>	<b>264</b>
	Pawara V.P.,Acharya P.S., Bramhapurkar P.P. , , Bhonsle A.V.	
<b>PO-18</b>	<b><i>In vitro</i> efficacy of bacteriophages against mastitis caused by antibiotic resistant bacteria</b>	<b>265</b>
	Dr. A.R. Deshpande, Dr. P.R. Suryawanshi, Dr. S.S. Gaikwad, Dr. O.S. Kashikar*	
<b>PO-19</b>	<b>Isolation and Identification of <i>Erysipelothrix rhusiopathiae</i> from an outbreak of Swine Erysipelas</b>	<b>266</b>
	A.R. Deshpande, P.R. Suryawanshi, S.S. Gaikwad, K.N. Kadam, O.S. Kashikar, Neha Singh	

<b>PO-20</b>	<b>Identification of phytobiotic potential of certain herbal extracts against mastitis causing bacteria</b>	<b>266</b>
	S.S.Vyawhare* Dr. A.R. Deshpande, Dr. P.R. Suryawanshi, Dr. S.S. Gaikwad and Dr. O.S. Kashikar	
<b>PO-21</b>	<b>Nutritional technology in livestock products: Eubiotic's role in broiler feed</b>	<b>267</b>
	Acharya P.S., Bramhapurkar P.P., Telange N.P., Dadke A.R.	
<b>PO-22</b>	<b>Antimicrobial activity of ginger extract on the bacteria isolated from pyometra case</b>	<b>268</b>
	Neha Singh*, A.R. Deshpande, P.R. Suryawanshi, S.S. Gaikwad, K.N. Kadam, S.R. Lawange	
<b>PO-23</b>	<b>Assessment of Shelf life of Wheat Flour Based Quail Meat Enriched Noodles at (35±2°C) Room Temperature Storage</b>	<b>268</b>
	D.M. More, S.V. Londhe, D.P. Patil, C.K. Choudhary, P.A. Shinde and P.N. Bhumre	
<b>PO-24</b>	<b>Evaluation of Cost Structure of Quail Meat Enriched Noodles</b>	<b>269</b>
	D.M. More, S.V. Londhe D.P. Patil, P.N. Bhumre and A.S. Nemade	
<b>PO-25</b>	<b>Development and Quality Evaluation of the Wheat Flour Based Quail Meat Enriched Noodles</b>	<b>269</b>
	D.M. More, S.V. Londhe, D.P. Patil, P.A. Shinde, C.K. Choudhary and A.S. Nemade	
<b>PO-26</b>	<b>Microscopic observations of small intestine in probiotic feed Japanese quail</b>	<b>270</b>
	Mamde C. S., Waghaye J. Y., Thakur P. N. Karad N. M. and S. D. Kadam	
<b>PO-27</b>	<b>Cultural and Molecular detection of viruses from clinical samples of poultry</b>	<b>271</b>
	Shilpi S. Singh, R.R. Pharande, Sonal Ingle, V. D. Thorat, R.S. Gandage, S.B. Majee	
<b>PO-28</b>	<b>Effect of <i>Andrographis paniculata</i> on experimentally induced <i>E. coli</i> pathology in broilers</b>	<b>271</b>
	Suvarna R. Sonwane, <u>R. S. Ingole*</u> , Madhuri S. Hedau, P. R. Rathod, S. W. Hajare and M. V. Ingawale	
<b>PO-29</b>	<b>MUTATIONAL ANALYSIS OF BRCA2 GENE IN CANINE MAMMARY TUMOR</b>	<b>272</b>
	Vaidya A.B <sup>1</sup> , Sawane M.P <sup>1</sup> , Chaudhari P.R <sup>2</sup> , Umrikar U.D <sup>1</sup> , Pawar V.D <sup>1</sup> , Deshmukh R.S <sup>1</sup> , Komatwar S.J <sup>1</sup> , Chopade M.M <sup>1</sup>	

<b>PO-30</b>	<b>Importance and Prevalence of Sexed Semen In Dairy Industry- A Review.</b>	<b>273</b>
	S. N. Solanke <sup>1</sup> , M. F. Siddiqui <sup>2</sup> , M. D. Kharwadkar <sup>3</sup> , V. S. Ingle <sup>4</sup> , A. S. Rathod <sup>5</sup> , M. B. Wakchaure <sup>6</sup> and N. P. Telange <sup>7</sup>	
<b>PO-31</b>	<b>Precision Dairy Farming (PDF) Technologies- Milk Yield and Milk Electrical Conductivity- A Review</b>	<b>274</b>
	A. S. Rathod <sup>1</sup> , M. F. Siddiqui <sup>2</sup> , M. D. Kharwadkar <sup>3</sup> , V. S. Ingle <sup>4</sup> , S. N. Solanke <sup>5</sup> , K. S. Mane.	
<b>PO-32</b>	<b>Karyological analysis of Boer breeding bucks using QFQ banding</b>	<b>275</b>
	<u>Kokani S.C.</u> , Pawar V. D., Sawane M. P., Chopade M. M., Khade S. B., Komatwar S.J., Deshmukh R.S. and C.B. Nimbkar	
<b>PO-33</b>	<b>Prolactin (bPRL) and Growth Hormone (bGH) genes polymorphism in Red Kandhari and Deoni cattle</b>	<b>276</b>
	SHAIKH GM, MAHENDRA KUMAR, SS DESHPANDE, SADHAWARE, MS VAIDYA AND PV NANDEDKAR	
<b>PO-34</b>	<b>SLAUGHTER WASTE: A SOURCE OF REGENERATIVE MEDICINE</b>	<b>277</b>
	NANDEDKAR P.V., MOHITE N.R., MAHENDRA KUMAR, SS DESHPANDE, SADHAWARE, AND MS VAIDYA	
<b>PO-35</b>	<b>Socio-economic profile of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>278</b>
	Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.	
<b>PO-36</b>	<b>Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>278</b>
	Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.	
<b>PO-37</b>	<b>Constraints faced by buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>279</b>
	Borade M.T, Deshmukh D.S., Patil A.P. and Jaybhaye N.M.	
<b>PO-38</b>	<b>PATHOLOGICAL STUDIES OF Haemonchus contortus (Barber Pole Worm) IN GOAT</b>	<b>279</b>
	<u>Utkarsh.B. Wankhede</u> Deepika.D.Ghodke, Roshni Padole, Priyanka Tikore, A.S.Rathod, P.R. Rathod	
<b>PO-39</b>	<b>Multiple organ failure in Labrador dog – A case report</b>	<b>280</b>
	Roshani M. Padole, A. S. Rathod, Priyanka Tikore, Deepika Ghodke, Madhuri Hedau, P.R. Rathod, N. M. Bhojane and R. S. Ingole	

<b>PO-40</b>	<b>Cardiac Cirrhosis In Horse -A Case Report</b>	<b>281</b>
	<u>Priyanka Tikore</u> , Deepika Ghodke, Roshani Padole ,Atul Rathod, Utkarsh Wankhede, N. M. Bhojane, R. S. Ingole and M.S. Headu	
<b>PO-41</b>	<b>ASPIRATION PNUMONIA IN GOAT KID – A CASE REPORT</b>	<b>281</b>
	Deepika D. Ghodke*, Roshani Padole, A. S. Rathod, Priyanka Tikore, U.B.Wankhede, P.R. Rathod, R. S. Ingole & M.S. Hedau	
<b>PO-42</b>	<b>TAPEWORM INFESTATION IN PEACOCK – A CASE REPORT</b>	<b>282</b>
	<u>Roshani Padole</u> , Atul S. Rathod, Priyanka Tikore, Deepika Ghodke, U. B. Wankhede, P. R. Rathod, Madhuri Hedau, N. M. Bhojne & R.S. Ingole	
<b>PO-43</b>	<b>Blood picture and immune response of broilers fed altered level of crude protein with supplementation of limiting amino acids</b>	<b>282</b>
	P.A. Sutar, K.Y. Deshpande*, R.S. Awhad, P.V. Nandedkar and P.R. Suryavanshi	
<b>PO-44</b>	<b>Effect of dietary inclusion of guar (<i>Cyamopsis tetragonoloba</i>) korma meal on gut health and immune response in broiler chicken</b>	<b>283</b>
	R.S. Awhad, K.Y. Deshpande, P.A. Sutar and P.R. Suryavanshi	
<b>PO-45</b>	<b>Molecular characterization and phylogenetic analysis of <i>Contagious ecthyma Virus</i> in Goats</b>	<b>284</b>
	Gosavi, P.D., P.R. Suryawanshi, A.R. Deshpande, S.S. Gaikwad, S.B. Majee and P.V. Nandedkar	
<b>PO-46</b>	<b>EXPERIMENTALLY INDUCED <i>E.coli</i> INFECTION IN QUAILS AND ITS AMELIORATION BY <i>Aegle marmelos</i>: IMMUNOPATHOLOGICAL STUDIES</b>	<b>284</b>
	<u>R.A. Kokare</u> , G.R.Gangane, G.B.Kulkarni, B.M.Kondre and B.G.Shingumare	
<b>PO-47</b>	<b>Haemato – biochemical alterations in <i>Ipomoea carnea</i> induced toxicity in Wistar rats</b>	<b>285</b>
	V.G. Khandare, G.R. Gangane, S.D.Moregaonkar, B.M. Kondre, <u>S. A. Londhe</u> and S.C. Bhatane	
<b>PO-48</b>	<b>Ameliorative effect of <i>Picrorhiza kurroa</i> against <i>Lantana camera</i> induced toxicity in Wistar rats: Haematological studies</b>	<b>286</b>
	<u>Birajdar B.W</u> , Gangane G.R, Moregoankar S.D, Kondre B.M, Aulwar B.S and Shingumare B. G.	
<b>PO-49</b>	<b>Prevalence of respiratory affections in goats</b>	<b>287</b>
	<u>Aulwar B. S.</u> , G.R. Gangane, S.D. Moregaonkar, P.R. Suryawanshi and B.W. Birajdar	

<b>PO-50</b>	<b>Ameliorative effect of <i>Swertia chirata</i> during experimental Aflatoxicosis in quails (<i>COTURNIX COTURNIX JAPONICA</i>): Biochemical evaluation</b>	<b>287</b>
	<u>Babar D. T.</u> , Gangane G.R., Kulkarni G.B., Moregaonkar S.D., Shingumare B. G. and Chavan P.A.	
<b>PO-51</b>	<b>Histoarchitectural alterations in Mercuric chloride induced toxicity and it's amelioration by <i>Allium sativum</i> in male Wistar rats</b>	<b>288</b>
	<u>A.U. Ranveer</u> , G.R. Gangane, B.M. Kondre, S.C. Bhatane, S.A. Londhe and R.U. Tandale	
<b>PO-52</b>	<b>CHARACTERIZATION OF FecG LOCI OF GDF-9 GENE IN THE OSMANABADI GOATS</b>	<b>289</b>
	Ukale P.S., Wagh P.T., Zunjarrao K.A., Bhawe K.G., Sawane M.P., Pawar V.D. and Doiphode A.Y.	
<b>PO-53</b>	<b>GENOTYPING OF OSMANABADI GOATS AT IGFBP-3 AND BMP-4 LOCI ASSOCIATED WITH GROWTH RATE</b>	<b>289</b>
	Tep G., Vairagade R.S., Wagh P.T., Zunjarrao K.A., Sawane M.P., Pawar V.D. and Doiphode A.Y.	
<b>PO-54</b>	<b>Therapeutic efficacy of tolfenamic acid 8% in clinical mastitis of bovines</b>	<b>290</b>
	<u>M. K. Patil</u> , P.V. Patil and A. P. Somkuwar	
<b>PO-55</b>	<b>GENOTYPING OF OSMANABADI GOAT AT FecX and FecB LOCI</b>	<b>291</b>
	Bhawe K. G., Sawane M. P., Wagh P. T., Pawar V. D., Umrikar U. D., Majee S. B. and Doiphode A. Y.	
<b>PO-56</b>	<b>BIOMARKERS OF EXPOSURE, EFFECT AND SUSCEPTIBILITY</b>	<b>291</b>
	D.P.PATIL <sup>1</sup> , S.V. LONDHE <sup>2</sup> , KALAKUMAR <sup>3</sup> , USHARANI <sup>4</sup> and B. ANILKUMAR <sup>5</sup>	
<b>PO-57</b>	<b>GENOTYPING OF <i>TOXOPLASMA GONDII</i> ASSOCIATED WITH ABORTED SHEEP</b>	<b>292</b>
	<u>Ajay S Satbige</u> ., C.Rajendran., N.A.Patil ., Vivek R. Kasaraliker and R.G. Bijurkar Thakur P. N., Waghaye J. Y. ,Mamde C. S., Karad N. M and S . D Kadam	
<b>PO-58</b>	<b>Histomorphology of Oviduct in Japanese quail</b>	<b>292</b>
	Thakur P. N., Waghaye J. Y. ,Mamde C. S., Karad N. M and S . D Kadam	
<b>PO-59</b>	<b>Constraints faced by buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state</b>	<b>293</b>
	Borade M.T, Deshmukh D.S., Patil A.P. and Jaybhaye N.M.	

<b>PO-60</b>	<b>Socio-economic profile of Osmanabadi goat entrepreneurs in Marathwada region of Maharashtra state</b>	<b>294</b>
	Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T	
<b>PO-61</b>	<b>Information needs of Osmanabadi goat entrepreneurs in Marathwada region of Maharashtra state</b>	<b>295</b>
	Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T	
<b>PO-62</b>	<b>Constraints faced by Osmanabadi goat entrepreneurs in Marathwada region of Maharashtra state</b>	<b>295</b>
	Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T	
<b>PO-63</b>	<b>A systematic review and Meta-analysis on prevalence of Methicillin-Resistant <i>Staphylococcus aureus</i> in Animals</b>	<b>296</b>
	Hamsapriya S, Rashmi R. Kurli, Krishnamoorthy P Suresh K.P and Parimal Roy	
<b>PO-64</b>	<b>Phenotypic Characterization and 16S rRNA sequence Analysis of Equine Isolates of <i>Streptococcus pluranimalium</i></b>	<b>297</b>
	R. Javed, A. K. Taku, M.A.Bhat , R. K. Sharma and G.A.Badroo	
<b>PO-65</b>	<b>GENOTYPING OF <i>TOXOPLASMA GONDII</i> ASSOCIATED WITH ABORTED SHEEP</b>	<b>298</b>
	<u>Ajay S Satbige.</u> , C.Rajendran., N.A.Patil ., Vivek R. Kasaralika and R.G. Bijurkar	
<b>PO-66</b>	<b>BIOMARKERS OF EXPOSURE, EFFECT AND SUSCEPTIBILITY</b>	<b>298</b>
	D.P.PATIL <sup>1</sup> , S.V. LONDHE <sup>2</sup> , KALAKUMAR <sup>3</sup> , USHARAN <sup>4</sup> and B. ANILKUMAR <sup>5</sup>	
<b>PO-67</b>	<b>GENOTYPING OF OSMANABADI GOAT AT FecX and FecB LOCI</b>	<b>299</b>
	Bhave K. G., Sawane M. P., Wagh P. T., Pawar V. D., Umrikar U. D., Majee S. B. and Doiphode A. Y.	
<b>PO-68</b>	<b>ISOLATION AND CULTURE OF RABBIT EMBRYONIC STEM CELLS</b>	<b>299</b>
	<u>B.K. Bhadane*</u> , S.K.Maiti, A.K.Sharma, Naveen Kumar, Amarpal, Sadan Bag and G. K. Das	
<b>PO-69</b>	<b>Detection and characterization of extended-spectrum beta-lactamase genes (<i>bla</i><sub>TEM</sub> and <i>bla</i><sub>SHV</sub>) conferred multidrug resistance to coliform bacteria isolated from faeces of dairy cattle in Chhattisgarh, India</b>	<b>300</b>
	Nidhi Rawat <sup>1*</sup> , S.D. Hirpurkar <sup>1</sup> , Fateh Singh <sup>2</sup> and Chandrahas Sannat <sup>1</sup>	

<b>PO-70</b>	<b>Detection and characterization of plasmid mediated extended-spectrum beta-lactamases and carbapenemases genes, including <i>bla</i><sub>NDM-1</sub> among Gram-negative bacteria isolated from drainage water, poultry farm environment and human urine</b>	<b>301</b>
	Fateh Singh <sup>a*</sup> , Sadanand D. Hirpurkar <sup>a</sup> , Nidhi Rawat <sup>a</sup> , Sanjay Shakya <sup>b</sup> , Satish Kumar <sup>c</sup> , and Rajiv Kumar <sup>c</sup>	
<b>SS-01</b>	<b>Current trends in diagnostic and clinico-therapeutic aspects of snake bite in animals</b>	<b>303</b>
	A.U. Bhikane and R.K. Jadhav	
<b>SS-02</b>	<b>Role of advances in biotechnology for animal breeding</b>	<b>307</b>
	Dr. M. S. Vaidya	
<b>SS-03</b>	<b>Surgical strike to combat endometritis through different therapies with special reference to immunomodulation in dairy animals</b>	<b>312</b>
	Dr. M. N. Rangnekar <sup>1</sup> and Dr. N. M. Markandeya <sup>2</sup>	
<b>SS-04</b>	<b>Recent Advances in control of Parasitism</b>	<b>317</b>
	<i>B. W. NARLADKAR</i>	
<b>SS-05</b>	<b>Herbal therapy in Veterinary Practice : Recent Advances</b>	<b>332</b>
	Sudhir R Rajurkar	
<b>SS-06</b>	<b>RECENT ADVANCES IN DIAGNOSTIC IMAGING TECHNIQUES IN VETERINARY PRACTICE</b>	<b>335</b>
	Dr. V. D. Aher	
<b>SS-07</b>	<b>Recent Advances in Poultry Management with special reference to backyard poultry</b>	<b>339</b>
	M.V. Dhumal and M G Nikam	
<b>SS-08</b>	<b>Recent advances in diagnosis of neoplasms in Animals : An Overview</b>	<b>348</b>
	S.D. Moregaonkar and G. R. Gangane	
<b>SS-09</b>	<b>Recent advances in Immuno-biotechnology</b>	<b>352</b>
	Dr. S.S. Gaikwad	

**Key Note Address**

**Merits and de-merits of avian influenza vaccination in poultry**

Diwakar D. Kulkarni

ICAR-National Institute of High Security Animal Diseases

OIE Reference laboratory for Avian Influenza, Bhopal

Avian influenza virus (AIV) infection is globally a big threat for animal health, productivity, food security, and the livelihood of people involved in poultry industry. The direct economic loss of the poultry farmers is due to mortality, production loss, effect on national trade involving poultry and poultry products, costs involved in control operations of the disease. Indirectly the disease has damaging effect on trade and traffic due to post AI outbreaks control measures inside the country; reduction in both profit and sale of the birds and the products etc. and the threat of human transmission. Due to this, the disease is considered as high-rated trans-boundary disease.

Some strains of highly pathogenic avian influenza (HPAI) virus cause severe zoonotic disease and may have pandemic potential. It is important to note that this is a disease of birds and not a human flu pandemic. However, Recognizing there are potential human health consequences if this virus enters bird populations, one need to address worker safety issues as well as that of public health. Till 16<sup>th</sup> March 2017 as per WHO record, the total number of persons died due to H5N1 from 16 countries was 453 out of 859 confirmed infections. Since 2016, the human deaths are reported only from Egypt in 2017. There are reports of nonfatal but serious human infections with LPAI viruses, particularly H9N2 and less commonly from AIV belonging to subtypes H4, H5, H6, H7, H10, and H11. Apart from H5N1, both fatal and nonfatal infections have been reported in significant number with HPAI viruses belonging to subtypes H5N6, H7N9. Currently, due to ongoing circulation of various strains (H5N1, H5N2, H5N8, H7N8, etc), outbreaks of avian influenza continue to be a global public health concern. Till now, there were more than 8400 outbreaks of highly pathogenic avian influenza (HPAI) all over the world in poultry of which India had 124.

Any country planning to control AI by way of vaccination may follow the path as given below-

- a. Carry out risk analysis exercise in detail, collect all the required surveillance data about incidence, characterization of local virus isolates, losses etc.
- b. Decide objectives of vaccination, target population, biosecurity related issues
- c. Study the source of vaccine, its QC parameters, immune response and one type of vaccine for one country
- d. Decide on exit policy, period to end the program and periodical review mechanism.

- e. Take into account the ducks and water-bodies as possible sources for checking the re-entry of the virus
- f. Research funding for changes in the field isolates and immune responses to older and newer viruses.

**Advantages of vaccination:** Properly used good quality vaccines can accomplish multiple goals:

- (1) Vaccine protects the birds against clinical signs and death, naturally reducing economic losses.
- (2) It reduces shedding of field virus even if vaccinated poultry become infected with the same virus
- (3) It reduces contact transmission of the field virus and its' environmental spread because of low shedding.

**Note of caution before commencement of vaccination program:**

- 1. In the case of countries that export poultry products, the most favorable status is country freedom from AIV. Countries that follow vaccination are considered as infected and usually lose their export markets. This is the biggest economic loss of the country.
- 2. Current commercially available vaccines do not prevent the infection completely. Domestic ducks that play an important role in the epidemiology of H5N1 HPAIV are generally not covered completely and continue to excrete the virus.
- 3. Improper vaccination without monitoring may reduce or prevent clinical signs, but may not reduce virus shedding. Hence, apparently healthy birds may shed some amount of virus that would increase the risk of virus spread to other birds, human exposure or environmental contamination.
- 4. As the vaccinated birds do not clinically suffer from the disease, there is likelihood of silent spread of the infection.
- 5. Apart from their short lifespan, broilers are more difficult to get good antibody titers; turkeys are difficult to get consistent titers.
- 6. Post-vaccination, it may not be possible to differentiate whether the antibodies developed in the birds are due to natural infection or vaccination thus interfering with serological surveillance of avian influenza and therefore DIVA strategy needs to be incorporated.
- 7. In most of the developing countries, vaccination campaigns are ill designed, slow and not properly executed. This can complicate disease control and may even prompt spread of disease and emergence of antigenic variants. This is mostly observed in partial post-vaccine immunity, which could be due to prime vaccination without boosters, vaccine of low potency, immunosuppressive diseases, antigenic mismatching, an inappropriate vaccination schedule and insufficient vaccine dose.
- 8. It has been observed that various clades arising due to mutations do not allow the vaccinal immunity to respond uniformly. In countries such as Egypt and Indonesia, it has created difficulties to make the

necessary changes to vaccines quickly. As the viruses continue to change, good vaccine of today may not work well next year. The post-vaccinal active surveillance along with mutational change monitoring mechanism need to be in place to detect antigenic variants rapidly and to make change/adjustments to vaccines. It has also been observed that cross-clade protective immune responses to influenza are very poor.

9. In case improper vaccination, incomplete population coverage or unwarranted vaccination, there may be disproportionate increase in genetic diversity of influenza viruses.
10. Vaccination increases management expenditure per bird.
11. Vaccination should not be considered as a permanent measure. The need for vaccination should be reviewed regularly & exit strategy must be developed prior to program. In practicality, it becomes difficult to exit as seen in all of the countries using vaccination since 6-10 years could not exit nor the disease was eradicated.
12. Poultry farmers consider AI vaccination as a guaranty to protect their birds from the disease (which is a myth); their biosecurity is slack and creates more serious situations.

**Concluding comments:** Every country understands its strength and weakness for the public health system; hence it is inappropriate to advice either in favor or against vaccination policy. The only word of caution would be the first decision to be weighed thoroughly and exit policy is firmed up. There should be enough evidence made available from systematic surveillance to say that the disease cannot be controlled by other means and the vaccination against AI is utmost essential. This assertion is important because, once a country enters into the vaccine trap; it takes to long time to get out of the situation. It may be noted with the global experience of 10 years with AI outbreaks that no vaccinating country could be exited as on today. Ideally, a vaccine developed by in-house research from a locally isolated LPAI strain can be used in targeted vaccination program with a clear objective. Such certified vaccine, if at all to be used, should be prioritized for high value breeding stocks, zoo birds and precious breeds of exotic birds in captivity. Post vaccinal quarantine, surveillance and monitoring along with periodical review of the situation should be continued.

**References cited:**

- Capua I, Marangon S. (2006) Control of avian influenza in poultry. *Emerg Infect Dis.* 12(9):1319-24 <http://dx.doi.org/10.3201/eid1209.060430>
- James-Berry C.M. (2013) Vaccine control of avian influenza H5N1 in poultry: need for a positive marker. *J Vaccines Vaccin* 4:168. doi: 10.4172/2157-7560.1000168

- Lee D. H., Fusaro A., Song C.S., Suarez, D.L., Swayne D.E. (2016). Poultry vaccination-directed evolution of H9N2 low pathogenicity avian influenza viruses in Korea. *Virology* 488:225–231
- Martins N.R. da S. (2012) An Overview on Avian Influenza Brazilian J. Poult. Sc. 14 (2):71-158
- Peyre, M. Fusheng, G. Desvaux S. and Roger F. (2009) Avian influenza vaccines: a practical review in relation to their application in the field with a focus on the Asian experience *Epidemiol. & Infect.* 137(1): 1-21.
- Read A.F., Baigent S.J., Powers C, Kgosana L.B., Blackwell L, Smith L.P., Kennedy, D. A., Walkden-Brown, S. W., Nair V.K. (2015) Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS Bio.* 113 (7): e1002198. <https://doi.org/10.1371/journal.pbio.1002198>
- Sims L., Tripodi A., von Dobschuetz S, Gardner E., Aguanno R. (2016) Rational use of vaccination for prevention and control of H5 highly pathogenic avian influenza FAO Publication Focus On: 10, 1-10 <http://www.fao.org/3/a-i5625e.pdf>
- Suarez D. L. (2005) Overview of avian influenza DIVA test strategies. *Biologicals.* 33(4):221-6.
- Swayne D. E., Pavade G. Hamilton K., Vallat B. & Miyagishima K. (2011) Assessment of national strategies for control of high-pathogenicity avian influenza and low pathogenicity notifiable avian influenza in poultry, with emphasis on vaccines and vaccination. *Rev. Sci. Tech. Off. Int. Epiz.* **30**, 839–870, doi: 10.20506/rst.30.3.2081
- World Organization for Animal Health (2016). Terrestrial Animal Health Code–2005. Chapter 2.7.12, avian influenza. Available from [http://www.oie.int/eng/normes/mcode/en\\_chapitre\\_2.7.12.htm](http://www.oie.int/eng/normes/mcode/en_chapitre_2.7.12.htm)
- World Organization for Animal Health. (2017) [http://www.oie.int/fileadmin/Home/eng/Animal\\_Health\\_in\\_the\\_World/docs/pdf/OIE\\_AI\\_situation\\_report/OIE\\_SituationReport\\_AI\\_18Sept2017.pdf](http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_report/OIE_SituationReport_AI_18Sept2017.pdf)

**T1**

**Anti-Microbial Resistance**

**T1-01**

## **Antimicrobial resistance: Current Status on its Containment Strategies in India**

S.D. Hirpurkar<sup>1</sup> and Fateh Singh<sup>2</sup>

<sup>1</sup>Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Anjora, Durg, Chhattisgarh

<sup>2</sup>ICAR-National Institute of High Security Animal Diseases, Anand Nagar, Bhopal

### **Introduction**

Antimicrobial resistance (AMR) is an emerging problem that is causing threat to public health due to ineffective control of communicable diseases. The discovery of antibiotics saved millions of lives from infectious diseases. In India, the unregulated use, uncontrolled sale without prescription has led to AMR development (Laxminarayan and Chaudhury. 2016). Antimicrobials are being used in prophylactic dose to enhance the productivity of food animals (Van Boeckel *et al.*, 2015). Surveillance on AMR, its environmental distribution and how anthropogenic, other biotic, abiotic inputs affect their spread, will aid in establishing the strategies to combat it (Khan and Nordmann, 2012). In 2014 an advisory was issued by Department of Animal Husbandry, Dairying and Fisheries, Government of India to all the States and Union territories for use of antimicrobials in food producing animals. However, there is no sound regulation so far in India and most of the countries, which is the most prior present need for the appropriate use of antimicrobials in food animals.

### **Mechanisms of antimicrobial resistance**

Consistent use of antimicrobials develops selective pressure on bacteria and induces their dynamic strategic evolution against the used antimicrobials resulting in survival and spread of resistant mutants. By means of complex evolution, the bacteria escape the inhibitory or bactericidal effects of antibiotics. Indiscriminate use of antibiotics, which leads to emergence of resistant strains, is mainly after irrational prescription of antibiotics by medical community (Roy *et al.*, 2007).

Bacteria acquire the antimicrobial resistance by means of spontaneous or induced genetic mutations. The genes confer resistance can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation and any antibiotic resistance genes reside on its plasmids, facilitate the gene transfer among different bacterial species (Livermore, 2003; Lupo *et al.*, 2012). The most common transferable antimicrobial resistance is developed by means of bacterial beta-lactamases such as extended spectrum beta-lactamases (ESBLs), AmpC-beta-lactamases and carbapenemases-which mediate deactivation of beta-lactam antibiotics (Thomson, 2010; Lupo *et al.*, 2012). Carbapenem resistance can

also be mediated by porin changes and changes in PBP (Tenover *et al.*, 2006). Moreover, the production of cephalosporinase (AmpC-beta-lactamase and ESBLs have low level carbapenemase activity) also develops enterobacteria resistant to carbapenems (Clinical and Laboratory Standards Institute, 2015).

Alteration in penicillin binding protein (PBP) is common in methicillin-resistant *Staphylococcus aureus* (MRSA) and other penicillin-resistant bacteria. Resistance to sulphonamides is mediated by alteration in metabolic pathway. Change in membrane porosity leads to reduced drug accumulation by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface also results in survival of bacteria. Efflux pump mediated carbapenem- resistance is common in *Enterobacteriaceae* bacterial isolates (Srinivasan *et al.*, 2015). One of the major causes of antimicrobial resistance is the decrease in effective drug concentration at the target site, due to the increased action of ABC transporters (Ponte-Sucre, 2009).

### **Antimicrobial use and resistance**

Earlier studies on AMR revealed low prevalence of antibiotic-resistant bacteria before the widespread use of antibiotics. The indiscriminate use antibiotics has increased now a days to maintain the human and animal health, and to increase the livestock production. Seventy-six percent of the overall increase in global antibiotic consumption between 2000 and 2010 was shared by BRICS countries (Brazil, Russia, India, China, and South Africa), in which upto 57% increased use was associated with hospital sector of China followed by 23% of the increase in the retail antibiotic sales in India (Van Boeckel *et al.*, 2014).

Reduction in the development of AMR can be achieved by an appropriate antibiotic, specific diagnosis of antimicrobial resistance is essential to deal with control, restricting the spread of antibiotic-resistant pathogens. Subsequently, surveillance of antibiotic resistance could be a valuable tool to screen the resistance trends on a population level (Kwak *et al.*, 2015).

### **AMR and its containment in India**

In India, the overuse of antimicrobials is attributed to high prevalence of antimicrobial resistant bacteria in humans (Datta *et al.*, 2012; Chakraborty *et al.*, 2013), animals (Singh, 2015), and environment (Kumarasamy *et al.*, 2010; Walsh *et al.*, 2011). Though, the antibiotic consumption pattern in human has been tagged in many countries; mapping of antibiotic consumption trend in animals is still incomplete. It is presumed that global consumption of antimicrobials in animals is double that of humans. One analysis on global antibiotic use in livestock indicated a total consumption of 63,151 tons in 2010 that will rise to 67% by 2030. It is predicted that the antimicrobial consumption by chicken and pig will increase by 129%

and 124%, respectively, in Asia, by 2030 (Van Boeckel *et al.*, 2015). Thus, rising consumer demand for food animal products and extensive farm management where antimicrobials are used routinely will likely to be responsible for exponential rise in antimicrobial consumption.

The National Action Plan on Antimicrobial Resistance (NAP-AMR) was drafted and further reviewed at the National Workshop on Development of National Action Plan on AMR. Six strategic priorities have been identified under the NAP-AMR

1. Improving awareness and understanding of AMR through effective communication, education and training;
2. Strengthening knowledge and evidence through surveillance;
3. Reducing the incidence of infection through effective infection prevention and control;
4. Optimizing the use of antimicrobial agents in health, animals and food;
5. Promoting investments for AMR activities, research and innovations; and
6. Strengthening India's leadership on AMR.

The efforts are needed to concentrate upon strategies, which aims to strengthen knowledge and evidence through surveillance of AMR, strengthening laboratories in human, animal, food and environment sectors. It also emphasize upon strengthening regulations for optimum use of antimicrobial agents in health, animals and food. Simultaneously, it aims to promote investments for AMR activities, research and innovations through new medicines and diagnostics, innovations to develop alternative approaches to manage infectious diseases, and sustainable financing to ensure adequate resources for containment of AMR. In addition, strengthening India's leadership on AMR through international collaborations to ensure India's contributions towards global efforts to contain AMR, is also needed.

#### AMR in man and animals

According to the NAP-AMR report (Anonymous, 2017), India is among the nations with the highest burden of bacterial infections. Consequently, the impact of AMR is likely to be higher in the Indian setting. Available data indicates to rising rates of AMR, across multiple pathogens of clinical importance, at the national scale. In 2008, about 29% of isolates of *Staphylococcus aureus* were methicillin resistant, and by 2014, this had risen to 47%. In contrast, in countries which have established effective antibiotic stewardship and/or infection prevention and control programs, the proportion of methicillin resistant *Staphylococcus aureus* (MRSA) isolates have been decreasing. Extended-spectrum beta-lactamases (ESBL) producing strains of *Enterobacteriaceae* have emerged as a challenge in hospitalized patients as well as in the community.

The report also documented various studies on development of AMR in some major pathogens viz *Klebsiella*, *E.coli*, *Pseudomonas*, *Enterococcus*, *Vibrio* etc. In Indian cities, 61% *E. coli* were ESBL producers. In the same study, 31-51% *Klebsiella* species were carbapenem resistant and 65% *Pseudomonas* sp. were resistant to ceftazidime, and 42% were resistant to imipenem.

The burden of AMR in livestock and food animals has been poorly documented in India. (Van Boeckel *et al.*, 2015). Drug resistant bacteria have been isolated from dairy cattle as early as the 1970s. One of the most common clinical issues encountered in the dairy farms is mastitis which maybe sub-clinical or overtly symptomatic. Commonly thought to be a disease of production, milk from mastitic cows and buffaloes have been shown to contain a wide range of bacteria, with a wide spectrum of resistance against commonly used antibiotics.

. In poultry sector, antibiotics are given as growth promoter through the premixed feed, which comes with added antibiotics that are not even mentioned on the label, it is difficult to exactly estimate the dose or the consumption levels of antibiotics in the poultry sector. Individual studies have consistently shown that bacteria isolated from animals or seafood has high levels of resistance.

#### Awareness and understanding of AMR

The initiative step constituting in effectively containing AMR is to improve awareness and understanding of AMR through effective communication, education and training. There is need to concentrate efforts to incorporate AMR as a core component in the professional education of medical and veterinary professionals and also to focus on awareness building, both in consumers and providers. This was highlighted by the results of the multi-country public awareness survey that was conducted by WHO in 2015.

#### Surveillance of AMR

Though Number of programmes are executed in India (viz Revised National Tuberculosis Control Programme, National Vector Borne Disease Control Programme (, and the National AIDS Control Programme etc), a cross-cutting programme dealing with antimicrobial resistance across multiple microbes has been lacking. The “National Programme on the Containment of Antimicrobial Resistance” was launched under the aegis of the National Centre for Disease Control (NCDC) under the 12th Five Year Plan (2012 – 2017). Network laboratories have been identified in the first phase of the programme, in course of which four pathogens of public health importance are being tracked: *Klebsiella* spp, *E. coli*, *Staphylococcus aureus*, and *Enterococcus* spp. The network intends to extend testing of resistance to two more index bacteria: *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Reporting from the laboratories puts overall resistance rates to be very high, against the commonly used fluoroquinolone, third generation cephalosporins and carbapenem. A strategy to scale the programme up in order to carry out surveillance of hospital acquired infections and antibiotic use patterns in healthcare settings has also been outlined with the help of national institutes. The Indian Council of Medical Research (ICMR) established a national network on surveillance of antimicrobial resistance in laboratories based at tertiary care academic centres, targeting medically important index microbes which have been identified by WHO.

The Antimicrobial Resistance Surveillance Research Network (AMRSN) established by the ICMR. The AMRSN incorporates in-depth understanding of clonality of drug resistant pathogens and the transmission dynamics to enable better understanding of AMR in Indian context and devise suitable interventions. The AMRSN, although currently limited to the human health side, plans to scale up on a national scale and expand its ambit to include samples from a wider spectrum of sources, including animal, environmental and food samples.

The critical comment is that, aside from the absence of approach to surveillance in animals, another weakness of the existing surveillance systems for AMR in India is that it does not account for antibiotic use. The existence of a surveillance system that can establish the relationship between the antibiotic consumption patterns and emergence of AMR is vital to producing evidence that may help in the designing and evaluation of effective interventions.

#### *Infection prevention and control*

The ICMR guidelines on infection control, which are locally relevant and implementable for India, were released last year at the international conference on AMR. It noted the need to establish functional hospital infection control committees (HICCs) to provide leadership to the IPC programs at the institutional level. NCDC has recently developed guidelines for hospital infection prevention and control and is also developing a policy on infection control, which is in the final phases of preparation.

#### *Use of antimicrobials*

With respect to consumption of antimicrobials in food animals, the global consumption was estimated to be 63,151 ( $\pm$ 1,560) units in 2010; India accounts for 3% of the global consumption and is the fourth highest in the world, behind China (23%), the United States (13%) and Brazil (9%). The consumption of antimicrobials in the food animals sector in India is expected to double by 2030.

The absence of stringently framed and implemented regulatory frameworks to limit the use of antimicrobials in livestock and food animals, especially for non-therapeutic purposes, like growth

promotions, has been one of the drivers of antibiotic overuse at the community level. A more recent directive has been issued in January 2015 by the FSSAI. The National AMR Containment Policy highlighted the need to establish a separate Schedule H1, under the Drugs and Cosmetics Rules, to regulate the sales of antibiotics. Guidelines for punitive actions against agencies that are in contravention of such policies are also outlined. The national policy also outlined the proposal for colour-coding antibiotic strips, and newer molecules (carbapenems, tigecycline, daptomycin, etc.), to eliminate their use outside of tertiary care settings.

### Research and innovations

It is observed that pharmaceutical companies have been reluctant to invest in research and development of antibiotics owing to the nature of the market, the current policies on conservation of newer classes of antimicrobials and the nature of antibiotic chemotherapy for infectious diseases. Despite the obvious need to develop newer classes of drugs to respond to the challenges of emerging AMR, there are few late stage candidates in the process of development.

Development of antibiotic resistance breakers (ARBs) to restore effectiveness of older classes of antibiotics has also emerged as an innovative way around the issue of resistance. ARBs can be known compounds as well as novel molecules which have no or minimal antibacterial activity, but which help in restoring the effectiveness of drugs to which the microbes would otherwise be resistant. The current consensus seems to be that there is a need to develop ARBs that are likely to salvage key members of each group of antibiotics, especially those that target Gram negative bacteria.

### National response

Acknowledging the problem of antimicrobial resistance, the Government of India has taken series of initiatives to tackle the growing antimicrobial resistance including constitution of a National Task Force on AMR Containment in 2010 leading to development of the national policy on AMR containment in early 2011. The Ministry of Health and Family Welfare, Government of India launched the National Programme on Containment of Antimicrobial Resistance under the Twelfth Five Year Plan (2012–2017). In India efforts are also being made to incorporate the One Health approach into these plans.

### **Alternates of antimicrobial therapy**

Now, it has been proved that bacteria dominated the human approach of antibiotic development. Due to shrinkage in effective spectrum of antibiotics against MDR pathogens, research and development of alternate approaches is future priority to tackle these emerging threats. Few alternatives of antibiotics

have been developed but they attract a little importance. One alternative is to prevent diseases by adding cytokines instead of antibiotics to animal feed. Few studies on using cytokines have shown that they also enhance the growth of animals without the harmful effect as of antibiotics on human health. Though bearing a less efficacy, another alternate may be the suitable vaccines which –unlike- to the antibiotics do not have the problem of resistance due to their ability to act by enhancing the body's natural defence (Sharma *et al.*, 2014).

Bacteriophages may be another alternate for treatment of bacterial diseases that have been extensively researched and used as a therapy for over 60 years, especially in the Soviet Union (Keen, 2012). Phage therapy has been widely used in the United States until the discovery of antibiotics, in the early 1940s. A review of studies that dealt with the therapeutic use of phages from 1966–1996 and recent research on phage therapy showed topical, oral or systemic use of phages in Polish and Soviet studies at a success rate of 80–95% with few gastrointestinal or allergic side effects. British studies also demonstrated significant efficacy of phages against *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas* spp. and *Staphylococcus aureus*. Phage therapy may prove as an important alternative to antibiotics for treating MDR pathogens (McGrath and van Sinderen, 2007).

One of the major causes of antibiotic resistance is the decrease in effective drug concentrations at their target site, due to the increased action of ABC transporters. Since ABC transporter blockers can be used in combination with current drugs to increase their effective intracellular concentration, the possible impact of ABC transporter inhibitors is of great clinical interest (Ponte-Sucre, 2009).

## Conclusion

Antimicrobial resistance has created a global crisis by use of all classes of antibiotics in humans and animals. Resistant bacteria in food-producing animals are of particular concern. The present potent crisis is further complicated by a marked decrease in the industry research and development of new antibiotics. Specific, rapid and reliable diagnostic techniques are primarily essential to meet with the requirements of the antimicrobial resistance control programs. Alternate approaches to antibiotics may be explored to reduce antimicrobial use. Establishment of coordination between all the stakeholders such as farmers, livestock owners, clinicians, entrepreneurs, government and non-government organisations, policy makers, diagnostic service providers, research organizations, and antibiotic-end-users, is a present priority to curb the indiscriminate use of antibiotics in near future. Since all kinds of antimicrobial uses in humans and animals contribute to the emergence, persistence, and spread of resistant bacteria, there is an urgent need of effective antibiotic stewardship program and Acts of regulation on antibiotic use to attain health security, and to safe and sustainable livestock production.

## References

- Anonymous (2017) National plan on antimicrobial resistance: WHO report co-ordinated by Ministry of Health and family welfare, Government of India
- Chakraborty, A., Adhikari, P., Shenoy, S., Baliga, S., Rao, S., Biranthabail, D. and Saralaya, V. (2013). Expression of ESBL, MBL and AmpC  $\beta$ -lactamases by extra intestinal *Escherichia coli* isolates: correlation with treatment and clinical outcome. J. Microbiol. Infect. Dis. 3(4):150-156.
- Clinical and Laboratory Standards Institute. (2015). Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.
- Datta, S., Wattal, C., Goel, N., Oberoi, J.K., Raveendran, R. and Prasad, K.J. (2012). A ten year analysis of multi-drug resistant blood stream infections caused by *Escherichia coli* & *Klebsiella pneumoniae* in a tertiary care hospital. Indian J. Med. Res. 135(6): 907-912.
- Keen, E.C. (2012). Phage therapy: concept to cure. Front. Microbiol. 3(238):1-3.
- Khan, A.U. and Nordmann, P. (2012). Spread of carbapenemase NDM-1 producers: the situation in India and what may be proposed. Scand. J. Infect. Dis. 44(7):531-535.
- Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F.A., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S., Krishnan, P., Kumar, A.V., Maharjan, S., Mushtaq, S., Noorie, T., Paterson, D.L., Pearson, A., Perry, C., Pike, R., Rao, B., Ray, U., Sarma, J.B., Sharma, M., Sheridan, E., Thirunarayan, M.A., Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore, D.M. and Woodford, N. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis. 10(9):597-602.
- Kwak, Y.K., Colque, P., Byfors, S., Giske, C.G., Mollby, R. and Kuhn, I. (2015). Surveillance of antimicrobial resistance among *Escherichia coli* in wastewater in Stockholm during 1 year: does it reflect the resistance trends in the society? Int. J. Antimicrob. Agents. 45(1):25-32.
- Laxminarayan, R. and Chaudhury, R.R. (2016). Antibiotic Resistance in India: Drivers and Opportunities for Action. PLoS Med. 13(3):e1001974.
- Livermore, D.M. (2003). Bacterial Resistance: Origins, Epidemiology, and Impact. Clin. Infect. Dis. 36(Supple. 1):S11-S23.
- Lupo, A., Coyne, S. and Berendonk, T.U. (2012). Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front. Microbiol. 3(18):1-13.
- McGrath, S. and van Sinderen, D. (2007). Bacteriophage: Genetics and Molecular Biology, 1st edn. Caister Academic Press. <http://www.caister.com/order>.

- Ponte-Sucre, A. (2009). ABC Transporters in Microorganisms. Caister Academic Press. Great Yarmouth, Norfolk, United Kingdom.
- Roy, N., Madhiwalla, N. and Pai, S.A. (2007). Drug promotional practices in Mumbai: a qualitative study. *Indian J. Med. Ethics.* 4(2):57-61.
- Sharma, C., Singh, C., Sharma, L.N., Purvia, R. and Adlakha, M. (2014). Antibiotic resistant organism: an emerging public health problem and role of Ayurveda (an overview). *Int. J. Ayur. Pharm. Res.* 2(1):17-29.
- Singh, F. (2015). Studies on extended-spectrum beta-lactamases and carbapenemases coding genes that confer multi-drug resistance to enteropathogenic bacteria. Ph.D. thesis, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh.
- Srinivasan, R., Bhaskar, M., Kalaiarasan, E. and Narasimha, H.B. (2015). Prevalence and characterization of carbapenemase producing isolates of Enterobacteriaceae obtained from clinical and environmental samples: Efflux pump inhibitor study. *African J. Microbiol. Res.* 9(17):1200-1204.
- Tenover, F.C., Kalsi, R.K., Williams, P.P., Carey, R.B., Stocker, S., Lonsway, D., Rasheed, J.K., Biddle, J.W., McGown, J.E. and Hanna, B. (2006). Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. *Emerg. Infect. Dis.* 12(8):1209-1213.
- Thomson, K.S. (2010). Extended-Spectrum-â-Lactamase, AmpC, and Carbapenemase Issues. *J. Clin. Microbiol.* 48(4):1019-1025.
- Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A. and Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci.* 112(18):5649-5654.
- Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A. and Laxminarayan, R. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* 14(8):742-750.
- Walsh, T.R., Weeks, J., Livermore, D.M. and Toleman, M.A. (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect. Dis.* 11(5):355-362.

**T1-02**

## **Antimicrobial Resistance : One Health Approach to combat**

Sandeep Chaudhari<sup>1</sup> and Shilpa Moon<sup>2</sup>

Department of Veterinary Public health, Nagpur Veterinary College  
Maharashtra Animal And Fishery Sciences University, Nagpur-440006

### **Antimicrobial Use**

The discovery of antimicrobials and their development to treat bacterial infections in humans and animals was one of the most important achievements of the 20th Century. Antimicrobials are used to treat or prevent infections (caused by bacteria, virus, fungi etc) in humans or animals (WHO, 2015). Since antimicrobials were first commercially produced, initially for use in human medicine and subsequently in veterinary medicine, their use has been associated with the risk of emergence of Antimicrobial resistance (AMR). AMR is the ability of microorganisms, such as bacteria, to become increasingly resistant to an antimicrobial to which they were previously susceptible. At the same time as the world has observed accelerated emergence of resistance, the discovery and development of new antimicrobial drugs has slowed down. The effectiveness of the existing antimicrobials should therefore be preserved as much as possible.

Antibiotics are used in many settings clinical medicine, communities, animal husbandry or aquaculture, horticulture etc. Increase in antimicrobial use is driving increase in AMR in both humans and animals (CDDEP, 2016). The efficacy of antimicrobials is declining rapidly; this is because antimicrobials are readily available over the counter without prescription in many countries, resulting in its indiscriminate use. The dissemination of counterfeit or substandard antibiotics by unscrupulous producers in the absence of credible drug regulations and law enforcement also contributes to increased resistance. Research and development of newer antimicrobials is inadequate. AMR is a dominant global health problem, placed on the agenda of national policy makers, international organisations, and financial institutions in developed and developing countries alike.

### **Antimicrobial resistance in food production environment**

The emergence of ABR in the food chain is considered a cross-sectoral problem, as antibiotics are widely used in aquaculture, livestock production, and crop culture (Acar and Moulin, 2006), antibiotic-resistant bacteria and antibiotic-resistant genes (ARGs) can easily spread at each stage of the food production chain (da Costa *et al.*, 2013), and can cause infections in humans (Chang *et al.*, 2015). There are several studies having reported food animals and products being infected and contaminated by antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Price *et al.*, 2012), antibiotic-resistant *Campylobacter* spp. (Ewnetu and Mihret, 2010) and extended spectrum-beta-lactamase (ESBL)

producing *Enterobacteriaceae* (viz. *Escherichia coli*, *Salmonella*, *Shigella* and *Klebsiella* spp., etc.) (Al-Bayssari *et al.*, 2015). The situation has been recently worsened with the emergence of antibiotic-resistant bacteria having significant pandemic potential, such as carbapenem-resistant *Enterobacteriaceae* (CRE) (harboring a VIM-1 carbapenemase resistant to the beta-lactam antibiotics family plus additional co-resistance) (Fischer *et al.*, 2012) and colistin-resistant *E. coli* (harboring mcr-1 gene and co-resistance genes) (Liu *et al.*, 2016), as well as emerging livestock associated-methicillin resistant *S. aureus* (LA-MRSA; Price *et al.*, 2012).

These antibiotic resistant bacteria or ARGs may reach humans indirectly along the food chain through consumption of contaminated food or food derived products or following direct contact with infected animals or biological substances such as blood, urine, feces, saliva, and semen among others (Chang *et al.*, 2015). The use of antimicrobial drugs in food-producing animals can result in antimicrobial drug resistant infections in humans. A good example is the use of fluoroquinolone in poultry and the emergence of fluoroquinolone-resistant *Campylobacter* infections among humans. A similar case is the emergence of a plasmid-mediated resistance gene (mcr-1) to colistin, a last resort antibiotic, identified in people and pigs in China (Liu *et al.*, 2016). This was followed by its rapid spread across Europe and Canada to the USA, where it has recently been identified (McGann *et al.*, 2016).

The contribution of animal production, to the global AMR crises is questioned by some on the grounds that we don't see so many animal associated infections in humans (Chang *et al.*, 2015). While this may be true, because of the way that many antibiotics are used in animal production, in sub-therapeutic doses and with long exposure periods, these production systems create ideal conditions for bacteria to fix genes that confer resistance. These genes can subsequently be transmitted to human adapted pathogens or to human gut microbiota via people, contaminated food or the environment. They also provide ideal conditions for the amplification of genes that may have arisen in people or the environment. The fact that the antibiotics used in human and animal health largely comprise the same or very similar molecules would be expected to drive the transmission of resistance between animals and people, either directly or via the environment.

### **Antimicrobial resistance a global problem**

Antimicrobial resistance has no geographical boundary; it tends to get transmitted between states, countries and even continents. The globalisation of the food system, with increasing movement of livestock and agricultural produce, combined with increasing human travel, facilitates the rapid spread and mixing of AMR genes that emerge (Robinson *et al.*, 2016). No matter how good a country's programme of antimicrobial stewardship in health and agriculture, they are laid bare to the importation of AMR genes that have emerged and spread from other parts of the world. The other factor making AMR a one world

issue is the greatest burden of AMR in low middle income countries (LMICs) because of their disease prone environments, poorer sanitary standards and, for the poorest, much reduced access to effective antibiotics (Laxminarayan *et al.*, 2015). Poor access to competent veterinary and extension services leave farmers in LMICs with antibiotics as their only resource to tackle endemic bacterial animal infections. Interventions must be based on an understanding of and respect for the different social and socioeconomic contexts in which they are to be implemented. AMR is a global problem needs global solutions but the solutions will not be the same in every country, or among different socioeconomic groups because some excessively use antimicrobials, some uses little and mostly use them unwisely, understanding patterns of use and incentives for changing these, and exploring alternative options, must underpin any reduction efforts.

### **Antimicrobial resistance in India**

In 2010, India was the world's largest consumer of antimicrobials for human health due to the convergence of factors such as poor public health infrastructure, rising incomes, a high burden of disease, cheap and unregulated sale of antimicrobials (Laxminarayan and Chaudhury 2016).

Growing antimicrobial use in the animal sector is resulting in a greater selection of pathogens (Van Boeckel *et al.*, 2015) and is being driven by increased demand for meat and poultry. The extreme growth in consumption of chickens is primarily the result of the expansion of this sector in India alone, where areas of high consumption (30 kg per km<sup>2</sup>) are expected to grow 312% by 2030 (Van Boeckel *et al.*, 2015). A recent Organisation for Economic Co-operation and Development (OECD) report indicated that the costs of withdrawing antimicrobial growth promoters in India would be roughly US \$1.1 billion (Laxminarayan *et al.*, 2015). However, widespread resistance may hold more consequence for India than for other countries because of India's high bacterial disease burden. Currently, India does not have regulatory provisions for the use of antimicrobials in cattle, chickens, and pigs raised for domestic consumption. Recent studies in various regions of India have discovered antimicrobial residues in food animal products (such as chicken meat and milk) (Laxminarayan *et al.*, 2015), indicating that antibiotic use in food animal production is widespread. There are no standards for tolerance of antibiotic residues in poultry, although such standards do exist for seafood including shrimps, prawns, or any other variety of fish and fishery products under the Food Safety and Standards (Contaminants, Toxins, and Residues) Regulations of 2011; more recently, standards for honey have also been developed (Sahu *et al.*, 2014). Effective limits on antimicrobial growth promoters in India could have knock-on effects on neighbors such as Bangladesh, Nepal, and Sri Lanka that are likely to be guided and influenced by regulatory action in India, given the interconnectedness of the region's pharmaceutical commerce (Basnyat, 2014).

On the positive side, efforts by groups like the Indian Association of Pediatrics, the Global Antibiotic Resistance Partnership (Ganguly *et al.*, 2013), and the Chennai Declaration (Ghafur, 2013) have helped build awareness about the problem among professional bodies, the media, policy makers, and the lay public. A high-level committee has been convened to address the issue at the Ministry of Health and is expected to issue recommendations soon.

### **The need of multisectoral approach**

Addressing the rising threat of AMR requires a holistic and multisectoral approach because antimicrobials used to treat various infectious diseases in animals may be the same or be similar to those used in humans. Resistant bacteria arising either in humans, animals or the environment may spread from one to the other, and from one country to another. The one health approach is the collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals and environment (AVMA, 2008).

AMR has clear links to each of these three domains. The global burden of AMR on the population has been estimated (WHO, 2014) and more specific studies have also been carried out. Out of the three domains, human health takes the spotlight, with multidrug-resistance genes now highly prevalent in many important and common pathogens like *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Such estimates of the burden of AMR have not been attempted for livestock or aquaculture, perhaps due to a perceived lower importance. Agricultural use contributes significantly to AMR. A recent review suggested that misuse of antimicrobials in animal production is a clear and substantial driver of AMR (Ji *et al.*, 2012) and there is a growing body of evidence linking antibiotic consumption in livestock to AMR in the clinic (Robinson, 2016). The third One Health pillar, environmental health, including that of crops where antimicrobials are also used in plant protection (Stockwell and Duffy, 2012), is the least well understood in this tangled web of antibiotic gene evolution, transmission and persistence. Environmental bacteria, being quantitatively the most prevalent organisms, serve as sources for AMR genes that can become incorporated, over time, into pathogens of people and animals. This naturally occurring phenomenon is exacerbated by the influx to the environment of AMR genes from livestock and human waste (Reinthler *et al.*, 2003) and by the vast quantities of antibiotic residues that enter the environment from the pharmaceutical industry (Larsson *et al.*, 2007) from intensive livestock farms (Ji *et al.*, 2012) and from hospitals (Diwan *et al.*, 2010). Because of this, it has been proposed that these activities represent effective hotspots for the development and spread of AMR genes (Martine *et al.*, 2009). The impact of such factors is likely to be more pronounced in developing countries with lax environmental legislation and enforcement.

One health science seems to fall short when it comes to understanding the economic forces behind many emerging infectious diseases; there is a further gap to be bridged between the biomedical,

environmental and animal sciences, and the social sciences. Integrated approaches to reduce selection pressure and disrupt AMR transmission cycles on a global scale must be sought that are founded not only on sound One Health principles, but also based on economic evidence and on principles of social equity and global access to effective healthcare for people and their animals.

Many organizations have committed to the idea that One Health can be an effective approach to address complex public health problems, especially antimicrobial resistance. By bringing together experts in many different fields, One Health encourages innovative problem solving and provides opportunities to enhance the tools and processes on which public health depends for designing, implementing and evaluating disease prevention and control programmes. One Health surveillance can draw data from multiple sources and monitoring systems, integrate, analyze and interpret it in a framework that crosses the domains of human and animal health and environmental science and serves as an example of both the potential benefits as well as the notable challenges in creating and maintaining effective One Health collaborations.

Whatever action we take will only be sustainable if it is based on a sound understanding of the relative roles of people, animals and the environment in the emergence, spread and persistence of AMR genes. This is not a problem that high income countries (HICs) will be able to solve alone. The important role that animal agriculture plays in livelihoods in LMICs is unknown or underestimated by many in HICs and needs to be an integral part of the thinking and negotiation if we want to avoid the pitfalls seen in climate negotiations, with LMICs often reluctant to take measures that may compromise their short term economic development.

## References

- Acar JF and Moulin G. (2013). Integrating animal health surveillance and food safety: the issue of antimicrobial resistance. *Rev. Sci. Tech.* **32**: 383–392.
- Al Bayssari C, Dabboussi F, Hamze M And Rolain JM. (2015). Emergence of carbapenemases producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in livestock animals in Lebanon. *J. Antimicrob. Chemother.* **70**: 950–51.
- AVMA. (2008). One health: A new professional imperative. One Health Initiative Task Force Final Report. Schaumburg, IL: American Veterinary Medical Association.
- Basnyat B. (2014). Antibiotic resistance needs global solutions. *Lancet Infect. Dis.* **14**(7): 549–50.
- Center for Disease Dynamics, Economics and Policy (CDDEP). (2016). Antibiotic use and resistance in food animal current policies and recommendations.
- Chang Q, Wang W and Regev-Yochay G. (2015). Antibiotics in agriculture and the risk to human health: how worried should we be? *Evol. Appl.* **8**: 240–5.

- Da Costa PM, Loureiro L and Matos AJF. (2013). Transfer of multi-drug resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. *Int. J. Environ. Res. Public Health* **10**: 278–94.
- Diwan V, Tamhankar AJ, Khandal RK *et al.* (2010). Antibiotics and antibiotic resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health* **10**: 414.
- Ewnetu D and Mihret A. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates from humans and chickens in Bahir Dar, Ethiopia. *Food borne Pathog. Dis.* **7**: 667–70.
- Fischer J, Rodriguez I, Schmoger S, Friese A, Roesler U, Helmuth R., *et al.* (2012). *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. *J. Antimicrob. Chemother.* **67**:1793–95.
- Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U, *et al.* (2011). Rationalizing antibiotic use to limit antibiotic resistance in India. *The Indian J. of medi. Res.* **134**: 281–94.
- Ghafur A. (2013). The Chennai Declaration: a solution to the antimicrobial resistance problem in the Indian subcontinent. *Clin Infect Dis.* **56**(8): 1190.
- Ji X, Shen O, Liu F *et al.* (2012). Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; *China. J. Hazard Mater.* **235–236**:178–85.
- Larsson DGJ, de Pedro C, Paxeus N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J. Hazard Mater* **148**:751–5.
- Laxminarayan R and Chaudhury RR. (2016). Antibiotic resistance in India: drivers and opportunities for action. *PLoS Med.* **13**(3): e1001974.doi:10.1371/journal.pmed.1001974
- Laxminarayan R, Matsoso P, Pant S *et al.* (2015). Access to effective antimicrobials: a worldwide challenge. *Lancet.* **387**:168–75.
- Laxminarayan R, Van Boeckel TP and Teillant A. (2015). The economic costs of withdrawing antimicrobial growth promoters from the livestock sector. Paris: Organization for Economic Cooperation and Development.
- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer, J., *et al.* (2016). Emergence of plasmid-mediated colistin resistance mechanism *mcr-1* in animals and human beings in China: amicrobiological and molecular biological study. *Lancet Infect. Dis.* **16**: 161–8.
- Martinez JL. (2009). The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc. Royal. Soc. B.* **276**: 2521–30.
- McGann P, Snesrud E, Maybank R *et al.* (2016). *Escherichia coli* harboring *mcr-1* and *blaCTX-M* on a novel IncF plasmid: First report of *mcr-1* in the USA. *Antimicrob. Agents. Chemother.* **60**: 4420–21.
- Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS., *et al.* (2012). *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock.

- Robinson TP, Wertheim HFL, Kakkar M *et al.* Animal production and antimicrobial resistance in the clinic. *Lancet* **387**:e1–e3. doi:10.1016/ S0140-6736(15)00730-8.
- Sahu R and Saxena P. (2014). Antibiotics in Chicken Meat. New Delhi, India: Centre for Science and Environment. PML/PR-48/2014.
- Stockwell VO and Duffy B. (2012). Use of antibiotics in plant agriculture. *Rev. Sci. Tech. Off. Int. Epiz.* **31**: 199–210
- Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, *et al.* (2014). Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **14** (8): 742–50.
- World Health Organization (WHO). (2014). Antimicrobial resistance: Global report on surveillance. Geneva: World Health Organization.
- World Health Organization (WHO). (2015). Global Action Plan on Antimicrobial Resistance. Geneva: World Health Organization.

**T1-03**

## **ANTIMICROBIAL RESISTANCE: POTENTIAL RISK TO HUMAN AND LIVESTOCK HEALTH**

V. C. Ingle, S. P. Awandkar and P.A. Tembhrune

Department of Veterinary Microbiology and Animal Biotechnology  
Nagpur Veterinary College, MAFSU, Nagpur-06

### **Abstract**

The acquired ability of bacteria to multiply and grow in presence of antibiotic(s) to which they were previously susceptible is termed as antibiotic resistance. The effective antibiotic becomes ineffective after gaining antibiotic resistance by the bacteria. Such antibiotic either cannot kill bacteria or limit their growth. Various mechanisms are involved in acquiring AMR. They include horizontal plasmid transfer and vertical evolution comprising genetic mutations and other mechanisms which confer upon the AMR to bacteria. Many bacteria of public health importance *viz.* *Salmonella*, *E. coli*, *S. aureus*, *Pseudomonas*, *Streptococcus*, *Mycobacterium* *etc* are showing increasing antimicrobial resistance. Moreover, *Mycobacterium spp.* showed multi-drug resistance (MDR) and many of them are extensively drug resistant (XDR). Many of them have potential to infect livestock also. The antimicrobial resistance (AMR) trends are indicative of increasing ineffectiveness of antibiotics. Very few antibiotics could be developed during last two decades. Development of a successful and effective antibiotic takes more than two decades. Therefore, emergence of AMR to multiple antibiotics is considered as a huge threat to global public

health. In the country like India, AMR pose not only serious public threat in terms of health, but in terms of food security, food safety, high disease burden, economic losses, livelihood and sustainability of marginal and landless livestock farmers, because the livelihood of a large Indian human population is dependent on livestock farming in rural and urban localities. Therefore, it is bound to impact achieving developmental goals.

### **Mechanisms of action of antibacterial agents**

To understand how the bacteria develop AMR, one should know how the antimicrobials work. The antibacterial agents may work on various principles. They are summarized as follows.

#### **1. Interference with cell wall synthesis**

$\beta$ -Lactams: Penicillins, Cephalosporins, Carbapenems, Monobactams

Glycopeptides: Vancomycin, Teicoplanin

#### **2. Protein synthesis inhibition**

Bind to 50S ribosomal subunit: Macrolides, Chloramphenicol, Clindamycin, Quinupristin-Dalfopristin, Linezolid

Bind to 30S ribosomal subunit: Aminoglycosides, Tetracyclines

Bind to bacterial isoleucyl-tRNA synthetase: Mupirocin

#### **3. Interference with nucleic acid synthesis**

Inhibit DNA synthesis: Fluoroquinolones

Inhibit RNA synthesis: Rifampin

#### **4. Inhibition of metabolic pathway**

Sulfonamides, Folic acid analogues

#### **5. Disruption of bacterial membrane structure**

Polymyxins, Daptomycin

### **Mechanisms of AMR**

A variety of mechanisms are involved in becoming the bacteria resistant to antibacterial agents. These mechanisms are documented in variety of bacterial genera. The innate resistance can be seen in some species of bacteria to certain antimicrobial agents. All the members from such species show resistance to the antimicrobials. However, acquired AMR by initially susceptible bacteria to a particular antibiotic is attracting greater concern due to its prolific spread under selection pressure. The mechanisms involved in evolution of AMR in bacteria are broadly classified in to following two groups.

#### **1. Horizontal transfer of AMR gene(s) / R plasmid**

The bacteria may acquire AMR gene(s) or R plasmid horizontally from donor bacteria or surrounding environment. The genetic mechanisms involved in horizontal gene transfer in bacteria are conjugation,

transduction and transformation. The bacteria can gain AMR through acceptance of resistance genes or extra-chromosomal genetic material from resistant strains of same or different species and genera. During conjugation, a gram negative donor bacterium (resistant) transfers plasmid containing resistance genes, to recipient bacterium (susceptible) via conjugation tube formed by elongated proteinaceous sex pilus, joining both of them. Gram positive bacteria also may exhibit conjugation by production of sex pheromones by the mating pair, facilitating the clumping and exchange of DNA from donor to recipient bacterium. Rarely, resistance genes are transferred from resistant bacterium to another via bacteriophage, which is termed as transduction. Bacteria may acquire DNA released by other bacteria in their environment, after cell lysis. This process often termed as transformation, can move resistance genes into previously susceptible bacteria.

These mechanisms are suggested for transfer of AMR genes horizontally. Once a bacterium becomes resistant to any antibiotic, its progeny maintains the AMR gene(s) or R plasmid under low level environmental antibiotic selection pressure.

## **2. Evolution of bacterial systems to make antibiotic ineffective (vertical evolution)**

The chromosomal mutation and adoption of genes responsible for antibacterial resistance by the bacteria is termed as vertical evolution. These genes may code certain enzymes degrading antimicrobials before exerting their effect on bacteria. *Staphylococcus* acquired genes for erythromycin ribosomal methylase enzyme which made it erythromycin resistant. The bacteria may acquire efflux pumps those extrude the antibacterial agent from the cell before it can reach its target site and exert its effect. *S. aureus* showed upregulation of pumps that expel the fluoroquinolones from the cells. Bacteria routinely acquire many genes for its metabolic pathway. These genes may lead to alteration in components of bacterial structure like cell wall. The antibacterial cannot recognize altered structure of cell wall and bacteria become resistant to such antimicrobials. Penicillin resistant *Pneumococcus* showed alteration in penicillin binding protein from its cell wall. The continuous selection pressure may force bacteria to mutate more frequently and precisely in order to modify their genome for developing AMR. The bacterial mutations may limit access of antimicrobial agents to the intracellular target site via down regulation of porin genes. *E. coli* down regulate an outer membrane protein channel (*OmpF*) necessary for entry of antimicrobials inside bacterial cells.

A single mutation in a key gene may be responsible for limited reduction in susceptibility of the host bacteria to antibacterial agent. Such mutation just allows bacteria for initial survival. Eventually, bacterium acquires additional mutations or genetic information conferring full-fledged resistance to the antibacterial agent. However, single mutation may be sufficient for high-level resistance, rarely, as documented in *S. aureus* for rifampin and *Campylobacter jejuni* for fluoroquinolone.

The mechanisms of vertical evolution result in selection of antibiotic resistant strains under selection pressure allowing them to survive and grow while the susceptible strains are killed.

### **Key reasons for development of AMR**

The AMR was documented as early as 1948 to penicillin, just eight years after its discovery. The story continues since then for different available antibiotics. Notably, the AMR is increasingly reported from different regions globally. The global dissemination of AMR has been demonstrated due to increased use of antibiotics, movement of people and animals, developing industrialization and trade.

The indiscriminate and irrational use of antimicrobials is considered as one of the major culprits for AMR. However, many others also are contributing to it. The overdosing and under-dosing along with incomplete course of antibiotic treatment in animals is responsible for transfer of AMR from animal pathogens to potential human pathogens. Though the antibiotics designed for veterinary use are exclusively designed, most of them are used in human therapy. Few belong to the antibiotic classes those are used in human medicine. Moreover, most of them are structurally identical. The purpose of antibiotic use in animals is not limited to treatment of infections. They are used as growth promoters also. Almost 70% of the antibiotics administered in animals are intended to non-therapeutic purpose. The overuse of antibiotics in livestock sector is mainly intended to secure food and reduce economic losses to the farmers. Due to growing concerns over AMR, many agencies banned use of antibiotics used in human therapy in animals. Despite this, the medically important antibiotics are used to feed livestock just to increase profit margin by curbing infections in highly stressed animals due to overcrowding, intensive farming, high production traits and environmental hygiene. The increasing use of antibiotics is indebted to increased emergence and re-emergence of pathogenic bacteria. The emergence of AMR in bacteria with zoonotic potential is serious threat to public as well as animal health. The advance human antibiotics are often used to treat companion animals. Penicillinase producing *Staphylococci* and EMBL producing *E. coli* are examples for this. The intensive and mixed farming practices are involved in transfer of AMR from pathogens of one animal species to the pathogens of other animal species.

Low doses of antibiotics used routinely in food producing animals favour emergence of resistant bacteria in animals. Sub-optimum doses help step-wise selection of resistance. Such non-therapeutic use for reasons such as growth promotion and mass disease prevention is rampant across India in intensive farming of food-producing animals such as poultry. Resistant bacteria proliferate and can make resistant other bacteria that are present in animals. Resistant bacteria can also be transferred to humans through several routes such as direct contact of handlers, live animals and carcasses at poultry farms and slaughterhouses; human consumption of meat, eggs and milk with resistant bacteria; and environmental contamination of soil, water and air through animal excreta and farm waste. Besides resistant bacteria,

antibiotic residues in environment and those entering into humans through food chain may also create selective pressure in bacteria.

### **Strategies for reducing AMR**

The AMR can be reduced by practicing ethics of using antibiotics.

1. Stop adding antibiotics in animal feed as growth promoters. Acidifiers, enzymes prebiotics and probiotics may be used instead.
2. The veterinarians should use the antibiotics those are not used in human medicine.
3. There should not be under dosing.
4. The antibiotic course must be completed considering their half life in blood.
5. Overdosing of antibiotics should be avoided.
6. Alternatives to antibiotics may be opted.
7. Viral and minor infections may not be treated with antibiotics.
8. The host immune system should be boosted by use of vaccines and balance feeding.
9. Stress on animals may be minimized.
10. The organic farming practices may be promoted.
11. Biosecurity measures shall be followed.
12. The individual and environmental hygiene and sanitation may be observed.
13. The carcass and animal waste disposal must be proper.
14. Antibiotic sensitivity testing may be performed for proper antimicrobial therapy.
15. Essential oils (eg. clove oil, tea oil, eucalyptus oil etc) can be used to prevent spread of AMR bacteria.
16. Possibilities in alternative medicinal approaches may be explored.
17. All the antibiotics should be traceable from manufacturer to end user.
18. Legal enforcement of guidelines may be of help.
19. Setting up standards for food and food products of animal origin.
20. Integrated monitoring of AMR in animals, fisheries and human shall be practiced.

### **Global initiative to combat AMR**

A political declaration was endorsed by Heads of State at the United Nations General Assembly in New York in September 2016 indicating world's commitment to deal with AMR by addressing its root causes across multiple sectors, especially human health, animal health and agriculture.

The World Health Organization (WHO) is providing technical assistance to national action plans for prevention and management of AMR and strengthens health and surveillance systems in various countries worldwide. Secondly, WHO is engaged with the World Organisation for Animal Health (OIE) and the

Food and Agriculture Organization (FAO) of the United Nations to promote best practices to avoid the emergence and spread of AMR in humans and animals.

WHO has been leading multiple initiatives to address antimicrobial resistance. The Global Antimicrobial Resistance Surveillance System (GLASS) is a WHO supported system that supports a standardized approach to the collection, analysis and sharing of data related to antimicrobial resistance at a global level to inform decision-making, drive local, national and regional action. Global Antibiotic Research and Development Partnership (GARDP) is a joint initiative of WHO and Drugs for Neglected Diseases initiative (DNDi), GARDP and encourages research and development through public-private partnerships. By 2023, the partnership aims to develop and deliver up to four new treatments, through improvement of existing antibiotics and acceleration of the entry of new antibiotic drugs. Inter-agency Coordination Group on Antimicrobial Resistance (IACG) has been established by the United Nations Secretary-General to improve coordination between international organizations and to ensure effective global action against this threat to health security. The IACG is co-chaired by the UN Deputy Secretary-General and the Director General of WHO and comprises high level representatives of relevant UN agencies, other international organizations, and individual experts across different sectors. Since 2015, WHO held World Antibiotic Awareness Week every November with the theme “Antibiotics: Handle with care”, the global, multi-year campaign has increasing volume of activities during the week of the campaign.

The OIE also advocates for a broad application of regulatory framework controlling production, import, distribution and use of antimicrobials in all member countries. It is involved in various actions for preventing AMR worldwide. These programs involve Harmonisation of national AMR surveillance and monitoring programs, Monitoring of quantities and usage patterns of antimicrobial agents in food animals, responsible and prudent use of antimicrobials in veterinary medicine, risk assessment for antimicrobial resistance arising from their use in animals, prevention of hazardous practices and condemning marketing and use of fake products.

### **National Antimicrobial Policy (NAP) of India**

India has set up its own NAP with following aims.

1. To understand the emergence and spread of AMR and factors influencing AMR.
2. To establish nationwide coordinated antimicrobial program.
3. To rationalize the usage of available antimicrobials.
4. To reduce antibiotic selection pressure by appropriate control measures.
5. To promote discovery of new and effective antimicrobials.
6. To diagnose the infectious diseases rapidly and accurately.

**T1-04**

## **ANTI-MICROBIAL RESISTANCE: ITS CURRENT INDIAN STATUS AND CONTROL STRATEGIES**

*R.J. Zende\** and D.P. Kshirsagar

Department of Veterinary Public Health & Epidemiology

Bombay Veterinary College, Mumbai

*ravindrazende@gmail.com*

### **Introduction**

The discovery of antimicrobials, commonly known as antibiotics, is hailed as one of the most significant discoveries in medical sciences. During the past seven decades, these agents have played a significant role in reducing the global burden of communicable diseases. Antibiotics also paved the way for the unprecedented medical and societal developments, and are today indispensable in all health systems. Unfortunately, these achievements and the potential of antibiotics to benefit mankind are now at risk. Due to extensive and inappropriate use of antibiotics in both human and animal health sectors, the microorganisms have adapted to survive by becoming resistant to these antimicrobials (Annon, 2016).

Antibiotics must be used judiciously in human and animals because both uses contribute to the emergence, persistence and spread of resistant bacteria. Food animals serve as a reservoir of resistant pathogens and resistance mechanisms that can directly or indirectly result in antibiotic resistant infections in humans. Antibiotic use is a major driver of resistance. In 2010, India was the world's largest consumer of antibiotics for human health at  $12.9 \times 10^9$  units (10.7 units per person). The next largest consumers were China at  $10.0 \times 10^9$  units (7.5 units per person) and the US at  $6.8 \times 10^9$  units (22.0 units per person). Seventy-six percent of the overall increase in global antibiotic consumption between 2000 and 2010 was attributable to BRICS countries, i.e., Brazil, Russia, India, China, and South Africa (Van Boeckel et al., 2014). In BRICS countries, 23% of the increase in the retail antibiotic sales volume was attributable to India, and up to 57% of the increase in the hospital sector was attributable to China. Access to antibiotics is rising, which portends well for the large proportion of India's population that has thus far had poor access to these life-saving drugs. The convergence of factors such as poor public health infrastructure, rising incomes, a high burden of disease, and cheap, unregulated sales of antibiotics has created ideal conditions for a rapid rise in resistant infections in India (Laxminarayan and Chaudhary, 2016).

### **AMR in Man and Animals in India**

India is among the nations with the highest burden of bacterial infections. An estimated 410 000 children aged 5 years or less die from pneumonia in India annually; accounting for almost 25% of all child deaths in India. The crude mortality from infectious diseases in India today is 417 per 100 000 persons.

Consequently, the impact of AMR is likely to be higher in the Indian setting. AMR is a major public health concern in India. The emergence of resistance is not limited to the older and more frequently used classes of drugs. There has also been a rapid increase in resistance to the newer and more expensive drugs, like carbapenems. Available data indicates rising rates of AMR across multiple pathogens of clinical importance. An indicator of the rising tide of AMR in India is the rapidly increasing proportion of isolates of *Staphylococcus aureus* that are resistant to methicillin. In 2008, about 29% of isolates were of methicillin-resistant *Staphylococcus aureus* (MRSA), and by 2014, this had risen to 47%. In contrast, countries that have established effective antibiotic stewardship and/or infection prevention and control programmes, the proportion of MRSA isolates has been decreasing.

The burden of AMR in livestock and food animals has been poorly documented in India. Aside from sporadic, small, localized studies, evidence that can be extrapolated to the national level is lacking. Given that there are few regulations against the use of antibiotics for non-therapeutic purposes in India, with no stringent implementation protocols even when there are regulations, the emergence of AMR from antibiotic overuse in the animal sector is likely to be an unmeasured burden in India.

### **Health System Factors**

Health system factors are also at fault. Doctors routinely receive compensation from pharmaceutical companies and pharmacists in exchange for antibiotic prescriptions. Infection control in hospitals is poorly monitored and could be improved. A point prevalence study in a large tertiary care hospital in India found an overall health-care-associated infection prevalence of 7%, with a third of these being surgical site infections. Half of all patients were receiving antimicrobials (Roy et al 2007).

Indeed, lack of access to effective and affordable antibiotics still kills more children in India than does drug resistance. However, to prevent over-the counter (OTC) sales of important antibiotics, the Central Drugs Standard Control Organization (CDSCO) implemented Schedule H1 in India starting March 1, 2014. The H1 list includes 24 antibiotics, such as third- and fourth-generation cephalosporins, carbapenems, antituberculous drugs, and newer fluoroquinolones. Antibiotics have previously been listed under Schedule H, which contained drugs that could be sold only with a valid prescription; almost all antibiotics were easily available over the counter in the country, leading to their rampant use (Kumar et al., 2014). Incorporating principles of antimicrobial stewardship and appropriate use into undergraduate and postgraduate medical education can be implemented and is under consideration by the Government of India. A more difficult problem is that of regulating the sales of substandard and illegitimate antimicrobials, the extent of which is poorly quantified (Bate et al., 2009).

## **Animal Production, Animal Health and AMR**

The Veterinary pharmaceutical drugs have been used for a long time in animal production as therapeutic agents to control infectious diseases or as prophylactic agents to prevent outbreaks of diseases (Dixon, 2001). The most commonly used antibiotics included are penicillin, streptomycin, tetracycline, erythromycin, oxytetracycline, neomycin and gentamycin. There are different sources of entry of antibiotics residues in meat like indiscriminate use of the drugs, non-adherence to the withdrawal period, lack of medication records in the farms, time interval between treatment and slaughtering of the animals for the meat purpose. The veterinary medicinal products are most common in foods of animal origin as are directly exposed to the animals. But this could be avoided, if used properly with sufficient withdrawal period of times. Drug residues in meat occur, when these are used via parental or oral route or as feed additives in food animals.

The range of veterinary medicinal products used in regular animal husbandry practices is extremely wide, ranging from teat dips to hormones. Approximately, 42% of all veterinary pharmaceuticals used worldwide are used as feed additives, 19% are used as anti-infective, 13% as parasiticides, 11% are used as biologicals and 15% represent other pharmaceuticals. All of them are administered to animals either by injections (intramuscularly, intravenous, subcutaneous) or orally in the feed and water, topically on the skin and by intramammary and intrauterine infusions. Injectable were responsible for 46% of the volatile residues in meat followed by oral administration at 20% (feed, water and bolus) and intra mammary infusions at 7%. Several other factors have contributed to the residues problem such as poor treatment records or failure to identify the animals and result from the use of a drug in same manner that is inconsistent with the labeling. This occurs primarily through not observing label of withdrawal times as well as extra label use of drugs.

Meanwhile, growth - promoting agents like the anabolic agents are added to improve the feed conversion efficiency by increasing the lean - to - fat ratio, while antimicrobial agents are added to make more nutrients available to the animal and not to the gut bacteria. In recent years, there has been an increasing concern regarding the development of increased bacterial resistance to certain antibiotics due to the abuse of antibiotics consumption (Butaye *et al.* 2001). Most veterinary drugs have been banned in the European Union for use in farm animals because of fears about health effects (genotoxic, immunotoxic, carcinogenic, or endocrine) from their residues in animal tissues. These substances can only be administered to animals for therapeutic purposes under strict control of a responsible veterinarian (Van Peteghem and Daeselaire, 2004). Antibiotics were banned due to concerns about the development of antimicrobial resistance (Reig and Toldra, 2009). The maximum acceptable level of antibiotics in meat is depicted in Table 1.

**Table 1: Maximum permissible level of antibiotics in meat (Heena *et al.*, 2015)**

Antibiotics	Animal Species	Level (mg/kg)
Penicillin	Cattle, calves	0.050
Tetracycline	Calves, sheep, goats, pig	0.25
Tylosine	Cattle, calves, pigs	0.20
Erythromycin	Pigs	0.10
Neomycin	Calves	0.25
Oxytetracycline	Cattle, calves, pigs	0.10
Chlortetracycline	Cattle Calves and Pig muscle	0.10
	Calf liver and kidney	1.00
		4.00
	Pig liver	2.00
Lincomycin	Pigs	0.10

In India FSSAI has set the tolerance limit for antibiotics and other pharmacologically active substances only for sea foods including shrimps, prawns or any other variety of fish and fishery products under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011. The maximum acceptable level of antibiotics in fish and fishery products by FSSAI is depicted in Table 2. No tolerance limit has been set for antibiotics and other pharmacologically active substances in meat and meat products by FSSAI.

**Table 2: Maximum permissible level of antibiotics in fish and fish products (FSSAI, 2011)**

Sr. No.	Name of antibiotics	Tolerance limit Mg/kg (PPM)
1.	Tetracycline	0.1
2.	Oxytetracycline	0.1
3.	Trimethoprim	0.05
4.	Oxolinic acid	0.3

The WHO has recommended that antibiotics which are also licensed in human medicine should not be used any more as growth promoters in livestock. Not only is there a risk to human health from direct toxicity and from allergic reactions in persons sensitized to the antibiotics involved but antibiotic residues may also interfere with any microbial examinations which may be necessary in assessing the fitness of the carcass. The age restrictions have been imposed for feeding of antibiotics in animals. If use of antibiotics is necessary as in treatment of animals a withholding period must be allowed till residues can no longer be detected. For determination of veterinary drug residues in foods, currently, 6 types of detection methods are commonly used. These include microbial growth inhibition assays, microbial receptor assays, enzymatic colorimetric assays, receptor binding assays, chromatographic methods and immunoassays. But microbial growth inhibition assays and later 2 methods are popular for monitoring of antimicrobial residues in meat and meat products as are capable of detecting a broad range of these drugs (Biswas *et al.*, 2007).

The screening for tetracycline was excellent with German three plate tests, the European Union four plate tests and new Dutch kidney test instead of Swab Test on Premises (STOP), Calf Antibiotic and Sulfa Test (CAST) and the Fast Antibiotic Screen Test (FAST). Most of the biosensors developed are aimed at determining them in biological or food samples.

To control residue of antibiotics in meat and meat products, one needs to bear in mind that the discontinuation of any antimicrobial used in food-producing animals without a full quantitative risk assessment may be unnecessary and even harmful to both animal and human health. Good hygiene practices should be insisted on farms, in abattoirs, during the distribution and marketing of foods, and during food preparation by consumers, and efforts concentrating on minimizing the transmission of all food-borne pathogens regardless of their antibiotic susceptibility are very important (Jeong *et al.*, 2010).

### **Surveillance Network of AMR in India**

The National Programme on the Containment of Antimicrobial Resistance was launched under the aegis of the National Centre for Disease Control (NCDC) under the Twelfth Five-Year Plan (2012–2017). The objectives of this programme were to establish a laboratory based AMR surveillance system of 30 network laboratories; generate quality data on AMR for pathogens of public health importance; strengthen infection control guidelines and practices, and promote rational use of antibiotics; and generate awareness about the use of antibiotics in both health care providers and in the community.

At present, 10 network laboratories have been identified in the first phase of the programme, in the course of which four pathogens of public health importance are being tracked: *Klebsiella spp*, *E. coli*, *Staphylococcus aureus*, and *Enterococcus spp*. The network intends to extend testing of resistance to two more index bacteria: *Pseudomonas aeruginosa* and *Acinetobacter spp*. Reporting from the 10 laboratories

puts overall resistance rates to be very high, against the commonly used fluoroquinolones, third generation cephalosporins and carbapenems, although resistance against reserve drugs like vancomycin was not noted in isolates of *Staphylococcus aureus*, or against colistin in Gram negative bacteria. A strategy to scale up the programme in order to carry out surveillance of hospital acquired infections and antibiotic use patterns in health care settings has also been outlined; additional focus on building awareness about rational use of drugs on a continuous basis is also being planned. Like most low- and middle-income countries, India is in the process of developing a national surveillance network. Aside from the absence of a One Health approach to surveillance, another weakness of the existing surveillance systems for AMR in India is that it does not account for antibiotic use. The existence of a surveillance system that can establish the relationship between the antibiotic consumption patterns and emergence of AMR is vital to produce evidence that may help in the designing and evaluation of effective interventions.

**FAO-ICAR conducted joint meetings on AMR in India, in the recent past. They include:**

- a) *National action plan on AMR* at ICAR New Delhi dated 5<sup>th</sup> December 2016
- b) *“Laboratory based surveillance of Anti-Microbial Resistance (AMR) in Health and Veterinary sectors”* dated 18 to 19<sup>th</sup> January 2017 at NIVEDI, Bengaluru.
- c) *‘Establishment of National Network of Veterinary Laboratories on Antimicrobial Resistance in India’* dated 7-8<sup>th</sup> March 2017 at Eastern Region station IVRI, Kolkata
- d) *“To identify Research priorities in veterinary sector for AMR”* dated 27-28<sup>th</sup> March 2017 at ICAR-CIFT, Kochi, Kerala

These meetings culminated in facilitating a network called INFAAR-Indian Network of Fishery and Animal Anti-Microbial Resistance. The NAP-AMR released by Govt. of India focused six strategic priorities for controlling AMR in India. They include:

1. Improve awareness and understanding of AMR through effective communication, education and training.
2. Strengthen knowledge and evidence through surveillance
3. Reduce the incidence of infection through effective infection prevention and control.
4. Optimize the use of antimicrobial agents in health, animals, and food.
5. Promote investments for AMR activities, research and innovations.
6. Strengthen India’s leadership on AMR.

## Conclusions:

Over the past decade, antimicrobial resistance has emerged as a major public health crisis. Integrated surveillance systems are essential to monitor the emergence and spread of antimicrobial resistance along the food production chain. Such systems require

1. Systematic sampling, harmonized laboratory methods, and good data management;
2. Detailed denominator data about the origin of the samples;
3. Subtyping of bacterial isolates, and molecular characterization of resistance genes;
4. Detailed antimicrobial usage data~ and,
5. Flawless collaboration and coordination, including sharing and comparing data.

Reducing antimicrobial usage requires collaboration between experts, regulatory authorities, and producers, and integrated monitoring of the effects of interventions is essential. There is no authentic data about the AMU in animals and aqua health sectors in India. AMU data is very crucial in designing any programme on AMR. There is no any Standard Operating Procedure (SOP) for Antibiotic Sensitivity Test (ABST) in animal disease diagnostics laboratories in India. Recently SOP was developed by ICMR for this purpose in human health diagnostic laboratories. On this line veterinary sector also need SOP for ABST. Extensive national surveillance on AMR with further “One Health” approach will be helpful in providing measures for control of AMR in India.

## REFERENCES

- Anonymous (2016). Antimicrobial resistance and its containment in India, Inter-Ministerial Review Meeting on Antimicrobial Resistance. Ministry of Health and Family Welfare, Govt. of India
- Bate R, Tren R, Mooney L, Hess K, Mitra B, Debroy B, (2009). Pilot study of essential drug quality in two major cities in India. *PLoS ONE*. 4(6):e6003
- Biswas, A.K., G.S. Rao, N. Kondaiah, A.S.R. Anjaneyulu and J.K. Malik, 2007. Simple multi residue method for monitoring of trimethoprim and sulfonamide residues in buffalo meat by high performance liquid chromatography. *J. Agric. Food Chem.*, 55: 8845-8850.
- Butaye, P., L.A. Devriese and F. Haesebrouck (2001). Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. *Antimicrobial Agents and Chemotherapy*, 45: 1374-1378.
- Dixon, S.N. (2001). Veterinary drug residues. In *Food Chemical Safety*, Vol. 1: Contaminants, edited by D. H. Watson. Cambridge, UK: Woodhead Publishing Ltd.

- Heena J, Parveez A P, Subha G, Moloya G, Mohammad M B, Praveen K and Syed A B, 2015 Chemical Residues In Meat And Meat Products: A Review, *World journal of pharmaceuticals and life sciences*, Vol 1(4): 106-122
- Jeong S.H., Kang. D, Lim M. W, Kang C. S and Sung H. J (2010) Risk Assessment of Growth Hormones and Antimicrobial Residues in Meat *Toxicol. Res.* 26(4), pp. 301-313
- Kumar A, Biswal M, Dhaliwal N, Mahesh R, Appannanavar SB, Gautam V, (2014). Point prevalence surveys of healthcare-associated infections and use of indwelling devices and antimicrobials over three years in a tertiary care hospital in India. *J Hosp Infect.* 86(4):272-4.
- Laxminarayan R and Chaudhury RR (2016) Antibiotic Resistance in India: Drivers and Opportunities for Action. *PLoS Med* 13(3): e1001974.
- Roy N, Madhiwalla N, Pai SA. (2007). Drug promotional practices in Mumbai: a qualitative study. *Indian journal of medical ethics.*; 4(2):57-61. PMID: 18630221.
- Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, (2014). Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis.*; 14 (8):742-50
- Van Peteghem, C. and E. Daeselaire. (2004). Residues of growth promoters. In Handbook of Food Analysis, 2nd ed., edited by L. M. L. Nollet. New York: Marcel Dekker.

**T1-05**

### **Antimicrobial Drug Resistance : Prospective Alternatives and Emerging Approaches**

Dr. Ashok V Bhosle

Department of Veterinary Microbiology,

College of Veterinary & Animal Sciences, Udgir

Maharashtra Animal & Fishery Sciences University, Nagpur

Since the introduction of very first antibiotic Penicillin, there has been an almost inevitable emergence of antibiotic-resistant bacteria, regardless of the mechanism of antimicrobial activity. No sooner is a new antibiotic is introduced, the bacteria develops a means, may be plasmid mediated preferably, to resist it.

Antimicrobials are used widely to prevent or treat disease in food animals. The major part of the usage is for prevention of disease, and their use has become an integral part of modern industrialized food-animal production, to the extent where nearly all feed for growing animals is supplemented with antimicrobials in various doses, ranging from so-called "sub therapeutic concentrations" to full therapeutic doses. It is estimated that the volumes of antimicrobials used in food animals exceeds the use in humans

worldwide, and nearly all the classes of antimicrobials that are used for humans are also being used in food animals, including the newest classes of drugs such as third- and fourth-generation cephalosporins, fluoroquinolones, glycopeptides, and streptogramins<sup>3</sup>.

The massive use of antimicrobial agents in agriculture has supported the intensification of modern food-animal production since the early 1960s by facilitating earlier weaning, higher animal densities, and the use of cheaper feed sources, among others, and has most likely contributed to increased outputs and lower prices of meat. However, the gains have come at a cost, which is being borne, in part, by other stakeholders, in particular public health. Furthermore, the production gains achieved by indiscriminate antimicrobial usage, production systems may to a large extent be achievable by other means in modern and more environmentally sustainable food-animal production systems, where higher emphasis is placed on animal welfare, a smaller environmental footprint, and disease prevention through hygiene and intelligent herd management<sup>1</sup>.

This paper focuses on the prospective alternatives and emerging approaches to curb antimicrobial resistance as well as describe different resistance mechanisms, means of gene dissemination and the epidemiology of antimicrobial resistance in the community.

### **Present Scenario**

Antibiotics are used to treat sick animals, and resistance in animal pathogens may lead to therapy failure. This has received little scientific attention, and therefore, in this article, we discuss examples that illustrate the possible impact of resistance on animal health and consequences thereof. For all animals, there may be a negative effect on health and welfare when diseases cannot be treated. Other consequences will vary depending on why and how different animal species are kept. Animals kept as companions or for sports often receive advanced care, and antibiotic resistance can lead to negative social and economic consequences for the owners. Further, spread of hospital-acquired infections can have an economic impact on the affected premises. As to animals kept for food production, antibiotics are not needed to promote growth, but, if infectious diseases cannot be treated when they occur, this can have a negative effect on the productivity and economy of affected businesses. Antibiotic resistance in animal bacteria can also have positive consequences by creating incentives for adoption of alternative regimes for treatment and prevention. It is probable that new antibiotic classes placed on the market in the future will not reach veterinary medicine, which further emphasizes the need to preserve the efficacy of currently available antibiotics through antibiotic stewardship. A cornerstone in this work is prevention, as healthy animals do not need antibiotics<sup>2</sup>.

Investigating the zoonotic antimicrobial resistance problem in its full complexity requires monitoring of antimicrobial usage and resistance in all relevant reservoirs and stages in the transmission route, and coherent analysis of the data (i.e., “integrated monitoring”). For the purpose of intervention, there are multiple potential points of control that may be used, depending on the specific nature of the problem. Identifying and intervening at the most efficient points of control requires a comprehensive assessment of the risk based on integrated monitoring, as well as good collaboration between all the stakeholders involved.

### **Losses due to Antimicrobial Resistance**

Worldwide, published estimates of the economic losses of clinical mastitis range from €61 to €97 per cow on a farm, with large differences between farms, e.g. losses due to clinical and subclinical mastitis varied between €17 and €198 per cow per year<sup>4</sup>.

### **Prospective Alternatives and Emerging Approaches**

According to ecoimmunological principles, ART prevalence should integrate evolutionary, ecological, and immunological aspects rather than only targeting bacteria. This approach requires further ecoimmunological points to illustrate prospective approaches based on recent studies of the functions of current alternatives to antibiotics.

***Phage therapies, AR gene transfer and editing:*** Bacteriophages have been identified from most, if not all, common bacterial species with ART. Using these viral phages through nurturing natural microbial ecology to control pathogenic bacteria would be a powerful approach. Nevertheless, disadvantages of phage therapies come from our incomplete understanding of the phage–bacteria parasitic relationship, including factors that determine phages’ parasitic specificity, virulence, and genetic exchange with infected bacterial hosts. All of these factors not only limit the efficacy of phage therapies, but also causes biosafety concerns. For example, the use of phages as an alternative to reduce AR may be hindered by the discovery that phages, in some cases, actually mediate AR gene transfer to host bacteria. Recent findings about the bacterial targeted nuclease system, CRISPR/Cas9, which consists of a Cas9 nuclease and a guided-RNA (gRNA) from the bacteria clustered regularly interspaced short palindromic repeats (CRISPR), show great potential of targeted gene-editing and bioengineering manipulation. Through this type of manipulation, some prospective phage therapies could control pathogenic bacteria and also maintain commensal homeostasis.<sup>5,6</sup>

***Multifunctional AMPs vs. only antimicrobial function:*** The AMP database (<http://aps.unmc.edu/AP/about.php>) contained over 2400 entries, 98.5% of which are from natural sources and have experimentally demonstrated sequence characteristics and antimicrobial activity. AMPs serve as “natural antibiotics”

and have been widely studied for development into a novel generation of antibiotics. Notably, most AMPs are multifunctional, exerting antimicrobial, lipid transfer, reproduction regulation, and immunomodulatory activities. Recent studies also indicate that AMPs are critical in regulating the homeostasis of animal gut microbiota. The multifunctional properties of AMPs (such as regulation of inflammation and modulation of immune cells) often hindered their clinical application, which focused on antibiotic action, but recent progress in computer-based design and structural separation of antimicrobial and immunomodulatory activities will facilitate the development of AMP-based antimicrobial therapies

***Pathogenic bacteria-targeting:*** Accompanied by the declining rate of discovery of novel antibiotics in recent years, several other antibacterial or bactericidal approaches have been postulated. A prominent idea is to use bacteriophages, a group of bacterial viruses that cause lysis in host bacteria in the late phase of the life cycle, to control pathogenic bacterial species. Phage therapies have been used to treat accessible topical infections and foodborne pathogens in the United States but are limited for clinic uses by regulatory hurdles because of biosafety concerns. Phages have been widely studied for their advantages of targeting specific bacteria, complementing the effect of antibiotics, and less resistance by bacteria; however, host specificity of phages limits a phage therapy to treat different bacterial species or sub-species . Recent studies have shown that bacterial phages actually mediated antibiotic gene transfer to preserve their host bacteria during antibiotic stress. One way to tailor therapeutic uses of phages in animal production is to engineer phages' lytic protein component of endolysins, which possess an ability to permeabilize bacterial cell walls and causes lysis. Phage endolysins resemble antimicrobial peptides (AMPs), which include several classes of innate immune peptides expressed in most organisms from bacteria and plants to animals.

**One Health approach** is useful when it comes to addressing zoonotic transmission of pathogens that are resistant to antimicrobials, because we need to engage a wide range of stakeholders including farmers, veterinarians, food safety professionals, medical doctors, as well as environment and wildlife experts in monitoring and control activities.<sup>12</sup>

## **Conclusion**

Integrated surveillance systems are essential to monitor the emergence and spread of antimicrobial resistance along the food production chain. Such systems require systematic sampling, harmonized laboratory methods, and good data management; detailed denominator data about the origin of the samples; subtyping of bacterial isolates, and molecular characterization of resistance genes; detailed antimicrobial usage data; and, flawless collaboration and coordination, including sharing and comparing data.

**Based on existing surveillance systems it is fair to conclude the following:**

There is a close relationship between the patterns of antimicrobial usage and the observed patterns of antimicrobial resistance in food animals; however, other factors such as co-selection and clonal spread also play a part.

There is a close relationship between levels and patterns of antimicrobial resistance in the food supply and antimicrobial resistance in human foodborne infections<sup>7</sup>, bearing in mind that some food is imported and other foods are consumed while travelling abroad, and that all sources need to be accounted for.

There is a great need to reduce the overall use of antimicrobials in agri- and aquaculture worldwide, and the experiences from different countries suggest that major reductions can be achieved without significant negative effects on animal health or productivity, and for the long-term benefit of public, environmental, and animal health.

A number of effective upstream interventions to reduce resistance have been documented, including banning nontherapeutic uses in food animals, enforcing prescription-only policies, removing financial incentives for prescribing therapeutic drugs, restricting the use of drugs considered critically important for human health, monitoring usage at the farm level and

**providing advice to high-end users, and establishing thresholds for resistant pathogens in food<sup>8</sup>.**

Reducing antimicrobial usage requires **collaboration between experts, regulatory authorities, and producers, and integrated monitoring of the effects of interventions** is essential. This maybe facilitated by establishing a coordinating body, for example, an antibiotic council, including all relevant stakeholders.

**The consequences of antibiotic resistance in bacteria are basically the same in human and veterinary medicine<sup>9</sup>.** Loss of effective antibiotic treatments through resistance will cause suffering for the affected individual, regardless of whether it is a human being or an animal. There will also be economic consequences through increased treatment costs in animal and human health care. These costs are likely to be much higher in human health care because of the more advanced procedures and treatments employed. However, in up-to-date companion animal health care the degree of knowledge and skill is high, and advanced and costly procedures and prolonged treatments are often used. Nevertheless, suffering of the individual animal and the overall costs in companion animal health care can be limited by the possible and relevant alternative to euthanize seriously sick or old animals. In the rearing of animals for food production,

it is a normal procedure to put animals down where the cost of treatment goes beyond the benefit in economic terms. Loss of access to effective therapy will also lead to economic losses due to reduced productivity of the animals, and loss of effective therapy in human health care is also associated with losses of productivity and subsequently to societal costs.

Although the consequences of resistance are mostly negative, the insight into the gravity of the problem and the focus from the scientific society and media on these issues have also had positive aspects. The emergence of resistance has been an incentive for development, evaluation, and adaptation of other regimes for treatment or prevention. Examples from companion animal health care are antibacterial shampoos for treatment of pyoderma in dogs, emphasis on debridement instead of antibiotics in wound care, and most importantly infection control in inpatient and outpatient care. Also, in the care of animals kept for food production, the emergence of resistance has brought on insights of the need to reduce morbidity by changes in husbandry and by effective biosecurity routines instead of by use of antimicrobials.

**Healthy animals do not need antibiotics.** In health care of companion animals and in animals kept for food production alike the main objective of efforts in the future should be to reduce the incidence of infectious diseases and thereby the need for antibiotics. This is even more relevant considering that it is highly unlikely that new antibiotic classes will be available for use in animals. If new antibiotic classes are placed on the market in the future, they will probably be restricted for use in human health care. This further emphasizes the need to mitigate emergence and spread of resistance to the antibiotics currently available in veterinary medicine through antibiotic stewardship, including measures to keep animals healthy without use of antibiotics.

#### **References:**

- Björn Bengtsson and Christina Greko 2014. Antibiotic resistance—consequences for animal health, welfare, and food production, *Ups J Med Sci.* 2014 May; 119(2): 96–102.
- D.M. Livermore, 2003. Bacterial resistance: Origins, epidemiology and impact, *Clin. Infect. Dis.* 36 11–23.
- Henrik C. Wegener 2014. Antibiotic Resistance—Linking Human And Animal Health Improving Food Safety Through a One Health Approach: Workshop Summary
- Hogeveen H<sup>1</sup>, Huijps K, Lam TJ 2011. Economic aspects of mastitis: new developments. *NZ Vet J.* Jan;59(1):16-23. doi: 10.1080/00480169.2011.547165.
- I.N. Okeke, K.P. Klugman, Z.A. Bhutta, A.G. Duse, P. Jenkins, T.F. O'Brien, A. Pablos-Mendez, R. Laxminarayan, 2005. Antimicrobial resistance in developing countries. Part II: Strategies for containment, *Lancet Infect. Dis.* 5, 568–580.

T. Barbosa, S.B. Levy, 2000. The impact of antibiotic use on resistance development and persistence, *Drug Resist. Update*, 3, 03–311.

The medical impact of the use of antimicrobials in food animals, *Report of a WHO Meeting*, WHO, Berlin, Germany 1997.

V. Perreten, F. Schwarz, L. Cresta, M. Boeglin, G. Dasen, M. Teuber, Antibiotic resistance spread in food, 1997. *Nature*, 38, 801–802.

[www.Antibiotic resistance\WHO\\_Antibiotic resistance.html](http://www.Antibiotic resistance\WHO_Antibiotic resistance.html)

**AB-01**

**Application of lytic bacteriophages in the treatment of  
*Pseudomonas aeruginosa* mastitis.**

Lakshmi kavitha K<sup>1</sup>, Sambasiva Rao K<sup>2</sup> and Srivani M<sup>3</sup>.

1 Associate Professor, 2 Junior Research Fellow, 3 Assistant Professor, Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Krishna dt. 521 102, Andhra Pradesh.

Mastitis by *Pseudomonas aeruginosa* is opportunistic and environmental in nature. The treatment often fails due to higher antibiotic resistance and many a times end with recurrence or chemical treatment of affected quarter. Hence lytic bacteriophages were studied as alternative therapeutic approach to antibiotics. For this purpose lytic bacteriophages were isolated using double agar overlay method using prophage free culture of *Pseudomonas aeruginosa*. Five lytic phages showing collective 100% host range were further characterized biophysically. The phages resisted pH 4,7,9 and temperature of 37°C, 42°C. On *in vitro* therapeutic study in mice the lytic phage mixture has decreased the inflammation of mammary gland effectively. The study revealed the application of lytic bacteriophages is worth as therapeutic agent and need further evaluation.

**AB-02**

**Emergence of Virulent and Antibiotic Resistant *Pasteurella multocida* and  
*Mannheimia hemolytica* in Sheep and Goats of Western Maharashtra, India**

Daphal S.H., Mhase P.P., Pawade M.M., Shelke P.R and Sangle J.D

Department of Microbiology, KNP College of Veterinary Science, Shirwal, Satara, Maharashtra

**Aim:** *Pasteurella* species are often encountered in small ruminants as major pathogens in respiratory illnesses resulting in heavy losses. Emergence of virulence and antibiotic resistance in *Pasteurella* species is further posing a threat to the sustainability of small ruminant farming. Study was aimed towards finding out the occurrence of *Pasteurella* spp. in small ruminants as respiratory pathogen and detection of antibiotic resistance and virulence in them by phenotypic and genotypic methods.

A total of 208 different samples like nasal swabs, lungs and heart blood were collected from diseased as well as healthy sheep (n=90) and goats (n=118) from the region of Western Maharashtra state of India. All the isolates (n=23) were processed for phenotypic characterization of *Pasteurella* spp. The isolates were confirmed with specific gene polymerase chain reaction by KMT and PHSSA gene PCR and CapA, CapD, and Lkt gene PCR for expression of virulence. All isolates were subjected to antibiotic sensitivity testing by disc diffusion technique with nine different drugs.

The bacteriological examination of samples revealed that, out of 208 samples 23 (11.05%) were found culturally positive for presence of *Pasteurella* spp. Out of total *Pasteurella* spp. isolated 16/23 (69.56%) were identified as *P. multocida* and 07/23 (30.43%) were identified as *M. haemolytica*. The isolates confirmed by Species specific KMT PCR for *P. multocida* resulted in cent percent positivity (100 %), while 57.12 percent of all phenotypically detected *M. haemolytica* were only confirmed by PHSSA PCR. The virulence of *P. multocida* organisms was determined by expression of capsular antigen CapA found in 81.25% isolates and CapD gene found in 50.00 % isolates, while virulence in *M. haemolytica* was determined by PHSSA and Lkt gene found in 57.12% and 42.85% isolates. Overall antibiotic sensitivity profile indicated that, enrofloxacin was most effective 91.3% isolates of *Pasteurella* spp. followed by 82.6% to ciprofloxacin and 78.26 % to gentamycin. Out of 23 isolates 65.2% were resistant to cotrimoxazole and penicillin each respectively, followed by 60.8% to ampicillin, 56.5% to tetracycline, 21.7% to streptomycin and 08.69% to ciprofloxacin, gentamicin and enrofloxacin each respectively, indicating emergence of multiple drug resistance.

The present study emphasized the occurrence of *Pasteurella multocida* and *Manheimia hemolytica* in diseased as well as healthy sheep and goats in the study area. PCR was found to be a valuable tool for detection and confirmation of both species rapidly as well as for determination of expression of virulence genes in the commensal isolates of respiratory tract of small ruminants. The emergence of multiple drug resistance in virulent *Pasteurella* species is suggestive of necessity to monitor continuously for the occurrence of these organisms in the study area in small ruminants.

**AB-03**

**Antimicrobial susceptibility of bacteria associated with mastitis  
in bovines around Western Maharashtra, India.**

Pawade M.M., Mhase P.P., Muglikar D.M., Shelke P.R and Sangle J.D

Department of Microbiology, KNP College of Veterinary Science, Shirwal, Satara, Maharashtra

Mastitis, acute as well as subclinical is an important disease of dairy animals leading to heavy economic losses to farmers and nation. Irrational use of antimicrobial drugs has increased to further complications of this condition and the indiscriminate use of antimicrobial is leading to evolution of multi drug resistant microbes. The management of mastitis which is a multi-factorial, multi-etiological syndrome needs to be addressed properly with adoption of precise antimicrobial therapy.

The present study was conducted on the milk samples received by Department of Microbiology for detection of mastitis in cattle and buffaloes from October 2015 to October 2017 period from the various

parts of Western Maharashtra region around Shirwal Dist. Satara. Total 160 milk samples of clinically positive dairy animals comprising 82 cows and 78 buffaloes were tested culturally and yielded positive for mastitis. The common isolates found in milk samples were species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *E.coli* and *Pseudomonas*.

The antibiotic sensitivity test result of mastitis affected cow milk indicated, highest sensitivity towards Enrofloxacin (48.72%), Gentamycin (36.58%), Amikacin (30.48%), ceftriaxone-tazo (12.19%), respectively. Intermediate sensitivity was reported in Ceftriaxone-clav (35%), Amoxy-clav (30.20%), Ciprofloxacin (30%) respectively, while Penicillin (39.04%), Oxytetracycline (18.29%), Amoxicillin (5.85%), Ampicillin (4.63%), Chloramphenicol (12.19%), Ceftriaxone (14.63%), Ampicillin salbactam (10.97%), showed resistance pattern.

While in case of buffalo, the antibiotic sensitivity test result indicated, high sensitivity towards Enrofloxacin (64.10%), Gentamycin (29.48%), Ciprofloxacin (17.94%), Ampicillin salbactam (19.23%), and Ceftriaxone-tazo (16.66%). Intermediate sensitivity was reported in Ceftriaxone-clave (25%), Chloramphenicol (24.32%), while Penicillin (52.56%), Oxytetracycline (41.02%), Amoxy-clav (28.20%), Ampicillin (15.38%), Amoxicillin (10.25%), Ceftriaxone (15.38%) were found resistant.

The conclusion of this study is, the antibiotic sensitivity test shows antibiotics like Enrofloxacin, Gentamycin, Amikacin, Ceftriaxon-tazo are sensitive while Penicillin, Oxytetracycline, Amoxycillin, Ampicillin, Chloramphenicol, Ceftriaxon, Ampicillin-salbactam are resistant to the mastitis affected milk samples of cow.

In case of milk samples of mastitis affected buffalo, high sensitivity was shown towards the antibiotics like Enrofloxacin, Gentamycin, Ciprofloxacin, Ampicillin salbactam and Ceftriaxon-tazo while Penicillin, Oxytetracycline, Amoxy-clav, Ampicillin, Amoxycillin and Ceftriaxon were found resistant.

**AB-04**

### **Isolation and Identification of *Pseudomonas* spp from mastitis cases along with antibiogram pattern**

Pawade M.M., Mhase P.P., Muglikar D.M., Shelke P.R and Sangle J.D

Department of Microbiology, KNP College of Veterinary Science, Shirwal, Satara, Maharashtra

The aim of present study were to isolate *Pseudomonas* species from mastitic milk samples of cow and buffalo in Satara district. Total 160 (cow-82; buffalo-78) samples were processed out of which 25(cow-12; buffalo-13) (15.62%) samples were culturally positive for *Pseudomonas* isolates.

The isolated *Pseudomonas* species produces characteristics colonies on selective agar i.e. on cetrimide agar. By Gram's staining, the morphology of isolated pseudomonas species showed Gram-negative, medium rod shaped appearance.

The antibiotic sensitivity pattern of the isolates of *Pseudomonas* showed highest sensitivity towards Ciprofloxacin (80%). Enrofloxacin (72%) and Gentamicin (60%), while highly resistant was observed towards Penicillin(100%), Oxytetracycline (76%), Amoxycillin (60%), Ampicillin(44%).

From above results it was concluded that *Pseudomonas* species is also one of the cause for bovine mastitis. and Ciprofloxacin and Enrofloxacin as the best drugs of choice for the same.

**AB-05**

### **Phenotypic and Genotypic Antibiotic Resistance of Salmonella serovar Newport isolated from Poultry**

R.N. Waghamare\*, A. M. Paturkar, R. J. Zende, V. M. Vaidya, Z. B Dubal and R. S. Khilari

Department of Veterinary Public Health

Bombay Veterinary College, Parel, Mumbai -12

\*Corresponding author : rupeshwaghamare@gmail.com

The rapid rise of MDR Salmonella serovar Newport isolates over the last decade as important causes of human salmonellosis and the antimicrobial resistant patterns of Salmonella circulating in the poultry farming and processing establishments are currently unknown. To address this issue, both the phenotypic and genotypic antibiotic resistance patterns of 06 Salmonella serovar Newport isolated from farm and processing establishments were analyzed by the disc diffusion, and PCR assays. Out of 06 Salmonella serovar Newport isolates 05 isolates showed resistance to five or more antimicrobial agents. Resistance to Ceftriaxone (CTX), Amoxycillin (AMC), Norfloxacin (NX), Oxytetracycline (O), Enrofloxacin (EX), Ciprofloxacin (CIP), Ampicillin (AMP), Neomycin (N), Erythromycin (E), Tetracycline (TE) was observed in 03 Salmonella isolates except one which was susceptible to Neomycin (N), while one isolates was found resistant only to Oxytetracycline (O), Neomycin (N) and Tetracycline (TE). Remaining 02 isolates showed resistance to 07 (NX, O, CL (Colistin), AMP, N, E, TE) and 05 (CTX, O, N, E, TE) antimicrobial agents, respectively. Amplification of antibiotic resistance genes showed that tetA (100%) correlated well with resistance to Tetracycline, whereas bla<sub>TEM</sub> (n=02) correlated well with resistance to Amoxicillin and Cephalosporin. It is concluded that Salmonella serovar Newport isolated from poultry farming and processing establishments showed MDR by both phenotypic and genotypic methods and they could be an important threat to human health. It is essential to establish the relationship between antibiotic resistance in Salmonella serovar Newport from poultry and clinical isolates.

**AB-06**

**QUORUM QUENCHING: A NOVEL THERAPEUTIC TOOL AND  
ANTIBIOTIC REPLACER**

D.P.PATIL<sup>1</sup>, USHARAN<sup>2</sup>, A.GOPALA REDDY<sup>3</sup> B. ANILKUMAR<sup>4</sup> and S.V. LONDHE<sup>5</sup>

<sup>1</sup>PhD Scholar,<sup>2,3,4</sup>Department of Veterinary Pharmacology and Toxicology  
College of Veterinary Science, Hyderabad (Telangana)

<sup>5</sup>Department of LPT, COVAS, Parbhani (M.S.)

Quorum sensing is cell to cell signaling mechanism that enables the bacteria to collectively control gene expression. This type of bacterial communication is achieved only at higher cell densities. Bacteria release various types of molecules called as autoinducers in the extracellular medium, these molecules are mediators of quorum sensing. When concentration of these signaling molecules exceed a particular threshold value, these molecules are internalized in the cell and activate particular set of genes in all bacterial population, such as genes responsible for virulence, competence, stationary phase etc. Quorum sensing occurs within a single bacterial species as well as between diverse species. Some social insects like ants and honeybees use quorum sensing to determine where to nest. Quorum sensing is identified first in *Vibrio fischeri* a marine gram negative bacteria controlling bioluminescence. Today QS of 25 gram negative and many gram positive bacteria is identified but only four gram negative and few gram positive is studied in depth.

**T2**

**Genomics And Proteomics**

**T2-01**

## **Role of Biotechnology in Livestock Development and Sustainability of Farmers**

Dr. Satish Kumar

Chief Scientist & Group Leader

CSIR-Centre for Cellular and Molecular Biology (CCMB)

Uppal Road, Hyderabad – 500 007

Livestock sector contributes 4.4% to the total GDP of the country and this contribution stands Rs. 500405 crores at current prices (National Accounts Statistics-2016). The total contribution of agriculture and allied sectors is gradually decreasing in the economy from 35% in 1981 to 15% in 2013. However, within the agricultural sector, the contribution of livestock has shown steady increase from 14.8% to 27% during the same period thus indicating the relative importance of the sector in generating economic growth. The total livestock population is around 512 million, out of which the bovines (cattle and buffalo) constitute approximately 300 million. While buffalo represents 36% of the total bovine population, it produces nearly 50% of the total milk in the country. Although, India is the highest milk producer in the world, the productivity per animal is extremely low as compared to the best world averages. Some of the obvious causes for the inferior productivity and low efficiency of production include lack of proper breed improvement programmes, poor nutrition, low coverage of veterinary services and extension backup in terms of management skills and technical knowhow. Given the biodiversity of different breeds of buffalo, cattle and other livestock species, there is a tremendous scope for enhancing the productivity per animal using biotechnological intervention. Thus far, as a nation, we have not been able to utilize this potential for the benefit of our farmers except limited success in using crossbreeding in cattle but with attendant issues like disease susceptibility and problems of fertility in crossbred animals. Some of the major challenges in making the livestock sector sustainable for the Indian farmer are - infusion of capital, modernization of production systems based on already existing traditional and scientific knowledge, specially advances in modern biotechnology. Further, organizational innovation in production systems and marketing is imperative for both attracting private sector investments and applications of modern biotechnology. This would enhance the profitability and sustainability of livestock production on one hand and ensure fair share of profit to farmers by removing the intermediaries from the marketing chain. While cooperative sector has been helpful to some extent, but much is needed to be desired; in this context, primary producer's company model may hold the promise. Finally, the author is of the opinion that sooner than later the corporatization of the livestock sector is imperative as has been happening for the poultry sector. These trends need to be utilized by the government by concentrating on providing the enabling policy framework and regulatory regimes so that the middle/small livestock farmer is an integral and sustainable beneficiary of such a system. Rather than the government directly participating in the developmental activities through animal

husbandry departments and/or livestock development boards, alternate developmental models needs to be developed. I shall present some of these during this talk.

*\* Based upon the presentation made by Dr. Satish Kumar during National Conference on “Rejuvenate Indian Agriculture for Sustainability”, 23<sup>rd</sup> & 24<sup>th</sup> September, 2017 at Indian Institute of Rice Research, PJTSAU Campus, Rajendranagar, Hyderabad*

**T2-02**

### **GENETIC STUDIES ON CAPRINE FECUNDITY**

Aakash Y. Doiphode, Priyanka T. Wagh, Kalpesh A. Zunjarrao and Ritesh S. Vairagade

Animal Genetics and Breeding

KNP College of Veterinary Science, Shirwal, Dist- Satara

#### **Introduction**

Goat is a symbol of economic hope for farmers because of their several unique features like cheaper rearing cost, higher fertility & fecundity along with ever rising demand for goat meat, skin/ hide & high manure value. There is continuous demand for goat meat in domestic market. The high level of production can only be possible either through the production of faster growing animals or through improving the reproductive efficiency of the animals.

#### **The genes influencing fecundity**

The genes influencing ovulation rate act principally in the ovary (Montgomery et al., 2001). Genetic studies in sheep have indicated that the ovulation rate and litter size can be genetically regulated either by a set of different genes each having a small effect as in the Romanov breeds (Mulsant et al., 2001), or alternatively by the action of single genes with major effect, named fecundity (Fec) genes (Davis, 2004). The elucidation of the aberrant reproductive phenotypes of animals with naturally occurring mutations or targeted deletions of certain Bone Morphogenetic Protein (BMP) and Growth and Differentiation Factor (GDF) family genes have highlighted the importance of genes regulating ovulation (reviewed in Shimasaki et al., 2004). GDF-9 and BMP-15 are obligatory for folliculogenesis through their ability to stimulate GC proliferation and modulate FSH dependent cytodifferentiation. The discovery in sheep that naturally occurring mutations in BMP-15 and GDF-9 (Montgomery et al. 2001) influence dramatically the number of ova ovulated, provides definitive evidence that the ovarian BMP system plays a central role in the mechanisms governing ovulation quota and litter size. This work has led to the concept that normal ovulation

quota requires the regulated expression of such genes (Erickson and Shimasaki, 2002). Another important Fec gene observed in sheep is Bone Morphogenetic Proterin Receptor 1 B (BMPR1B), also known as Booroola or Actin like kinase 6 located on ovine chromosome 6. The FecB locus of BMR1B, if mutated (Q249R) exhibits codominant expression with additive effect on ovulation rate (Souza et al., 2001; Wilson et al., 2001). FecB mutation was observed in many sheep breeds worldwide including Garole, Kendrapada and Bonpala sheep of India. (Roy et al., 2011). The FecB mutation was also reported in the highly prolific Black Bengal breed of goat in India (Polley et al., 2009; Kumari et al., 2015).

Several studies have been undertaken worldwide on other candidate genes regulating fecundity in goats including; follicle stimulating hormone (FSH) (An et al., 2010), FSH receptor (FSHR) (Guo et al., 2013), Tissue-specific POU-containing transcription factor (POU1F1) (Feng et al., 2011), Prolactin receptor (PRLR) (Li et al., 2010; Li et al., 2011a; Di et al., 2011), Kisspeptin (KiSS-1) (Feng et al., 2009; Cao et al., 2010), G protein-coupled receptors 54 (GPR54) (Cao et al., 2011; Maitra et al., 2014; Ahlawat et al., 2015), Growth hormone (GH) (Silva et al., 2009; Zhang et al., 2011), Inhibin (INH) (He et al., 2010; Chu et al., 2012; Sharma et al., 2015), Cocaine-amphetamine-regulated transcript (CART) (Wang et al., 2011), Gonadotropin releasing hormone (GnRH) (An et al., 2012), Gonadotropin-releasing hormone receptor (GnRHR) (Han et al., 2009; An et al., 2009; Yang et al., 2011), Luteinizing hormone beta-subunit (LH $\beta$ ) (Di et al., 2009; Sun, 2009), Bone morphogenetic protein 4 (BMP4) (Chu et al., 2010; Sharma et al., 2013), Kit ligand (KITLG) (Silva et al., 2006; An et al., 2011), Melatonin receptor 1b (MT2) (Jia et al., 2012), Steroid 21-hydroxylase (CYP21) (Yan, 2010) and Arylalkylamine-N-acetyltransferase (AA-NAT) (Chu et al., 2013; Sharma et al., 2015). Various polymorphic sequence variations in fecundity related genes associated with prolificacy trait in goats observed in these studies are summarized in Table 1 and polymorphic sequence variations in fecundity related genes observed in Indian goats is presented in Table2.

**Table1.** Polymorphic sequence variations in fecundity related genes associated with prolificacy trait in goats

Gene	Mutation	Region	Breed	Reference
BMPR1B	Q249R (FecB)	Exonic	Black Bengal	Polley et al., 2009
GDF-9	G4093A	Exonic	Xinong Saanen Guanzhong Boer	An et al., 2012
	A792G Homozygous	Exonic	Big foot black Jintang black	Zhu et al., 2013
BMP-15	G1100T (FecX <sup>B</sup> )	Exonic	White goat population of Guizhou	Lin et al., 2007
	BB genotype (T456G, C466G, C510T, T511C)	Exonic	Funiu white goat	Wang et al., 2011

<b>Gene</b>	<b>Mutation</b>	<b>Region</b>	<b>Breed</b>	<b>Reference</b>
FSH $\beta$	G40A, T148C	Exonic	Xinong Boer	An et al., 2010
FSHR	CC and CD genotype T70A, G130C	Exonic	Jining Grey	Guo et al., 2013
PIT-1/ POU1F1/ GHF-1	T allele C256T, G682T	Exonic	Jining Grey	Feng et al., 2011
PRLR	HH, HK, AH and AK genotypes	Intron 1	Jining Grey	Di et al., 2011
	CD genotype	Intron 2	Jining Grey	Di et al., 2011
KiSS-1	C allele G296C, Deletion 1960-1977	Exonic	Jining Grey	Cao et al., 2010
	T2643C	Intron 2	Jining Grey	Hou et al., 2011
	G384A, T2489C, G2510A, C2540T	Exonic	Xining Saanen Guanhong Boer	An et al., 2013
	G296C, G2510A, C2540T	Intronic	Indian goat breeds	Maitra et al., 2014
GPR54	Allele T C4152T	Exonic	Jining Grey	Cao et al., 2011
GH	A781G, A1575G	Exonic	Matou Boer	Zhang et al., 2011
INH $\alpha$	C865T	Exonic	3 Chinese goats	He et al., 2010
INH $\beta$	A782G	Exonic	Jining Grey	Chu et al., 2012
INH $\beta$ B	G693A, C840T	Exonic	Indian goats	Sharma et al., 2015
CART	C593A	Intronic	Chuangdong White Guizhou White Gulin Ma	Wang et al., 2011
GnRH	A3548G	Exonic	Xinong Saanen Guanzhong Boer	An et al., 2012
GnRHR	GT genotype G891T	Exonic	Boer	Yang et al., 2012
	GG genotype G757A	Exonic	Boer	Yang et al., 2012

Gene	Mutation	Region	Breed	Reference
	AA genotype	Exonic	Shaanan Boer	Li et al., 2011
	AA genotype G154A	Exonic	Chuandong White Gulin Ma Guizhou White	Huang et al., 2012
LH $\beta$	DD and CD genotype C1124T	Exonic	Jining Grey Liaoning Cashmere	Sun et al., 2009
BMP-4	CC genotype CA dinucleotide repeat	3' flanking region	Jining Grey	Chu et al., 2010
KITLG	T769C, G817T, G9769C	Exonic	Xiong Saanen Guanzhong Boer	An et al., 2011
CYP21	BB and AB genotype	Exonic	Jining grey	Yan, 2010
AA-NAT	D Allele C265T	Exonic	Jining grey	Chu et al., 2013

**Table2.** Polymorphic sequence variations in fecundity related genes observed in Indian goats

Gene	Mutation	Region	Breed	Reference
BMPR1B	Q249R (FecB)	Exonic	Black Bengal	Polley et al., 2009
	GG, GA and AA genotypes G(-623)A	Promoter	Osmanabadi, Jakhra	Ahlawat et al., 2013
	GG, GA genotypes G(-623)A	Promoter	Beetal, Barbari, Black-Bengal, Malabari	
	GG genotype G(-623)A	Promoter	Ganjam and Sirohi	
	TT and TC Genotypes T(-242)C	Promoter	Malabari and Black-Bengal	
	TT Genotype T(-242)C	Promoter	Beetal, Ganjam and Sirohi	
KiSS1	G296C, G2510A and C2540T	Intron 1	Black Bengal	Maitra et al., 2014a
GPR54	CC and CT genotypes	Exon 1	Beetal, Black Bengal, Barbari,	Maitra et al., 2014b.
	C1122T TT, CT, CC genotypes T1830C	Intron 1	Malabari, Osmanabadi, Sangamneri, Jakhra, Ganjam, Sirohi	

Gene	Mutation	Region	Breed	Reference
INHBB	GG and GA genotype G693A	Exon 2	Beetal, Sangamneri, Ganjam	Sharma et al., 2015a
	GG, GA and AA genotype G693A	Exon 2	Osmanabadi	
	CT genotype C840T	Exon 2	Osmanabadi	
BMP4	GA G1534A	Exon 2	Black Bengal Jakhrana	Sharma et al., 2013
AA-NAT	C825T	Exon 2	Beetal, Barbari,	Sharma et al. 2015b
	C1249T	Exon 3	Malabari, Osmanabadi, Sangamneri, J akhrana, Ganjam, Sirohi	

### Studies on Osmanabadi goat

Osmanabadi goat is one of the most important breeds of goat found in Maharashtra, India. The breed is large in size. The colour pattern varies from white, brown and spotted but mostly it is black. The breed is considered useful for both meat and milk. Kidding is observed twice a year and twinning is common. There is wide variation in the kidding size. The important question is whether Osmanabadi goats are multi-ovulator at all occasion or the variation in the kidding size is a reflection of underlying variation in the ovulation rate.

We intended to identify the genetically superior animals favouring multi-ovulation. Keeping in view the importance of Fec genes and Osmanabadi breed of goat, we have targeted nine loci in GDF-9, two loci in BMP-15 and one locus of BMPR1B gene in the Osmanabadi breed of goat, which are known to be polymorphic in the sheep, influencing ovulation. In addition we have for the first time targeted the mature peptide region of BMP- 15 and GDF-9 gene for detection of novel polymorphisms. The mature peptide region of BMP-15 and GDF-9 gene in the genomic DNA of Osmanabadi breed of goat was characterized using PCR-SSCP, the most sensitive method of polymorphism detection. Locus G1, G4, G6 and G8 of GDF-9 gene and FecX<sup>H</sup>, FecX<sup>I</sup> and FecX<sup>L</sup> locus of BMP-15 gene was genotyped using Tetra primer amplification refractory mutation system (T-ARMS PCR). FecB locus of Osmanabadi goats was genotyped using PCR-RFLP. The FecX<sup>B</sup> and FecX<sup>G</sup> loci of BMP-15 gene and G7, FecG<sup>E</sup>, FecTT and G1189A loci of GDF-9 gene were genotyped using direct sequencing of representative PCR products of each locus. Osmanabadi goats were found to be monomorphic carrying wild type allele at the FecX<sup>B</sup>, FecX<sup>H</sup>, FecX<sup>I</sup>, FecX<sup>L</sup> and FecX<sup>G</sup> loci of BMP-15. FecB locus of the BMPR1B gene was found to be wild

type monomorphic. Also the goats were carrying monomorphic wild type G1, G4, G6, G7, G8 (FecG<sup>H</sup>), FecG<sup>F</sup>, FecTT and G1189A loci in the GDF-9 gene.

## **Conclusions**

Based on the present investigation it is observed that the fecundity of Osmanabadi goats is found to be independent of FecX, FecB and FecG mutations. However recent studies have observed many point mutations (SNP's) in the Fec genes in Indian goats. Polygenic nature of quantitative traits limits the application of marker assisted selection (MAS) in livestock. The reproductive traits involve multiple genes and their interactions and therefore genomic selection or genome wide association studies can be a better choice over MAS. Considering the research findings on polymorphic nature of fec genes in Indian goats, there is scope for development of SNP chips suitable to the Indian goats. The SNP chip based selection in Indian goats will facilitate the early stage selection in goats. The possibility of producing mutated caprine embryos by inducing the known mutations of the BMP-15, GDF-9 or BMPRI1B gene can also be explored in near future. The desired mutations can be induced using homologous recombination or zinc finger nuclease method or CRISPR method of genome editing.

## **Acknowledgement:**

This research work was carried out as a part of the project objectives of Science and Engineering Research Board, New Delhi, funded project in the form of consumables and equipments. The authors also thank Associate Dean, Bombay Veterinary College, Mumbai and Hon'ble Vice Chancellor, Maharashtra Animal and Fishery Sciences University, Nagpur, MS, India India, for providing the necessary facilities.

## **References:**

- Ahlawat, S., Sharma, R., Maitra, A., Tantia, M.S. and Prakash, V. (2015). Association analysis of a novel SNP in GPR54 gene with reproductive traits in Indian goats. *Indian J. Dairy Sci.* 68 (1), 39–44.
- An, X.P., Han, D., Hou, J.X., Li, G., Wang, J.G., Yang, M.M., Song, Y.X., Zhou, G.Q., Wang, Y.N., Ling, L., Yan, Q.M., Cao, B.Y., (2009). GnRHR gene polymorphisms and their effects on reproductive performance in Chinese goats. *Small Ruminant Res.* 85, 130–134.
- An, X.P., Han, D., Hou, J.X., Li, G., Wang, Y.N., Li, L., Zhu, G.Q., Wang, J.G., Song, Y.X., Cao, B.Y., (2010). Polymorphism of exon 2 of FSHB gene and its relationship with reproduction performance in two goat breeds. *Agric. Sci. China* 9, 880–886.
- An, X.P., Han, D., Hou, J.X., Li, G., Wang, Y.N., Li, L., Zhu, G.Q., Wang, J.G., Song, Y.X., Cao, B.Y., (2010). Polymorphism of exon 2 of FSHB gene and its relationship with reproduction performance in two goat breeds. *Agric. Sci. China* 9, 880–886.

- An, X.P., Hou, J.X., Li, G., Song, Y.X., Wang, J.G., Chen, Q.J., Cui, Y.H., Wang, Y.F., Cao, B.Y., (2011). Polymorphism identification in the goat KITLG gene and association analysis with litter size. *Anim. Genet.* 43, 104–107.
- An, X.P., Hou, J.X., Zhao, H.B., Li, G., Bai, L., Peng, J.Y., Yan, Q.M., Song, Y.X., Wang, J.G., Cao, B.Y., (2012). Polymorphism identification in goat GnRH1 and GDF9 genes and their association analysis with litter size. *Anim. Genet.* 44, 234–238.
- An, X.P., Ma, T., Hou, J.X., Fang, F., Han, P., Yan, Y., Zhao, H., Song, Y.X., Wang, J.G., Cao, B.Y., (2013). Association analysis between variants in KiSS1 gene and litter size in goats. *BMC Genet.* 14, 63–68
- Cao, G.L., Chu, M.X., Fang, L., Di, R., Feng, T., Li, N., (2010). Analysis on DNA sequence of KiSS-1 gene and its association with litter size in goats. *Mol. Biol. Rep.* 37 (8), 3921–3929.
- Cao, G.L., Chu, M.X., Fang, L., Feng, T., Di, R., Li, N., (2011). Analysis on DNA sequence of GPR54 gene and its association with litter size in goats. *Mol. Biol. Rep.* 38, 3839–3848.
- Chu, M.X., Lu, L., Feng, T., Di, R., Cao, G.L., (2010b). Polymorphism of bone morphogenetic protein 4 gene and its relationship with litter size of Jining Grey goats. *Mol. Biol. Rep.* 38, 4315–4320.
- Chu, M.X., Peng, Z.L., Chen, H.Q., Zhang, Y.J., Fang, L., Di, R., Cao, G.L., Feng, T., Li, N., (2012). Polymorphism in exon 2 of INHBB gene and its relationship with litter size in Jining Grey goats. *Anim. Sci. Pap. Rep.* 30 (1), 57–63.
- Chu, M.X., Yan, Y., Wang, P.Q., Yang, H.G., Hao, G., Yu, J.G., Tang, Q.Q., Feng, T., Cao, G., Huang, D.W., Di, R., Liu, Q.Y., Li, N., (2013). Polymorphism of AA-NAT gene and its relationship with litter size of Jining Grey goat of China. *Anim. Sci. Pap. Rep.* 31, 15–26.
- Chu, M.X., Zhao, X.H., Zhang, Y.J., Jin, M., Wang, J.Y., Di, R., Cao, G.L., Feng, T., Fang, L., Ma, Y.H., Li, K., (2010a). Polymorphisms of BMPR-IB gene and their relationship with litter size in goats. *Mol. Biol. Rep.* 37, 4033–4039.
- Davis, G.H. (2004). Fecundity genes in sheep. *Animal Reproduction Science.*, 82-83: 247- 253.
- Di, R., Liang, C., Chu, M.X., Liu, W.Z., Fang, L., Ma, Y.H., Li, K., (2009). Polymorphism of Luteinizing Hormone beta-subunit gene and its relationship with litter size of Jining Grey Goats. *Acta Veterinaria et Zootechnica Sinica* 40 (8), 1171–1178.
- Di, R., Yin, J., Chu, M.X., Cao, G.L., Feng, T., Fang, L., Zhou, Z.X., (2011). DNA polymorphism of introns 1 and 2 of Prolactin Receptor Gene and its association with litter size in goats. *Anim. Sci. Pap. Rep.* 29 (4), 343–350.
- Erickson, G.F. and Shimasaki, S. (2002). Morphologic and physiologic correlates of the PCOS ovary. In: Chang RJ, Dunaif A, Heindel JJ, eds. Polycystic ovary syndrome. New York: Marcel Dekker, Inc., 55–62.
- Feng, T., Chu, M.X., Cao, G.L., Tang, Q.Q., Di, R., Fang, L. and Li, N. (2011). Polymorphisms of caprine POU1F1 gene and their association with litter size in Jining Grey goats. *Mol. Biol. Rep.* 39 (4), 4029–4038.

- Feng, T., Zhao, Y.Z., Chu, M.X., Zhang, Y.J., Fang, L., Di, R., Cao, G.L., Li, N., (2009). Association between sexual precocity and alleles of KISS-1 and GPR54 genes in goats. *Anim. Biotechnol.* 20, 172–176.
- Guo, X.H., Li, Y., Chu, M.X., Feng, C.J., Di, R., Liu, Q.Y., Feng, T., Cao, G., Huang, D.W., Fang, L., Tang, Q.Q., Ma, Y.H., Li, K., (2013). Polymorphism of 5 regulatory region of caprine FSHR gene and its association with litter size in Jining Grey goat. *Turkish J. Vet. Anim. Sci.* 37, 497–503.
- Han, D., Li, G., Cao, B.Y., Wang, Y.N., Li, L., (2009). Polymorphism of GnRHR gene and its relationship with litter size trait of Saanen dairy goat. *J. China Agric. Univ.* 14, 93–97.
- He, Y., Ma, X., Liu, X., Zhang, C., Li, J., (2010). Candidate genes polymorphism and its association to prolificacy in Chinese Goats. *J. Agric. Sci.* 2, 88–92.
- Hou, J.X., An, X.P., Wang, J.G., Song, Y.X., Cui, Y.H., Wang, Y.F., Chen, Q.J., Cao, B.Y., (2011). New genetic polymorphisms of KiSS-1 gene and their association with litter size in goats. *Small Ruminant Res.* 96 (2), 106–110.
- Huang, Y.H., Wang, P.Q., Yang, L., Chu, M.X., Zhang, B.Y., Deng, L.M., Tan, Y., Fan, Q., (2012). Polymorphism of gonadotropin releasing hormone receptor (GnRHR) gene and its relationship with litter size in goats. *Acta Veterinaria et Zootechnica Sinica* 43 (1), 22–28.
- Jia, L., Chu, M.X., Di, R., Cao, G., Feng, T., Chen, H.Q., Sun, J., Fang, L., (2012). Polymorphism and structure of exon 2 of caprine melatonin receptor 1b gene and its relations to fertility and seasonal estrus. *Anim. Sci. Pap. Rep.* 30 (2), 169–179.
- Kumari R., Dayal S., Kumar S, Lal S. V., Chakraborti A., Barari S. K. and Dey A., (2015). Genetic polymorphism of bone morphogenetic protein receptor type 1 gene in Black Bengal goat and its association with litter size *Indian J. Anim. Sci.* 85 (5): 469-471
- Li, G., An, X.P., Fu, M.Z., Hou, J.X., Sun, R.P., Zhu, G.Q., Wang, J.G., Cao, B.Y., (2011a). Polymorphism of PRLR and LH genes by SSCP marker and their association with litter size in Boer goats. *Livestock Sci.* 36 (2), 281–286.
- Li, G., Wu, H.P., Fu, M.Z., Zhou, Z.Q., (2011b). Novel single nucleotide polymorphisms of GnRHR gene and their association with litter size in goats. *Archiv Tierzucht* 54 (6), 618–624.
- Li, Y.J., Zhang, L., Shang, L.Q., Wang, H.F., Zou, H., Zhang, H., Ji, D.J., (2010). Genetic polymorphism at three loci of PRLR and FSHR gene correlate with litter size in Chinese Haimen goat. *J. Anim. Vet. Adv.* 9 (22), 2835–2838.
- Lin, J.B., Du, Z.Y., Qin, C., Wang, J.F., Ran, X.Q., (2007). Polymorphism of BMP15 gene in Guizhou White goats. *Anim. Husbandry Vet. Med.* 39 (12), 21–24.
- Maitra, A., Sharma, R., Ahlawat, S., Tantia, M.S., (2014b). Novel genetic polymorphisms in caprine GPR54 gene associated with reproductive functions. *Indian J. Anim. Sci.* 84 (11), 1196–1201.
- Maitra, A., Sharma, R., Ahlawat, S., Tantia, M.S., Roy, M., Prakash, V., (2014a). Association analysis of polymorphisms in caprine KiSS1 gene with reproductive traits. *Anim. Reprod. Sci.* 151, 71–77.

- Montgomery, G.W., Galloway, S.M., Davis, G.H. and McNatty, K.P. (2001). Genes controlling ovulation rate in sheep. *Reproduction.*, 121: 843–852.
- Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., Pisselet, C., Riquet, J., Monniaux, D., Callebaut, I., Cribiu, E., Thimonier, J., Teyssier, J., Bodin, L., Cognie, Y., Chitour, N. and Elsen, J.M. (2001). Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. *Proc Natl Acad Sci USA.*, 98: 5104-5109.
- Polley, S., De, S., Batabyal, S., Kaushik, R., Yadav, P., Arora, J.S., Chat-topadhyay, S., Pan, S., Brahma, B., Datta, T.K., Goswami, S.L., (2009). Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. *Small Ruminant Res.* 85, 122–129.
- Roy, J., Polley, S., De, S., Mukherjee, A., Batabyal, S., Pan, S., Brahma, B., Datta, T.K., Goswami, S.L. Polymorphism of fecundity genes (FecB, FecX, and FecG) in the Indian Bonpala sheep. *Anim. Biotechnol.* 2011;22:151–162.
- Sharma, R., Ahlawat, S., Maitra, A., Roy, M., Mandakmale, S., Tantia, M.S., 2015b. Diversity in Indian goat leads to identification of polymorphism in candidate gene of reproductive trait. In: International Symposium on “Sustainable Management of Animal Genetic Resources for Livelihood Security in Developing Countries”. Madras Veterinary College, Chennai, India.
- Sharma, R., Ahlawat, S., Maitra, A., Roy, M., Mandakmale, S., Tantia, M.S., (2013). Polymorphism of BMP4 gene in Indian goat breeds differing in prolificacy. *Gene* 532, 140–145.
- Sharma, R., Maitra, A., Ahlawat, S., Roy, M., Mandakmale, S., Tantia, M.S., (2015a). Identification of novel SNPs in INHBB gene of Indian goat. *Indian J. Anim. Sci.* 85 (1), 53–57.
- Shimasaki, S., Moore, R.K., Otsuka, F. and Erickson, G.F. (2004). The bone morphogenetic protein system in mammalian reproduction. *Endocrine Reviews.*, 25: 72–101.
- Silva, J.R.V., Figueiredo, J.R., VandenHurk, R., (2009). Review: involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. *Theriogenology* 71, 1193–1208.
- Silva, J.R.V., van den Hurk, R., van Tol, H.T.A., Roelen, B.A.J., Figueiredo, J.R., (2006). The Kit ligand/c-Kit receptor system in goat ovaries: gene expression and protein localization. *Zygote*14,317–328.
- Souza C J, MacDougall C, Campbell B K, McNeilly A S and Baird D T. (2001). The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. *Journal of Endocrinology* 169(2): R1–R6.
- Sun, R.P., (2009). Polymorphism of LH gene and its relationship with litter number of goat. *J. Northwest Agric. Forest. Univ.* 37, 53–64.
- Wang, P.Q., Deng, L.M., Zhang, B.Y., Chu, M.X., Hou, J.Z., (2011b). Poly-morphisms of the cocaine-amphetamine-regulated transcript (CART) gene and their association with reproductive traits in Chinese goats. *Genet. Mol. Res.* 10 (2), 731–738.

- Wang, Y.Q., Li, Y.X., Zhang, N., Wang, Z.B., Bai, J.Y., (2011a). Polymorphism of exon 2 of BMP15 gene and its relationship with litter size of two Chinese goats. *Asian-Australas. J. Anim. Sci.* 24 (7), 905–911.
- Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Connell, A.R., McNatty, K.P., Montgomery, G.W., (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol. Reprod.* 64, 1225–1235.
- Yan, Y., (2010). Steroid 21-hydroxylase gene (*cyp21*) as a candidate gene for prolificacy of Jining grey goat. *J. Agric. Biotechnol.* 18, 917–924.
- Yang, W., Tang, K., Zhang, C., Xu, D., Wen, Q., Yang, L., (2011). Polymorphism of the GnRHR gene and its association with litter size in Boer goats. *S. Afr. J. Anim. Sci.* 41 (4), 398–402.
- Zhang, C., Liu, Y., Huang, K., Zeng, W., Xu, D., Wen, Q., Yang, L., (2011b). The association of two single nucleotide polymorphisms (SNPs) in growth hormone (GH) gene with litter size and superovulation response in goat-breeds. *Genet. Mol. Biol.* 34 (1), 49–55.
- Zhang, C.Y., Wu, C.J., Zeng, W.B., Huang, K.K., Li, X., Feng, J.H., Wang, D., Hua, G.H., Xu, D.Q., Wen, Q.Y., Yang, L.G., (2011a). Polymorphism in exon 3 of follicle stimulating hormone beta (FSHB) subunit gene and its association with litter traits and superovulation in the goat. *Small Ruminant Res.* 96, 53–57.
- Zhang, H.Y., Ding, X.L., Ying, S.J., Wang, Z.Y., Pang, X.S., Wang, R.F., Chen, Q.K., Shi, J.F., Zhang, H., Wang, F., (2008). SSCP analysis on exon 2 of GDF9 gene in local goat breeds of Jiangsu province. *Jiangsu Agric. Sci.* 5, 51–53.
- Zhu, G.Q., Wang, Q.I., Kang, Y.G., Li, Y.Z., Cao, B.Y., (2013). Polymorphisms in GDF9 gene and its relationship with litter size in five breeds of black goats. *Iranian J. Appl. Anim. Sci.* 3 (3), 625–628.

**AB-07**

### **Pan-genome codon usage pattern in Brucella**

Gaikwad S S, Usharani J., Suryawanshi P R, Deshpande A R, Majee S B

Department of Veterinary Microbiology,

College of Veterinary and Animal Sciences, Parbhani 431 402.

Analysis of relative synonymous codon usage data has both practical and theoretical applications in understanding the virulence, host preference and molecular biology of pathogen. Differences in codon usage patterns among genes reflect variations in base compositional biases and the intensity of natural selection. Here, we report genome wide codon usage pattern in Nine Brucella species: Brucella abortus, Brucella melitensis, Brucella ovis, Brucella suis, Brucella inopinata, Brucella canis, Brucella vulpis, Brucella microti, Brucella ceti which may be helpful in revealing the constraint factors such as mutation or selection pressure and improving the cloning, expression, and characterization of some special genes. The median GC content of all the genomes is GC rich (above 50%). The results of neutrality and effective number of codons plot analyses showed that both mutational bias and natural selection affect codon usage. We found that gene length is strongly correlated with codon usage and may contribute to the codon usage patterns observed in genes. Nucleotide composition and gene expression levels also shape codon usage bias. We have performed principal component analysis on the relative synonymous codon usage (RSCU) values of 61 sense codons. We identified codons with higher RSCU values and also identified optimal codons.

**AB-08**

### **EFFECT OF DIFFERENT TROPICAL STRESS CONDITIONS ON HSP-70 PROTEIN EXPRESSION IN VARIOUS BROILER STRAINS**

S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 and Suhas Amrutkar 5

Central Avian Research Institute, IZATNAGAR (U. P.)- 243122. INDIA

The present experiment was conducted to study the effect of different tropical stress conditions on molecular traits (HSP 70) in various broiler strains during 3 to 4 weeks of age. High ambient temperature is an important hindering factor for poultry production in the tropical and subtropical regions. Heat stress causes serious losses in poultry production because it increases mortality and reduces performance of chicken. Several nutritional and managemental practices could not completely prevent the negative effect of heat stress. Introgressing some important major genes like Naked neck (Na), and Frizzle (F) into broiler germplasm may substantially improve the heat tolerance. Hence, the experiment was designed to evaluate three indigenously developed broilers viz. CARIBRO-Tropicana (Naked neck and Frizzle gene bearing), CARIBRO-Mritunjai (Naked neck gene bearing) and CARIBRO-Vishal (Normal plumaged) under different THI (i.e. 72, 85 and 91) for 4 hours daily for 7 days. Total 324 broiler chicks (i.e. 36

chicks in each group) of 3 weeks of age were used in this study. The difference among three genetic groups on mRNA expression on HSP-70 were also significant ( $P < 0.05$ ) at different days of experiment at THI 72, 85 and 91. The higher mRNA expression levels of HSP-70 at all the time points under different THIs were exhibited in CARIBRO-Tropicana ( $1.27 \pm 0.01$  to  $2.51 \pm 0.019$ ) as compared to CARIBRO-Vishal ( $1.037 \pm 0.016$  to  $2.118 \pm 0.020$ ) and CARIBRO- Mritunjai ( $1.164 \pm 0.005$  to  $2.321 \pm 0.010$ ). The effect of THI was also found significant ( $P < 0.05$ ) on mRNA expression of HSP-70 at different days of experiment in each of the genetic groups. The mRNA expression levels of HSP-70 were significantly ( $P < 0.05$ ) higher at THI 91 than at THI 72 and 85 in each genetic groups. Significantly lower ( $P < 0.05$ ) mRNA expression of HSP-70 was observed on 0 th day than at 3 rd day under THI 85 in CARIBRO-Tropicana. mRNA expression of HSP-70 in CARIBRO-Tropicana under THI 91 was observed significantly lower at 0 th day than at 3 rd and 7 th day. In CARIBRO-Mritunjai and CARIBRO-Vishal, the mean mRNA expression of HSP-70 were observed significantly lower on at 0 th day than at 3 rd and 7 th day of experiment under THI 85 and THI 91.

**AB-09**

**Development of Assisted Reproductive Biotechnologies for Species**

**Conservation and Livestock Production: from microRNAs to ex situ approaches**

Sadanand D. Sontakke

Principal Scientist & Project Leader

Laboratory for the Conservation of Endangered Species (LaCONES),

CSIR-Centre for Cellular and Molecular Biology Annexe-I,

Attapur ring road, Hyderabad 500 048

Reproduction and fertility are central to the survival of a species. Understanding basic reproductive processes including ovarian follicular development and ovulation is critical for normal ovarian function. First part of talk would overview systematic development and successful application of variety of reproductive biotechnologies including semen banking and non-surgical artificial insemination to produce live births of Indian wild ungulates, as an alternative to in situ conservation and genetic management strategies that can be directly applied for conservation breeding programs in critically endangered species as newer dimension to wildlife conservation in India.

In second part, I would discuss work carried out partly at the Roslin Institute, UK on understanding involvement of new players (microRNAs) of fertility regulation in farmanimals. Microarray profiling and RT-qPCR identified, for the first time, a set of microRNAs associated with follicular growth and granulosa cell function, and as many as 33 microRNAs involved in follicle-to- luteal transition were identified in bovine that may have potential regulatory roles in ovulation and steroidogenesis, and female fertility in general.

**AB-10** **EFFECT OF THERMAL CONDITIONING DURING EMBRYOGENESIS ON EPIGENETIC CHANGES IN BDNF GENE IN BROILER CHICKS**

S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 and Suhas Amrutkar 5  
Central Avian Research Institute, IZATNAGAR (U. P.)- 243122. INDIA

The present experiment was conducted to study the epigenetic changes under extreme stress condition during embryogenesis. The total 100 eggs of Caribro Vishal (i.e. 50 eggs in each group) were incubated. In control group, normal incubator temperature in setter during 0-18 th day of incubation was 37.8°C and in hatcher during 19-21 st day of incubation was 36.5°C. In treatment group, the temperature in setter and hatcher were same except during 14-18 th day, incubation temperature were raised from 37.5°C to 40°C for 4 hours. The ability of chicks of both control and experimental groups (heat conditioning group) for epigenetic temperature adaptation was assessed by evaluating the expression of BDNF gene and fold increase of DNA methylation. BDNF genes in brain at day old stage were studied by using real time PCR. The expression analysis of BDNF gene in brain exhibited higher expression levels in thermally conditioned chicks as compared to control group. Approximately 2 fold increase in DNA methylation was exhibited by thermally conditioned chicks in BDNF gene promoter over the control group chicks. Overall, the thermally conditioned chicks exhibited better performance and tolerance under stress.

**AB-11** **INFLUENCE OF DIFFERENT TROPICAL STRESS CONDITIONS ON PRODUCTION TRAITS IN VARIOUS BROILER STRAINS**

S. A. Amrutkar 1 ; V. K. Saxena 2 ; Simmi Tomar 3 , Bharti Deshmukh 4 and Suhas Amrutkar 5  
Central Avian Research Institute, IZATNAGAR (U. P.)- 243122. INDIA

The present experiment was conducted to study the effect of different tropical stress conditions on production traits in various broiler strains during 3 to 4 weeks of age. High ambient temperature is an important hindering factor for poultry production in the tropical and subtropical regions. Heat stress causes serious losses in poultry production because it increases mortality and reduces performance of chicken. Several nutritional and managerial practices could not completely prevent the negative effect of heat stress. Introgressing some important major genes likes Naked neck (Na), and Frizzle (F) into broiler germplasm may substantially improve the heat tolerance. Hence, the experiment was designed to evaluate three indigenously developed broilers viz. CARIBRO-Tropicana (Naked neck and Frizzle gene bearing), CARIBRO-Mritunjai (Naked neck gene bearing) and CARIBRO-Vishal (Normal plumaged) under different THI (i.e. 72, 85 and 91) for 4 hours daily for 7 days. Total 324 broiler chicks (i.e. 36 chicks in each group) of 3 weeks of age were used in this study. At THI 72, 85 and 91; boy weight, weight

gain, FCR were observed highest in Naked neck birds and lowest in normal plumaged birds under different THI. The CARIBRO-Vishal showed highest stress as compared to other group. Higher the THI more severe was the effect on the traits. Among all the three genetic groups CARIBRO-Mritunjai exhibited highest means for production traits as well as tolerated the heat stress in a better way; therefore CARIBRO-Mritunjai was adjudged to be the best genetic group for production under hot-tropical climate.

**T3**

**Stem Cells And Regenerative  
Medicine**

**T3-01** ***EX-VIVO* DERIVATION OF CAPRINE FETAL ADNEXA MESENCHYMAL STEM CELLS: A COMPARATIVE IMMUNOMODULATORY ANALYSIS**

G.Taru Sharma

[gts553@gmail.com](mailto:gts553@gmail.com)

Principal Scientist and Head, Director CAFT,  
Division of Physiology and Climatology,  
ICAR-Indian Veterinary Research Institute, Izatnagar

To analyze the comparative immunomodulatory properties of stimulated caprine fetal adnexa MSCs, stem cells were derived and expanded *ex-vivo* from {amniotic fluid (cAF), amniotic sac (cAS), Wharton's jelly (cWJ) and cord blood (cCB)}. A homogenous population of all the caprine fetal adnexa derived MSCs, at third passage, were subjected to comparative immunomodulatory analysis after the tri-lineage differentiation and phenotypic characterization. On stimulation with inflammatory cytokines (INF- $\alpha$  and TNF- $\alpha$ ), the mRNA levels of different cytokines and growth factors in caprine fetal adnexa MSCs were found to be modulated. Activated PBMCs were significantly inhibited in cWJ MSCs compared to non-activated PBMC than that of other fetal adnexa MSCs. Maximum inhibition on PBMC proliferation was produced by cWJ MSCs followed by cAS MSCs than the other two cell types. All the four fetal adnexa MSCs differentiated into the tri-lineages as well as expressed surface antigens and pluripotency markers. Outcome of this work witnessed IDO as the major immuno-modulator in cWJ MSCs, whereas iNOS emerged to be the major player for cAS MSCs. Therefore, it is concluded that cWJ MSCs exert relatively maximum immuno-modulation compared to all the other fetal adnexa derived MSCs, hence it is considered the best option amidst all the fetal adnexa MSCs for further clinical applications. Details of the above work shall be discussed during the talk with the long term goals of the inference drawn out of this work.

**T3-02**

### **Stem Cells : As Regenerative Medicine**

C. H. Pawshe, S. A. Ingle, V.S. Dhaygude and R. R. Shelar

Stem Cell Laboratory, Bombay Veterinary College, Parel, Mumbai – 12

Maharashtra Animal and Fishery Sciences University, Nagpur.

Stem cells are referred to as undifferentiated cells which are capable of indefinite cell divisions and giving rise to more cells of same type or differentiate into other cell types. Usually these are of embryonic (Embryonic Stem Cells) or adult (Adult or Somatic Stem Cells) origin. Both these stem cell types vary greatly in their potency to differentiate into different cell types. Stem cells (SCs) also play a role in internal repair system of the body by differentiating into the desired cell type and thereby replacing the damaged cells. They remain inactive in the body and divide only under special conditions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart they proliferate and differentiate into specialized cell types. Thus, they act as building blocks of the body by making and repairing the body structures.

Since many decades, scientists have been exploiting this characteristic of the Stem Cells for treatment of diseases. Bone marrow transplant has been used in patients with leukemia and lymphoma since over 30 years and is still the most widely practiced form of stem cell therapy (Karanes et al, 2008). Besides these, stem cells have been used and are being studied by various workers in human and Veterinary medicine to exploit their potential for the treatment of a variety of conditions. A number of conditions have been described where the stem cells have a potential to regenerate the damaged tissue and revert the normal function of the respective organs. Studies have demonstrated that the use of stem cells as therapeutic agents in conditions of brain damage, cancer, spinal cord injury, heart damage, conditions involving blood cells, etc. show convincing results. Stem cells are being exploited for treatment and relief of lot more conditions in humans. They are being studied for the treatment of baldness, missing teeth, deafness, vision impairment, neural and behavioral birth defects, transplantations, treatment of wounds, etc. where the stem cells of the patient himself could be used for treatment so as to avoid graft rejection.

#### **Stem cell use in animals:**

Stem cell research has also been extended to the Veterinary sciences to treat similar disease conditions in animals. Research conducted on horses, dogs and cats can benefit the development of stem-cell treatments in Veterinary medicine and can target a wide range of injuries and diseases such as myocardial infarction, stroke, tendon and ligament damage, osteoarthritis, osteochondrosis and muscular dystrophy both in large animals, as well as humans. The high degree of frequency and severity of certain injuries in race horses has put Veterinary medicine at the forefront of this novel regenerative approach. Autologous

adipose derived stem cells have been shown to be effective in clinical trials of dogs with osteoarthritis of the hip and elbow in dogs. One of the most common traumas brought to Veterinary hospitals are the spinal cord injuries. Dogs treated with stem cells after injuries and traumas have shown improvements over conventional therapies. Clinical trials for the treatment and regeneration of peripheral nerves are also being done. Haematopoietic stem cells are also being studied for the potential to treat the corneal ulcers in horses and dog.

### **Research work at Stem Cell Laboratory, Bombay Veterinary College, Mumbai**

The isolation and phenotypic characterization of mesenchymal stem cells (MSCs) have been demonstrated in several vertebrate species including murine, canine, ovine, avian, bovine, equine, and primates, however, the study on therapeutic aspect of stem cell use is still limited domestic animals. Stem Cell Laboratory has been established at Bombay Veterinary College, Mumbai, with objective to develop clinical grade cGMP compliant mesenchymal stem cells (MSC) for therapeutic use in canines and the research work on this aspect is in progress. The stem cell research work was conducted in collaboration between Stem Cell Laboratory, Bombay Veterinary College, Parel, Mumbai and Kasiak Research Laboratory, Thane.

### **Canine Adipose Derived Mesenchymal Stem Cells – Impact of site of adipose tissue collection on cell yield and characterization**

The MSCs have been isolated conventionally from bone marrow. However, there is enough evidence that MSCs exist not only in bone marrow, but virtually in all the organs of body. Therefore, different tissue source of the MSC may be critically important in determining biological activity and may generate MSC products with different cytokine expression profile (Horwitz and Dominici, 2008). Hence different MSC tissue sources may be especially suited for specific clinical applications. In canine, isolation of MSC have been reported from bone marrow, adipose tissue, umbilical cord blood, Wharton's Jelly. However, adipose tissue is thought to be a promising source because it can be harvested in relatively large quantities from small amount of adipose tissue, easy to isolate, with minimum morbidity and is of great value to culture and expand safely and effectively for development of cell based therapies. In a recent report it was demonstrated that adipose tissue had pluripotent population cells that showed pluripotent markers expression like SSEA-3, TR-1-60, Oct3/4, Nanog, Sox-2 and spontaneously differentiated into all the cell lineages (Heneidi, *et al.*, 2013). Adipose derived mesenchymal stem cells (AD-MSC) derived from fat obtained from different anatomical sites showed considerable variations in term of cell yield and cellular multi-potent properties (Wouter, *et al.*, 2008). Therefore, we investigated whether different anatomical sites in an individual donor influence the nature, growth characteristics and differentiation potential of their AD-MSC.

In our study we found that AD-MSC derived from inguinal and mesenteric fat failed to grow beyond first passage. Cells progressively lose the proliferation capacity, their stemness characteristics, cell surface expression and their tri-lineage differentiation potential after first passage. Whereas, AD- MSC derived from subcutaneous fat had consistent proliferation rate, higher cell yield and consistent population doubling time up to passage-5 before they became senescence. These results are in agreement with other investigators where, MSC derived from subcutaneous fat showed significantly higher proliferation rate, higher cell yield, and uniform multi-potential properties than inguinal, omental and intra-abdominal fat tissues (Jung, *et al.*, 2014, Guercio, *et al.*, 2013). The difference in the replicating capacity in adipose tissues from different anatomical sites may be due to the presence of abundant number of more rapidly replicating pre-adipocyte subtype in subcutaneous fat than fat from other anatomical sites (Tchkonina, *et al.*, 2005).

#### **Effect of media and seeding densities on ex vivo expansion of allogeneic clinical grade canine adipose derived mesenchymal stem cells**

Adipose tissue derived MSC are found to be effective in several different pre-clinical animal models of human disease as well as in clinical applications. Few reports suggested significant improvement after stem cells transplantation in disease conditions in dogs such as spinal cord injury (Chung *et al.*, 2013, Ryu *et al.*, 2012), osteoarthritis (Guercio, *et al.*, 2012), and dilated cardiomyopathy (Pogue, *at al.*, 2013). Stem cell therapy may be an option for such indications that could relieve the pain, suffering and increase the quality of life of dog and owner. To take this initiative to the next step, first, it is important to generate a large quantity of clinical grade MSCs while retaining stem cell characteristics, immunosuppressive capacities and multi-lineage differentiation potential. Reaching the estimated clinical dose of 5 to 100 million cells for adult dogs can be not only challenging but could also hamper the progress of clinical grade MSC applications. As per earlier literature suggested that sequential sub-passage of canine AD-MSC decrease multi-potential properties including cell morphology, doubling time, cell yield, differential potential, pluripotent markers, cell surface markers and make them clinically ineligible (Lee *et al.*, 2014). Media used for tissue culture would have an important impact on growth and differentiation of MSCs. Not much has been reported about therapeutic expansion of canine AD-MSCs.

In the our laboratory, we attempted to identify the optimal basal culture medium for large scale expansion of canine AD-MSCs which could lead to the production of large numbers of MSCs for subsequent cellular therapeutic approaches. We have also performed analysis of seeding densities, growth kinetics, and surface marker phenotype and tri-lineage differentiation potential. Our attempt was to establish an off-the shelf product for canine clinical applications.

We have evaluated two major basal medium viz. DMEM-KO and  $\alpha$ -MEM alone or in 5 different combinations. Evaluation of different basal media led us to conclude that  $\alpha$ -MEM or 25-75  $\alpha$ -MEM-DMEM-KO and seeding density at the rate of 5000 cells/cm<sup>2</sup> are most suitable for the expansion of canine AD-MSC as evident from the proliferation rate, total cell numbers, population doubling time, the number of population doublings. Particularly, combination of 25: 75 DMEM-KO:  $\alpha$ -MEM is most ideal combination to achieve all the parameters including the highest cell yield of 256,000 cells / cm<sup>2</sup> keeping all the safety parameters intact for clinical application. Canine AD-MSCs started losing their adherent potential after passage-5 in all the media combinations that were tested in this experiment. It is not known whether present culture conditions and serum concentration sufficiently support adhesion protein and extra-cellular matrix synthesis. This needs to be further analyzed. Therefore, production of large quantity of canine AD-MSCs with minimal manipulation while retaining their stemness, self-renewal potential, ability to differentiate into multi-lineage tissue is challenging.

Another important factor that affected the overall yield of MSCs is seeding density. We evaluated seeding densities at 1000, 2000, 3000, 4000 and 5000 cells/cm<sup>2</sup> in a different basal medium. It is known that lower densities had a faster proliferation and higher PD than higher densities. We observed that canine AD-MSCs at seeding density of 5000 cells /cm<sup>2</sup> in all medium had a faster proliferation rate with lesser PD and PDT with significantly higher cell yield. Particularly, 25:75 DMEM-KO:  $\alpha$ -MEM combination was most optimal and yielded as high as 256,000 cells / cm<sup>2</sup> and 166,000 cells/cm<sup>2</sup> in passage-1 and 2 respectively.

Effect of seeding densities on canine AD-MSC was not observed in the literature. However, in one of the reports, investigator used 7000 to 8000 amniotic membranes MSC per cm<sup>2</sup> but details were lacking (Park, *et al.*, 2012). More population doublings occurred at lower density seeding may affect the senescence of cells, characterization, and up-regulation of transformation markers. Our finding of optimum seeding density at 5000 cells/cm<sup>2</sup> for maximal expansion is useful for potential clinical applications as cell culturing procedure can be less time consuming, less risk of contamination, loss of characteristics, cell transformation, abnormality and affordable. During our large scale production method we could be able to produce high cell yield within 2 passages while retaining their stemness, tri-lineage differentiation potential and immunosuppression activity.

### **Wound Healing Activity of Canine Adipose Tissue Derived Mesenchymal Stem Cells in Wistar Rats**

Wound healing occurs with restoration of skin integrity and function, leaving minimal scarring compared to the uninjured surrounding tissue (Wu et al, 2007). However, there are often obstacles to a successful healing process, including the crucial problem of insufficient skin tissue in the area of injury. Several studies have shown the efficacy of stem cells in promoting faster and superior wound healing (Wu

et al, 2007). Alexaki et al. (2012) successfully used adipose derived mesenchymal stem cells in wound healing in mice and compared their effect with dermal fibroblasts. Ansari et al. (2013) reported that canine bone marrow Mesenchymal stem cells and stem cell conditioned media help for diabetic wound healing in rat. The application of stem cells in wounds promoted more efficient reepithelialization by their proliferative effect on keratinocytes. With this hypothesize, that the use of ASCs will improve healing in a mouse, present study was undertaken in our laboratory to explore the efficacy of canine adipose tissue derived mesenchymal stem cells on wound healing in wistar rats model.

The mesenchymal stem cells were isolated from adipose tissue of donor dogs and stem cell suspended in the 1.5 cc normal saline having concentration  $2 \times 10^6$ /ml used for the wound healing treatment. Male wistar rats weighing 130-220 gm were selected and wounds were produced on dorsal thoracic region of anaesthetized rat by cutting away approximately 300 mm<sup>2</sup> full thickness of skin from predetermined area. The rats were divided into three groups viz. Group I (treatment of stem cells @  $2 \times 10^6$  cells /wound injected subcutaneously at five different places around the wound), group II (Soframycin topical application on the wound surface) and group III (normal saline injected subcutaneously at five different places around wound). The wounds were observed for progressive changes in size of wound for the period of 18 days at 3 days interval and calculated the percent retraction in wound size. There was no significant difference in percent wound retraction in Group I (stem cell injected ) and group II (Soframycin topical application) as compared to group III (normal saline injected) at 3<sup>rd</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup> and 18<sup>th</sup> days intervals. Histopathological evaluations with respect to reepithelization, PMNL (polymorphonuclear leucocytes) infiltration, proliferation of fibroblasts, new vessels and new collagen in Group I, group II and group III not showed any significant difference at 7<sup>th</sup>, 14<sup>th</sup> and 18<sup>th</sup>days intervals. In this study we were not observed any significant effect of stem cell on wound healing. A similar type of finding was noted by Loder et al. (2015) where they also tested the effect of adipose derived stem cells in the treatment of burns wound. The mice with inflicted burns that received stem cells injections showed no significant difference in comparison to controls (received saline injections) with respect to proliferation and vascularization (Loder et al., 2015). Kim (2012) reported that the Adipose-derived mesenchymal stem cells (ASCs) are attractive cell source for wound healing, however, one obstacle to this approach is that the transplanted ASC population can decline rapidly in the recipient tissue.

Stem cell therapies have shown promising results over the last decades. However, there is currently controversy regarding the direct use of stem cells the wound area or use other biological material as barrier for stem cell based therapy in clinical practice. Eppley (2001) documented that implementing stem cells without providing a proper skeletal basis will not result in functional tissue because a biological matrix is highly required to integrate all cells and molecules to interact properly. The research work regarding the therapeutic use of canine adipose derived mesenchymal stem cell for the treatment of osteoarthritis, atopic dermatitis, renal failure and wound healing in canines is in progress in our laboratory.

## References:

- Alexaki, V.-I., Simantiraki, D., Panayiotopoulou M. et al. (2012). *Cell Transplantation*; vol. 21, no. 11, pp. 2441–2454.
- Ansari MM, Sreekumar TR, Chandra V, Dubey PK, Kumar GS, et al. (2013) *J Stem Cell Res Ther* 3: 141. doi:10.4172/2157-7633.1000141
- Chung CS, Fujita N, Kawahara N., J. (2013). *Vet Med Sci*; available at <http://dx.doi.org/10.1292/jvms.12-0470>.
- Eppley BL, (2001). *Plast Reconstr Surg*; 107:757-62.
- Guercio A, Di Marco P, Casella S, et al. (2012). *Cell Biol Int*. 36, 189–194.
- Guercio A, Di Bella S, Casella S, et al. (2013) *Cell Biol Int*; Mar 15.
- Heneidi S, Simerman AA, Keller E, et al. (2013) *PLoS One*; 8(6): e64752. doi:10.1371/journal.pone.0064752.
- Horwitz EM, Dominici M. (2008). *Cytotherapy*; 10:771-774.
- Jung HG, Ahn EK, Lee JH, Kim YH, Leem SH, Heo J, Kim H, (2014). *Tissue Engineering and Regenerative Medicine*, 11 (2):137-142
- Karanes C, Nelson GO, Chitphakdithai P, Agura E. et al. (2012). *J Dermatol Sci*; 68: 149-56.
- Kim H, Choi K, Kweon OK, Kim WH. (2012). *J Dermatol Sci*; 68: 149-56.
- Lee KS, Kang HW, Lee HT, Kim HJ, Kim CL, Song JY, Lee KW, Cha SH. (2014). *Res Vet Sci*. Apr;96(2):267-75. doi: 10.1016/j.rvsc.2013.12.011. Epub 2013 Dec 30.
- Loder S, Peterson, J. R., Agarwal, S. et al. (2015). *Journal of Burn Care & Research*; vol. 36, no. 1, pp. 70–76.
- Park SB, Seo MS, Kim HS, et al. (2012). *PLoS One*; 7:e44693. Epub Sep 14.
- Pogue B, Estrada AH, Sosa-Samper I, et al. (2013). *J Small Anim Pract*; Jun 3. doi: 10.1111/jsap.12098. [Epub ahead of print].
- Ryu HH, Kang BJ, Park SS, et al. (2012) *J Vet Med Sci*;74: 1617–1630.
- Tchkonina T, Tchoukalova YD, Giorgadze N, et al. (2005). *Am J Physiol Endocrinol Metab*. 288(1):E267-77.
- Wouter J. F. M. Jurgens, Maikel J., et. (2008). *Cell Tissue Res*. Jun; 332(3): 415–426.
- Wu Y, Chen L, Scott PG, Tredget EE (2007) *Stem Cells*; 25: 2648-59.

**AB-12**

**ROLE OF EMBRYONIC STEM CELLS FOR OSTEOGENESIS  
IN THE REPAIR OF BONE DEFECTS IN RABBIT MODEL**

B.K. Bhadane\*, S.K.Maiti, A.K.Sharma, Naveen Kumar, Amarpal, Sadan Bag and G. K. Das  
Division of Vet. Surgery & Radiology, IVRI, Izatnagar, Bareilly (UP) 243 122

\* Presently working as Assistant Professor, Department of Surgery & Radiology, COVAS,  
Parbhani (MS)

**ABSTRACT**

Bone is the only tissue in the body that is able to heal without microscopical scarring. Further healing involves numerous cells, regulation of cell function and biochemical interactions in the repair process. Specific bone induction proteins growth factors can induce bone formation and healing in- vivo ideally known as bone morphogenic proteins (BMP). Stem cells have the therapeutic potential in the realm of orthopaedic surgery because of their capacity to self renew and differentiate into various types of mature cells and including bone. Embryonic stem cells (ESC) are pluripotent cells derived from inner cell mass of pre-implantation embryos and represent embryonic pre-cursors that give rise to any cell type in the embryo, specifically differentiated into osteogenic cells under selective culture conditions. The present study was conducted to investigate the potentiality of ESC for osteogenesis in the repair of bone defects.

Experimental model of critical segmental bone defect in diaphysis of radius(5mm ) was created in 36 rabbit. Tissue engineered construct (TEC) was prepared with crushed granules of hydroxyapatite (HA) alone (group A), HA and ESC (group C), Bone morphogenetic protein2 (group D), transforming growth factors (TGF beta 1) (group E), and Insulin growth factors (IGF) (group F). Clinically all the animals showed recovery with primary cutaneous healing in all the animals. Gross observations revealed that filling of the defect was found better in treatment groups. The radiological and histological observations depicted more signs of new bone formation and periosteal reactions in the treatment groups. ESC induces osteogenesis for repair of bone defect with growth factors of BMP-2, TGF beta-1, IGF and additive effect of transplanted ESC.

**T4**

**Cutting Edge Innovations In  
Reproductive Biotechnology**

**T4-01**

## **Testes-mediated gene transfer (TMGT) - a potential tool for producing transgenic livestock**

Abhijit Mitra<sup>1</sup> and Pramod Kumar R<sup>2</sup>

<sup>1</sup>Director, ICAR-National Research Center on Mithun, Medziphema, Dimapur -797 106 (Nagaland), India; (email: drabhijitmitra@gmail.com)

<sup>2</sup> SciGenom Labs Pvt. Ltd., Kakkanad, Cochin, Kerala, India

The advent of transgenic livestock technology has revolutionized the course of biological research and opened up countless opportunities in both the basic and applied area of research. It offers a more direct approach for enhancing quality (e.g., milk quality) and efficiency (e.g., growth) of livestock production, developing livestock with resistance against diseases, as well as, providing a system for producing pharmaceuticals or nutraceutically important recombinant proteins, and xenoplantation products. In comparison to plants and other laboratory animal species, the progress of the application of this technology in livestock species for agricultural use has been very slow.

In 1980, Gordon and coworkers reported the first successful report of making transgenic mice using pronuclear microinjection. But, its application in livestock species was initiated only after the demonstration of a dramatic increase (nearly 50%) in body weight and size of transgenic mice carrying human growth hormone gene (Palmiter et al. 1982). In 1985, the first transgenic livestock, pig expressing human growth hormone, was produced (Hammer et al. 1985). This followed a series of reports demonstrating the generation of transgenic and by the next decade, transgenic animals in almost all the livestock species including cow, sheep, pig, and goat were produced. In the meantime, though retroviral vector came into the existence, the pronuclear microinjection remained as the most popular and used method of producing transgenic animals. In spite of the significant refinement in the existing technology of pronuclear microinjection, the application of transgenic animals for agricultural use remained constrained by three factors: 1) the lack of knowledge of the genetic basis of factors controlling the production traits, 2) the lack for tissue and developmentally appropriate promoters, and 3) the poor efficiency (0.1-3%) of production of transgenic livestock. Besides, the another most important problem was the random integration of the transgene resulting in either off-target effect and/or inefficient expression of the transgene due to the position effect.

In mid 90's the technological breakthrough enabling cloning of mammalian species using somatic cells (Campbell et al. 1996) as well as adult cells (Wilmot et al. 1997) resulted in a paradigm shift. The consequent advent of somatic cell nuclear transfer (SCNT) allowed the application homologous recombination to genetically modify the cultured cells that could be further employed for generating transgenic animals with 100% efficiency. The application of SCNT resulted in the production of transgenic sheep (Schnieke et al. 1997) and the first knockout (KO) sheep (Denning et al. 2001) and pigs (Dai et al. 2002). These methods, however, are often afflicted by the requirement of highly specialized laboratory techniques and a very skilled early embryonic manipulation.

Almost at the time when “Dolly the sheep” was hitting the headline, Naldini and co-workers(1996) demonstrated that ability of nondividing lentiviral vector for stable in vivo gene transfer. The lentiviral-mediated gene transfer method is safer than the retroviral method and shows a comparatively higher efficiency but suffers from insertional mutagenesis (Modlich et al. 2009; Biffi et al. 2011) and limitation in transgene-carrying capacity (Thomas et al. 2003; Meng et al., 2016).

The above-mentioned technologies, including pronuclear microinjection, embryonic stem cell-mediated method, and viral-mediated transfection, are predominantly relied on manipulation of the female germ cells. During the early 2000's, targeting male germ cells emerged as an alternative for transgenic animal production. Generally, two strategies of gene transfer to male germ cells are employed; 1) sperm-mediated gene transfer (SMGT), and 2) testis-mediated gene transfer (TMGT). SMGT includes the direct transfer of genes into sperm cells, whereas TMGT involves in vivo introduction of foreign DNA into testicular germ cells to produce transgenic sperm cells. SMGT though appeared as a straightforward method, but poor repeatability (Lavitrano et al. 2002, 2003). On the other hand, the TMGT allows mass gene transfer by natural mating exempting the use of cumbersome procedures like in vitro fertilization and embryo transfer. Furthermore, TMGT ensures a greater possibility of stable integration of transgenes into the genome of the host animal (Dhup and Majumdar, 2008; Yamazaki et al. 2000).

Sperm-mediated gene transfer (SMGT), exploits the ability of sperm cells to bind, internalize and transport the exogenous DNA into an oocyte during the process of fertilization, is considered as a much interesting and promising alternative (Lavitrano et al, 1989). The method of SMGT appears to be a simple, efficient and applicable to a variety of species that uses spermatozoa for its propagation. The DNA binding efficiency, however, varies widely ranging from 0.3 to 78% among the sperms of different species. The success of SMGT is influenced by several factors including the donors of spermatozoa, incubation media, size and type of the exogenous DNA, and the kind of assisted reproductive technique used. In order to improve the DNA uptake efficiency of sperm cells, several strategies have been employed which include DNA–liposome complexes (Lai et al., 2001), electroporation (Gagne et al., 1991), virus-mediated transfection (Takehashi et al., 2007), linker (receptor) based method (Wu and Wu, 1987), combination of restriction enzyme-mediated integration (REMI) with SMGT (Kroll and Amaya, 1996), as well as combination of intracytoplasmic sperm injection (ICSI) with sperm/DNA interaction (Perry et al., 1999). Amongst those, electroporation-aided SMGT is considered to be cheaper and more efficient method. Using electroporation-aided SMGT, transgenic offsprings are successfully produced in finfish and shellfish, and also in Rohu fish.

Recently we have reported, for the first time, a protocol for electroporation aided SMGT in goat (Pramod et al, 2016). Our study demonstrated that under the optimized condition, electroporation can result in maximum DNA uptake by the caprine sperm cells with minimum adverse effect on their vital parameters including fertilizing ability. We also produced transgenic fluorescent embryos using transfected sperms.

Traditional SMGT experiments are potentially characterized by lack of reproducibility. *In vivo* gene transfer to introduce the transgene into testicular (sperm) stem cells namely testis-mediated gene transfer (TMGT) could be an alternative approach (Dhup and Majumdar, 2008) to solve this problem. Some scientists describe TMGT as an independent technique from SMGT, but others consider it as just a modification or simple variation of it, because, in both cases, sperm cells are undertaking the process of gene transfer. This technique would, in principle, remove the need to collect, manipulate or transfer eggs, thus providing a major streamlining of germline transgenesis (Smith, 2002). This *in vivo* technology introduces foreign DNA directly into testis by injection. In order to increase the efficiency, several strategies including virus-mediated as well as non-viral physical and chemical methods have been employed for TMGT (Dhup and Majumdar, 2008; Sehgal et al., 2011). Owing to higher efficiency, virus-aided TMGT offers an attractive proposition, but it is constrained by biohazard risk and possible harmful effects such as uncontrolled infection or inflammation (Scobey et al., 2001). Amongst the non-viral methods, both lipofection and electroporation aided TMGT are considered as an easier and safer method (Umamoto et al., 2005).

The available literature indicates that electroporation method of TMGT has immense potential to produce transgenic laboratory animals (Huang et al., 2000; Dhup and Majumdar, 2008). However, till now there is no report of TGMT in large animals. In our laboratory (at Genome Analysis Lab, IVRI, Izatnagar), under the ICAR-National Fellow programme, we developed a successful method of transgenesis in goat by gene transfer into testicular cells using electroporation. Initially, we demonstrated that electroporation under optimized condition can efficiently transfer the foreign gene into the testicular tissues, particularly in somniferous tubules (Raina et al. 2015). Subsequently, we demonstrated the foreign gene expression in embryo and spermatogonial stem cells (SSCs) and the successful production of a transgenic goat using this method (*under publication*).

In conclusion, SMGT appears to be simple, efficient, and relatively inexpensive methods for modifying animals and the genome of animals. However, its underlying molecular basis is generally neglected and the inconsistencies concerning the reproducibility associated with this method remain unsolved. In spite of it is having a well-accepted and established method, SMGT still needs further refinement to make the technique even more widespread and reliable. Given the wide availability of livestock semen, SMGT can be considered as the method of choice for the production of genetically modified farm animals. As the TMGT method has high success rate in the production of transgenic laboratory animals, we can try this technique in farm animals using optimized conditions. Another problem in transgenic livestock production is the strict ethical regime and difficulties in obtaining the required permission due to lack of precision in transgene integration. However, the advent of genome editing technologies namely zinc finger nucleases (ZFNs; Cui, 2011), transcription activator-like effector nucleases (TALENs; Park et al. 2014) and clustered regularly interspersed short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) (Dow, 2015) has improved the precision ensuring the targeted gene integration in the host genome. In future, the combination of any of the above gene editing technologies and TMGT offers efficient

gene transfer in large animals. It is expected that the recent technological advancement in gene delivery techniques along with genome editing will bring the desired success in producing agriculturally important and commercially viable transgenic livestock.

## **References**

- Biffi, A. et al. 2011. *Blood* 117, 5332-5339.
- Campbell KH, , et al., 1996. *Nature*. 380:64–68
- Chen, X. et al., 2014. *Mol. Med. Rep.* 9, 955–960.
- Cui, X. 2011. *Nat. Biotechnol.* 29, 64–67 (2011).
- Dai Y. et al., 2002. *Nat Biotechnol.* 20:251–255.
- Denning C. et al. 2001. *Nat Biotechnol.* 19:559–562
- Dhup, S. and Majumdar, S.S. 2008. *Nat. Methods.* 5: 601–603.
- Dow, L.E. 2015. *Nat. Biotechnol.* 33, 390–394 (2015).
- Gagne et al., 1991. *Mol. Reprod. Devel.* 29: 6 -15.
- Gordon, J.W., 1980. *Proc. Natl. Acad. Sci. USA.* 77: 7380.
- Hammer R. E. et al. 1985. *Nature* (315):680–683
- Kroll K.L. and Amaya.E. 1996. *Development.* 122: 3173 -3183.
- Lai et al., 2001. *Zygote*, 9, pp. 339–346.
- Lavitrano, M et al., 1989.. *Cell.* 57: 717 -723.
- Lavitrano, M. et al., 2002. *Proc Natl Acad Sci USA.* 99, 14230-14235.
- Lavitrano, M. et al., 2003. *Mol Reprod Dev.* 64, 284–291.
- Meng, J. et al. 2016. *Sci. Rep.* 6, 19750 (2016).
- Modlich, U. et al. 2009. *Mol. Ther.* 17, 1919–1928.
- Palmiter R. D. et al., 1982. *Nature.* 300:611–615
- Park, T.S. et al., 2014. *Proc. Natl. Acad. Sci. USA* 111, 12716–12721 (2014).
- Perry, A.C.F. et al, 1999. *Science.* 284: 1180 -1183.
- Pramod R. K., Kumar R., Mitra A. 2016. *Gene* 576 (1), 505-511
- Raina, A., Kumar, S., Shrivastava, R., and A Mitra. 2015. Testis mediated gene transfer: In vitro transfection in goat testis by electroporation. *Gene* 554 (1), 96-100
- Schnieke A. E., et al. 1997. *Science.* 278:2130–2133
- Scobey et al., 2001. *Endocrinology.* 142:948–954.
- Sehgal, L et al., 2011. *PLoS ONE* 6, e21975.
- Simões, R. et al. 2013. *Zygote.* 18, 1–5 .
- Smith, K. R. 2004. *International Journal of Medical Sciences* 1(2): 76-91.
- Takehashi, M. et al., 2007. *Proc. Natl. Acad. Sci. USA.* 104: 2596-2601.
- Thomas, C. E et al. 2003. *Nat. Rev. Genet.* 4, 346–358.
- Umamoto Y. et al., 2005. *J Androl.* 26:264-271.
- Wilmot I, et al., 1997.. *Nature.* 385:810–813
- Wu, G.Y. and Wu, C.H. 1987. *J. Biol. Chem.*, 262: 4429 -4432.
- Yamazaki, Y., et al. 2000. *J. Exp.. Zool.* 286, 212–218.

**T4-02 ADVANCE DIAGNOSTIC TECHNIQUES IN ANIMAL REPRODUCTION**

Dr. Sarita U. Gulavane- M.VSc. Ph. D.

Associate Professor, Dept. of Animal Reproduction, Obstetrics &Gynaecology.

Bombay Veterinary College, Parel, Mumbai 400 012

Production and reproduction is closely related in production animals. It is important to diagnose reproductive problems as early as possible to maintain optimum reproductive status. Various advance diagnostic techniques are available for diagnosis of reproductive problems. Some of them are still in early stages of development or some though available; are not widely used due to various constrains.

The various advance diagnostic techniques used in animal reproduction are ultrasonography, Doppler ultrasonography, endoscopy, laparoscopy, uterine cytology etc.

**Ultrasonography:**

Transrectal real-time ultrasonography has been developed as a research and practical tool in large and small animal reproduction. This non-invasive technique has made it possible to provide real-time and serial analyses of ovarian morphological changes, fetal development and has helped in generating new information on reproductive physiology during the oestrous cycle and pregnancy. This has greatly contributed to an understanding of the real-time dynamics of follicular development and more accurate diagnosis compared with rectal palpation in reproductive management in cattle. Practical applications of ultrasonography include early diagnosis of pregnancy, identification of twin fetuses, detection of ovarian and uterine pathologies, determination of fetal sex, foetal aging in unknown mating dates in different species etc. In recent years, local blood flow has been analysed in individual ovarian follicles and the corpus luteum (CL) in the cow using colour Doppler ultrasonography (Matsui and Akia ,2009). From these observations, it has been found that the blood supply to follicles is closely related to follicular growth, atresia and ovulation, the blood supply to the CL increases in parallel with its growth, and there is an acute increase in blood flow in the mature CL prior to luteal regression. Colour Doppler ultrasonography may provide an estimate of the physiological status of follicles and corpora lutea. For example, images of blood flow can be used to assess the thickness of the follicular wall and provide a differential diagnosis of follicular and luteal cysts. Assessment of the area of blood flow in the CL using colour Doppler imaging may offer a useful adjunct in estimating CL function, which could be applied to the diagnosis of non-pregnancy and fetal loss. The number of small follicles which have blood flow at the start of gonadotrophin treatment may be a useful index to predict the superovulatory response. With improvements in portability and cost-effectiveness, the evaluation of ovarian blood flow by colour Doppler

ultrasonography is likely to become widely used as a diagnostic tool for monitoring ovarian function in dairy cattle. It is now clear that color Doppler ultrasound is very useful for observing echogenicity with local blood flow thereby providing an easily obtained estimation of the physiological status of follicles, CLs and early conceptus. Widespread commercial application of color Doppler ultrasonography will depend on further technological developments that will reduce the cost, improve performance and ease-of-use. Colour Doppler studies can be useful in clarifying potential problems in high-producing dairy cattle during the postpartum period. This imaging technique can be used to obtain blood flow velocity waveforms from the uterine arteries at any time during the oestrous cycle, pregnancy and puerperium in different species. During all these phases, characteristic changes in the uterine blood flow could be observed. In studies in canines uterine blood flow was found to be low during diestrus and high during proestrus and oestrus. (Freeman *et al.* (2013)

Doppler sonography can be used to differentiate temporary from persistent ovarian follicles and follicles with luteal tissue from follicles without luteal tissue and to assess the response of follicular cysts to administration of a gonadotropin releasing hormone (GnRH) analogue. The results showed that the perfused area more accurately reflects active luteal tissue than wall thickness. Thus, colour Doppler sonography is superior to B-mode sonography for differentiating follicular and luteal cysts and aids in the selection of treatment (Rauch *et al.* 2008).

By far the largest initial application for Doppler instruments is in obstetrics, which is due to the ease with which one can emit and receive ultrasound in a pregnant uterus. In order to achieve better assessment of foetal health and avoid pregnancy losses by timely intervention, Doppler ultrasonographic evaluation of the pregnant uterus is the need of the day. It is a non invasive technique and it is possible to evaluate both the physiology and the patho-physiology of utero-feto-placental hemodynamics during the course of pregnancy. The technique is practically without any ill effects on the foetus or the dam (Blanco *et al.* 2008). Obstetrical colour Doppler gives the clinician a reliable opportunity to review foeto-maternal hemodynamics by investigating the vessels like the umbilical artery and vein, uteroplacental arteries, fetal thoracic aorta, fetal caudal vena cava and fetal cerebral artery (Lees *et al.* 2003, Scotti *et al.* 2008, Abdelheim *et al.* (2013).

In few studies in cattle during pregnancy, an exponential rise in uterine blood supply could be detected. There was a positive relationship between the uterine blood flow volume at the end of gestation and the birth weight of calves. (Bollwein *et al.* 2002). In studies during puerperium, the uterine blood flow volume declined tremendously, especially during the first week after birth. In cows, with pathological disturbances of the puerperium a delayed decrease in the uterine blood flow volume was observed. Characteristic alterations occurred also in the luteal blood flow during the oestrous cycle, which were highly related to those of the progesterone levels. Furthermore, it has been detected by the colour Doppler

technique that there is no decrease, but an increase of the luteal blood flow at the beginning of luteolysis in cows. Another group has found that there are close relationships between the LH-surge and the follicular blood flow before ovulation. In conclusion, these studies show that transrectal colour Doppler sonography is a useful technique for the investigation of the genital blood flow and provides new information about physiological changes of the genital organs during, all reproductive phases. The influence of the genital blood flow on fertility in cows needs to be examined further in future studies (Herzog and Bollwein (2007).

Resistance indices of uteroplacental and umbilical arteries, fetal aorta and fetal common carotid arteries progressively decrease throughout normal canine gestation (Blanco *et al.*, 2010; Miranda and Domingues, 2010) guaranteeing an appropriate perfusion of the placenta and fetal viscera. An experimental canine model of abnormal gestation suggested that RI of uterine artery could also be a predictor of compromised pregnancy and impending abortion in this species (Blanco *et al.*, 2009). In canine pregnancy, the application of Doppler ultrasonography technique can be explored in various areas like, timely intervention for threatened abortions, deciding the time for caesarean section in prolonged pregnancies, protracted dystokias etc. Doppler parameters and indices can be used for the same. Although Doppler exams have been done in different species recently, it is thought that it would be a reliable diagnostic tool in future.

There are studies on blood flow of uterine arteries during uterine torsion which indicate that in cases of uterine torsion the uterine flow velocity waveform show high systolic flow and absence of early diastolic flow and poor uterine and placentomal blood perfusion. It was concluded that, depicting blood flow within the middle uterine artery using color Doppler sonography could be helpful in correct diagnosis of duration and degree of uterine torsion and concurrently predicting the viability of the fetus and dam (Husain, 2013).

### **Laparoscopy**

Laparoscopy is a minimally invasive surgical technique for viewing the internal structures of the abdominal cavity by means of a telescope through a small incision made in the abdominal wall. It is an endoscopic procedure that bridges the gap between clinical evaluation and surgical exploration. The technique of laparoscopy has been used extensively in human medicine for its simplicity and accuracy. Limited abdominal exploration (keyhole) for laparoscope guided organ examination permits precise and accurate site localization of the various internal organs and excision biopsy is indicated to ascertain a correct diagnosis, specific therapy and accurate prognosis. Direct visualization of the organ with a token invasive method also helps the clinicians to imply an assiduous control over the technique without invasive exploratory surgery and proves its superiority over other non invasive diagnostic techniques like X - ray,

ultrasound, MRI etc. Moreover, laparoscopy requires minor surgical intervention; it provides one of the only available practical means of making repeated direct examination of abdominal viscera .

### **Diagnostic laparoscopy**

Diagnostic laparoscopy is a procedure that allows a health care provider to look directly at the contents of a patient's abdomen or pelvis, including the fallopian tubes, ovaries, uterus. Results indicate that laparoscopy may be useful diagnostic procedure for mummified fetus, polycystic ovarian and uterine growth, persistent corpus luteum, etc. Serial laparoscopy is used in studying patterns of follicular development involving the measurement, counting and histological evaluation of ovaries of animals at various stages during the oestrous cycle, or marking of follicles. In companion animals laparoscopy has wider applications in diagnosis and treatment of various reproductive disorders like retained testis, ovarian cysts, ovarian remnants. Hysteroscopy is chosen as the reference method to evaluate endometrium in cows with clinical endometritis for comparative study of various techniques for diagnosis as it is the only direct method used to evaluate the endometrium (Mudoz *et al.* 2010) . Laparoscopy helps in direct visualization of the size, colour, external texture and peripheral blood circulation of the abdominal and pelvic organs. Minimally invasive surgery has particular advantages for biopsy procedures when a guided biopsy can be taken and the site of biopsy directly observed after collection, with minimal postoperative discomfort.

### **Uterine Cytology:**

Cytological examination of the reproductive tract is often used to evaluate possible reproductive lesions. An inconsistent success following conventional therapies is achieved due to lack of diagnostic standards specially in sub clinical endometritis. Comparative studies have been done by intrauterine bacteriology and endometrial cytology in cows diagnosed with clinical endometritis with findings obtained by vaginoscopy. It was observed that with vaginoscopy there were false positive cases indicating need of going beyond routine examination of vaginal discharge for diagnosis of endometritis. (Westermann *et al.* (2010). Therefore, most of the recent studies have been focused on sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. The accumulation of intrauterine fluid has been shown to be associated with bacterial growth and delayed uterine involution .Polymorphonuclear cells (PMNs) are the predominant inflammatory cell type found in intrauterine fluid accumulations and the determination of the relative proportion of PMNs has been shown to be predictive of reproductive performance in the postpartum cow (Kasimanickam *et al.*, 2004 and Gilbert *et al.*, 2005). Based on this a novel approach for uterine cytological examination is carried out by estimating percentage of PMN cells in uterine cytology. Routine methods for diagnosing endometritis involve uterine biopsies, lavage and swabs but these may cause irritation and distortion of cells. The methos used for endometrial cytology samples include cotton swab ,cytobrush technique, and low volume flush . Various studies have indicated cytobrush technique

being more reliable and accurate technique for diagnosis of endometritis (Barlund *et al.*, 2008 and Honparkhe *et al.*, 2014). In studies in buffaloes it was concluded that endometrial cytology by cytobrush technique was easy and accurate method to diagnose subclinical endometritis in postpartum Murrah buffaloes (Bajaj *et al.*, 2016).

The advances in various diagnostic techniques in animal reproduction are very promising. However more research work is required to make these technologies user friendly, easy to perform on field and reduce the cost so that there application will increase at field level.

### **References:**

- Abdelheim EM, Kishk EAF, Atwa KA, Metawea MAH (2013) Validity of umbilical artery Doppler waveform versus umbilical vein Doppler waveform in the prediction of neonatal outcome in intrauterine growth restriction cases. *J Vet Med Sci*, 10.1016/J. MEFS.2013.09.005.
- Bajaj Nitin Kumar, Salil Kumar Jain, MadhuSwamy and OmprakashShrivastava (2016) Diagnosing Subclinical Endometritis in Postpartum Murrah Buffaloes Using Cytobrush Technique. 32nd Meeting of the European Embryo Transfer Association (AETE); Barcelona, Spain, September 9th and 10th, 2016. Conference abstracts. *Anim. Reprod.*, v.13, n.3, p.365, Jul./Sept. 2016 365
- Barlund C.S., T. D. Carruthers, C. L. Waldner, and C. W. Palmer.(2008). A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* 69:714–723.
- Blanco, P.G., D.O. Arias, C. Gobello (2008) Doppler Ultrasound in Canine Pregnancy. *J Ultrasound Med* 27: 1745–1750.
- Blanco, P.G., Arias, D., Rube, A., Barrena, J.P., Corrada, Y., Gobello, C., 2009. An experimental model to study resistance index and systolic/ diastolic ratio of uterine arteries in adverse canine pregnancy outcome. *Reprod. Domest. Anim.* 44, 164–166.
- Blanco, P.G., Tórtora, M., Rodríguez, R., Arias, D., Gobello, C.,(2010). Ultrasonographic assessment of maternal cardiac function and peripheral circulation during normal gestation in dogs. *Vet. J.*, doi:10.1016/j.tvjl.2010.08.013.
- Bollwein H, Baumgartner U, Stolla R. Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenology* 2002; 57:2053–2061.
- Freeman SL, Russo M, England GCW (2013): Uterine artery blood flow characteristics assessed during oestrus and the early luteal phase of pregnant and non-pregnant bitches; *The Veterinary Journal*, 197: 205-210.
- Gilbert R. O., C. L. Shin, H. N. Erb. Guard, and M. Frajblat (2005). Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* 64:1879–1888.

- Hassain A. Hussein (2013) Validation of color Doppler ultrasonography for evaluating the uterine blood flow and perfusion during late normal pregnancy and uterine torsion in buffaloes. *Theriogenology* 79(2), 1045-1053
- Honparkhe M., S. P. S. Ghuman, J. Singh, S. S. Dhindsa, A. Kumar, M. Chandra and P. S. Brar, (2014) Diagnosing subclinical endometritis through uterine cytobrush cytology and its treatment with proteolytic enzymes in buffaloes. In: XXIX Annual Convention of ISSAR on “Frontier reproductive Biotechnologies for Enhancing Animal Fertility and Fecundity: Global Perspective”, held at Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, Jan. 8-10, RFF 024, pp 131. RFF 055 pp 147.
- Herzog K, H Bollwein (2007) Application of Doppler Ultrasonography in Cattle Reproduction. *Reproduction in domestic animals*, 42(2) 51–58
- Kasimanickam R. T. F., R. A. Duffield, C. J. Foster, K. E. Leslie, J. S. Walton, and W. H. Johnson. (2004). Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* 62:9–23.
- Lees C, Deane C, Albaiges G (2003).: Integrating uterine and fetal Doppler into obstetrics. In, Lees C, Deane C, Albaiges G (Eds): *Making Sense of Obstetric Doppler Ultrasound A Hands-on Guide*. 53-59, Arnold, London,
- Miranda, S.A., Domingues, S.F., (2010). Conceptusecobiometry and triplex Doppler ultrasonography of uterine and umbilical arteries for assessment of fetal viability in dogs. *Theriogenology* 74: 608–617.
- Motozumi Matsuia Akio Miyamotob (2009). Evaluation of ovarian blood flow by colour Doppler ultrasound: Practical use for reproductive management in the cow. *The Veterinary Journal* Volume 181, Issue 3.
- Madoz L.V, R. L. De la Sota, K. Suzuki, W. Heuwieser, M. Drillich (2010). Use of hysteroscopy for the diagnosis of postpartum clinical endometritis in dairy cows. *Veterinary Record* 167, 142-143
- Rauch Alexandra, Lars Krüger, Akio Miyamoto, Heinrich Bollwein (2008). Colour Doppler Sonography of Cystic Ovarian Follicles in Cows. *Journal of Reproduction and Development* Vol. 54 No. 6 P 447-453
- Scotti, L., P. Di Salvo and F. Bocci (2008) Doppler evaluation of maternal and foetal vessels during normal gestation in queen. *Theriogenology* 69: 1111 – 1119.
- Westermann, S., M. Drillich, T.B. Kaufmann, L.V. Mado and W. Heuwieser (2010). A clinical approach to determine false positive findings of clinical endometritis by vaginoscopy by the use of uterine bacteriology and cytology in dairy cows. *Theriogenology*, 74(7): 1248–1255.

**T4-03**

## **Pioneering innovations in Reproductive Biotechnology for Improved livestock Productivity**

Dr Vikas D Dighe

Scientist D and Head,

National Centre for Preclinical Reproductive and Genetic Toxicology,

ICMR-National Institute for Research in Reproductive Health, Parel, Mumbai- 400 012, India Email:

[dighev@nirrh.res.in](mailto:dighev@nirrh.res.in), [dighevikas@hotmail.com](mailto:dighevikas@hotmail.com)

Biotechnology is the integrated application of scientific and engineering principles in order to achieve the useful products and services by using the biological systems. In the second millennium, the cooperations between the scientific branches that are much more open to novelty such as molecular genetics, genetic engineering, proteomics, and targeted production technologies, have generated great opportunities for human and animal welfare, nutrition and health issues. This sphere of science is increasingly becoming sustainable means of improving livestock production by influencing animal health, nutrition, reproduction, genetics and breeding.

Biotechnological achievements of recent years have emerged as powerful tool to improve various livestock products including milk and meat products. The biotechnological tools can be applied in production of high yielding animal, improvement in the quality attributes of animal origin products, production of hormones, functional and designer livestock products, enzymes, bio-preservation of livestock products, efficient byproduct utilization, quality control and meat authentication

New reproductive technologies present farmers with many opportunities; such technologies include artificial insemination (AI), sperm sexing, oestrus control, multiple ovulation and embryo transfer (MOET), *in vitro* production (IVP) of embryos, cloning and cloning for the production of GM animals (Faber *et al.*, 2003). Many of the current reproductive technologies are concerned with animal breeding programmes; most of these are concerned with the selection of animals based on an evaluation of their genetic quality and the dissemination of the superior genetic material from the nucleus to the commercial population.

### **Artificial insemination**

Artificial insemination is not merely a novel method of bringing about impregnation in females. Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germplasm of the bulls of superior quality can be effectively utilized with the least regard for their location in faraway places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

Semen can be easily transported; allowing multiple females in different geographical locations to be inseminated simultaneously, and semen can be stored for long periods of time, meaning that males can produce offspring long after their natural reproductive lives end. One can choose only the few best males for use as parents, increasing the selection intensity. Furthermore, because males can have more offspring, their offspring can be used in a progeny test program to more accurately evaluate the genetic value of the male.

### **Semen-sorting technology/Semen sexing**

The ability to predetermine the sex of offspring prior to conception is a highly desired technological tool for assisted female breeding programs specifically for milk production, and in males, for meat production and increasing livestock numbers. The current technology is based on the well-known differences in X- and Y-sperm in the amount of DNA. The technology uses modified flow cytometric instrumentation for sorting X- and Y-bearing sperm.

### **Cryopreservation and freeze-drying of sperm**

There are those who believe that the differences among species in the ability of their sperm to survive freeze–thawing is related to their tolerance of osmotic stress (Guthrie *et al.*, 2002). These workers see it as critically important that the osmotic behaviour of sperm be determined and that cryopreservation protocols are adjusted to make it possible for sperm with appropriate motility and survival ability to be inseminated. It is likely that future research will continue to be concerned with semen storage (Vishwanath, 2003).

The production of mouse pups from freeze-dried sperm has recently been reported by Japanese researchers; they used low temperature and pressures to remove water from the sperm and stored them at 4°C for periods of up to 3 months.

### **Sperm encapsulation**

Microcapsules with a semi-permeable membrane, designed to release sperm over an extended period of time, may be a means of ensuring that they are in the cow's oviduct at the optimal time for fertilization to be achieved. Bovine sperm microencapsulation is done using capsules of components, *viz.*, calcium alginate, cellulose sulfate-poly-diallyl-dimethyl-ammonium chloride (CS-pDADMAC), poly-l-lysine, polyvinylamine and protamine sulfate membrane using standard encapsulation procedure. Thus, encapsulation helps not only in sustained release of sperm as well as prevents cryocapacitation and also reported to have increased conception rate. It may be possible to increase sperm lifespan during

microencapsulation by adding agents that stabilize membranes, inhibit peroxidation and decrease calcium uptake.

### ***In vitro* spermatogenesis**

Sperm production involves complex endocrine and autocrine/paracrine regulation of germ cell proliferation and differentiation. Such studies will enable the molecular mechanisms regulating the process of spermatogenesis to be determined. In practical terms, *in vitro* spermatogenesis may provide the basis for treating certain forms of male infertility in humans and of genetically modifying the male germ line in farm animals.

### **Sperm Transcriptomics**

Transcriptomics involves the study of mRNA at various developmental stages including spermatogenesis. Sperm deliver paternal genes to the oocyte and also carry remnant messenger RNA arising out of spermatogenesis. These transcripts are associated with different cellular and biological processes. Profiling of these transcripts using microarrays or by next generation sequencing technologies has proved highly effective tool for studying sperm mRNA expression profile analysis and polymorphism in related genes. Studies have shown that there is a difference of expression profile transcripts in high-fertility bulls with higher concentrations of transcripts for membrane and extracellular space protein locations as compared to the low-fertility bulls. These transcripts include protamine 1, casein beta 2 and thrombospondin receptor CD36 molecule. These studies shall pave way for elucidating transcriptomic changes associated with abnormal development in spermatogenesis and facilitate the improvement of assisted reproductive technologies and also serve as fertility markers.

### ***In Vitro* Maturation, Fertilization and Culture (IVMFC)**

This involves oocyte collection from slaughterhouse ovaries or from live animals followed by maturation and fertilization *in vitro* for the production of viable embryos. IVMFC has provided an excellent source of embryos for embryo transfer, cloning, transgenesis, and other advanced *in vitro* techniques. It has also allowed the analysis of the developmental potential of embryos, pattern of gene expression, epigenetic modifications and cytogenetic disorders in various domestic species and has been used as a model for human embryogenesis studies.

### **Intracytoplasmic Sperm Injection (ICSI)**

ICSI is a micromanipulation technique used for treating male infertility. It involves mechanical insertion of a selected sperm into the cytoplasm of an oocyte to produce desirable embryo. This technique

helps in elucidating the different steps of fertilization. Since the first report of ICSI success, ICSI has been done in species such as rabbits, mice, sheep, humans, horses, cattle, and pigs including buffaloes. This technique is also used for sperm vector system for animal transgenic. ICSI has also been done with sexed semen with a success rate of 80% in cattle and 48-63% in small ruminants using fresh and frozen-thawed semen.

### **Embryo Transfer Technology**

ETT is an important tool to improve livestock at faster rate as well as provides an opportunity to utilize the genetic contribution of both male and female. ETT involves superovulation, an important step for increasing the number of oocyte from superior donors. It was evident that MOET programs could result in increased selection intensity and reduced generation intervals, resulting in increased genetic gain.

ET came to play an increasingly important role in the genetic improvement of dairy cattle in many countries. The technology has been used in many aspects of reproductive research since the 1950s. Embryo transfer offers breeders of farm animals unique possibilities that will ultimately result in more readily available food and by-products of higher quality. As with most of the new animal breeding technologies, embryo transfer helps to increase efficiency so that the same quantities of animal products can be produced with less feed, energy, and land.

### ***In Vitro* Embryo Production**

Although oocytes and embryos can come from superovulated donor animals, this is likely to be expensive and not always free from animal welfare concerns. For such reasons, ***in vitro* production (IVP)** techniques, particularly those based on ovaries recovered after the donor's demise, have received much attention in the past 10–15 years (Galli et al., 2003). Europe has been at the forefront of applying such technologies. In the commercial **cattle embryo transfer (ET)** industry, *in vitro* embryo production is now an alternative to conventional means of obtaining embryos for transfer, using immature oocytes collected from the ovaries by **ovum pick-up (OPU)** of donor cattle of differing ages and physiological states. Reliable methods are now available for the maturation and fertilization of bovine oocytes *in vitro*; culture methods, although still imperfect, enable embryos to be grown to the stage at which they are suitable for transfer or cryopreservation. *In vitro* embryo production involves three steps, which have been developed to the greatest extent in cattle: oocyte *in vitro* maturation (IVM), *in vitro* fertilization (IVF).

### **Embryo Cryopreservation**

The mouse embryo was the first to be cryopreserved. Using the vitrification which involves the use of highly concentrated aqueous solution of cryoprotective agents, *viz.*, glycerol, ethylene glycol, and non-permeating agents such as sucrose, glucose, and fructose during no freezing equipment and hence considered superior as compared to slow freezing. Vitrification has been used successfully for cryopreservation of cattle embryos at various developmental stages with the recovery rate of 88-89%. This technique is advantageous as it reduces the risk and expense in the transportation of expensive animals; reduce disease transmission and conservation of endangered species germplasm but the survival rate of frozen embryos diminishes thereby causes poor pregnancy rates following embryo transfer.

### **Controlling Oestrus and Ovulation**

Research in oestrus control in cattle has been directed towards providing the farmer with a method whereby his cows can be inseminated at a predetermined time (fixed time AI); by this means, so the theory goes, the farmer is saved the time and labour involved in heat detection. The fact remains, however, that, despite advances in the development of short-term progestogen treatments and in combination treatments involving progestogens, oestrogens, prostaglandins and GnRH, there may still be some way to go in meeting the needs of farmers seeking an optimally high calving rate after a single, fixed-time insemination.

### **Cloning: Somatic Cell Nuclear Transfer**

Somatic cell nuclear transfer also termed as “cloning” involves utilization of micromanipulation technique and cell fusion to transfer blastomeres of multicellular embryo or somatic cell into enucleated oocytes]. In this technique, nucleus of blastomere is reprogrammed for development of new embryo. It is useful technique used for multiplication of elite animals with minimal genetic variation. Animal cloning was used for propagation of valuable genotypes, induce genetic modifications, and for producing transgenics. The first animal obtained by somatic cloning was a sheep - “Dolly”. Cloning holds the promise of bypassing conventional breeding procedures by allowing the creation of thousands of duplicates of genetically engineered animals. It can be used for the conservation as well as propagation of endangered species. It may be used as a tool for the production of stem cells for therapeutic cloning. This technique has opened novel opportunities for genetic engineering, animal genetic diversity conservation, tissue regeneration, and development of targeted ES cells for therapeutics. This technique can also be used in local breeds containing genes that confer adaptation, heat tolerance and disease resistance. In future, the scope of cloning can be in xenotransplantation, as it would allow multiplication of humanized animal models for research and therapeutics.

## **Production of Transgenics**

The health of human beings is directly affected by food. Genetic modification of livestock for increased food production may pave way to improve public health via enhanced nutrition. All over the world, traditionally, farmers have tried to improve the breeds of livestock to obtain nutritious, and cost-effective animal products. In this era of biotechnology, transgenesis allows us to obtain these products in a short span of time. Modification of the genome of livestock by introducing desired genes in it, improves the nutritional quality and the quantity of food. It can also be used to specifically increase the percentage composition of nutrients with respect to demand. **Transgenesis** has enabled us to produce **transgenic cows** that yield more milk, milk with less cholesterol or with any other desired character. Similarly, transgenic **pigs, chicken, fish** and **cattle** have been produced for their enhanced nutritious meat. Transgenic **sheep** and goats can be raised for more wool or fiber production.

### **Increased meat on their bones**

The ability to produce transgenic pigs and cattle with enhanced muscle growth is an area of increasing interest. Researchers have been studying the effects of targeting myostatin, the only secreted protein known to negatively affect muscle mass in vivo, as well as genes for growth-related hormones and lean muscle mass. Transgenic myostatin knockout cows have been produced in the US. Transgenic pigs containing insulin-like growth factor-1 and a desaturase gene from spinach have been shown to have increased growth rates and increased levels of polyunsaturated fatty acids, respectively.

### **Improved Milk quality**

The researchers use miRNA technology to silence the expression of  $\beta$ -lactoglobulin (BLG) in the milk, making it potentially less allergenic. In addition, high casein levels were reported in the BLG-deficient milk. Casein makes up 80% of milk protein in conventional cows and is an extremely valuable component of milk because of its nutritional value and processing properties. The increased casein levels associated with this BLG knockout cow could provide increased calcium levels and higher cheese yields.

### **Improvement in Disease Resistance**

Genetic modification of livestock by transgenesis will produce healthier animals. Healthy animals are an asset to anyone involved in the rearing of livestock. Thus, the technique of animal biotechnology should give more emphasis on producing animals with higher resistance to diseases.

For example, **mastitis** is a condition developed due to infection of breast tissue by a pathogen that results in inflammation like symptoms like swelling, redness, warmth and breast pain. This results in low

milk production in the affected cattle. The occurrence of this disease can be reduced by producing transgenic cows that carry gene for lysozyme and secrete the protein in their milk. Lysozyme protein provides higher resistance to mastitis by killing the bacteria *Staphylococcus aureus*. However, the resistance offered is dose-dependent. Lysozyme is an antimicrobial peptide that protects the mammary gland against this major mastitis-causing pathogen.

### **Reproductive fitness**

The improvement in reproductive fitness of healthy livestock is always a positive signal for people associated with livestock rearing. And, such transgenic animals producing desired products or exhibiting superior characters are more suitable to meet the requirement of human population, be it food or therapeutics.

Numerous potential genes have been identified that can greatly affect the litter size. An engineered **estrogen receptor (ESR) gene** can be introduced in the genome of transgenic cattle that can increase the litter size. For example, introduction of this gene in numerous breeds of pigs effectively increases the number of progeny and thus the availability of food.

A gene for fecundity has been identified called the **Boroolla fecundity (FecB) gene**, in Merino sheep which allows for increased ovulation rate. The gene increases the ovulation rate by approximately 1.5 ova. The transgenic sheep carrying FecB gene can be produced which will increase the fecundity in different breeds of sheep.

### **Biotechnology helps to enhances Selective Breeding**

#### **Genetic Screening of Breeding Stock**

Traditional animal breeding method (TABM) exploited variations that existed within breeds and animal populations to bring about genetic improvement in traits of economic importance such as milk yield, growth traits, and egg numbers. TABM has been very successful over the years by utilizing records of the phenotype of an animal and a number of its relatives to estimate the likelihood that an animal will pass on its good traits to its offspring. However, for traits that are difficult to measure such as disease resistance, fertility, and feed efficiency, these traditional breeding methods have not been successful. The idea of using marker-assisted selection (MAS) to overcome the shortfalls of TABM has been around since 1923. MAS is the selection of traits of interest indirectly by selecting genetic markers associated with desired qualities, as opposed to traditional methods of finding desired qualities by observing phenotypic traits. By estimating breeding values based on marker, pedigree and phenotypic information, MAS can bring genetic improvement in traits of animals where TAB alone has failed. Beginning in the late 1970's many molecular genetic markers were discovered and developed, including allozymes, restriction fragment

length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), microsatellite DNA and SNPs. The ability to analyze these markers was developed over several decades and has made the mapping of quantitative trait loci (QTL) feasible on a large scale. Out of these genetic markers, SNPs are currently the marker of choice because of their large numbers spaced across the genome. The decreasing cost and rapid progress in next-generation sequencing methods that employ massively parallel approaches in sequencing several hundred thousand to millions of reads simultaneously have led to the identification of many SNPs in livestock species. SNP arrays containing tens of thousands of SNPs distributed throughout the genome are now available for several livestock species and support the interrogation of hundreds of loci at a low cost. Many markers are now known across the genomes of many livestock species, including cows, sheep, and pigs. Genomic selection, can use all these markers simultaneously to predict the genomic estimated breeding value (GEBV) for traits of animals without needing to know the location of genes in the genome.

### **Conclusion:**

Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely influenced by commercial considerations and socio-economic goals. Advances in reproductive biotechnology to support livestock production is an integral part of viable agriculture in multi-enterprise systems. Livestock are integral part of a vulnerable 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 the particular genes that lead to these desirable traits and it is now possible to select superior germ plasm 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 buffaloes, 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 in the production of cheap and dependable diagnostic kits and vaccines. Several obstacles limit the application of biotechnology at present: there is a lack of infrastructure and insufficient manpower, so funding is needed if resource-poor farmers are to benefit from biotechnology.

### **References:**

- Faber et al., 2003 *Theriogenology* 2003;59:125-38.
- Guthrie et al., 2002 *Biol. Reprod.* 2002;67:1811–1816.
- Galli et al., (2003) *Theriogenology.* 3;59:599–616
- Choudhary et al., 2016 *Veterinary World*, 9(4): 388-395.
- Madan ML (2005) *Rev Sci Tech.* 24(1):127-39.
- Vishmanath R. (2003) *Theriogenology* 2003;59:571-84.

**AB-13 Fertility response in Indian Zebu cattle (*Bos indicus*) at synchronized ovulation by Ovsynch protocol and timed AI**

B. L. Kumawat, N. M. Markandeya and A. B. Mali

Department of Animal Reproduction, Gynecology and Obstetrics

College of Veterinary and Animal Sciences, Parbhani-431402, MAFSU (MS)

The present investigation was intended to study the fertility response of Indian cows (*Bos indicus*) to the hormonal stimulus using ovsynch protocol. A total of 90 cyclic native cows were selected and categorized in two equal groups. One group (n=45) was assigned with standard ovsynch protocol composed of GnRH (Day 0), PGF2 $\alpha$  (Day 7) and GnRH (Day 9) schedule whereas another group was kept untreated as control. Timed artificial insemination was done 16-20 hours after second Gynarich injection (Day 10). All cows in both the groups were monitored for estrus and cows detected in estrus were inseminated artificially. Upon pregnancy diagnosis by transrectal palpation in synchronized cows after 60 days of insemination, 73.33% (33/45) and 15.55 % (07/45) conceptions were recorded on first and second service, respectively with 88.89 % (40/45) cumulative pregnancies. On the other hand in control group, 58.62% (17/29) and 13.79% (04/29) conceptions were recorded on first and second service, respectively with an overall 46.67% (21/45) pregnancy rate. A significant difference is appreciable upon comparison of reproductive performance of synchronized (88.89%) vs. non-synchronized (46.67%) cows in terms of overall pregnancies. These outcomes of trial yielded promising results in terms of pregnancy rate in deshi cows treated with ovsynch protocol.

**AB-14 COMPARATIVE STUDIES OF BOVINE PRE-IMPLANTATION EMBRYO SEXING USING LAMP AND PCR BASED METHODS**

P. Raja\*, K. Vijayarani, T.M.A. Senthilkumar and K. Kumanan

Centralized Embryo Biotechnology Unit,

Department of Animal Biotechnology,

Madras Veterinary College,

TANUVAS, Chennai-07.

Sex determination in bovine embryos is a useful technique in reproductive biotechnology. The purpose of this study was to compare the embryo sexing methods between Loop Mediated Isothermal Amplification System (LAMP) and Polymerase Chain Reaction (PCR) of in-vitro produced bovine embryos at morula stage. LAMP is a novel DNA amplification method that amplifies a target sequence specifically under isothermal conditions. For rapid sexing of bovine pre-implantation embryos using the LAMP reaction,

two set of primers have been designed targeting Male specific Y chromosomal region and Male – Female common satellite I (1.715) region and the reaction was carried out at 60°C for 45 min. The amplified LAMP product was visualized using SYBR Green I without Agarose electrophoresis. The inner cell mass (ICM) was collected from in-vitro produced morula stage bovine embryos using micromanipulator. After biopsy, the remaining part of the embryo was used to confirm the sex by PCR with already established primers. The LAMP reaction was compared with PCR based sex determination methods and we found that LAMP based embryo sexing accurately determined the gender and was proven to be suitable for field application. This study showed that the present method can be applied in bovine breeding programs to facilitate manipulation of the sex ratio of offspring.

**T5**

**Nutraceuticals and  
Biotechnology In Livestock  
Products**

**T5-01**

## **Herbal Nutraceuticals: Livestock Dairy Products Application**

S.P. Changade, P. D. Sawale & V.K.Lule

College of Dairy Technology, Warud (Pusad)- 445204

Maharashtra Animal and Fishery Science University, Nagpur

Email id: spchangade@gmail.com, addtcwarud@gmail.com

### **Introduction**

Nutraceuticals are nutritionally or medicinally enhanced foods with health benefits of recent origin and marketed in developed countries. This is a term of recent origin (1979) and comprises nutritionally or medicinally enhanced foods with health benefits (Brower, 1998). These include 'engineered grain', cereals supplemented with vitamins or minerals or genetically manipulated soybean and canola oil without trans-fatty acids, etc.

Herbal preparations are being marketed as nutraceuticals or health foods. It is pertinent to mention that herbal medicines are therapeutics of the indigenous/traditional systems of medicine and it is unethical to classify them as health foods. Even the minimum standards laid down by WHO are not followed. Nutraceuticals are in great demand in the developed world particularly USA and Japan. Nutraceutical market in USA alone is about \$ 80–250 billion, with a similar market size in Europe and Japanese sales worth \$ 1.5 billion (Brower, 1998). As per available records, the herbal medicine market in 1991 in the countries of the European Union was about \$ 6 billion (may be over \$ 20 billion now), with Germany accounting for \$ 3 billion, France \$ 1.6 billion and Italy \$ 0.6 billion (Brower, 1998). Incidentally in Germany and France, herbal extracts are sold as prescription drugs and are covered by national health insurance. In 1996, the US herbal medicine market was about \$ 4 billion and with the current growth rate may be more than double by the turn of century. Thus a reasonable guesstimate for current herbal medicine market worldwide may be around \$ 30–60 billion. The Indian herbal drug market is about \$ one billion and the export of herbal crude extracts is about \$ 80 million. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine.

Rapid urbanization and industrialization have produced advancement on the social and economic fronts in developing countries such as India which has resulted in dramatic lifestyle changes leading to lifestyle related diseases. Consumption of diets rich in fat and calories besides a high level of mental stress has compounded the problem further. This changing health scenario, has made people more prone to diabetes, cardiovascular diseases (CVD), obesity and other nutritional disorders (Knowler *et al.*, 2002). Due to the appearance of several lifestyle-related health disorders, there has been increasing interest in the study of functional foods with supplemented with functional components or substances. Functional foods provide some health benefit beyond their nutritional value (Hasler 1991).

Functional foods are foods that offer unique health benefits that go beyond simply meeting basic nutrient needs. Many also help to reduce chronic disease risk. Functional foods contain “bioactive compounds,” or naturally occurring chemicals that act on our bodies. It is these bioactive compounds that offer the health and wellness benefits that have been linked to functional foods (Dietitians of Canada, 2010). By 2014, the international functional food market is expected to reach a value of about \$29.75 billion (Singh et al., 2012). Recent has been estimated that the sector growth increased from 8.5 percent to 20 percent per year, ahead of the overall industry, where growth is estimated at 1 percent to 4 percent per year (<http://www.pwc.com/enUS/us/transaction-services/publications/assets/functionalfoods.pdf>).

Some bioactive ingredients targeted for functional dairy/food products are omega-3 fatty acids, plant sterols, polyphenol, dietary fibres, probiotics, and prebiotics. Today, we are witnessing a great deal of public interest in the use of herbal remedies. Herbs have been used as food and medicine for centuries. Herbal medicine is based on the concept that plants contain certain natural substances that can promote health and alleviate illness. About 20% of plant species found in India are used for medicinal purpose. In herbal medicine the term herb is used loosely to refer not only to herbaceous plants, but also to bark, roots, leaves, seeds, flowers and fruits of trees, shrubs and woody vines and extracts of suave that are valued for their savory, aromatic or medicinal qualities.

A wide variety of active phytochemicals including the flavonoids, terpenoids, lignans, sulfides, polyphenols, carotenoids, coumarins, saponins, plant sterols, curcumins and phthalides have been identified. In the recent past, research interest has been focused on various herbs that possess hypolipidemic, anti-platelet, anti-tumor or immune - stimulating properties that may be useful adjuncts in reducing the risk of CVD and cancer. Presently, the herbal products being marketed in the country are mostly sold as medicine for cure of certain ailments and are, therefore, classified as ‘medicines’. Their recent boom in herbal crop production throughout India will not succeed if there is little attention on value- addition to new product development.

*Ayurveda* is one of the world’s most ancient holistic health philosophy and system that cover almost every aspect of wellbeing. *Ayurveda* has mentioned several ways in which the medicinal benefits of herbs could be conveyed via certain foods as carriers. Milk is one of the most important of such carriers (Sawale et al., 2011, 2013). In India, a significant amount of milk produced is utilized for traditional dairy products (Pal and Raju, 2007). Conversion of liquid milk into traditional dairy products not only preserves milk solids for longer time but also adds value to milk. Indian traditional dairy products have a huge demand and their domestic markets are well established. However, in order to compete and to sustain with the ever increasing functional food market, Indian dairy industry should find ways to induce or to improve the functionality in traditional dairy products.

## Application of herb into traditional Indian dairy products

### *Butter and ghee*

In India about 39 % of total milk produce is converted into ghee and butter.. Ghee consist 98% glycerides and 0.3% moisture. The consumers are, therefore, now always looking for complementary or alternative products. The clarified milk fat, particularly ghee has the characteristics to absorb all the medicinal properties of the herbs with which it is fortified, without losing its own qualities. Exploiting this concept several medicated ghee preparations have been developed and about 55-60 medicated ghee types are reported in *Ayurvedic* literatures and they have also been used in the treatment of various diseases (Pandya and Kanwajia, 2002).

Herbs contain high amounts of phenolic compounds which possess great antioxidant properties. The natural antioxidant properties of herbs have made their use in the formulation of functional foods specifically targeted for the people suffering from cardio vascular diseases (Najgebauer-Lejko et al. 2009). The antioxidant properties of herbs led their use into fat rich dairy products for retarding auto-oxidation there by prolonging the shelf-life. Moreover, it was found that the artificial antioxidants, like BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisole) are not safe for human consumption (suspected to have carcinogenic activity). Presently, the herbal ghee being marketed in the global market is mostly sold as medicine (medicinal ghee). These products possess typical flavour, bitter or pungent taste and a dark colour. Such therapeutic preparations are therefore not acceptable for regular consumption. At NDRI, Karnal herbal ghee incorporating functional attributes of *arjuna* has been developed for providing beneficial effects against cardiovascular diseases and the product was more stable to oxidative deterioration as compared to conventional ghee. The consumer acceptability of this product was also very good (Rajanikant, 2006). Unlike in case of medicated *ghee* preparations, *arjuna ghee* can be replaced with normal *ghee* in the daily diet.

On the other hand, increasing sensitivity of consumers to synthetic ingredients as well as their increasing awareness about the effect of diet on their health contributed to the increasing trend to use natural additives like herbal extracts for the stabilization of fat rich dairy foods like *ghee*, butter oil, butter etc.. Sage (*Salvia officinalis*) and Rosemary (*Rosmarinus officinalis*) extracts are the most widely used for this purpose (Ozcan 2003). These extracts have antioxidant activity many times stronger than synthetic antioxidants like BHA or BHT (Estévez et al. 2007). The some of the reported study's related to herbal ghee is depicted into table 1 where the herb fortified ghee has shown good antioxidants activity as compared to ghee supplemented with synthetic antioxidant.

**Table 1. Reported examples of herbal supplemented ghee**

Type of Ghee	Medicinal Plants	Synthetic antioxidant	References
Sheep Ghee	Rosemary ( <i>Rosmarinus officinalis</i> ) ground sage and rosemary	Mixture BHA and BHT(1:1) Mixture BHA and BHT(1:1)	Amr 1990 Shahidi et al. 1995
Butteroil	Clove ( <i>Syzygium aromaticum</i> L.), caraway ( <i>Carum carvil</i> L.) and coriander ( <i>Coriandum sativum</i> L.)		Ali, 2009
Cow ghee	Satavari( <i>Asparagus racemosus</i> )	BHT	Pawar et al. 2011
Cow ghee		BHT	Gadhi et al. 2011
Cow ghee	<i>Ashavgandha</i> ( <i>Withania somnifera</i> )	BHT	Purohit et al.2011

The study of egarska and Rafa<sup>3</sup>owski (1997) revealed that addition of the Ethanolic extract of sage in the amount of 0.1-0.2 to the sweet cream directly before churning affected lower level of peroxide value during storage of butter and butter fat at 20 and 60°C, respectively. Methanolic extracts of sage, rosemary and oregano were also found to be effective in retardation of oxidation (TBA test, peroxide value) and lypolysis (FFA) processes in butter (Ayar et al., 2001). Ozkan et al. (2007) reported that addition of *Satureja cilicica* EO (at 0.5, 1.0 and 2.0% levels) in butter exhibited strong antioxidant activity in a concentration dependant manner. However, the addition of dried sage and rosemary did not prevent the lipolysis and formation of peroxides during low temperature storage of the sour-cream butter (Najgebauer-Lejko et al., 2009). Freeze-dried hydrodistilled extract of clove (*Syzygium aromaticum* L.), caraway (*Carum carvil* L.) and coriander (*Coriandum sativum* L.) when added at 400 ppm level exhibited antioxidant effect in butter oil (Ali, 2009). Recently, Pawar et al. (2012) has successfully increased the oxidative stability of *ghee* by incorporating the alcoholic and aqueous extracts of Satavari herb.

### ***Dahi, Lassi and Shrikhand***

*Lassi*, a ready-to-serve traditional fermented milk beverage has got wide popularity in India as well as in overseas markets. Sweet *lassi* with its characteristic sweet and slightly sour taste can be used as a food carrier for herbal bioactives like *Aloe vera* juice. Hussain et al. (2011) has developed functional *lassi* using the herb *Aloe vera* (*Aloe barbadensis* Miller). A culture combination containing NCDC 60 and *Lactobacillus paracasei* ssp *paracasei* L at an inoculum rate of 2 percent was used for functional *lassi*

preparation. Animal study of functional *lassi* revealed that it has better immunoprotective effects compared to control *lassi*. Recently, Pal et al. (2012) also supplemented *Aloe vera* juice into *lassi* to enhance its health benefits. The authors have reported that supplementation of *Aloe vera* juice at 15% level into *lassi* has obtained optimum sensory scores. Herbal supplemented probiotic *dahi* using the herb *Aloe barbadensis* Miller was also prepared by Hussain et al. (2011). The authors have reported that *Aloe vera* supplementation has supported the growth of probiotic strain *Lactobacillus paracasei* ssp *paracasei* L in *dahi*. The probiotic viability was more than 7 log cfu/ml during 12 days storage period.

Shrikhand being a semi soft, sweetish sour, whole milk product prepared from lactic fermented curd can easily harbor herbs/herbal extracts without undergoing significant changes in sensory quality. Landge et al. (2011) successfully prepared *shrikhand* using Ashwagandha herb powder as an additive. The authors have found that addition of 0.5% Ashwagandha powder to *shrikhand* has improved the organoleptic quality and the product was remained acceptable up to 52 days at refrigerated temperature.

### **Sandesh**

*Sandesh* is a very popular heat-desiccated product of coagulated milk protein mass called *chhana*. Bandyopadhyay et al. (2007) incorporated herbs such as turmeric (*Curcuma longa* L.), coriander (*Coriandrum sativum* L.), curry leaf (*Murraya koenigii* L.), spinach (*Spinacia oleracea*) and aonla (*Emblica officinalis*), separately as a paste, at the 10% level into *Sandesh* to induce the antioxidant properties into the product. The authors have also reported that the use of coriander herb with its antimicrobial and antioxidant properties increased the shelf-life of herbal *sandesh* up to 8 days and 30 days when stored at 30±1°C and 7±1°C, respectively when compared with the remaining samples.

### **Incorporation of herbs into other milk products**

Sawale et al., (2011; 2013) studied the effect of herb (*Pueraria tuberosa*) on physicochemical and therapeutic properties of milk who observed that milk supplemented with herb at sensory acceptable level (0.4%) was significantly ( $p < 0.05$ ) increased antioxidant activity while reduced the browning, pH and heat stability as compared to control. In *in vivo* study, it also shown that the immunomodulatory and antioxidative potential of herb-milk model system was improved in experimental mice.

Kamble et al., (2010) found that the addition of pineapple pulp @15% into burfi was significantly ( $p < 0.05$ ) improved acceptability (flavor, body texture and colour and appearance) than all others treatments.

Landge et al., (2011) was successfully prepared shrikhand using ashwagandha herb powder as additive who found that 0.5% ashwagandha treated shrikhand with 40% sugar has not only better organoleptic quality but also remain acceptable up to 52 days of storage under refrigerated temperature.

Indian herbs such as turmeric (*Curcuma longa* L.), coriander (*Coriandrum sativum* L.), curry leaf (*Murraya koenigii* L.), spinach (*Spinacia oleracea*) and aonla (*Emblica officinalis*), as a paste, were incorporated separately at the 10% level into sandesh (a heat-desiccated product of coagulated milk protein mass or chhana, which is analogous to cottage cheese). Their total antioxidative status was assessed in vitro using Randox's method. The antioxidative levels of the various herbs were compared with the synthetic antioxidants TBHQ and BHA: BHT (1:1) at 100 and 200 mg/kg levels. The antioxidative effect of herbal sandesh decreased in the order: turmeric > curry leaf > aonla > spinach > coriander leaf. Total antioxidative status of herbal sandesh was lower than samples with TBHQ but similar to those with 200 mg/kg BHA: BHT (1:1) (Bandyopadhyay et al., 2007). He has also revealed that the use of coriander herb with its antimicrobial and antioxidant properties increase the shelf-life of herbal sandesh up to 8 days at (30+10C) and 30 days at (7+10C) using HDPE bags as packaging materials as compared to others herb used for preparation.

Singh et al., (2005) prepared the chhana by using two herbal coagulants. Herbal coagulants chhana was found to have difference sensory (flavour, body and texture, colour and appearances) and chemical composition (fat, protein, lactose and ash) as compared to that conventional chhana.

### **Challenges for development of traditional herbal dairy products**

1. The consumer awareness is an important for marketability for newly develops traditional herbal dairy foods as compared to exist traditional products. 2. Need to develop/adopt new technology such as microencapsulation, emulsion etc concerning to reduce colour and bitterness/ astringency taste of herb. 3. Pay more attention toward extraction of bioactive. This will avoid the addition whole plant components, 4. It is also important to get stability of herb bioactive into products at different processing condition and storage. 5. The extent of beneficial effects of herbs may vary with the geographical variations.

### **Conclusion**

Herbs and nutraceuticals are natural substances with wide range of health attributes. In India, most of milk is converted into traditional dairy products which is consumed by people of all age can act as potent carrier for the herbs which can add functional attributes to the product and consumers well being. However, there are several technological challenges that have to be overcome to develop milk products enriched with herbs. Process modifications are needed to reduce the undesirable effects of herbs incorporation on palatability of foods. Research should be focussed to find out the effect of processing conditions on the bioavailability of functional components present in herbs. Knowledge of interactions of herbal and food constituents and their effect on human health has to be studied thoroughly. Scientific

community must apply modern techniques to assure the efficacy and safety of herbs and their bioactive components for their safe use in food formulations.

## References:

- Ađaođlu S, Dostbil N, Alemdar S (2005) The antibacterial efficiency of some herbs used in herby cheese. *YYÜ Vet Fak Derg* 16(2):39-41
- Agboola SO, Chan HH, Zhao J, Rehman A (2009) Can the use of Australian cardoon (*Cynara cardunculus* L.) coagulant overcome the quality problems associated with cheese made from ultrafiltered milk?. *LWT - Food Sci Tech* 42:1352-1359
- Akahoshi R, Takahashi Y (1996) Yoghurt containing *Bifidobacterium* and process for producing the same. PCT-International Patent Application, WO96/37113A1 (cited from FSTA 1997-08-P0149)
- Bakirci I (1999) The effects of some herbs on the activities of thermophilic dairy cultures. *Food / Nahrung* 43(5):333-335
- Bandyopadhyay M, Chakraborty R, Raychaudhuri U (2007) Incorporation of herbs into sandesh, an Indian sweet dairy product, as a source of natural antioxidants. *Int J Dairy Tech* 60(3):228-233
- Bender AE, Bender DA (2005) *A Dictionary of Food and Nutrition*. Oxford University Press, New York
- Benzie, I. F. and Wachtel-Galor, S. (Eds.). (2011). *Herbal medicine: biomolecular and clinical aspects*. CRC Press, Boca Raton.
- Boza JJ, Martinez-Augustin O, Gil A (1995) Nutritional and antigenic characterization of an enzymatic protein hydrolysate. *J Agric Food Chem* 43:872-875
- Bullerman H, Gourma LB (1987) Effects of oleuropein on the growth and aflatoxin production by *Aspergillus parasiticus*. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 20:226-228
- ChingYun K, MiaoLing W, MeiJen L, ChienJung H (2009) Studies on the manufacture of functional fermented milk with Chinese herbs. *J Taiwan Livestock Res* 42(2):109-120
- Dan, B. (2003). *Chinese Herbal Medicine: Materia Medica* (3rd edn). Eastland Press, Vista, CA, USA.
- Dietitians of Canada. (2010). What are Functional Foods and Nutraceuticals; ([http://www.dietitians.ca/Nutrition-ResourcesAZ/FactSheetPages\(HTML\)/Miscellaneous/Functional-foods-andnutraceuticals.aspx](http://www.dietitians.ca/Nutrition-ResourcesAZ/FactSheetPages(HTML)/Miscellaneous/Functional-foods-andnutraceuticals.aspx). Accessed by 2012 July 20.)
- Esteves CLC, Lucey JA, Pires EMV (2002) Rheological properties of milk gels made with coagulants of plant origin and chymosin. *Int Dairy J* 12:427-434
- Estévez M, Ramirez R, Ventanas S, Cava R (2007) Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *LWT: Food Sci Tech* 40:58-65
- Hasler, C.M. (1998). Functional Foods: Their role in disease prevention and health promotion. *Food Technology*, 52(11):63-70.
- Hassan FAM, Helmy WA, Anab AK, Bayoumi HM, Amer H (2010) Production of healthy yoghurt by using aqueous extract of garlic. *Arab Univ J Agric Sci* 18(1):171-177

Hayaloglu AA, Fox PF (2008) Cheeses of Turkey: 3 Varieties containing herbs or spices. Dairy Sci Tech 88:245-256

[http://www.pwc.com/en\\_US/us/transaction-services/publications/assets/functionalfoods.pdf](http://www.pwc.com/en_US/us/transaction-services/publications/assets/functionalfoods.pdf)

Hussain SA, Sharma P, Singh RRB (2011) Functional Dairy Foods-An overview. In: Souvenir of "International conference on functional dairy foods" held during Nov 16-19 at Karnal, India, pp vii-iii

Jarvis B (1983) Mould and mycotoxins in mouldy cheeses. Microbiologie Aliments Nutr 1:187-191

Kaefer CM, Milner JA (2009) The role of herbs and spices in cancer prevention. J Nutr Biochem 19:347-361

Kamble K, Kahate PA, Chavan SD and Thakare VM (2010) Effect of Pine-apple Pulp on Sensory and Chemical Properties of Burfi. Veterinary World 3(7): 329-331

Knowler, W.C., Barret-connor, E., Fowler, S.E., Hamman, R.F., Lachin, J. M., Walker, E.A., (2003). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England journal of medicine*, 346:393-403.

Lamasa EM, Barrosa RM, Balcao VM, Malcata FX (2001) Hydrolysis of whey proteins by proteases extracted from *Cynara cardunculus* and immobilized onto highly activated supports. Enzyme and Microbial Technol 28:642-652

Landg UB, Pawar BK and Choudhari DM (2011) Preparation of shrikhand using ashwagandha powder as additive. Dairying, Foods & Home Sciences 30 (2):79- 84

Mocanu GD, Rotaru G, Botez E, Vasile A, Gîtin L, Andronoiu D, Nistor O (2009) Research concerning the production of a probiotic dairy product with added medicinal plant extracts. Food Tech 32:37-44

Najgebauer-Lejko D, Grega T, Sady M, Domaga<sup>3</sup>a J (2009) The quality and storage stability of butter made from sour cream with addition of dried sage and rosemary. Biotechnology in Animal Husbandry 25(5-6):753-761

O'Connell JE, Fox PF (2001) Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. Int Dairy J 11:103-120

Özcan M (2003) Antioxidant activity of rosemary, sage and sumac extracts and their combinations on stability of natural peanut oil. J Medicinal Food 6(3):267-270

Pandya NC, Kanawjia SK (2002) *Ghee*: A Traditional Nutraceutical. Ind Dairyman 54(10):67-75

Pawar N Arora, S, Singh RRB, Wadhwa BK.(2012) The effects of Asparagus racemosus (shatavari) extract on oxidative stability of ghee, in relation to added natural and synthetic antioxidants. International Journal of Dairy Technology 65(2):293-299.

Peng D, YaDong Z, Ying M, Yuan G, Dong L (2010) Production and function of dealcohol yogurt beverage. China Dairy Industry 38(1):26-28

Peterson DG, Totlani VM (2005) Influence of flavonoids on the thermal generation of aroma compounds. In: Phenolics in foods and natural health products, Shahidi F (ed), American Chemical Society, Washington, DC.

PricewaterhouseCoopers. (2009). foods industry: Trends and market opportunities [http://www.pwc.com/en\\_US/us/transaction-services/publications/assets/functional-foods.pdf](http://www.pwc.com/en_US/us/transaction-services/publications/assets/functional-foods.pdf)

Rajnikant (2006). Development of process for herbal ghee (*Arjuna* ghee). M.Sc thesis submitted in NDRI, Karnal.

- Rosenthal I, Bernstein S, Nakimbugwe DN (1999) Effects of tea solids on milk. *Milchwissenschaft* 54:149-152
- Sadeghi E, Basti AA, Misaghi A, Salehi TZ, Osgoii SB (2010) Evaluation of effects of *Cuminum cyminum* and probiotic on *Staphylococcus aureus* in Feta cheese. *J Medicinal Plants* 9(34):131-141
- Sarabi-Jamab M, Niazmand R (2009) Effect of essential oil of *Mentha piperita* and *Ziziphora clinopodioides* on *Lactobacillus acidophilus* activity as bioyogurt starter culture. *American-Eurasian J Agric Environ Sci* 6(2):129-131
- Sawale PD, Singh RRB & Arora S (2013) Stability and quality of Herb (*Pueraria tuberosa*) - milk model. *Journal of Food Science and Technology* (DOI: 10.1007/s13197-013-1067-y / 11<sup>th</sup> July).
- Sawale PD, Singh RRB, Kapila S, Arora S, Rastogi S & Rawat, AKS 2013a Immunomodulatory and antioxidative potential of herb (*Pueraria tuberosa*) in mice using milk as the carrier. *International Journal of Dairy Technology* 66 (2):202-206
- Schaller F, Rahalison L, Islam N, Potterat O, Hostettmann K, Stoeckli-Evans H, Mavi S (2000) A new potent antifungal 'quinone methide' diterpene with a cassane skeleton from *Bobgunnia madagascariensis*. *Helvetica Chimica Acta* 83:407-413
- Schamberger GP, Labuza TP (2007) Effect of green tea flavonoids on Maillard browning in UHT milk. *LWT-Food Sci Tech* 40:1410-1417
- Singh SK, Dwivedi HB, Kumar R and Yadav MP (2005) Use of chemical andherbal coaglatants in chhana preparation from cow milk. *Farm science journal* 14(2):71-71
- Singh, RRB. and Hussain, SA (2011). Application of herbs in development of functional milk and milk products. In compendium of National Training Programme on "Basic and Applied Approaches in Designing of Dairy based Nutraceuticals and Functional Foods". 18-27<sup>th</sup> July, 2011, NDRI, Karnal, India, pp: 19-24.
- Tarakci Z (2004) The influence of helis (*Prangos* sp.) on characteristics of vacuum-packed Van herby cheese during ripening. *Milchwissenschaft* 59:619-623
- Tarakci Z, Temiz H, Ugur A (2011) The effect of adding herbs to labneh on physicochemical and organoleptic quality during storage. *Int J Dairy Technol* 64(1):108-116
- Tekin<sup>o</sup>en OC (1997) Süt Ürünleri Teknolojisi. *S.Ü.Vet Fak Yay, Konya* s:224-226
- Trevelyan J (1993) Herbal medicine. *Nursing Times* 89:36-38
- Winston D (1992) Medicine and Ethnobotany. In: American Herbalism, Tierra M (ed), The Crossing Press, Freedom, CA.

**T5-02**

### **Nutraceuticals: A New Vista for Health and Medicine**

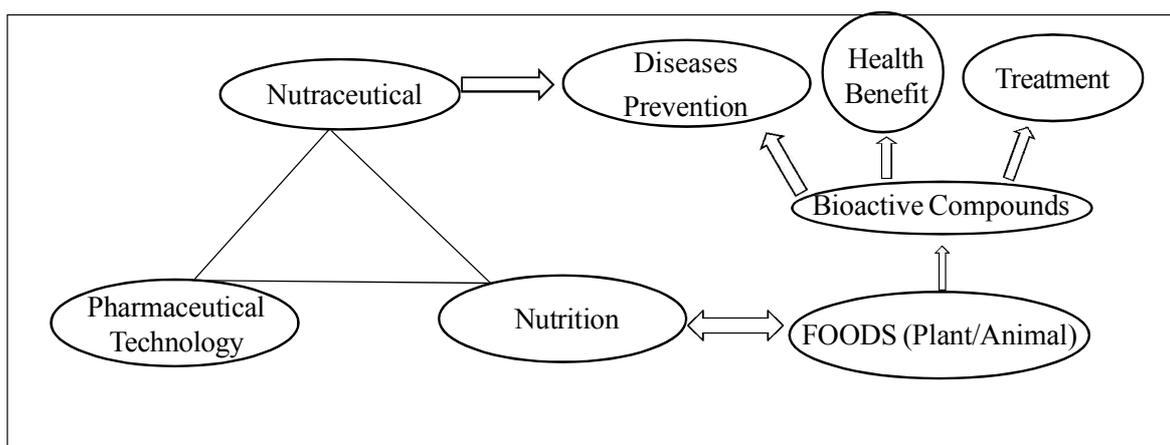
Manish Kumar Chatli\*, Kumbhar Vishaland Sandeep Rindhe

Department of Livestock Products Technology,

College of Veterinary Science, GADVASU, Ludhiana-141004

Email: \*hodlptgadvasu@gmail.com

Life without food is not possible as it provides nutrient that nourish our body and keep our system working in good condition. The quality of life mainly depends on what we eat, and hence nutrition and its impact on health are very important. Nutraceutical is the term derived from the combination of nutrition and pharmaceutical technology. Nutraceuticals are naturally derived bioactive compounds that help in improving health, preventing chronic diseases, delaying the aging process, increasing life expectancy, and supporting the structure or function of the body. The development of functional processed meat products with enhanced 'healthiness' through the use of nutraceuticals is essential for the industry to compete with other food sectors. The development of novel concept of nutraceuticals is considered to play a vital role in disease prevention in addition to being nutrient providers.



### **HISTORY OF NUTRACEUTICALS**

The term “nutraceutical” was coined in 1989 by the Foundation for Innovation in Medicine (New York, US), to provide a name for this rapidly growing area of biomedical research. A nutraceutical was defined as any substance that may be considered a food or part of a food and provides medical or health benefits including the prevention and treatment of disease. Nutraceuticals may range from isolated nutrients, dietary supplements and diets to genetically engineered “designer” foods, herbal products and processed products such as cereals, soups and beverages. Doubtlessly, many of these products possess pertinent physiological functions and valuable biological activities. The ongoing research will lead to a new generation of foods, which will certainly cause the interface between food and drug to become increasingly

permeable. The present accumulated knowledge about nutraceuticals represents undoubtedly a great challenge for nutritionists, physicians, food technologists and food chemists. Public health authorities consider prevention and treatment with nutraceuticals as a powerful instrument in maintaining health and to act against nutritionally induced acute and chronic diseases, thereby promoting optimal health, longevity and quality of life.

### **Types of nutraceuticals**

There are numerous classifications and categorizations of nutraceuticals, functional foods and dietary supplements. Previously, they have been categorized as either potential or established nutraceuticals (Pandey et al., 2010), on the basis of the food material and nutrients (Singh & Sinha, 2012) or in terms of their effects on the body (Das & Sen, 2014). Most commonly, however, their classification is based on the chemical constituents and/or active ingredients (Srivastava et al., 2015) as described in detail below Table.

**Table :** Classes and active ingredients of nutraceuticals, some of the most commonly claimed health benefits are also listed.

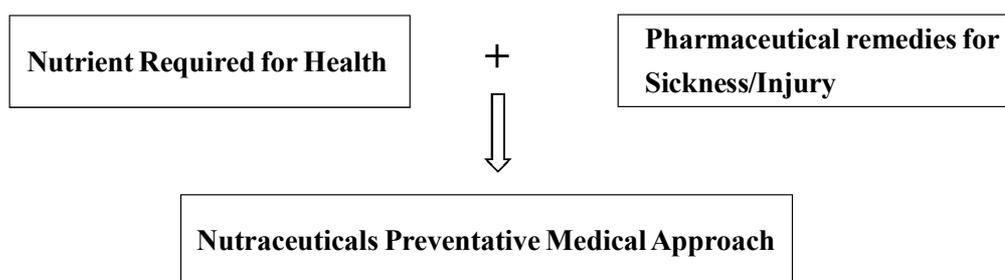
<b>Class</b>	<b>Chemical/ Active ingredients</b>	<b>Potential benefits</b>	<b>Reference</b>
Polyphenolic compounds	Flavones	Anti-inflammatory, antioxidant, anti-carcinogenic and antimetastatic.	Yang et al., 2008
	Isoflavones	Ameliorate postmenopausal symptoms, antioxidant, anticarcinogenic, radioprotective, immunomodulatory	Zhou et al., 2015
	Flavonones	Anti-tumoral, antioxidant, anti-inflammatory, vasoprotective and anti-atherosclerosis effects	Khan et al., 2014
	Flavonols	Ameliorate chronic inflammatory disease symptoms, antioxidant, neuro- and, genoprotective	Arredondo et al., 2015
	Anthocyanins	Antioxidant, chemopreventive (anti-metastatic), immunomodulatory.	Smeriglio et al 2016
	Proanthocyanidins	Anti-obesity, improvement of lead-induced cognitive impairments, decreased cardiovascular mortality, antioxidant activity	Liu et al., 2014

Class	Chemical/ Active ingredients	Potential benefits	Reference
	Resveratrol	Anticarcinogenic, improvement of bone density, reversal of age related cognitive impairments, protection against retinal ganglion cell dendrite loss.	Zhao et al., 2015
	Curcumin	Anticarcinogenic, radioprotective, antioxidant, antidotal against neurotoxic agents, anti-inflammatory	Lopez-Jornet et al., 2016
Carotenoids	Lutein	Improvements in neural functions and respiratory health protective.	Peng et al., 2016
	Lycopene	Radioprotective, attenuation of high-fat diet-induced cognitive impairments, antioxidant.	Wang et al., 2016
	a and b carotene	Improvement of bone density, anticarcinogenic, anti-ischemic, chemo-sensitizer	Zhang et al., 2016
	a-cryptoxanthin	Anticarcinogenic, anti-inflammatory, chemopreventive, antiarteriosclerotic	Chaudhari et al., 2014
Anthraquinones	Barbaloin	Antidiabetic, anti-helminthic, antiviral	Rajat et al., 2012
	Capsaicin	Hepatoprotective, antioxidant, anticarcinogenic, vasorelaxative (hypertension prevention).	Giri, Mukherjee, Barman, & Maity, 2016
	Hypericin	Analgesic, antioxidant, anti-depressant	Rajat et al., 2012
	Piperine	Anticarcinogenic, angiogenesis inhibitor, anti-biofilm formation effect, cholesterol gallstones formation prevention.	Song et al., 2015
Terpenes	Borneol	Bone metabolism modulation, anticarcinogenic, cholesterol gallstones formation prevention	Tatman & Mo, 2002
	Santonin	Antidiabetic, anti-helminthic	Vonthron-Senecheau, 2016
	Gossypol I	Contraceptive, antiparasitic	Rajat et al., 2012
	Menthol	Cholesterol gallstones formation prevention, antimicrobial, antioxidant, analgesic	Kamatou, et al., 2013
Alkaloids	Quinine	Improvement in body weight (anti-obesity), anti-malarial.	Rajat et al., 2012
	Morphine	Analgesic, antidepressant.	Rajat et al., 2012
	Ergot alkaloids	Antidiabetic	Atta Ur & Zaman, 1989
	Coumarin	Antioxidant, anti-inflammatory, antidiabetic, anti-thrombotic	Zaragoza et al., 2016

Class	Chemical/ Active ingredients	Potential benefits	Reference
Gulcosinolates		Antioxidant	Sun et al., 2012
Saponins		Anticarcinogenic, anti-inflammatory, hypo-cholesterolaemic	Jaramillo et al., 2016
Isothiocyanates	Sulporaphane	Anti-inflammatory	Nallasamy et al., 2014

### Concept of nutraceuticals

In the pharmaceutical development process, it is a requirement to have clinical test results from animal tests and studies, for verification of their therapeutic effects. But in the case of nutrition, there was no verification method for foods in preventing diseases in the past. In recent years however, as food composition has been scientifically proven to cause life stylerelated diseases, and has become a social issue. The nutraceutical products are recognized and produce health benefits like alleviating the risk of cancer and heart disease and also to prevent or treat hypertension, high cholesterol, excessive weight, osteoporosis, diabetes, arthritis, macular degeneration (leading to irreversible blindness), cataracts, menopausal symptoms, insomnia, diminished memory and concentration, digestive upsets and constipation, not to mention headaches; other products are touted as cures for thinning hair, lack of confidence, poor complexion, varicose veins, alcoholism, depression, and lethargy. The concept of Nutraceuticals has started to be acknowledged as one of the measures for preventing such diseases (Prabu et al., 2012), the concept of nutraceuticals is shown in figure 2.



### Motivating the Development of Functional Foods and Nutraceuticals:

The genesis of the functional foods and nutraceuticals industry has occurred for a number of reasons. Possible positive role diet can play in disease risk management, an increase in public health consciousness, aging population, escalating health care costs, recent advances in research and technology, changes in government regulations and accountability, expansion of the global marketplace, sympathetic media, and science-based evidence is contributing to the popularity of nutraceuticals foods. Increased education may

also be partly responsible, as well as an overall heightening of interest in the general area of preventive health. Second, regulatory bodies have become increasingly cognizant and supportive of the public health benefits of functional foods.

In the global marketplace nutraceuticals and functional foods have become a multi-billion dollar industry. Internationally, significant limitations to growth in this area are resulting from a necessity to properly label and assess the health effects of nutraceutical and functional foods. Selection for consistent production of high and low productivity of active plant components within specific ecological regions will allow development of alternative nutraceuticals and functional foods with distinctive and more reliable health and food properties. (Nissar et al., 2008)

India is the home of a large number of medicinal herbs, spices and tree species that have a substantially large domestic market. The functional foods and nutraceuticals are available as traditional Indian Ayurvedic Medicines in India and marketed in different brand names. However, no strict pharmaceutical regulations are available for the Ayurvedic and nutraceutical health products in India; they are available to the public as over the counter without any medical prescription. India has a large share of the international functional food and nutraceutical market, and exports products to various countries. However, India's major export destination is the USA and Japan

### **Functional Foods from Animal Sources**

Some animal products with potential beneficial effects on human health are considered as functional foods. Meat systems have great potential for delivering important nutrients into the diet. The nutritional composition of meat products can be altered by direct addition of bioactive food ingredients or by the inclusion of bioactive compounds into animal diets. The latter technique has the advantage that the bioactive compounds would be biologically introduced into the food and thus would not have to be declared as a food additive. Meat and poultry products are a food category with both positive and negative nutritional attributes. Muscle foods are major sources for many bioactive compounds including iron, zinc, conjugated linoleic acid (mainly ruminants) and B vitamins (Jimenez-Colmenero et al., 2001). However, meats and processed meats are also associated with nutrients and nutritional profiles that are often considered negative including high levels of saturated fatty acids, cholesterol, sodium and high fat and caloric contents (Whitney & Rolfes, 2002). Some of these negative nutrients in meats can be minimized by selection of lean meat cuts, removal of adipose fat, dietary manipulation to alter fatty acid composition and proper portion control to decrease fat consumption and caloric intake. In addition, the nutritional profile of meat products could be further improved by addition of potentially health promoting nutrients. These products would be categorized as functional foods which are defined as foods with nutritional profiles that exceed conventional products. In deciding proper nutrients for functional foods, several factors should be considered including

the bioactive compound's current intake level in the diet (e.g. would the consumer benefit from an increase in the bioactive compound in the diet), biological efficacy in humans, stability in the food product and impact on quality parameters such as color, flavor and texture.

#### *Unsaturated fatty acids:*

In many countries, consumers are over consuming saturated fatty acids and under consuming polyunsaturated fatty acids especially the omega-3 fatty acids (Dietary Guidelines for Americans, 2005). The fatty acid composition of meat from ruminants is generally more saturated due to the fact that unsaturated fatty acids are subjected to biohydrogenation in the rumen. The lack of a rumen means that the fatty acid composition of muscle foods from animals such as pigs, poultry and fish can be altered by diet as many papers have been published on increasing the unsaturated fatty acids composition of pigs and poultry (Bou et al., 2009). However, these practices are limited by the fact that increasing levels of unsaturated fatty acids decreases the oxidative stability of the meat product. In contrast, increasing unsaturated fatty acids by dietary manipulation has been very successful in eggs. This is because eggs are naturally very antioxidative with the lipids packaged in oxidative stable lipoproteins, the iron inactivated by binding to proteins such as phosvitin and maintenance of low oxygen environment (Bou et al., 2009). Thus, alteration of dietary fatty acids to change muscle composition is most easily accomplished with oleic acid since it is at least 10 times more oxidatively stable than polyunsaturated fatty acids such as linolenic (McClements & Decker, 2008). However, if dietary manipulation is performed to increase polyunsaturated fatty acids, then antioxidant technologies must also be employed to minimize oxidative deterioration. Inhibition of oxidative deterioration could be accomplished by increasing muscle antioxidants by diet or food ingredients, decreasing storage temperature and/or oxygen exclusion by vacuum packaging.

#### *Dietary fiber*

Dietary fiber can be classified as soluble and insoluble fiber. Both types of fiber have numerous health benefits including maintaining bowel integrity and health, lowering blood cholesterol levels, controlling blood sugar levels and providing a non-caloric bulking agent that can aid in weight loss by replacing caloric food components such as fat. According to the Dietary Guidelines for Americans (2005), dietary fibers are under consumed by most adults indicating that fiber fortification in meat products could have health benefits. In addition to the benefit of increased fiber consumption, dietary fibers in meat products also have other advantages such as fat replacement, increased water-holding capacity and improved oxidative stability when the fiber source is associated with phenolic antioxidants (Sayago-Ayerdi et al., 2009).

### *Minerals*

Dietary mineral are essential for bone health, hypertension, muscle and nerve function, regulation of blood sugar levels and thus are important in diseases such as hypertension, cardiovascular disease, osteoporosis and diabetes (Whitney & Rolfes, 2002). Calcium, potassium and magnesium are the most commonly under consumed minerals in the diet (Dietary Guidelines for Americans, 2005). Fortification of processed meats with calcium, potassium and magnesium can be accomplished without major changes in sensory profile (Selgas et al., 2009). An additional advantage of calcium, potassium and magnesium fortification is that these minerals can be used to reduce sodium levels in processed meats thus further improving the nutritional profile of the product (Moon et al., 2008). Salt such as potassium lactate can also be advantageous since it inhibits lipid oxidation and microbial growth (Moon et al., 2008). Calcium can activate the protease, calpain, which increases meat tenderness. Research has shown that calcium infusion into beef cattle can both increase muscle calcium concentrations (Dikeman et al., 2003) and improve beef tenderness after 14 days of aging (Diles et al., 1994). However, in some processed meats, calcium, potassium and magnesium inhibit proteolytic enzymes important in the flavor development of dry-cured meats (Armenteros et al., 2009) although they do not negatively impact salty flavor (Armenteros et al., 2009).

### *Antioxidant vitamins*

Dietary antioxidants have been suggested to be beneficial to immune function, heart disease and cancer. Vitamins A, C and E are consumed at levels below their recommended dietary intake levels by many consumers (Dietary Guidelines for Americans, 2005). Addition of vitamin C to meats is difficult because it is not very stable at meat pH and it tends to promote lipid oxidation (Haak et al., 2009). In addition, vitamin C addition to meats is often prohibited since it can stabilize meat color and thus is considered adulteration.  $\beta$ -carotene is an important source of dietary Vitamin A. In general, meats are not a good source of  $\beta$ -carotene or other carotenoids with the exception of chicken.  $\beta$ -carotene concentrations can be increased by dietary supplementation (for review see Bou et al., 2009). Addition of  $\beta$ -carotene to meats as a food ingredient is often difficult due to its chemical instability. Both exogenous addition and dietary fortification of  $\beta$ -carotene is also limited since it will alter the color of the muscle (Torrissen, 2000). Meats are an important dietary source of vitamin E with poultry and ground beef being the second and sixth most important sources of  $\alpha$ -tocopherol in men while poultry is the third most important source in women (Ma et al., 2000). Muscle foods could be even better sources of vitamin E through meat fortification or dietary supplementation with  $\alpha$ -tocopheryl acetate. Vitamin E fortification in foods is most effective when  $\alpha$ -tocopheryl acetate is used as the ingredient.

### *Conjugated linoleic acid (CLA)*

One fatty acid that has drawn significant attention for its potential health benefits in the last two decades is conjugated linoleic acid (CLA). CLA is naturally found in milk and dairy products (Chin et al., 1992) and was originally identified as an anti-cancer component from ground beef extract (Pariza & Hargraves, 1985). The cis-9,trans-11 CLA isomer in food can originate by one of two pathways; from the incomplete biohydrogenation of linoleic acid to stearic acid by rumen bacteria or from the delta-9 desaturation of trans-11 vaccenic acid (a primary intermediate for ruminant biohydrogenation) in mammalian tissues (Kay et al., 2004). This isomer consists of up to 80–85% of total CLA in food (Chin et al., 1992).

Typical CLA concentrations in beef range from 6 to 9 mg/g fat. Since CLA is found in adipose fat, CLA concentrations are highly influenced by fat concentrations. Dietary CLA from beef can be increased by manipulation of animal diets and direct CLA addition to meats. CLA concentrations in beef can be influenced by diets containing oils or oilseeds high in polyunsaturated fatty acids (usually linoleic or linolenic). These dietary practices can increase CLA concentrations up to 3 fold (Madron et al., 2002). Muscle CLA concentrations can also be increased by the direct inclusion of CLA into animal feeds which has the added benefit of decreasing muscle adipose fat concentrations (Dugan et al., 1997). Since CLA is also available as a food additive, it can be directly added to foods. The most significant bioactivity of the cis-9,trans-11 CLA isomer is its anti-cancer property, as shown in a number of animal cancer models such as breast, epidermis, prostate, colon, liver, kidney, and lung (Kelley et al., 2007). Overall, it is not conclusive if naturally occurring CLA has a significant health impact on prevention of cancer. Thus, further studies are needed to confirm the implications of CLA on human cancer prevention.

### *Bioactive peptides:*

Food-based strategies to control high blood pressure would be an excellent intervention to improve health and wellness and decrease health care costs. Naturally occurring peptides with ACE (Angiotensin I-converting Enzyme) inhibitory activity have been identified from the proteolytic degradation products of food proteins. Proteins that can produce ACE inhibitory peptides can be of animal (e.g. dairy, fish, meat and eggs), plant (e.g. soy, rice and garlic) or microbial (e.g. yeast) origin (Hong et al., 2008).

Bioactive peptides have been identified from the hydrolysis of skeletal muscle proteins including myosin, tropomyosin, troponin, actin and collagen (Vercruyse et al., 2005). The ACE inhibitory activity of peptides from sources such as dairy proteins has been the most extensively studied. For example, the casein polypeptides (e.g.  $\alpha$ s1,  $\alpha$ s2 and  $\beta$ ) can be hydrolyzed into over 65 different peptides with ACE inhibitory activity. The ACE inhibitory activity of these peptides varies extensively with half maximal inhibitory concentrations (IC<sub>50</sub>) ranging from 2 to N1000  $\mu$ M when measured with in vitro tests. The

peptides also vary greatly in size ranging from 2 to over 80 amino acids (Hong et al., 2008). A large number of studies have shown that peptides originating from food proteins have ACE inhibitory activity in vitro and can decrease blood pressure in laboratory animal studies (Hong et al., 2008). However, evidence also exists for the ability of these peptides to decrease blood pressure in human populations.

### Marine-based nutraceuticals

Many marine-based food ingredients fall under the category of nutraceuticals, which are bioactive substances with medicinal characteristics or added health benefits such as anticancer or anti-inflammatory activity. Fortification of foods with nutraceuticals has become an increasingly popular method for providing nutritional food products to health-conscious consumers. Marine based nutraceuticals are already an active industry in Japan and Europe, and the US market has experienced significant growth over the past decade (Ohr, 2005). Some examples of marine nutraceuticals currently marketed in the United States include products such as fish and algal oils rich in omega-3 fatty acids, chitin and chitosan, fish and shark liver oil, marine enzymes and chondroitin from shark cartilage, sea cucumbers, and mussels. Omega-3 fatty acids are well known for their wide range of health benefits, including reduced risk of cardiovascular disease and enhanced brain development in infants, while chondroitin has been shown to have anti-inflammatory and anticancer properties. Marine-based food ingredients and nutraceuticals can be derived from a vast array of sources, including marine plants, microorganisms, and sponges, all of which contain their own sets of unique biomolecules that allow them to thrive in their respective habitats. Another growing source for marine-based food ingredients has been fish and seafood by-products resulting from postharvest processing.

**Table:** Some major categories of marine-derived food ingredients used commercially shown in table

Category	Food ingredient	Application	Major marine sources	Health benefits and other advantages	Ref.
Photo synthetic pigments	Carotenoids: b-carotene, astaxanthin, and lutein	Natural food colorings, nutraceutical agents, farmed salmon pigmentation	Microalgae: <i>D. salina</i> , <i>S. maxima</i>	Vitamin A precursors, inflammatory, antioxidants, anticarcinogenic, anti-natural pigments	Maeda et al., 2005
Lipids	Omega-3 fatty acids: SDA, EPA, DHA	Nutraceuticals, fish oil capsules, fortification of livestock, feed and infant formula	Fish (e.g., salmon, sardine, tuna, herring), microalgae ( <i>Navicula</i> spp., <i>N. frustulum</i> , <i>B. sinensis</i> , <i>P. tricorutum</i> ), fungi (phycomycetes)	Numerous health benefits (e.g. visual and neuro development, reduce risk of cardiovascular problems)	Maeda et al., 2005

Category	Food ingredient	Application	Major marine sources	Health benefits and other advantages	Ref.
Proteins	Collagen	Edible casings in the meat industry (e.g., sausages)	Fish (albacore tuna, silver-line grunt, bigeye snapper, brown-backed toadfish, hake, trout)	Can be extracted from processing by-products	Jongjareonrak et al., 2005
	Albumin	Replacement for egg albumin as a whipping	Mollusks, crustaceans, low-fat fish	High flexibility and strength, health benefits (anticoagulant, Antioxidant)	Nicholson et al., 2000
Enzymes	Lipases	Numerous uses in the fats and oils industry (e.g., production of omega-3-enriched triglycerides)	Atlantic cod, seal, salmon, sardine, Indian mackerel	Higher specificity for omega-3 fatty acids	Shahidi and Janak Kamil, 2001
	Red algae enzymes in the starch degradation pathway (e.g., α-1,4-glucan lyase and others)	Production of the natural sugar 1, 5-anhydro-D-fructose and the antifungal compound micro thecin	Red algae (genera <i>Gracilariales</i> )	Production of compounds that exhibit antioxidant, antimicrobial, and/or anti-tumor properties	Yu, 2005

### Challenges and Opportunities:

The field of nutraceuticals and functional foods is new and many gaps exist in the knowledge base, for example, it is widely accepted that the health-promoting properties of foods are not necessarily due to single components, but rather a few or several active ingredients. This creates a significant shift from the pharmaceutical model, which is based on paradigm shift from the pharmaceutical model, which is based on the efficacy of single agents. Manufacturers wish to make specific claims of health benefits on their product labels. Food companies and research institutes still develop foods with potential functional properties without assessing their chronic and acute effects in humans to attest the alleged potentiality. In a related field, namely ethnopharmacology, the development of new products with pharmacological activity in certain organs/glands and/or biomarker in humans, the study of traditional herbs and foods, which are known to be safe from the toxicological standpoint, is generally the first step to be taken after some in vitro measurements (Abbasi et al., 2015). However, precaution should always be taken when evaluating natural products: for example, Abd-ElSalam et al. (2016) evaluated the antioxidant activity in vitro (test tubes) and in vivo (rats previously subjected to oxidative stress) of green tea-derived catechins, including

epigallocatechin gallate (EGCG). Authors verified no correlation between free radical scavenging activity and the content of EGCG in vitro and the in vivo data showed not only loss of antioxidant activity but also the formation of harmful pro-oxidants in hepatocytes, corroborating the fact that a careful design of quality control protocols requires correlation between chemical assays and bioassays to verify efficacy, stability, and most importantly safety of nutraceuticals.

### **Biotechnological Applications in Nutraceuticals:**

Biotechnology is a diverse field of science, which has been instrumental in human development ever since life has evolved. Basically, it integrates human, animals, and microorganisms with the technology for the betterment of life. There are various food processing sectors where the biotechnological tools can be applied for betterment of the food products. These aspects include increasing the yield of food, improve the nutrition value, use of fermentation process to yield different food products, producing important enzymes, increase the shelf life, improving the organoleptic properties of food, and to enhance the food safety. With the advances in the biotechnology, bio-fortification of foods using technologies such as recombinant DNA technology and fermentation procedures is gaining advantage in the industry. Designer foods are normal foods developed by using the biotechnology tools, fortified with health promoting ingredients.

### ***What the future holds?***

There are positive results and indications that some of the aforementioned nutraceuticals hold promising outcomes to clinical pathological conditions. Nonetheless, such promises should take into consideration the experimental designs themselves (Arredondo et al., 2015). Attention must be paid, particularly relating to the effective dosages used, namely, when carriers are utilized. Furthermore, considering the ongoing debate about the efficacy of these products, studies have to focus in determining whether nutritional low and chronic administration of nutraceuticals do, in fact, play a role in health. However, this dramatic change has not only failed to materialize, but, to a large extent, it has become discredited. There are compounds, such as polyphenols, that, in essence, are true nutraceuticals, with real observable health boons. However, the vast number of compounds that have appeared in the market, each claiming broader and more of benefits than the previous, has led to a general skepticism from large segments of the population. The progress in technological tools applied to the evaluation of the effects of nutraceuticals, such as metabolomics as well as the advent of new fields of research, namely, nutrigenomics (Pavlidis et al., 2015), will no doubt lead to new and different health claims, which must be carefully evaluated. The current status quo cannot be sustained for much longer and there is the need to adequately evaluate the dietary health benefits, if any, of these heavily marketed products. Regulatory bodies should be proactive and must assume a preventive and pre-emptive position, in order to safeguard against multiple

inclusions into the categories of “foods for special dietary use” and “medical foods”, which the framework for admission has not been soundly established (Burdock et al., 2006).

Furthermore, the current legal void in which these products are marketed has further contributed to this disbelief, while companies keep on making “healthy profits” from supposedly “healthy foods”. The challenges facing this industry are difficult and numerous, but perhaps the key to overcoming these lies in the laboratory: it is necessary to ascertain the exact mechanisms by which many food-derived substances exert a beneficial effect, as it has become clear that the same isolated and concentrated forms frequently exhibit equivocal effects. A keen understanding of the bio- and pharmacokinetics will help to unravel the compounds that can ultimately lead to true, established nutraceuticals, while simultaneously allowing for the establishment of relevant legislation and efficient regulation. From the industry’s perspective, such research will give credit to the health claims and will identify which compounds are, in fact, nutraceuticals worthy for commercialization, and, maybe, lead to “healthier” profits.

### **References**

- Abbasi A, Emam-Djomeh Z, Mousavi MAE, Davoodi D (2015) Stability of vitamin D3 encapsulated in nanoparticles of whey protein isolate. *Food Chem* 143: 379-383
- Armenteros M, Aristoy M, & Toldra F. (2009). Effect of sodium, potassium, calcium and magnesium chloride salts on porcine muscle proteases. *European Food Research and Technology*, 229(1), 93-98.
- Arredondo F, Echeverry C, Blasina F, Vaamonde L, Díaz M, Rivera F, et al. (2015). Chapter 25-flavones and flavonols in brain and disease: Facts and pitfalls A2-watson, Ronald Ross. In V. R. Preedy (Ed.), *Bioactive nutraceuticals and dietary supplements in neurological and brain disease* (pp. 229-236). San Diego: Academic Press.
- Arredondo F, Echeverry C, Blasina F, Vaamonde L, Díaz M, Rivera F. (2015). Chapter 25-flavones and flavonols in brain and disease: Facts and pitfalls A2-watson, Ronald Ross. In V. R. Preedy (Ed.), *Bioactive nutraceuticals and dietary supplements in neurological and brain disease* (pp. 229e236). San Diego: Academic Press.
- Atta Ur. R. & Zaman K. (1989). Medicinal plants with hypoglycemic activity. *Journal of Ethnopharmacology*, 26, 1-55.
- Bou R., Codony R., Tres A., Decker E. A., & Guardiola, F. (2009). Dietary strategies to improve nutritional value, oxidative stability, and sensory properties of poultry products. *Critical Reviews in Food Science and Nutrition*, 49(9), 800-822.
- Burdock, G. A., Carabin, I. G., & Griffiths, J. C. (2006). The importance of GRAS to the functional food and nutraceutical industries. *Toxicology*, 221, 17-27.

- Chaudhari, R. S., Mankumare, V. B., Shankaranarayanan, J., Deshpande, J. V., Sunilkumar, T. K., & Sathaye, S. (2014). b-Cryptoxanthin rich paprika extract prevents ovariectomy induced bone loss in Wistar rats. *PharmaNutrition*, 2, 149-154.
- Chin, S. F., Storkson, J. M., Ha, Y. L., & Pariza, M. W. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid. *Journal of Food Composition and Analysis*, 5, 185-197.
- Das, A., & Sen, C. K. (2014). Chapter 2-nutritional supplements and functional Foods: Functional significance and global regulations A2-bagchi, debasis. In *nutraceutical and functional food regulations in the United States and around the world* (2nd ed.). San Diego: Academic Press.
- Dietary guidelines for Americans (2005). U.S. Department of Health and Human Services, & U.S. Department of Agriculture. <http://www.health.gov/dietaryguidelines/dga2005/document/>.
- Dikeman, M. E., Hunt, M. C., Addis, P. B., Schoenbeck, H. J., Pullen, M., Katsanidis, E., et al. (2003). Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. *Journal of Animal Science*, 81(1), 156-166.
- Diles, J. J. B., Miller, M. F., & Owen, B. L. (1994). Calcium-chloride concentration, injection time, and aging period effects on tenderness, sensory, and retail color attributes of loin steaks from mature cows. *Journal of Animal Science*, 72(8), 2017-2021.
- Dugan, M. E. R., Aalhus, J. L., Schaefer, A. L., & Kramer, J. K. G. (1997). The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Canadian Journal of Animal Science*, 77(4), 723-725.
- Haak, L., Raes, K., & De Smet, S. (2009). Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties. *Journal of the Science of Food and Agriculture*, 89(8), 1360-1365.
- Heba-Alla H. Abd-ElSalam, Medhat A. Al-Ghobashy, Hala E. Zaazaa & Mohamed A. Ibrahim. 2016. On-column decaffeination and HPLC analysis of epigallocatechin gallate in green tea nutraceuticals. *Journal, Separation Science and Technology* Volume 51, 2016 - Issue 4.
- Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: a novel alternative to drugs? *Peptides*, 29(6), 1062-1071.
- Jaramillo, S., Muriana, F. J. G., Guillen, R., Jimenez-Araujo, A., Rodriguez-Arcos, R., & Lopez, S. (2016). Saponins from edible spears of wild asparagus inhibit AKT, p70S6K, and ERK signalling, and induce apoptosis through G0/G1 cell cycle arrest in human colon cancer HCT-116 cells. *Journal of Functional Foods*, 26, 1-10.
- Jongjareonrak, A., Benjakul, S., Visessanguan, W., and Tanaka, M. 2005. Isolation and characterization of collagen from bigeye snapper (*Priacanthus macracanthus*) skin. *J. Sci. Food Agric.* 85, 1203-1210.
- Kamatou, G. P. P., Vermaak, I., Viljoen, A. M., & Lawrence, B. M. (2013). Menthol: A simple monoterpene with remarkable biological properties. *Phytochemistry*, 96, 15-25.

- Kay, J. K., Mackle, T. R., Auld, M. J., Thomson, N. A., & Bauman, D. E. (2004). Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. *Journal of Dairy Science*, 87(2), 369-378.
- Kelley, N. S., Hubbard, N. E., & Erickson, K. L. (2007). Conjugated linoleic acid isomers and cancer. *Journal of Nutrition*, 137(12), 2599-2607.
- Khan, M. K., Zill, E. H., & Dangles, O. (2014). A comprehensive review on flavanones, the major citrus polyphenols. *Journal of Food Composition and Analysis*, 33, 85-104.
- Liu, C.-M., Ma, J.-Q., Liu, S.-S., Zheng, G.-H., Feng, Z.-J., & Sun, J.-M. (2014). Proanthocyanidins improves lead-induced cognitive impairments by blocking endoplasmic reticulum stress and nuclear factor- $\kappa$ B-mediated inflammatory pathways in rats. *Food and Chemical Toxicology*, 72, 295-302.
- Lopez-Jornet, P., Gomez-García, F., García Carrillo, N., Valle-Rodríguez, E., Xerafin, A., & Vicente-Ortega, V. (2016). Radioprotective effects of lycopene and curcumin during local irradiation of parotid glands in Sprague Dawley rats. *British Journal of Oral and Maxillofacial Surgery*, 54, 275-279.
- Ma, J., Hampl, J. S., & Betts, N. M. (2000). Antioxidant intakes and smoking status: data from the continuing survey of food intakes by individuals 1994–1996. *American Journal of Clinical Nutrition*, 71(3), 774-780.
- Madron, M. S., Peterson, D. G., Dwyer, D. A., Corl, B. A., Baumgard, L. H., Beeraman, D. H., et al. (2002). Effect of extruded full-fat soybeans on conjugated linoleic acid content of intramuscular, intermuscular, and subcutaneous fat in beef steers. *Journal of Animal Science*, 80(4), 1135-1143.
- Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., and Miyashita, K. 2005. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.* 332, 392–397.
- McClements, D. J., & Decker, E. A. (2008). Lipids. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Food chemistry* (pp. 155-216). Boca Raton, FL: CRC Press.
- Moon, S. S., Kim, Y. T., Jin, S., & Kim, I. (2008). Effects of sodium chloride, potassium chloride, potassium lactate and calcium ascorbate on the physico-chemical properties and sensory characteristics of sodium-reduced pork patties. *Korean Journal for Food Science of Animal Resources*, 28(5), 567-573.
- Nallasamy, P., Si, H., Babu, P. V. A., Pan, D., Fu, Y., Brooke, E. A. S., et al. (2014). Sulforaphane reduces vascular inflammation in mice and prevents TNF- $\alpha$ -induced monocyte adhesion to primary endothelial cells through interfering with the NF- $\kappa$ B pathway. *The Journal of Nutritional Biochemistry*, 25, 824-833.
- Nicholson, J.P., Wolmarans, M.R., and Park, G.R. 2000. The role of albumin in critical illness. *Br. J. Anaesth.* 85, 599–610.

- Nissar, M., Chatli, M. K., Biswas, A. K., Sharma, D. K. and Sahoo, J. (2008). Functional Foods of animal origin: A New Vista for Human Health. *Indian Food Industry* Nov-Dec: 56-63
- Ohr, L.M. 2005. Riding the nutraceuticals wave. *Food Technol.* 59, 95–96.
- Pandey, M., Verma, R. K., & Saraf, S. A. (2010). Nutraceuticals: New era of medicine and health. *Asian Journal of Pharmaceutical and Clinical Research*, 3, 11-15.
- Pariza, M. W. & Hargraves, W. A. (1985). A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7, 12-dimethylbenz[a]anthracene. *Carcinogenesis*, 6(4), 591-593.
- Pavlidis, C., Patrinos, G. P., & Katsila, T. (2015). Nutrigenomics: A controversy. *Applied & Translational Genomics*, 4, 50-53.
- Peng, M.-L., Chiu, H.-F., Chou, H., Liao, H.-J., Chen, S.-T., Wong, Y.-C., et al. (2016). Influence/impact of lutein complex (marigold flower and wolfberry) on visual function with early age-related macular degeneration subjects: A randomized clinical trial. *Journal of Functional Foods*, 24, 122-130.
- Prabu S L, Suriyaprakash T.N.K., C D Kumar, SureshKumar S, and Ragavendran T. 2012. *Elixir Pharmacy* 46, 8372-8377.
- Rajat, S., Manisha, S., Robin, S., & Sunil, K. (2012). Nutraceuticals: A review. *International Research Journal of Pharmacy*, 3, 4.
- Sayago-Ayerdi, S. G., Brenes, A., & Goni, I. (2009). Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. *Lwt-Food Science and Technology*, 42(5), 971-976.
- Selgas, M. D., Salazar, P., & Garcia, M. L. (2009). Usefulness of calcium lactate, citrate and gluconate for calcium enrichment of dry fermented sausages. *Meat Science*, 82(4), 478-480.
- Shahidi, F. and JanakKamil, Y.V.A. 2001. Enzymes from fish and aquatic invertebrates and their application in the food industry. *Trends Food Sci. Technol.* 12, 435–464.
- Singh, J., & Sinha, S. (2012). Classification, regulatory acts and applications of nutraceuticals for health. *International Journal of Pharma and Bio Sciences*, 2, 177-187.
- Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2016). Chemistry, pharmacology and health benefits of anthocyanins. *Phytotherapy Research*, 1265-1286.
- Song, X.-Y., Xu, S., Hu, J.-F., Tang, J., Chu, S.-F., Liu, H., et al. (2015). Piperine prevents cholesterol gallstones formation in mice. *European Journal of Pharmacology*, 751, 112-117.
- Srivastava, S., Sharma, P. K., & Kumara, S. (2015). Nutraceuticals: A review. *Journal of Chronotherapy and Drug Delivery*, 6, 1-10.
- Sun, B., Yan, H., Zhang, F., & Wang, Q. (2012). Effects of plant hormones on main health-promoting compounds and antioxidant capacity of Chinese kale. *Food Research International*, 48, 359-366.

- Tatman, D., & Mo, H. (2002). Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. *Cancer Letters*, 175, 129-139.
- Torrissen, O. J. (2000). Chapter 11: Dietary delivery of carotenoids. In E. A. Decker, C. Faustman, & C. J. Lopez-Bote (Eds.), *Antioxidants in muscle foods* (pp. 289-313). : Wiley-Interscience.
- Vercruyse, L., Van Camp, J., & Smagghe, G. (2005). ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: a review. *Journal of Agricultural and Food Chemistry*, 53(21), 8106-8115.
- Vonthron-Senecheau, C. (2016). Chapter 11-medicinal Properties: Antibiotic, tonic, and antiparasitic properties. In *seaweed in health and disease prevention* (pp. 369-388). San Diego: Academic Press.
- Wang, Z., Fan, J., Wang, J., Li, Y., Xiao, L., Duan, D., et al. (2016). Protective effect of lycopene on high-fat diet-induced cognitive impairment in rats. *Neuroscience Letters*, 627, 185-191.
- Whitney, E. N., & Rolfes, S. R. (2002). *Understanding nutrition*, Ninth edn Belmont, CA: Wadsworth.
- Yang, S.-F., Yang, W.-E., Kuo, W.-H., Chang, H.-R., Chu, S.-C., & Hsieh, Y.-S. (2008). Antimetastatic potentials of flavones on oral cancer cell via an inhibition of matrix-degrading proteases. *Archives of Oral Biology*, 53, 287-294.
- Yu, S. 2005. "The Discovery of New Enzymes, New Products and a New Pathway from Marine Red Algae". *Marine Bio-Manipulation Frontier for Food Production*, Hokkaido, Japan.
- Zaragoza, C., Monserrat, J., Mantecon, C., Villaescusa, L., Zaragoza, F., & Alvarez- Mon, M. (2016). Antiplatelet activity of flavonoid and coumarin drugs. *Vascular Pharmacology*, 87, 139-149.
- Zhang, Y., Zhu, X., Huang, T., Chen, L., Liu, Y., Li, Q., et al. (2016). Beta-Carotene synergistically enhances the anti-tumor effect of 5-Fluorouracil on esophageal squamous cell carcinoma in vivo and in vitro. *Toxicology Letters*, 261, 49-58.
- Zhao, D. (2016). Challenges associated with elucidating the mechanisms of the hypocholesterolaemic activity of saponins. *Journal of Functional Foods*, 23, 52-65.
- Zhou, C., Lin, H., Ge, X., Niu, J., Wang, J., Wang, Y., et al. (2015). The Effects of dietary soybean isoflavones on growth, innate immune responses, hepatic antioxidant abilities and disease resistance of juvenile golden pompano *Trachinotus ovatus*. *Fish & Shellfish Immunology*, 43, 158-166.

**T5-03 Magnitude and current status of biotechnology in food processing**

Dr. Vikas S. Waskar

Professor & Head

Department of Veterinary Public Health

College of Veterinary and Animal Sciences, Udgir, Dist. Latur

*Abstract*

Biotechnology is being harnessed in various aspects of the livestock industry to hasten breed development for improved animal health and welfare, enhanced reproduction, and improved nutritional quality and safety of animal-derived foods. Biotechnology in the food processing sector makes use of micro-organisms for the preservation of food and for the production of a range of value-added products such as enzymes, flavour compounds, vitamins, microbial cultures and food ingredients. Biotechnology applications in the food-processing sector, therefore, target the selection and manipulation of micro-organisms with the objective of improving process control, product quality, safety, consistency and yield, while increasing process efficiency. The use of modern biotechnology has proved to be rapid, sensitive and accurate methods for detection and analysis of bacterial contaminants and pathogens or their toxins.

**Keywords :** Biotechnology, food processing, food safety, livestock foods

Biotechnology is the use of living systems and organisms to develop or make products, or “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use” (UN Convention on Biological Diversity). For thousands of years, mankind has used biotechnology in agriculture, food production and medicine. Biotechnology includes a wide range of diverse technologies and they may be applied in each of the different food and agriculture sectors.

Biotechnology in the food processing sector makes use of microorganisms for the preservation of food and for the production of a range of value-added products such as enzymes, flavour compounds, vitamins, microbial cultures and food ingredients. Biotechnology applications in the food-processing sector, therefore, target the selection and manipulation of microorganisms with the objective of improving process control, product quality, safety, consistency and yield, while increasing process efficiency<sup>1</sup>.

Biotechnology is also widely employed as a tool in diagnostics in order to monitor food safety, prevent and diagnose food-borne illnesses and verify the origins of foods. Techniques applied in the assurance of food safety focus on the detection and monitoring of hazards whether biological, chemical or physical. These applications have been explored and discussed in subsequent sections.

## **1. Biotechnology in food fermentation -**

Fermentation is the process of bioconversion of organic substances by microorganisms and/or enzymes (complex proteins) of microbial, plant or animal origin. Microbial cultures can be genetically improved using both traditional and molecular approaches, and improvement of bacteria, yeasts and moulds is the subject of much academic and industrial research. Traits considered for commercial food applications include sensory quality (flavour, aroma, visual appearance, texture and consistency), virus (bacteriophage) resistance in the case of dairy fermentations, and the ability to produce antimicrobial compounds (e.g. bacteriocins, hydrogen peroxide) for the inhibition of undesirable microorganisms. In many developing countries, the focus is on the degradation or inactivation of natural toxins (e.g. cyanogenic glucosides in cassava), mycotoxins (in cereal fermentations) and anti-nutritional factors (e.g. phytates)<sup>2</sup>.

Methods most commonly deployed for microbial inoculation in food fermentations include<sup>2</sup> a) Spontaneous inoculation of fermentation processes, b) “Appropriate” starter cultures as inoculants of fermentation processes, c) Defined starter cultures as inoculants of fermentation processes, d) Defined starter cultures developed using the diagnostic tools of advanced biotechnologies and e) GM starter cultures.

## **2. Biotechnology in the production of enzymes -**

Enzymes are essential in the metabolism of all living organisms and are widely applied as processing aids in the food and beverage industry. Enzymes were primarily isolated from plant and animal sources, and thus a relatively limited number of enzymes were available to the food processor at a high cost. Today, bacteria and fungi are exploited and used for the commercial production of a diversity of enzymes. Several strains of microorganisms have been selected or genetically modified to increase the efficiency with which they produce enzymes. In most cases, the modified genes are of microbial origin, although they may also come from different kingdoms. For example, the DNA coding for chymosin, an enzyme found in the stomach of calves, that causes milk to curdle during the production of cheese, has been successfully cloned into yeasts (*Kluyveromyces lactis*), bacteria (*Escherichia coli*) and moulds (*Aspergillus niger* var. *awamori*)<sup>3</sup>. Chymosin produced by these recombinant microorganisms is commercially produced and is widely used in cheese processing.

Genetic technologies have improved the efficiency with which enzymes can be produced, increased their availability, reduced their cost and improved their quality. Additionally, through protein engineering, it is possible to generate novel enzymes with modified structures that confer novel desired properties, such as improved activity or thermostability or the ability to work on a new substrate or at a higher pH.

### **3. *Biotechnology in the production of food processing aids -***

Food processing is a complex procedure involving blend of activities related to mechanical processes, structural and textural changes, rheological alterations, physical interventions, complex chemical and biochemical reactions, microbial metabolism and so on. Many food processing aids like colours, flavours, stabilizers, additives, enhancers, humectants, organic acids, amino acids, etc. are either suitably added during the processing or generated/synthesized during the fermentation process. Various types of flavouring agents, organic acids, food additives and amino acids are all metabolites of microorganisms during fermentation processes. A diversity of metabolites produced include enzymes which are capable of breaking down carbohydrates, proteins and lipids present within the substrate and/or fermentation medium; vitamins; antimicrobial compounds (e.g. bacteriocins and lysozyme); texture-forming agents (e.g. xanthan gum); amino acids; organic acids (e.g. citric acid, lactic acid) and flavour compounds (e.g. esters and aldehydes)<sup>4</sup>.

### **4. *Biotechnology for enhancing animal productivity –***

Despite great advances in agricultural productivity and economic well-being in much of the world over the past 50 years, food insecurity and poverty continue to be serious issues in many regions<sup>5</sup>. The application of in-genuine research is important in order to respond to the biological principles in manipulating living organisms pressure of producing more food from animals to cater or their derivatives to either improve or multiply a food requirement of the ever-growing human product is biotechnology in simple means.

Transgenic animals such as mice, rats, rabbits, pigs, sheep and cows have been developed with the help of biotechnology. Through transgenic technique, the manipulation of genes of one organism into genome of another organism of same or other species is carried out in such a way that the genes are not only expressed but also gets transmitted to its progeny<sup>6</sup>. Transgenic animals have been developed with aim of increasing growth rate, sustaining adaptability and improved food quality. Some breakthroughs in this technique can be elucidated as production of transgenic cows giving milk containing specific human proteins for the treatment against human emphysema; cloned transgenic cattle for increased amount of  $\alpha$  and  $\beta$  casein in milk fat and increased levels of human origin lacto-ferrin<sup>7</sup>. Similarly, transgenic cows have been developed that will give more milk or milk with less amount of lactose or cholesterol; transgenic pigs having more meat or 30% more loin mass, 10% more carcass lean tissue and 20% less total carcass fat<sup>8</sup> and transgenic sheep that yield more wool are other interventions. In the future, it will be possible to obtain information on the genetic constitution of the animals that will allow a prediction of the production potential of an animal at birth, or perhaps even as a fetus, as well as the selection of animals best suited to a specific production environment.

## 5. ***Biotechnology for quality improvisation of livestock products -***

Biotechnology as applied to food processing in most developing countries makes use of microbial inoculants to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of foods. Value added livestock products have been developed using the important aspects of biotechnology by identification, isolation and modification of useful genes and inserting them into suitable microorganisms through appropriate “vectors” to get the desirable results. For instance, the quality of carcass can be improved by manipulating the lipoprotein receptor and leptin genes thereby the cholesterol and fat content of meat can be adjusted. Certain genes have been decoded responsible for quality of meat which offer excellent opportunities for enhancing level of meat quality and decreasing variability. It is been proved by many researchers that tenderness and Pale Soft Exudative (PSE) condition in pigs are genetically governed characters. Many such decoded gene sequences are now available as gene that affects tenderness of meat before slaughter are CLPG in sheep, myostatin in beef, RN in pork in addition to RYRI gene in pork governs rate of rigor mortis and calpain:calpastatin ratio and cathepsin during storage<sup>9</sup>.

Many ethnic products have been gained entry into international cuisine and with advent of biotechnology, multiples of processing alterations have been done in these products to suit the food safety norms at par with global standards. Indonesian *tempe*, Oriental soy sauce, fermented pork sausage (*nham*), fermented fish paste – *Som Fug* and *dawadawa* (fermented locust bean) are well known examples of indigenous fermented foods that have been industrialized and marketed globally with intervention of biotechnological tools through modification of starter cultures<sup>2</sup>. The need to assure the safety and quality of these products in compliance with requirements of importing markets has been a driving force for the upgrading of starter cultures as well as for diagnostic methodologies for verification of their quality and safety.

## 6. ***Biotechnology for enhancing keeping quality of the food products –***

Food preservation has remained the focus of scientific studies since prolonged period owing to perishable nature of livestock origin food commodities. Incorporation of certain antimicrobial agents to livestock products viz bacteriocin, nisin, H<sub>2</sub>O<sub>2</sub>, etc. has yielded highly promising results of enhancing the shelf life of the products. Thus, manufactures and consumers, both are benefitted economically, nutritionally and therapeutically. It is possible to develop new strains of lactic acid bacteria capable of production of natural food grade preservative(s) and/or new strains of LAB capable of resisting to common foodborne pathogens by extending their antibacterial spectrum. Lactic acid bacteria can act antagonistically against a wide range of food pathogens and spoilage organisms like *Salmonella*, *Staphylococcus*, *Clostridium*, *L.monocytogenes*, *Y.enterocolitica* and *Psuedomonas*<sup>10</sup>. Suppression of spoilage and food borne pathogens

by lactic acid bacteria could be beneficial to human health and dairy industry as these attributes can considerably improve the shelf life and safety of fermented foods.

#### 7. **Biotechnology in food safety and quality control –**

Food safety encompasses not only the microbiological quality control but also presence of physical and chemical contaminants. Although, conventional techniques have been successfully employed for detection of food-borne pathogens and chemical residues from food products, the techniques are cumbersome, laborious and time consuming. Biotechnology plays a vital role to improve the quality of livestock products alongwith identification of organisms at the genus, species, sub-species and even strain levels, to pinpoint sources of food contamination. A selected few applications of biotechnology have been highlighted below–

Rapid detection of foodborne pathogens and emerging pathogens	PCR assay for detection of foodborne pathogens like Salmonella, <i>S.aureus</i> , <i>C.perfringens</i> , <i>L.monocytogenes</i> , <i>C.jejuni</i> , <i>C.coli</i> , <i>Y.enterocolitica</i> , Hepatitis A virus <sup>11</sup> and multiplex real-time PCR for <i>E.coli</i> O157:H7 serogroup <sup>12</sup> . rDNA technology, genetic engineering, microarray etc. Random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) molecular marker systems can also be used for the comparison of genetic differences between species, subspecies and strains <sup>4</sup>
Mycotoxin detection	ELISA kits detection of aflatoxins, deoxynivalenol, fumonisins, ochratoxins and zearalenone <sup>13</sup>
Detection and identification of foods and food ingredients	Species differentiation by amplification of characteristic DNA fragments with species-specific primers or the consensus PCR approach, by use of universal primers followed by sequencing <sup>14</sup> or restriction fragment length polymorphism (RFLP) analysis <sup>15, 16</sup> RFLP technique for identification of unique polymorphisms in food commodities for identification of their source or origin through DNA barcodes <sup>17</sup>

#### References

- Springham, D.; Springham, G.; Moses, V. and Cape, R.E. (1999). *Biotechnology: The Science and the Business*. CRC Press. ISBN 978-90-5702-407-8.
- Proceedings of FAO International Technical Conference on “Agricultural biotechnologies in developing countries: Options and opportunities in crops, forestry, livestock, fisheries and agro-industry to face the challenges of food insecurity and climate change” (ABDC-10) held at Guadalajara, Mexico during 1–4 March 2010.

- Valyasevi, R. and Rolle, R.S. (2002). An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope of their improvement. *Int. J of Food Microbiol.* **75** : 231–239.
- Berger, R.G. (2009). Biotechnology of flavours – the next generation. *Biotechnology Letters* **31** : 1651–1659.
- FAO (2008) 'Hunger on the rise' (<http://www.fao.org/newsroom/EN/news/2008/1000923/index.html>).
- Srinivasa V. and Goswami S.L. (2007). Transgenic farm animals– A mobile pharmaceutical industry. *Indian Dairyman* **59** : 26-32.
- Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'Huilier, P. and Liable, G. (2003). Cloned transgenic cattle produce milk with higher levels of b-casein and k-casein. *Nature Biotech.*, **21** : 157-162.
- Pursel, V.G., Wall, R.J., Mitchell, A.D., Elsasser, T.H., Solomon, M.B., Coleman, M.E., Mayo, F. and Schwartz, R.J. (1999). Expression of insulin-like growth factor-I in skeletal muscle of transgenic pigs. In transgenic animals in agriculture (J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin, eds). CABI Publishing, New York,
- Murthy T.R.K. (2004). Biotechnological approaches for enhancing meat quality. *Indian Food Industry* **23** : 24-26.
- Batish, V.K., Grover, S. and Neelakantan, S. (1993). Improving shelf life and safety of fermented milk products through genetically improved microorganism. *Indian Dairyman*, **45** : 51-57.
- Barrett, T., Fang, P. and Swaminathan, B. 1997. Amplification methods for detection of food-borne pathogens. In: H. Lee, S. Morse and O. Slovak, eds. *Nucleic Acid Amplification Techniques: Application to Disease Diagnosis*, pp. 171–181. Eaton Publishing. Boston, USA.
- Yoshitomi, K.J., Jinneman, K.C. & Weagant, S.D. 2003. Optimization of 3'-Minor Groove Binder-DNA probe for the rapid detection of *Escherichia coli* O157:H7 using real-time PCR. *Mol. and Cell. Probes.* **17** : 275–280.
- Schmale III, D.G. and Munkvold, G.P. 2009. Mycotoxins in crops: a threat to human and domestic animal health. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2009-0715-01.
- Chen D., Bai F., Zhou M.L., Zhang X.Y. and Wu D.J. (2008). Differentiation of *Bos grunniens*, *Bos taurus*, and *Bubalus* from meat products mixture based on mitochondrion 12S rRNA gene. *Yichuan* **30** : 1008–1014.
- Chen S.Y., Liu Y.P. and Yao Y.G. (2010). Species authentication of commercial beef jerky based on PCR-RFLP analysis of the mitochondrial 12S rRNA. *J. Genet Genomics* **37** : 763–769.
- Wang Q., Zhang X., Zhang H.Y., Zhang J., Chen G.Q., Zhao D.H., Ma H.P. and Liao W.J. (2010). Identification of 12 animal species meat by T-RFLP on the 12SrRNA gene. *Meat Sci.* **85** : 265–269.
- Teletchea, F., Maudet, C. and Hänni, C. (2005). Food and forensic molecular identification: update and challenges. *Trends Biotech.* **23** : 359–366.

## Milk protein as carrier of micronutrient (vitamin A) for milk fortification

Chitra Gupta<sup>1</sup>, Sumit Arora<sup>2</sup>, Sneha Shende<sup>1</sup>, Madhav Patil<sup>1</sup> and Prasad Patil<sup>1</sup>

<sup>1</sup>College of Dairy Technology, Warud (Pusad) and <sup>2</sup>National Dairy Research Institute, Karnal, Haryana

### Introduction

Micronutrients are nutrients required by humans and other living creatures in small quantities to orchestrate a whole range of physiological functions, which the organisms themselves cannot produce. Micronutrient malnutrition is a serious problem among children in developing countries, caused by lack of food and improper diet. Deficiency of micronutrients such as iron, iodine and vitamins leads to malnutrition (Muller and Krawinkel 2005). Vitamin and mineral deficiencies affect more than 2 billion people worldwide and are especially prevalent in developing countries including India (Ezzati *et al.* 2004). Vitamin A deficiency (VAD) affects an estimated 190 million pre-school aged children and 19 million pregnant and breastfeeding women globally. In India, more than a quarter of the world's vitamin A deficient preschool children reside who suffer from subclinical VAD and one-third of the preschool children show clinical signs and symptoms of VAD (WHO 2009).

### Milk protein as carrier of micronutrients

Milk proteins have important functional properties such as the ability to bind hydrophobic molecules, interact with other biopolymers, stabilize emulsions, form gels and to some extent, retard oxidation. Due to these properties, milk proteins are ideal materials for the entrapment and delivery of micronutrients and bioactive compounds (Livney 2010). Binding of vitamin with various milk proteins reduce vitamin degradation during heat treatments, high pressure processing and UV-light induced degradation process (Saiz-Abajo *et al.* 2013).

Belatik *et al.* (2012) and Bourassa *et al.* (2013) reported the complexation of retinol and retinoic acid with bovine serum albumin (BSA),  $\alpha$ - and  $\beta$ -CN. They analysed retinol and retinoic acid binding sites, binding constant and the effect of retinoid complexation on the stability and conformation of BSA,  $\alpha$ - and  $\beta$ -CN using FTIR, UV-visible and fluorescence spectroscopic methods. Structural analysis showed that retinoids bind BSA,  $\alpha$ - and  $\beta$ -CN via both hydrophilic and hydrophobic contacts with overall binding constants of  $K_{\text{ret-BSA}} = 5.3 (\pm 0.8) \times 10^6 \text{ M}^{-1}$ ,  $K_{\text{retac-BSA}} = 2.3 (\pm 0.4) \times 10^6 \text{ M}^{-1}$ ,  $K_{\text{ret-}\alpha\text{-CN}} = 1.21 (\pm 0.4) \times 10^5 \text{ M}^{-1}$ ,  $K_{\text{ret-}\beta\text{-CN}} = 1.11 (\pm 0.5) \times 10^5 \text{ M}^{-1}$ ,  $K_{\text{retac-}\alpha\text{-CN}} = 6.2 (\pm 0.6) \times 10^4 \text{ M}^{-1}$  and  $K_{\text{retac-}\beta\text{-CN}} = 6.3 (\pm 0.6) \times 10^4 \text{ M}^{-1}$ . The number of bound retinol molecules per protein (n) was 0.8 ( $\pm 0.2$ ) for BSA, 1.5 ( $\pm 0.1$ ) for  $\alpha$ -CN and 1.0 ( $\pm 0.1$ ) for  $\beta$ -CN, while 0.5, 1 and 1 molecule of retinoic acid was bound to BSA,  $\alpha$ - and  $\beta$ -CN, respectively. This complexation induced only minor alterations to protein conformation.

Forrest *et al.* (2005) studied the interactions of vitamin D<sub>3</sub> with  $\alpha$ -lg A and  $\alpha$ -CN under various environmental conditions (i.e., pH and ionic strength) using fluorescence and circular dichroism spectroscopic techniques. It was observed that apparent dissociation constant was 0.02 to 0.29  $\mu$ M and 0.06 to 0.26  $\mu$ M and apparent mole ratios 0.51-2.04 and 1.16-2.05 (mol of vitamin D<sub>3</sub> bound per mole of protein) for  $\alpha$ -lg A and  $\alpha$ -CN, respectively. According to this study interactions may strongly influence vitamin D<sub>3</sub> stability and, hence, bioavailability in processed dairy products. Semo *et al.* (2007) studied the incorporation of vitamin D<sub>2</sub> in re-assembled casein micelles and observed that this binding improved the stability of vitamin against UV-light induced degradation without affecting the size and morphology of casein micelles. These complexes can be used as nano vehicles for entrapment, protection and delivery of sensitive hydrophobic nutraceuticals within food products. Diarrassouba *et al.* (2014) also studied the stability of  $\alpha$ -lg-vitamin D<sub>3</sub> complex at 4°C, upon exposure to UV light and in simulated intestinal conditions using Caco-2 cells. Binding of  $\alpha$ -lg to vitamin D<sub>3</sub> significantly improved the vitamin stability at 4°C and upon exposure to UV light. Complexation of  $\alpha$ -lg to vitamin D<sub>3</sub> also improved the crossing of vitamin D<sub>3</sub> across monolayer, hence its bioavailability.

Liang and Subirade (2010) studied the binding of folic acid to  $\alpha$ -lg and observed it to be bound on the surface of  $\alpha$ -lg possibly in the groove between the  $\alpha$ -helix and the  $\alpha$ -barrel. Complexation of folic acid to  $\alpha$ -lg improves its photostability. Zhang *et al.* (2014) also studied the interaction of  $\alpha$ -CN with folic acid using fluorescence, absorption spectroscopy and circular dichroism. It was found that folic acid bound to  $\alpha$ -CN by hydrophobic contacts with a dissociation constant of  $\sim 10^{-5}$  M. It was also observed that binding to  $\alpha$ -CN inhibits the photodecomposition of folic acid. Perez *et al.* (2014a) studied the formation of nano-complexes with folic acid and  $\alpha$ -lg and their impact on in-vitro gastro-duodenal proteolysis. Colloidal stability tests ( $3 < \text{pH} < 10$ ) revealed that nano-complex formation improved  $\alpha$ -lg dissolution near its iso electric point and at low pH values. SDS-PAGE analysis of digesta collected from gastric and duodenal in-vitro digestion of  $\alpha$ -lg and its nano-complexes, revealed no marked alterations in the proteolytic susceptibility of  $\alpha$ -lg. These nano-complexes can be used for the delivery of folic acid in clear beverages with minimal effects to the protein's sensitivity to proteolysis.

Liang *et al.* (2011) studied the complexation of  $\alpha$ -lg with  $\alpha$ -tocopherol and evaluated its impact on  $\alpha$ -tocopherol stability. This interaction did not disrupt the secondary & tertiary structures of  $\alpha$ -lg. Complex formation improved the solubility as well as stability of  $\alpha$ -tocopherol. Sáiz-Abajo *et al.* (2013) studied the effect of different processing treatments on stability of  $\alpha$ -carotene in re-assembled casein micelles. It was observed that binding of  $\alpha$ -carotene in re-assembled casein micelles improves its stability towards sterilisation, pasteurisation, high hydrostatic pressure and baking.

Belatik *et al.* (2012) and Bourassa *et al.* (2013) studied the binding of retinol and retinoic acid with BSA, human serum albumin (HSA),  $\alpha$ - and  $\beta$ -casein. In retinol-BSA complexes, retinol is surrounded

by \*Arg-209 (2.50 Å = H-bond), Leu-162 (2.38 and 2.42 Å), Phe-150 (1.89 Å), Phe-157 (2.14 and 2.42 Å), Trp-134 (2.52 and 2.39 Å) and Tyr-161 (1.81 Å) with the binding energy of 13.10 kcal/mol. Similarly, retinoic acid is surrounded by \*Arg-209 (2.53 Å = H-bond), Ile-165 (2.10 Å), Leu-162 (2.46 and 2.31 Å), Phe-150 (2.16 Å), Phe-157 (1.99 Å), Trp-134 (2.55 Å) and Tyr-161 (2.14 Å) with the free binding energy of 12.90 kcal/mol.

Reports are also available regarding the binding of various tea polyphenols (catechin (C), epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG)), curcumin and polyunsaturated fatty acid to  $\alpha$ -,  $\beta$ -CN,  $\beta$ -lg and re-assembled casein micelles (Hasni *et al.* 2011; Perez *et al.* 2014b; Staszewski *et al.* 2014).

### **Vitamin A deficiency**

VAD is the second largest cause of global blindness next to cataracts. The major cause of VAD is inadequate dietary intake of the preformed retinol or precursors of vitamin A. Increased vitamin A requirement in certain physiological or pathological conditions, inadequate absorption or loss of intestinal contents in diarrhea are often contributory factors in establishing VAD. An estimated 250 million preschool children are vitamin A deficient and this deficiency is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections (Allen *et al.* 2006). Clinical symptoms of VAD such as night blindness are seen among women of reproductive age and in pregnant women (Gragnolati *et al.* 2005).

### **Fortification of milk with vitamin A**

Government of India is committed to tackle VAD through milk fortification. Food Safety and Standards Authority of India (FSSAI) approved the fortification of toned/double toned/skimmed milk with vitamin A. Fortified milk should contain minimum 770 IU of vitamin A per liter of milk (FSSR, 2016 draft standards). Milk is an oil-in-water emulsion, hence, possibility exists for addition of vitamins in their oily form or use water dispersible forms. Ease of mixing has been identified as an advantage of using dry, water dispersible forms of the fortificant. However, the disadvantage of this is that vitamins are less stable in this form after addition to milk as the protective coating dissolves leaving the vitamin susceptible to degradation (O'Brien and Robertson 1993). Addition of oily vitamin preparations is recommended after dilution and pre-homogenisation with a suitable quantity of milk. These oily vitamin preparations also have a tendency to adhere on the walls of containers and pipelines. Binding of vitamin A to milk protein improves the stability and solubility of vitamin A.

## Conclusion

Micronutrient malnutrition is a serious threat to the health and productivity of billions of people worldwide. Among micronutrients, vitamin A deficiency (VAD) is the second largest cause of global blindness next to cataracts. Binding of milk protein to micronutrient improves stability of the micronutrients. Fortification of milk with milk protein-Vit A complexes can be method for fortification milk with vit.A.

## References

- Allen, L., Benoist, B. D., Dary, O. & Hurrell, R. (2006). Guidelines on food fortification with micronutrients. World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO).
- Belatik, A., Hotchandani, S., Bariyanga, J. & Tajmir-Riahi, H. A. (2012). Binding sites of retinol and retinoic acid with serum albumins. *European Journal of Medicinal Chemistry*, 48, 114-123.
- Bourassa, P., N'soukpoé-Kossi, C. N. & Tajmir-Riahi, H. A. (2013). Binding of vitamin A with milk  $\alpha$ - and  $\beta$ -caseins. *Food Chemistry*, 138, 444-453.
- Diarrassouba, F., Garrait, G., Remondetto, G., Alvarez, P., Beyssac, E. & Subirade, M. (2014). Increased stability and protease resistance of the  $\beta$ -lactoglobulin/vitamin D<sub>3</sub> complex. *Food Chemistry*, 145, 646-652.
- Ezzati, M., Lopez, A. D., Rodgers, A. & Murray, C. J. (2004). Comparative quantification of health risks: The global and regional burden of disease attributable to selected major risk factors. World Health organization. Geneva, Switzerland.
- Forrest, S. A., Yada, R. Y. & Rousseau, D. R. (2005). Interactions of vitamin D<sub>3</sub> with bovine  $\beta$ -lactoglobulin A and  $\beta$ -Casein. *Journal of Agricultural and Food Chemistry*, 53, 8003-8009.
- FSSR (2016). Food Safety and Standards (Fortification of Foods) Regulations (draft standards). Food Safety and Standards Authority of India. Ministry of Health and Family Welfare, Government of India, New Delhi, India.
- Gragnolati, M., Shekar, M., Das Gupta, M., Bredenkamp, C. & Lee, Y. K. (2005). India's undernourished children. A call for reform and action, World Bank Report.
- Hasni, I., Bourassa, P., Hamdani, S., Samson, G., Carpentier, R. & Tajmir-Riahi, H. A. (2011). Interaction of milk  $\alpha$ - and  $\beta$ -caseins with tea polyphenols. *Food Chemistry*, 126, 630-639.
- Liang, L. & Subirade, M. (2010).  $\beta$ -Lactoglobulin/Folic Acid Complexes: Formation, Characterization, and Biological Implication. *Journal of Physical Chemistry B*, 114(19), 6707-6712.
- Liang, L., Tremblay-Hébert, V. & Subirade, M. (2011). Characterisation of the  $\beta$ -lactoglobulin/ $\alpha$ -tocopherol complex and its impact on  $\alpha$ -tocopherol stability. *Food Chemistry*, 126, 821-826.
- Livney, Y. D. (2010). Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Sciences*, 15(1-2), 73-83.

- Muller, O. & Krawinkel, M. (2005). Malnutrition and health in developing countries. *Canadian Medical Association Journal*, 173(3), 279-286.
- O'Brien, A. & Robertson, D. (1993). Vitamin Fortification of Foods (Specific applications). In: Ottaway, P. B., Efitor. *The Technology of Vitamins in Food*. (1<sup>st</sup> ed.). Blackie Academic & Professional, London. pp. 114-142.
- Perez, A. A., Andermatten, R. B., Rubiolo, A. C. & Santiago, L. G. (2014b).  $\alpha$ -lactoglobulin heat-induced aggregates as carriers of polyunsaturated fatty acids. *Food Chemistry*, 158, 66–72
- Perez, O. E., David-Birman, T., Kesselman, E., Levi-Tal, S. & Lesmes, U. (2014a). Milk protein-vitamin interactions: Formation of beta-lactoglobulin/folic acid nano-complexes and their impact on in vitro gastro-duodenal proteolysis. *Food Hydrocolloids*, 38, 40-47.
- Saiz-Abajo, M. J., González-Ferrero, C., Moreno-Ruiz, A., Romo-Hualde, A. & González-Navarro, C. J. (2013). Thermal protection of  $\alpha$ -carotene in re-assembled casein micelles during different processing technologies applied in food industry. *Food Chemistry*, 138(2), 1581-1587.
- Semo, E., Kesselman, E., Danino, D. & Livney, Y. D. (2007). Casein micelle as a natural nano-capsular vehicle for nutraceuticals. *Food Hydrocolloids*, 21, 936–942.
- WHO (2009). Global prevalence of vitamin A deficiency in populations at risk 1995-2005. Global database on vitamin A deficiency. World Health Organization. Geneva, Switzerland.
- Zhang, J., Liu, Y., Liu, X., Li, Y., Yin, X., Subirade, M., Zhou, P. & Liang, L. (2014). The folic acid/ $\alpha$ -casein complex: Characteristics and physicochemical implications. *Food Research International*, 57, 162–167.

**AB-15** **Efficacy of Herbal Liver Tonic (Superliv.Conc.)in Improving Growth Performance, Nutrient Utilization and Carcass Traits in Broiler Chicken**

M.V. Dhumal\*, M G Nikam<sup>1</sup> and P V Nandedkar<sup>2</sup>

\*Professor, Department of Poultry Science College of Veterinary and Animal Science, Parbhani, Maharashtra Animal and Fishery University Nagpur (M.S.) India

Corresponding author: dhumalmv@gmail.com

**ABSTRACT**

The investigation was carried out to evaluate the efficacy of herbal liver tonic in improving growth performance, carcass qualities, nutrient utilization and economics in broilers. The experiment was carried out on commercial on 375 days old Vencobb400 broiler straight run chicks. The chicks were weighed and distributed randomly into three treatment groups viz, A, B, and C with five replicates of 25 chicks each. For treatment group A ration was prepared as per BIS (2007) and for treatment group B ration was formulated with reduced energy (2.5%) with supplementation of Superliv @ 500gm/ton. Similarly, for treatment group C ration was formulated with reduced energy (5%) with supplementation of Superliv. @ 500gm/ton. The cumulative weight gain, feed consumption, FCR and mortality for different treatments groups were non-significant. The non-significant differences among the treatment groups observed for edible carcass yield, breast yield, heart, liver, gizzard, giblet weight. The significant decreased values for SGOT and SGPT in Superliv con. supplemented groups at 42nd day was observed. Significant increased values for total serum protein and globulin at 42nd day for Superliv. Con. supplemented groups were noticed, however, the values for albumin for different groups were non-significant. Significant improvement in the percentmetabolizable energy and protein retention values in 2.5% energy reduction and supplementation of Superliv con. were recorded. An improvement in the percentage (%) retention of phosphorus was noticed in herbal liver tonic(Superliv concentrate) supplemented groups. An increase in profitability might be due to lower feed cost, thereby reduced cost of production and higher body weight. It was concluded that Superliv @ 500 gm/tonne of feed is used efficiently for compensating the performance of broiler and beneficial for optimum feed utilization without any adverse effect on health

**AB-16**

## **HOLISTIC APPROACH TO RESOLVE CURRENT ISSUES AND FUTURE THREATS IN POULTRY INDUSTRY THROUGH BIOTECHNOLOGY**

V R Kulkarni,

VRK NUTRITIONAL SOLUTIONS,Pune

Livestock production can fulfil not only food requirements of Indian population but also have potential to provide quality food. Green revolution and white revolution have done wonders and contributed to India's development, however some upcoming issues have to be tackled for further quality food security for healthy life. In this context, veterinarians in general and BIOTECHNOLOGIST in specific have to play a bigger role. All future health issues will be solved and or treated only through food. In achieving this passionate biotechnological research and panchant innovations have to play important role.

Immunosuppression due to stress right from chick transportation and handling environmental distress, vaccination stress to anti-nutritional factors in feed is impacting negatively. Gut health being challenged due to very innovative new technologies like Pelletting vertical and hatchery contaminations, Quality and quantity of water metabolic disorders due to high quality nutrition for faster growth, litter and bedding material contamination are few things mentioned here

Respiratory problems associated with drastic environmental changes, Ammonia load in housing sheds, housing density, and vaccination stress are being always challenged leading to number of respiratory diseases like CRD, ILT, ND, VVND, etc. Increasing input costs and volatile finished product prices have really put our industry into dilemma

Bio-methane production from poultry litter have not only solved the pollution and social issues but also added revenue to the farmers by producing green energy from waste

Further, we could found out protein factors required in definite quantity to boost immunity, our early nutrition programme successfully not only developed visceral organs early but also improved performance beyond imagination. Economical and feasible functional fibres introduced by us are not only solving issues of gut health but also proved the solution for AGPBAN. Last and most important, our study and discovery is our conditioned bedding material with acids and probiotic have given answer to respiratory issues, animal welfare and health conscious future generation and also it can prove much better solution to cage ban with cleaner and healthy chicken and eggs and off course tranquil enjoyment to birds.

**AB-17**

**Optimisation of analytical conditions for estimation of unbound vitamin A in milk-protein Vitamin A complexes prepared for milk fortification**

Chitra Gupta 1 , Sumit Arora 2 & Madhav Patil 1

1 College of Dairy technology, Warud (Pusad) & 2 National Dairy Research Institute,  
Karnal, Haryana, India, 132001  
email: chitragupta97@gmail.com

**Abstract**

The recent trends for consumption of low fat and fat free foods have led to an increase in deficiencies of vitamin A. It is susceptible to light and heat and thus require stabilization in aqueous medium. Stability can be improved by binding of vitamin A to milk protein. Native, reassembled and succinylated milk proteins were used for the preparation of milk protein- Vitamin A (Vit A) complexes. Different methods were evaluated for separation of unbound vitamin A from milk protein- Vit A complexes. It was hypothesized that vitamin bound to the protein will precipitate along with the milk proteins and the unbound vitamin will remain in the solution, hence, different methods of protein precipitation were evaluated for analyzing unbound vitamin A. Centrifugation and ultrafiltration techniques were utilized for estimation of unbound vitamin A. Protein was precipitated by isoelectric precipitation and also by ammonium sulphate precipitation. TCA precipitation and careez precipitation disrupt the structure of protein or may affect the binding of vitamin A to milk protein, therefore these were not tried for the estimation of unbound vitamin A in the sample. Among all the methods, protein precipitation with ammonium sulphate gave most suitable results for estimation of vitamin A in milk protein-Vit A complexes.

**AB-18**

**Zinc enriched Lactic Acid Bacteria: A Novel Approach for Zinc Supplementation**

V. K. Lule\*, S. P. Changade, P. D. Sawale & A. R. Sarode

Affiliations: College of Dairy Technology, Warud (Pusad), MAFSU-Nagpur

Email: vaibhao\_only@rediffmail.com

Email id: spchangade@gmail.com, addtcwarud@gmail.com

Zinc (Zn) is one of the essential metal ions to life. After iron, it is the second most abundant transition metal ion in the living organisms, including humans. Though an essential metal ion, it is toxic at higher concentrations, yet the toxicity of zinc has been found to be quite low and rare as compared to other metals. Severe zinc deficiency causes a number of adverse physiological consequences on the epidermal,

gastrointestinal, central nervous, immune, skeletal, and reproductive systems. It has been demonstrated that the form of the trace elements affects the intake efficiency in animals. Several studies have reported that certain organic compounds of trace elements (including iron, zinc, magnesium, and selenium) are more bioavailable than the inorganic forms, possibly because the mechanisms for absorption have been adapted to these kinds of nutrients during species evolution. The deficiency of zinc in humans due to nutritional factors and several disease states has been well recognized. The high phytate content of cereal proteins is known to decrease the availability of zinc, thus the prevalence of zinc deficiency is likely to be high in a population consuming large quantities of cereal proteins. Alcoholism, malabsorption, sickle cell anemia, chronic renal disease, and chronically debilitating diseases are also known to be predisposing factors for zinc deficiency. Supplementation of Zn-deficient groups with Zn-enriched LAB may be a new promising application of LAB in addition to their techno-functional and probiotic properties. Delivering Zn in a highly bioavailable form through a Zn bioenriched fermented dairy food/food supplement using gut friendly microbial cell factories could be a novel nutritional intervention at “Man” level in “One Health Mission” (Soil-Plant- Animal-Man). As a functional validation there is need of further research to assemble much information on mechanistic inside of bioavailability of Zn-enriched LAB in the host system using advanced molecular and proteomic tools.

**AB-19**

**Effect of supplementing chelated Zn, Cu and Mn on growth performance of Murrah buffalo calves**

Vishal Mudgal\*, N. Saxena, K. Kumar, S. S. Dahiya, B. S. Punia and M. L. Sharma  
Division of Animal Nutrition and Feed Technology  
ICAR – Central Institute for Research on Buffaloes, Hisar 125 001, Haryana, India

Feeds commonly used for dairy animals ration needs to be supplement regularly by mineral supplements to fulfil their daily mineral requirement for different physiological purposes. Availability of different minerals supplied through mineral mixture depends on their sources. It has been observed that availability of minerals is very less when supplemented through their inorganic source, which remains a cause of large amount of minerals get excreted through faeces and remains a cause of environment pollution. Organic sources of minerals had been considered for their better bioavailability then routinely used inorganic sources. In search of the same twenty four male Murrah buffalo (*Bubalus bubalis*) calves ( $318.54 \pm 8.85$  kg body weight) were divided equally into four groups and fed either a control diet where Zn, Cu and Mn were supplemented through inorganic source or supplemented these minerals with organic sources @ 50, 75 or 100% levels as supplemented in control group. Experimental feeding was continued for a period of 180 days with recording of body weights at day zero and followed by fortnightly intervals. At the end of

feeding trial body weights of four groups were 416.28, 413.64, 421.57 and 416.16 kg, respectively, which indicated that replacement of inorganic trace minerals with organic one did not adversely affect the growth performance of buffalo calves with reduction of amount of these minerals up to 50% level as compared to its inorganic counterpart. On the basis of above study it may be concluded that, no difference in the growth performance ( $P>0.05$ ) of buffalo calves in a long term study, with reduction of the level of supplementation (up to 50%) indicated higher bioavailability of these minerals when supplemented through their organic sources, which definitely will be environment friendly too.

**T6**

**Application Of Veterinary  
Pharmaceuticals And Drug  
Delivery**

T6-01

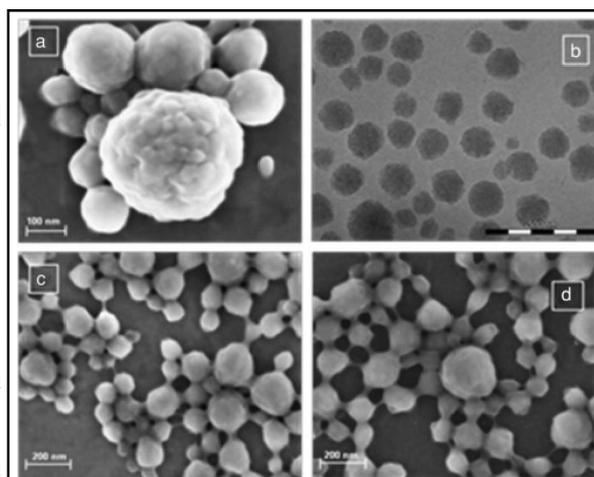
## Recent Advances in Nano-technology Based Novel Drug Delivery Veterinary Drugs

Dr. Abdul Samad\*, Isha Naik\*\*, Kaveri Kalola\*\* and Dr. Mala D. Menon\*\*

\*Research and Development Consultant and Ex-Dean and Director MAFSU; \*\*Bombay College of Pharmacy, Kalina, Mumbai (address email- drasamad11@gmail.com)

**I- Advances in Nanotechnology based veterinary drugs:** Animal infections are ubiquitous and difficult to treat. Many diseases, especially the one having chronic course are also communicable to human hence of public health importance. Conventionally the approach has been to reformulate the drugs developed in medical research for animal use. Such drugs when administered gets distributed throughout the body. In situations where the infections is multi-organ in spread such a strategy is fruitful but where infection is localized or a particular organ is the hot spot for multiplication and spread, conventional approach is not ideal. During last two decades scientists have focussed on developing novel ways to deliver drugs, vaccine to targeted organs. One of such approaches is nanotechnology wherein drug is internalized into nanosizecarrier particles and depending on the size, structure and ligand these could be delivered to desired site or organ. Advantage is that the nanoparticles can mimic or alter biological processes especially the bio-distribution and destination of biomolecules such as drug and vaccines, DNA parts and DNA scaffolds. Targeted drug delivery to organ on interest afflicted primarily with infection or the disease is an area of immense interest to veterinary medicine since this will obviate problems of residues in products and reduction of dose and frequency of administration. Thus as against conventional drugs targeted drugs have several critical advantages, especially in veterinary sciences.

**Nano-particulate carriers:** The general principle of the targeted drug delivery is that the drug is encapsulated in micro- or nanoparticles (NPs) made from biodegradable polymers. The chemical and physical nature of the nanoparticles decide its targeting destination and bio-distribution behaviour. Polymeric nanoparticles made from natural and synthetic polymers have received considerable attention as these have been reported to be stable and amenable to surface and shape modifications. Because of these characteristics NPs can be tailor-made to achieve controlled drug release and disease-specific localization. It is now proved that NPs can become concentrated preferentially in tumours, inflammatory sites, intracellular, and at antigen



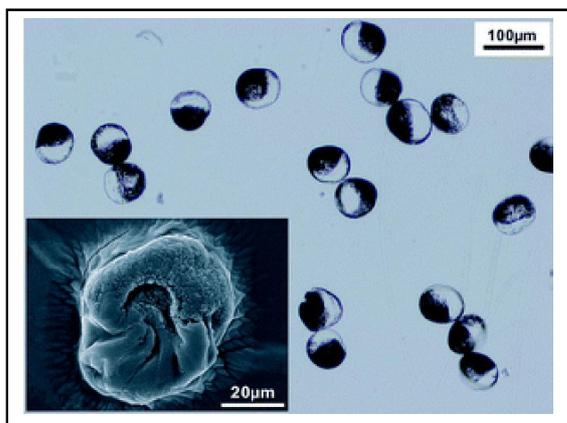
processing sites. Another advantage of biodegradable polymeric nanoparticles is that once accumulated at the site it can become like a local drug depot providing continuous supply of encapsulated therapeutic compounds.

During development stages many potential molecules are abandoned due to sub-optimal intestinal absorption, unacceptable kinetics and toxicity and safety studies. Quite a few are not pursued because of rapid metabolic degradation by biological enzymes before these could reach the destination. Even for such kind of molecules nanotechnology can be harnessed to overcome these deficiencies. For example Amphotericin B when given parentally has been found to have toxic side effects. But when the same drug is encapsulated in lipid NPs (such as liposomes) the toxicity reduces considerably.

**Intracellular Animal Infections:** A large number of infections in domestic animals are intracellular, especially cells of reticuloendothelial system. These cells in general do not have efficient drug uptake mechanism hence only few drugs are able to permeate and accumulate in therapeutic concentrations. Many microbes such as, mycobacteria, brucella, listeria, staphylococci, salmonella, rhodococci, theilaria, anaplasma, rickettsia, reside inside the cell away from any immune attack. Few organisms like mycobacteria, brucella and listeria reside in the phagosome preventing its coalescing with lysosomal enzymes. Delivering drugs to such sites is a big challenge as the molecule after reaching to the infection site has to pass through lipid bilayer, cytoplasm and phagosomal membrane, unharmed. It is also known that organelle such as phagosome have limited drug uptake mechanisms. For these reasons infections caused by these microbes are chronic, difficult to treat and eliminate hence treatment attempts, if any, are prolonged. Nano-technology based carrier systems can address such issues since nano-carriers can be designed to deliver drugs inside macrophage phagosome. Thus, the advantages of using nanoparticles for drug delivery are a result of two main basic properties: small size and use of biodegradable materials. Many studies have demonstrated that nanoparticles are advantageous over microparticles (>1  $\mu\text{m}$ ) because the later can't be given intravenous. The smallest capillaries in the body are 5–6  $\mu\text{m}$  in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5  $\mu\text{m}$ , without forming aggregates, to ensure that the particles do not cause an embolism.

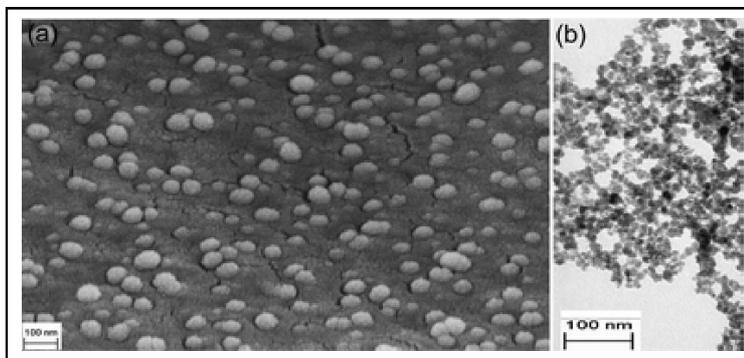
The use of biodegradable materials for nanoparticle preparation allows for sustained drug release within the target site over a period of days or even weeks. There are various types of carriers employed in targeted drug delivery system.

**Hydrogels:** Hydrogel-nanoparticles are based on technology that uses hydrophobic polysaccharides for encapsulation and delivery of drugs and vaccine antigens. For example, in one system four cholesterol molecules aggregate to form a self-aggregating hydrophobic core with pullulan outside and the resulting nanoparticles stabilize entrapped proteins. These particles stimulate the immune system and are readily



taken up by dendritic cells. Curcumin, a substance found in turmeric is known to have anti-cancer properties but its clinical application has been limited due to its poor solubility and minimal systemic bioavailability. But when curcumin is encapsulated in a polymeric nanoparticle creating curcumin-NP which provides an opportunity to expand the clinical repertoire of this efficacious agent by enabling soluble dispersion.

**Micelles and liposomes:** These are micelles / spherical super-molecular assemblies of amphiphilic copolymer. The core of micelles accommodates hydrophobic drugs, and the shell is a hydrophilic brush-like corona that makes the micelle water soluble, thereby allowing delivery of the poorly



soluble contents. Liposomes has been used in solubilizing hydrophobic or poorly soluble drugs. Another class of liposomes have been produced by treating with PEG to create stealth micelle formulations which minimize opsonization of micelles and thus maximize serum half-life. Such drugs are also called long-circulating NP formulations and can be used where infections are in blood and drug is not intended to go intra- or intercellular. Examples doxorubicin (DOX)-encapsulated pluronic micelles from a copolymer of PEG-DOX-conjugated poly(aspartic acid), and paclitaxel-encapsulated PEG-PLA micelle formulation, both used in treating cancer in human and dogs.

The Institute of Chemical Technology, Mumbai in collaboration with Bombay Veterinary College Veterinary Nuclear Medicine Centre has been able to focus on use of nanotechnology based targeted drug delivery system to develop novel formulations for important animal infections.

**NP-buparvaquone:** This is the only drug available to treat *T. annulata* infection in crossbred cattle. The problem with the current formulation is that it does not eliminate infection, dose is high hence there are side effects and many treated animals turn carrier for life which perform sub-optimally. In many cases relapse is common. The novel formulation is polymeric NP developed using a simple but ingenious technology developed at Institute of Chemical Technology. This technology is called in situ nano-technology.

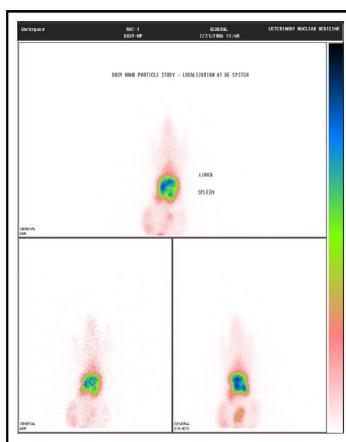


Fig. Scintigraphy evidence of bio-distribution of rifampicin in RE system

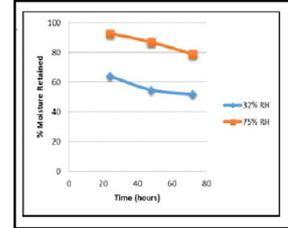
The difference here is that the available formulation is pre-concentrate and once this is mixed with dextrose 5% it instantly forms uniform NPs of spherical shape of size 400 – 500 nm. Thus the manufacturing process is simple for easy commercialization. In vitro studies have shown that the NPs can kill the parasitic stages intracellular. Scintigraphy studies have shown that when given intravenous drug is targeted to reticuloendothelial cells predominantly. Because of this the dose of drug has reduced to 0.5 mg per kg as against 2.5 mg per kg with nascent drug. Clinical trials have proved that the NP-buparvaquone is highly effective as single injection in eliminating infection. The NP drug has also been found to be effective in eradicating sub-clinical infection, much more common than clinical syndrome.

**NP-rifampicin:** The formulation has been developed employing in situ technology. The nano-particulate drug is used in first steps, the first being adding rifampicin and a polymer and then adding this to 5% dextrose to form in situ nano-particulate rifampicin. The formulation has been designed in such a way that a definite proportion of rifampicin is in free non-encapsulated form. Such a hybrid formulation has been found to be useful in treating intracellular and transient extracellular *Brucella*. This formulation has been developed to treat brucellosis in domestic animals. The drug is now ready for multicentre clinical trials in goat and cows.

**Nano-silver Based in situ Bandage For Animal Wounds** This is the first crafted formulation for treating wounds in domestic animals. The basic principle is that animal wounds tend to be more exposed to environmental infections and moisture evaporation. This results in delayed healing. Another complication is maggots due to laying of eggs by flies. We therefore sought to develop a novel formulation which would promote a barrier film covering entire wound but releasing trapped moisture in a programmed way. The novel formulation is available in solution form as spray. The formulation consists of two sprays to be used one after the other. The first is a disinfectant cleaning spray that would take care of existing infections whereas the second spray containing nano-silver rapidly forms a barrier film which is hydrophobic and does not permit wound contamination with bacteria or maggots. Silver nanoparticles have been interlinked chemically which is released in a controlled way over days. The *in situ* film forming solutions were found to be odourless, opaque, greyish green in colour and free of any bubbles or fibres having pH 7.4 and viscosity of 740 cps. About 0.11ml of volume of formulation was required per area of wound. The time for film formation was 1- 2 minutes. SEM images showed homogenous distribution of AgNPs in the film formed which has a tensile strength  $2.309 \pm 0.050$  g/cm<sup>2</sup>. Other critical parameters values studied were folding endurance, silver content assay and percent swelling which were  $294 \pm 38$ ,  $88.1 \pm 1.64$  % and

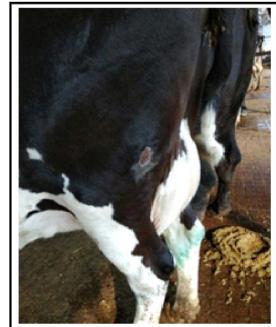
112.37 %, respectively. The barrier film in wet and dry condition was able to retain around 85% of moisture for 3 days. The films showed good antimicrobial activity with zone of inhibition of more than 3mm for 3 days. Limited clinical trials in field revealed that even under field conditions the wound after spray application remained moist which resulted in fast healing. In most of the cases only one spray application was found to be sufficient to stimulate healing within 5-7 days (please see the photographs).

In conclusion, nanotechnology and targeted drug delivery system would be the future way of developing novel drug for animals.



**Initial**

**One week after application of the formulation (No reapplication required)**



T6-02

## Nano Particles : Green synthesis, characterisation and its use in Drug delivery system : A Review

Sudhir R Rajurkar

Professor,

Department of Vet. Pharmacology & Toxicology, COVAS, Parbhani

### INTRODUCTION :

The term “nano” is derived from the Greek word “nanos” which means small and it is used as the prefix for one billionth parts ( $10^{-9}$ ). According to American Society for Testing and Materials (ASTM international 2006), nanoparticles are those particles which have two or more than two dimensions and are in the size range of 1 – 100 nm (Alanazi *et al.*, 2010). These particles have special and enhanced physical and chemical properties as compared to their bulk materials due to their large reactive and exposed surface area and quantum size effect as a result of specific electronic structures. These particles have been widely used in many fields such as electronics, photochemical, biomedicine and chemistry (Di Guglielmo *et al.*, 2010).

Metallic nanoparticles and combination of Metallic nanoparticles with organic materials to form hybrids possess unique physical, chemical, optical and electrical properties which make them different and more applicable than large size metals. Nanoparticles are proved to be promising multi-directional utilities because they can be used for imaging and many therapeutic functions like anti diabetic, anti-cancer therapy and effective drug delivery system. These nanoparticles can be synthesized from different organic, inorganic or hybrid of organic and inorganic materials but among all these, inorganic (Metallic) nanoparticles are of most important for diagnosis and simultaneous therapy (Theranostics) due to their easy modification, stability and high drug loading capacity (Kim and Jon, 2011).

Nanoparticles have wide applications in the field of biomedicine such as to deliver pharmaceuticals, for diagnostic approaches as well as for the therapeutic purposes because nanoparticles have very small sized particles, they can be used for targeted drug delivery and the metallic nanoparticles respond resonantly to the magnetic field which varies with time so they transfer enough toxic thermal energy to the tumor cells as hyperthermic agents (Khlebtsov and Dykman, 2010).

Production of nanoparticles can be achieved through mainly three methods such as, Chemical, Physical and Biological methods. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmental friendly processes of nanoparticles synthesis that should be nontoxic. Recently, a study has been demonstrated synthesis of gold nano-triangles and silver NPs using *Aloe Vera* plant extracts (Chandran

*et al.*, 2006). Already some works have been reported on synthesis of gold nanoparticles by *Azadirachta indica* (Thirumurugan *et al.*, 2010).

Gold nanoparticles have extremely small size and high surface area hence their surfaces has been available for further modification with hydrophobic, hydrophilic, cationic, anionic or any neutral moieties to the surrounding environment so they have many application in biological sciences. Surface modification of the nano materials have strong effect on the interaction of these nano materials with cells.

### **Medicinal uses of gold nanoparticles A review :**

Finkelstein *et al.* (1976) reported Bulk gold is well known to be “safe” and chemically inert, and gold based compounds have been used in the clinic as anti-inflammatory agents to treat rheumatoid arthritis (Auranofin<sup>®</sup> and Tauredon<sup>®</sup>).

Antonovych (1981) reviewed that the use of gold for medicinal purposes dates back to 2500 BC to the ancient Chinese and Egyptians.

Metz *et al.* (1982) used radioactive gold micro-particles effectively in local radioisotope cancer therapy.

Pricker (1996) reviewed that in medieval Europe, numerous recipes for gold elixirs existed and in the 17<sup>th</sup> and 19<sup>th</sup> century gold was used to treat fevers and syphilis, respectively.

Armendariz *et al.* (2002) reviewed that the use of gold nanoparticles dates back to 16<sup>th</sup> century, for both medical and staining purposes. Thereafter, gold nanoparticles have found application in analytical methods such as colorimetric techniques for the determination of heavy metal ions in aqueous solutions.

Mukherjee *et al.* (2005) reported that the inhibitory activity of gold nanoparticles against VPF/VEGF165 induced proliferation of endothelial cells provides clear evidence over their therapeutic potential in the treatment of diseases like chronic inflammation, pathological neo-vascularisation, rheumatoid arthritis, and neoplastic disorders.

Tsai *et al.* (2007) reported that an intra-articular administration of 180- $\mu$ g/mL 13-nm gold nanoparticles (AuNPs) ameliorates the course of Collagen induced arthritis in rats.

Christopher *et al.* (2007) studies on nanosized gold particles (27 nm) had been proven to be effective in ameliorating the symptoms of mycobacterial-, collagen- and pristane-induced arthritis in rat models. This contrasts with the drug sodium aurothiomalate that was only effective against mycobacterial-

induced arthritis but not to the same extent as Au<sup>0</sup>. Gold in the traditional Indian Ayurvedic medicine, Swarnabhasma (gold ash), has been characterized as globular particles of gold with an average size of 56-57 nm.

Zhou *et al.* (2008) reported a method to detect copper (II) by azide and alkyne functionalized AuNPs based on the fact that the extinction efficient of gold nanoparticles is several orders of magnitude larger than those of traditional organic chromophores.

Gurunathan *et al.* (2009) reported that metal nanoparticle have tremendous application in the area of catalysis, optoelectronics, diagnostics biological probes display devices.

Pooja *et al.* (2011) reviewed various methods of functionalization of GNPs and their applications in biomedical research. Functionalization facilitates targeted delivery of these nanoparticles to various cell types, bio imaging, gene delivery, drug delivery and other therapeutic and diagnostic applications. This review is an amalgamation of recent advances in the field of functionalization of gold nanoparticles and their potential applications in the field of medicine and biology.

Siddiqi *et al.* (2012) reported that low doses of AuNPs used in the study caused a significant change in oxidative stress and antioxidant defense indices only in liver. Therefore they offer potential to be exploited for various *in-vivo* uses.

### **Green synthesis, Coating and characterisation of gold nanoparticles**

For the synthesis of gold nanoparticles (GNPs), two clean test tubes were taken, one containing 10ml of 1mM Hydrogen tetra chloro-aurate (Himedia Chemicals, Mumbai) solution as control and the second tube containing 9ml of 1mM Hydrogen tetra chloro-aurate solution and 1ml of *Azadirachta indica* plant leaf extract which was used in all the groups except Group - I animals for the evaluation of toxicity which were coated with *Morinda citrifolia* fruit extract for Group – V, VI, VII, VIII, IX to be used. Both the tubes were incubated at room temperature for 1-2 hours. The change in colour from light yellow to ruby red was observed. The gold nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant was discarded and the pellet then was dissolved in deionised water. The synthesis of gold nanoparticles was confirmed by colour changes and qualitatively characterized by UV-vis spectrophotometry, XRD, FTIR, zeta potential, SEM and TEM images.

For the synthesis of Gold nanoparticles (GNPs) can be coated with medicinal plant extract, the nanoparticle solution already formed was taken (as per dose) and mixed with the aqueous fruit extract (as per dose). The resulting solution was maintained at room temperature and stirred for 1 hour to covalently modify the surface of GNPs with the phytochemicals present in plant extract.

Gold nanoparticles formed were characterized by UV-Vis spectrometry method, x-ray diffraction method, fourier transform infrared spectroscopy, zeta potential, dynamic light scattering, scanning electron microscopy and transmission electron microscopy imaging method.

Spectrophotometry was done using Systronics UV-visible spectrophotometer (Model-118, 2nm band width, Si photodiode). Absorbances were taken at different wavelengths (350nm to 700nm) against 1mM chloroauric acid solution blank, after 30 minutes and 2 hours, 6 hours and 24 hours of gold nanoparticles preparation. Findings were plotted on graph to observe the wavelength which showed peak absorbance.

X-ray diffraction analysis is the most useful method by which X-rays of a known wavelength are passed through a sample to identify the crystalline structure. The X-rays were diffracted by the lattice of crystal to give a unique pattern of peaks of 'reflections' at different angles and of different intensity, just as light can be diffracted by a grating of suitably spaced lines. The green synthesized GNPs pellets were grounded using a mortar and pestle into powder. X-ray powder diffraction measurement was carried out by using Philips X'Pert MPD powder diffractometer with long fine focus Cu K $\alpha$  anode operated at 40 KV and 30 mA in Bragg-Brentano geometry. The X-Ray detector moves around the sample and measures the intensity of these peaks and the position of these peaks [diffraction angle 2 $^\circ$  / sec]. The highest peak is defined as the 100% peak and the intensity of all the other peaks are measured as a percentage of the 100% peak. The powder XRD data were obtained in the 2 $^\circ$  range from 5 $^\circ$  to 80 $^\circ$  in step-scan mode with 2 $\theta$  step size of 0.03 $^\circ$ .

Fourier transform infrared spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance. FTIR has become important tool in understanding the involvement of functional groups in interactions between metal particles and biomolecules. In the current study, FTIR measurements were performed to identify the biomolecules responsible for capping and stabilizing the gold nanoparticles and its coating with plant extract. FTIR analysis of the dried AuNPs was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio using transmittance modes operating at a resolution of 4 cm $^{-1}$ . In this method AuNPs solution mixed with KBr and convert into a pellet form by using bench press. Thereafter the formed pellet was used for functional groups identification by IR spectroscopy (SHIMADZU FTIR-8400, Japan) with spectra recorded between 4000 and 400 cm $^{-1}$ . In addition, to identify which functional group is present in the aqueous leaf extract causing the reduction? For that, the Neem leaf extract was pelletized and FTIR spectra were recorded between. Same procedure was also followed for *Morinda citrifolia* fruit extract coated AuNPs. The spectrums of AuNPs in the aqueous extract, pure neem leaf extract and fruit extract coated AuNPs were compiled.

The surface charge (zeta potential) and particle size of AuNPs and fruit extract coated AuNPs were determined by electrophoretic light scattering (ELS), using a Zetasizer Nano ZS90 analyser (Malvern Instruments Inc., UK). A He–Ne laser with fixed wavelength of 633 nm was used as a light source and the intensity of scattered light was measured by a detector at 90°. This instrument passes laser which provides 90° scattering angle. This instrument read the sample with appropriate medium i.e. water. The temperature was set at 25<sup>o</sup> c. the samples were put into the cuvette (clean disposable zeta cells). The charges applied on the sample solution and results were read by Zetasizer software after 12 consecutive recording (Zeta run). XRD, FTIR and zeta potential studies were performed at UICT, NMU, Jalgaon, Maharashtra, India.

For TEM imaging dip preparation for nanoparticles by flotation method (Bozzola and Russell, 1998) was done. A drop of sample was placed on a piece of parafilm and the carbon coated copper grid was placed. After 5-10 minutes the excess was drained with the help of filter paper. The sample was washed with distilled water and then stained with 2% uranyl acetate. Then it was air dried and observed under transmission electron microscope (Model- Hitachi, H-7500) at various magnifications as per the standard protocol practising at RUSKA lab, college of veterinary science, SPVNRTSUVAFS, Rajendranagar, Hyderabad, India.

For SEM Samples were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hrs at 4<sup>o</sup>C and post fixed 2% in aqueous osmium tetroxide for 4 h. Dehydrated in series of graded alcohols and dried to critical point drying with CPD (EMS-850) unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model - JEOL JFC-1600) for 3 minutes and scanned under scanning electron microscope (SEM - Model: JOEL-JSM 5600) at required magnifications as per the standard procedures at RUSKA Lab, College of Veterinary Science, SPVNRTSUVAFS, Rajendranagar, Hyderabad, India.

## **References :**

- Alanazi F.K., A.A. Radwan, and I.A. Alsarra (2010) Biopharmaceutical applications of nanogold. Saudi Pharmaceutical Journal; 18: 179-193.
- Antonovych T. T. (1981) Gold Nephropathy. Annals of Clinical and Laboratory Science, 11 (5): 386–391.
- Armendariz V., I. Herrera, J. Peralta-Videa, M.J. Yacaman, H. Troiani, P. Santiago and J. L. GardeaTorresdey (2004). Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. Journal of Nanoparticle Research, 6: 377–382.
- Bozzola J.J. and L.D. Russell (1998) In: Electron microscopy principles and techniques for biologists, 2<sup>nd</sup> Edn., Jones and Bartlett publishers. Sudbury, Massachusetts, 132-133.
- Chandran S.P., M. Chaudhary, R. Pasricha, A. Ahmad, and M. Sastry (2006) of Gold Nanotriangles and Silver Nanoparticles using Aloe vera plant extract. Biotechnology Progress, 577 – 583.

- Chatterjee S., Bandyopadhyay A. and Sarkar K. (2011). Effect of iron oxide and gold nanoparticles on bacterial growth leading towards biological application. *Journal of Nanobiotechnology* 2011, 9:34 <http://www.jnanobiotechnology.com/content/9/1/34>
- Christopher L.B., G. Bushell, M.W. Whitehouse, D.S. Agrawal, S.G. Tupe, K.M. Paknikar and E.R.T. Tiekink (2007) Nanogoldpharmaceutics (i) The use of colloidal gold to treat experimentally-induced arthritis in rat models; (ii) Characterization of the gold in Swarna bhasma, a microparticulate used in traditional Indian medicine. *Gold Bulletin* 2007, 40/3.
- Di Guglielmo C, D.R. Lopez, J. De Lapuente, J.M. Mallafre and M.B. Suarez (2010) Embryotoxicity of cobalt ferrite and gold nanoparticles: a first in vitro approach. *Reproduct Toxicol*; 30: 271-276.
- Finkelstein A. E., D. T. Walz, V. Batista, M. Mizraji, F. Roisman and A. Misher (1976) Auranofin: new oral gold compound for treatment of rheumatoid-arthritis. *Ann Rheum Dis* 35:251–257.
- Gurunathan S., K. Kalishwaralal and R. Vaidyanathan (2009) Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloid Surf B Biointerfaces*. 74 ;328–335.
- Khlebtsov N.G. and L.A. Dykman (2010). Optical properties and biomedical applications of plasmonic nanoparticles. *J Quantitat Spectroscop Radiat Transf*; 111: 1-35.
- Kim D., S. Park, J.H. Lee, Y.Y. Jeong, and S. Jon (2007) Antibiofouling Polymer-Coated Gold Nanoparticles as a Contrast Agent for in Vivo X-ray Computed Tomography Imaging, *J. AM. CHEM. SOC.* 129, 7661-7665.
- Metz O., W. Stoll and W. Plenert (1982) Meningitis prophylaxis with intrathecal Au-198-colloid and methotrexate in childhood acute lymphocytic-leukemia. *Cancer* 49:224 – 228.
- Mukherjee P., R. Bhattacharya, P. Wang, L. Wang, S. Basu, J. A. Nagy, A. Atala, D. Mukhopadhyay, S. Soker (2005) Antiangiogenic properties of gold nanoparticles. *Clin Cancer Res* 2005, 11:3530-3534.
- Pooja M., K. V. Tiwari, V. A. Dennis and S. R. Singh. (2011). Functionalized Gold Nanoparticles and Their Biomedical Applications. *Nanomaterials* 2011, 1, 31-63; doi:10.3390/nano1010031.
- Pricker S.P. (1996) Medical uses of gold compounds: Past, present and future. *Gold Bulletin*, 29 (2): 53-60.
- Siddiqi N. J., M. A. K. Abdelhalim, Y. A. AlYafee and A. S. Alhomida (2012) Studies on the effect of gold nanoparticles on oxidative stress and antioxidants defence indices in various rat tissues. *African Journal of Pharmacy and Pharmacology*:6(47): 3246-3251.
- Song J.Y. and B.S. Kim (2008) Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst Eng*.
- Suman T.Y., S.R. Radhika Rajasree, R. Ramkumar, C. Rajthilak, P. Perumal (2013) The Green synthesis of gold nanoparticles using an aqueous root extract of *Morinda citrifolia* L. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 118: 11–16.
- Tsai C. Y., A.L. Shiau, S.Y. Chen, Y. H. Chen, P.C. Cheng and M. Y. Chang (2007) Amelioration of collagen-induced arthritis in rats by nanogold. *Arthritis Rheum*;56(2):544-54.
- Zhou Y., S. Wang, K. Zhang and X. Jiang (2008) Visual Detection of Copper(II) by Azide- and Alkyne-Functionalized Gold Nanoparticles Using Click Chemistry, *Angew. Chem. Int. Ed.* 2008, 47, 7454-7456.

**T6-03**

**EVALUATION OF VETERINARY PHARMACEUTICAL PRODUCT  
DEVELOPMENT BY GAMMA SCINTIGRAPHY**

**Dr. RAJIV V.GAIKWAD**

In charge, Veterinary Nuclear Medicine; Professor and Head, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence and Hospital Superintendent, Teaching Veterinary Clinical Complex, Bombay Veterinary College, Parel, Mumbai- 400 012.

Email: [drrajivgaikwad@gmail.com](mailto:drrajivgaikwad@gmail.com), [drvvg2001@yahoo.com](mailto:drvvg2001@yahoo.com)

Pharmaceutical product development is a long process to come-out with single entity in market. This process has many hurdles starting with identification of promising molecule, purification, evaluation for promising results, development of formulation evaluation by applying various *in vitro* techniques and then *in vivo* evaluation of product for their route of administration, release pattern, absorption pattern with various interventions etc. hurdles of regional absorption testing in laboratory and volunteer subjects etc. by invasive techniques and collecting blood at different time intervals to assess drug levels etc. Therefore development of new molecule of pharmaceutical / therapeutic interest may take minimum of 10-12 years with huge investment of man-power, money etc, and only 8-10% of all screened molecules are reaching at final stage of clinical relevance as a market product. The duration (by 2-3 years), cost of development can be minimized by adopting scintigraphy as a tool for evaluation of new promising molecule for their regional absorption pattern or absorption pattern etc. The common examples are: *in vivo* assessment of biodistribution of molecule in laboratory animal model. For comparisons of *in vivo* and *in vitro* assessment, studies can be undertaken after administration of very small doses of radiolabelled compound with designated routes of administration. This tracer kinetic study would help in planning, evaluation, timely assessment of molecule.

Gamma scintigraphy is widely used as a diagnostic modality and now being used for pharmaceutical evaluation in development of product to visualize / assess drug delivery system. The distribution of radiolabelled formulation can be assessed by planar imaging (2D) / SPECT (3D). Technetium-99m (radiopharmaceutical) has energy of 140keV and half-life of 6 hrs is being used for a broad range of diagnostic applications and assessment of drug delivery. About 85% of nuclear medical units are using 99mTc labeled pharmaceuticals. The various radiopharmaceuticals can be used for the specified purposes of drug development evaluation or assessment of its effect on targeted site or organ to prove the claim.

Gamma Scintigraphy is a non invasive evaluation technique to provide relevant information of release pattern, adjunct in providing information to check various peaks of drug release; it will be suitable method for analyzing regional absorption studies of promising molecule. If planning of release of drug at different locations and its evaluation / assessment of plasma levels in blood would give better information

of GI transit, lag period, regional release and absorption time, suitability in plasma level in few animal models. This information is to assess regional absorption pattern, variables such as pH, time, repeat dosing etc.

Role in formulation development: Different types of delivery formulations can be evaluated *in vivo* in three to four animals at a time to assess its repeatability and evaluation of formulation to choose which better one is. This will help to minimize time in development of molecule.

Potential alternative portals of drug entry to the systemic circulation include the buccal, sublingual, nasal, pulmonary and vaginal routes. These routes are also being studied for the local delivery of drugs directly to the site of action, thereby reducing the dose needed to produce a pharmacological effect and also possibly minimizing systemic side-effects / toxicities.

Many a time's formulations can be evaluated for its beneficial effects as well as detrimental effects (toxicological) on various blood components, alteration or change in bio-distribution behavior of known radiopharmaceuticals. This will be possible to demonstrate by *in vitro* and *in vivo* studies in animal model. Many ayurvedic or herbal formulations can be evaluated for the same purposes to demonstrate their efficacy to prove the claims.

Microdose studies are designed to evaluate pharmacokinetics or imaging of specific targets and are designed not to induce pharmacologic effects. Because of this, the risk to human subjects is very limited. A *microdose* is defined as less than 1/100th of the dose of a test substance calculated (based on animal data) to yield a pharmacologic effect of the test substance with a maximum dose of <100 micrograms (for imaging agents).

Gamma scintigraphy or tracer kinetic study can be used for the sequential imaging after radiolabelling of molecule for:

**Release of the drug from:**

- o Stomach (micro particulate, muco-adhesive formulations etc.),
- o Conjunctival sac (microtab etc.)
- o Per-rectal formulations for residence
- o Colonic targeting etc.
- o Distribution and the retention time of a novel bioadhesive intravaginal delivery system

- o Assessment of behavior of new molecule
- o Distribution pattern of drug (*in vivo*) after intravenous administration (will help to assess affinity of molecule to particular organ, may help in assessment of beneficial effects of function).
- o Regional deposition / absorption studies,
- o Scintikinetetic studies (Pharmacokinetic)
- o Therapeutic target
- o Receptor, monoclonal antibody and peptide imaging

Sequential imaging would help to assess the initial rate of localization, optimum time (T max) for localization and clearance (T<sub>1/2</sub>) in target organ.

Therefore gamma scintigraphy has potential for refined pharmaceutical industrial application with available imaging methods, which fit into the process of drug development. The appropriate use of molecular imaging in drug discovery and drug development can significantly speed up the development process and save money. These procedures have two more spin-off advantages: (a) since same animal can be used for timescale study, the variability is minimum and (b) animal-friendly since less number of animals required hence recommended method for animal welfare.

**AB-20**

**Antimicrobial and immunological studies of curcumin against *E. coli* induced infection in broilers**

Godbole PV, Hajare SW, Poonam Bhosale, Madhuri Hedau, Ingole RS and Ingawale MV

Post Graduate Institute of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences

University, Akola 444 104, M.S. INDIA

Place of work –Dept of Pharmacology and Toxicology, PGIVAS, Akola.

Presenting author – Dr. S.W. Hajare,

Email – sunilwhajare@rediffmail.com

The present study was conducted to evaluate the therapeutic and immunomodulatory activity of curcumin against *Escherichia coli* induced infection in broilers. Curcumin is an active ingredient of *Curcuma longa* responsible for various pharmacological activities of turmeric. Ninety birds were divided into six groups randomly and equally. Group I was normal control, Group II was *E. coli* control, Group III and IV received curcumin prophylactically and therapeutically @ 200 mg/kg and 400 mg/kg feed, respectively. Group V received cephalixin and Group VI served as curcumin control. In general performance, the average weekly body weight gain of broilers from group I, III, IV and group V showed significant ( $P < 0.05$ ) increase as compared to *E. coli* control group. The maximum body weight gain was observed in curcumin control group. The average weekly feed consumption consumption in *E. coli* control group was decreased as compared to normal control and other treatment groups. In clinical observations the birds from *E. coli* control showed most of the signs and symptoms of colibacillosis such as blood tinged diarrhoea, depression, loss of appetite, dyspnoea, and sneezing 2-3 days post infection till the end of 3<sup>rd</sup> week. The curcumin received groups showed mild symptoms of colibacillosis with less duration of illness. In *in-vitro* antimicrobial screening test, curcumin inhibited the growth of *E. coli* in a dose dependant manner. In disc diffusion test the maximum zone of inhibition was  $15.33 \pm 1.00$  mm at 20 mg/disc concentration. The minimum inhibitory concentration (MIC) of curcumin was found to be 1.5 mg/ml. In immunological study, the Geometric Means of HA/HI titre of different groups against Ranikhet disease birds at 3<sup>rd</sup> and 6<sup>th</sup> week of age were better in curcumin treatment groups. In *E. coli* re-isolation studies, *E. coli* were re-isolated from most of the samples of heart, lung, liver and intestine from *E. coli* control group. In curcumin prophylactic group only one sample each from liver and intestine received *E. coli*. In curcumin therapeutic and Cephalixin group one sample each from heart, lung and liver and two samples from intestine received re-isolation of *E. coli*. In conclusion, curcumin has got antimicrobial and immunomodulatory activities and it could be used in future as alternatives to antibiotics growth promoters to control *E. coli* infection in broilers.

**T7**

**Immunology,  
Immunodiagnosics And  
Vaccinology**

**T7-01**

**Subviral particle based infectious bursal disease vaccine: a way forward towards translation from lab to land**

Sohini Dey<sup>#</sup>, C. Madhan Mohan, R. Saravanan, Dinesh C. Pathak, Ashis Debnath, Rahul, R. Narayan, M. Asok Kumar, A. Gulhane\* and B. Piruthiviraj Kumar\*

<sup>#</sup>Principal Scientist, Recombinant DNA Laboratory, Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Bareilly 243 122, UP

\* Research and Development, Globion India Private Limited, Telangana, 502281.

### **Introduction**

Infectious bursal disease (IBD) is a highly contagious viral disease of young chicken, causing immune suppression and a serious threat to the poultry industry. Control of the OIE listed disease is dependent on effective vaccination programme that can induce active immune response, so that the birds can be protected in their susceptible age of the disease at 3-6 weeks old. Based upon the field strains, the unvaccinated flocks can suffer from mild immune suppressions to high mortality up to 90-100%. The modified live vaccines (MLVs) have been developed and classified as “mild”, “intermediate”, “intermediate plus” IBD vaccines according to the extent of interference and neutralizing ability of the maternally derived antibodies (MDA) to the vaccine virus. The live attenuated virus is used worldwide to protect the broiler chickens. However, the major drawback of the attenuated IBD vaccines is that they are sensitive to the maternally derived antibodies (MDA) which are always present at the time of vaccination. Moreover, the “hot” or intermediate plus vaccines can themselves be responsible for vaccine induced lesions in the bursa of Fabricius and immunosuppression that can lead to increased susceptibility of the chicken to other diseases. Thus, the disadvantages of MLVs have led to the development of recombinant DNA-IBDV vaccines, wherein one or more immunogenic genes are expressed by recombinant DNA technology.

IBDV, a bi-segmented double stranded RNA virus belongs to the genus Avibirna virus, family Birnaviridae. VP2 is considered as the major host-protective antigen eliciting most of the neutralizing epitopes and is responsible for antigenic vaccination, cell tropism and viral virulence. Till date, a recombinant vaccine namely herpes virus vector of turkey expressing the VP2 gene of IBDV has been licensed for use. In this context, our research group has developed the subviral particle based IBD vaccine being generated by expressing the full length VP2 gene of IBDV in *Saccharomyces cerevisiae*, and evaluated its role in protective immunity and efficacy in the presence of MDA against IBDV in the field trial.

### **Infectious Bursal Disease and its control**

Infectious bursal disease (IBD), also known as Gumboro disease is a highly contagious, immunosuppressive disease of immature chickens caused by a birnavirus, infectious bursal disease virus (IBDV) which is responsible for major economic losses in the poultry industry worldwide. IBDV infects

the bursa of Fabricius of particularly the actively dividing and differentiating lymphocytes of the B-cell lineage of young chickens, resulting in morbidity, mortality and immunosuppression. The double stranded RNA virus exists in two serotypes but only serotype 1 causes the disease in young chickens.

Immunization of chickens is the primary method to control IBD in chickens. It is mandatory to protect chickens against the infection during the first weeks after hatch. To induce high titres of maternally derived antibodies that persist over the whole laying period, layers are vaccinated with inactivated oil emulsified vaccines. After hatching, chickens are immunized with live vaccines. In India, IBD has been controlled by vaccination with intermediate strain vaccines coupled with biosecurity measures. An “immune complex” vaccine has been developed, in which the vaccine virus is complexed *in vitro* with an optimum amount of antibodies that is used for *in ovo* vaccination. It has been suggested that the immune complex is taken up by follicular dendritic cells (macrophages) wherein the virus resides until the time of drop of maternal antibody. A number of experimental recombinant IBD vaccines have been developed which use fowl poxvirus, fowl adenovirus, Marek's disease virus and Semliki Forest virus as the vector.

### **Characteristics and immunogenicity of the SVP based IBD vaccine**

The IBDV major capsid protein VP2 led to the formation of 22 nm subviral particles (SVPs) in *Saccharomyces cerevisiae* that is structurally similar to IBDV. In a laboratory vaccine trial, these SVPs, in addition to their ability to stimulate the B-cell mediated immune responses, were highly effective in stimulating CD4 proliferative response and cytotoxic T lymphocyte responses. Further, to develop the vaccine candidate as a technology: “Sub viral particle (SVP) based Infectious bursal disease vaccine” clinical trials were performed with the help of an industrial partner that manufactures poultry vaccines, M/ S. Globion India Pvt. Ltd., Hyderabad to answer the following key questions:

- 1. Is the SVP based vaccine formulation safe and effective when administered in the poultry farms?**
- 2. Does the SVP based vaccine formulation protect the day old chicken in the presence of maternal antibodies?**

Traditionally the protective efficacy is evaluated in specific pathogen free (SPF) chickens (SPF). The SVPs induced complete protection with or without adjuvant in the susceptible SPF birds on challenge with vvIBDV from day 7 post-vaccination onwards. However, under field conditions, residual MDA levels might interfere with vaccine efficacy. Hence, the commercial broilers with various levels of MDA were vaccinated to one day old age chickens with SVP based IBD vaccine. After the expected decrease of the maternal immunity, a clear serological conversion to IBDV antigen was evidenced in the vaccinated birds. The expression profiles of various genes associated with cell mediated immunity were subsequently analyzed 14 days post vaccination by real time PCR analysis. The vaccine could induce the expression of IL-4 as well as IFN- $\alpha$  transcripts that supports the induction of humoral and cell mediated immune responses.

Lymphocyte proliferation assay using MTT dye was performed on days 21 and 35 post-vaccination to assess the increased cellular immune responses in the vaccinated birds and the SVP based vaccine induced significantly higher antigen specific proliferation responses. Overall, these results support the hypothesis that stimulation of specific cell-mediated immunity contributes to the response against IBDV in SVP based IBD vaccinated chickens.

Protection afforded by the vaccine was evaluated by conducting the challenge study with vvIBDV at day 35 post vaccination. The birds in the unchallenged control groups remained healthy and exhibited normal sized bursa, whereas the control challenged group showed typical clinical signs with 90% mortality with severe haemorrhagic bursa when challenged with vvIBDV. In contrast, the vaccinated birds were completely protected against vvIBDV challenge. The vaccinated chickens with and without adjuvant had significantly ( $P < 0.01$ ) higher bursa body weight ratios and lower bursal lesion scores in comparison to the control group 10 days post challenge.

**Conclusion :** Most of the poultry diseases can be prevented these days due to advancement of science in the field of disease diagnosis and treatment using modern tools as enlisted above. Virus-like particles are one of the highly effective types of subunit vaccines that mimic the authentic structure of virus particles without the genetic material. The present study investigated the efficacy of the SVPs as an ideal vaccine as measured by antibody titres, bursal body weight ratio, clearance of IBDV from the bursa, percentage of mortality, cytokine response, stimulation index (SI) in LTT and estimation of CD4/CD8 T cell subsets in SPF as well as broiler chickens in the presence of various levels of maternal antibodies. The results obtained at the clinical trials conducted in the poultry vaccine company were consistent with the laboratory scale trials supporting an effective vaccine development and take even in the presence of MDA, together with an absence of bursal lesions after vaccination. Thus, the data indicate that SVPs reveal excellent safety and broad efficacy profiles that are superior to the commercially available IBD MLVs. Further, a single dose of vaccine could be used in day old birds in the hatchery in presence of MDA against IBD.

The research programme was being supported by an extramural grant awarded to Sohini Dey (Principal Investigator) by the Department of Biotechnology, Government of India (Grant No. BT/PR13554/ADV/90/186/2015).

#### **References :**

- Dey Sohini, Chitra Upadhyay, C. Madhan Mohan, Jag Mohan Kataria, Vikram N. Vakharia (2009). Formation of subviral particles of the capsid protein VP2 of infectious bursal disease virus and its application in serological diagnosis. *Journal of Virological Methods* 157: 84-89.
- Gupta, S.K., Deb, R., Dey, S., Chellappa, M.M (2014). Toll like receptor-based adjuvants: enhancing the immune response to vaccines against infectious diseases of chicken. *Expert Reviews in Vaccines* 13(7):909-25.
- Maity, H.K., Dey, S., Madhan Mohan, C., Khulape, S.A., Pathak, D.C. and Vakharia, V.N. (2015). Protective efficacy of a DNA construct encoding the VP2 gene of infectious bursal disease and a truncated HSP70 of Mycobacterium tuberculosis in chickens. *Vaccine* 33: 1033-39.

## Changing epidemiological patterns of Japanese encephalitis infection and impact on animal health in India

Baldev R. Gulati

ICAR-National Research Centre on Equines, Hisar, Haryana

Japanese encephalitis (JE) is mosquito-borne zoonotic viral infection of horses, pigs and humans. It is estimated to cause 67900 human cases annually and over 10 million cases estimated during last 60 years. JEV causes reproductive disorders in pregnant sows and encephalitis in horses. JE is a disease of rural agricultural areas where vector mosquitoes proliferate in close association with pigs, wading birds, and ducks. The principal vector species is *Culex tritaeniorhynchus*, which is present in great density in rainy season in both tropical and temperate regions. In this paper, epidemiological factors associated with the spread of JE infection in human and animals in different parts of the country have been discussed, delineating the role of pigs. This paper also gives the overview of current diagnostic techniques and options for controlling JE infection in animals.

JEV belongs to the genus *Flavivirus* in the family *Flaviviridae*. JEV is an enveloped virus, approximately 50 nm in diameter with a plus-sense, single-stranded RNA of ~11 kb, encoding single open reading frame (ORF) that codes for a polyprotein of 3432 amino acids that is subsequently cleaved into 3 structural [capsid (C), premembrane (prM), and envelope (E)] and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. The E protein is the most important structural protein and induces virus-neutralizing (VN) antibodies, which impart protective immunity against JEV. Based on E gene phylogenetic analysis, JEV strains have been classified into five genotypes (G1-V). Genotype GIII is most widely distributed in Asian countries and most of the JEV strains isolated in India belong to GIII. During the past decade, JEV GI has been introduced into South Korea, Thailand and China and also has been reported since 2007 in India. In animals, GIII is widely distributed in pigs from several countries, including India while GI from China, Cambodia, Korea & Thailand. Equine JEV isolates from Japan, Hong Kong and Taiwan have been typed as GI, GII and GIII. We isolated JEV from a horse in India that belongs to GIII.

A definitive diagnosis of JE depends on isolation or demonstration of virus in sick or dead animals. The routine laboratory diagnosis of JEV infection is based on culture and serology followed by identification of the viral genome by reverse transcription-polymerase chain reaction (RT-PCR). Virus isolation is the “gold standard” but has generally been unsuccessful, owing to the low level of transient viraemia associated with the disease process, which in turn requires viable virus in samples. JE virus can be isolated by a variety of methods, the classical being intra-cerebral inoculation of clinical specimens in suckling mice. Various cell lines such as Vero, BHK21, LLCMK2, C6/36 have been used for virus isolation. Molecular diagnostic methods have been used for sensitive and specific assays for the identification of JEV, including

reverse transcriptase PCR (RT-PCR), fluorogenic real-time (TaqMan) or SYBR green-based real-time PCR, and loop-mediated isothermal amplification (LAMP). Serological tests are useful to determine the prevalence of infection in animals include virus neutralization (VN), haemagglutination inhibition (HI), complement fixation (CF) and enzyme-linked immunosorbent assay (ELISA). Serologically, JEV infection can be detected by immunoglobulin M (IgM). The IgG-based serological assays require significant rise in antibody titre in paired sera collected during acute and convalescent phases to confirm the diagnosis. The specificity of serological tests should always be considered for JEV diagnosis, especially in some regions of the world, where two or more antigenically closely related viruses are circulating. The diagnosis of JE is difficult in these areas due to high degree of cross-reactive antibody among related flaviviruses.

JEV is transmitted in a zoonotic cycle among mosquitoes and vertebrate-amplifying hosts, chiefly pigs and wading birds. Pigs serve as amplifying hosts because they develop viremia that remains high enough to infect mosquitoes for up to 4-14 days. Humans and horses are considered as the dead-end hosts as they have brief periods of viremia with low virus titres. The ardeid wading birds (heron, egrets) are considered the primary enzootic hosts of JEV and play important role in epizootic viral amplification. Mosquitoes that commonly transmit JEV are most heavily concentrated in rural, rice growing areas and feed most actively in the late afternoon and early evening. Although JEV has been isolated from over 30 species, paddy-breeding mosquitoes of the *Culex vishnui* subgroup, particularly *Cx. tritaeniorhynchus*, are the major vectors of the virus. Till recently, JEV transmission has been exclusively described as being mosquito-mediated. However, recently it has been demonstrated that JEV can be transmitted between pigs in the absence of arthropod vectors. Pigs shed virus in oronasal secretions and are highly susceptible to oronasal infection. Further, virus persists in pig tonsils for at least 25 days. These findings could have a major impact on the ecology of JEV in temperate regions with short mosquito seasons. The duration of viremia of JEV in birds and pigs is too short for these animals to effectively maintain the virus during adverse conditions. Persistence of viral infection under low environmental temperature has been demonstrated in insectivorous bats. JEV isolation from *Murina aurata* confirmed that the bats can act as reservoir and amplifier host of JE virus in nature.

The pigs develop pyrexia and anorexia following JEV infection. The most common symptom of JEV infection in pigs is the birth of stillborn or mummified fetuses, usually at term. Piglets born alive often have tremors and convulsions and die soon after birth. Hydrocephalus, cerebellar hypoplasia and spinal hypomyelinogenesis may also be seen in pigs. In a serosurveillance in pig population in two districts of Assa, we reported 22.99% of 335 pigs JEV sero-positive during 2015-16. There was significantly higher JEV positivity in pigs from Sivasagar district (31.31%) than in Kamrup Rural district (11.51%). The ecology of Assam represents a classical example of reasons for upsurge of JE in human and animal population. JE has been reported from human population and Animals in sporadic outbreaks or epidemic

forms in upper Assam since 1976. Assam has been reporting highest number of human JEV cases in India continuously since 2011. The reasons for proliferation of disease in Assam are many fold. The economy of Assam depends mainly on agricultural and allied activities. Rice is the main crop and occupies about two-third of the total cropped area in the state. Assam also has the largest pig population accounting for 17.96% of the country's total pig population and state has witnessed over 6% growth in pig population during 1994-2012. Abundance of rainfall and mean temperature of 25°C (10-32°C) supports mosquito breeding. Environment changes resulting in frequent floods, increasing rice & pig farming, and abundance of mosquitoes makes Assam the most vulnerable state for spread of Japanese encephalitis.

Alongside infection in pigs, JEV infection in horses can occur although it is considered very rare and again. Clinical signs in horses may include fever, impaired locomotion, stupor, teeth grinding, blindness, coma and death. In some cases, the only symptom may be fever and short period of lethargy. Sporadic cases of JE in horses have been reported from India. Other animal species like cattle, goats, dogs and cats can be sub-clinically infected. Sero-surveillance among equines in 13 different states of India revealed maximum JE positivity in Manipur followed by those in Gujarat, Madhya Pradesh and Uttar Pradesh. JEV has been isolated from a horse showing neurological signs of encephalitis from Haryana. Phylogenetic analysis of the full-length sequence indicated that the JEV strain isolated from horse in India belongs to genotype III.

The failure to halt the spread of JE in Asia and the Pacific region, despite the availability of an effective and inexpensive vaccine for 40 years, is of considerable public health concern. Various factors are responsible for proliferation of JE in animals. The population explosion in the JEV endemic areas, area under rice cultivation and rice productivity has grown exponentially. As per FAO STAT database, the World rice production in 2007 is 650.2 million tons and of this, 590.2 million tons is produced in Asia where JEV is endemic. Rice production has increased nearly three times during last fifty years. One of the main reasons for the proliferation of JE is the transformation caused by water resources development and management that create suitable breeding sites for vectors, which in turn influence the frequency and transmission dynamics of the disease. Area under rice cultivation has increased from 115.3 million ha to 156.7 million ha during last 50 years. The largest irrigated rice area in 2005 was found in India (41.9 million ha), followed by China (29.0 million ha), Bangladesh (10.5 million ha), and Thailand (10.0 million ha).

Swine population in the JEV-endemic countries has grown consistently in last 50 years. Swine population in India is concentrated mainly in northeastern states and Uttar Pradesh –states where JEV is highly endemic. According to 18<sup>th</sup> Livestock Census of India, the total swine population has grown consistently over the past 50 years, except with marginal decline from 14 million head in 2003 to 12 million head in 2007.

Climate change has been implicated in the increase of transmission of several vector-borne diseases, including JE. With the global warming, there has been increase in JE vector proliferation and longevity. The changing climate has affected agricultural practices such as irrigation, resulting in increase in irrigated rice-production area, which in turn result in JE outbreaks. Climate change is also influencing migration patterns of birds, which may result in introduction of JEV into new areas.

The virological factors contributing to the spread of JEV include the emergence of more virulent strains and replacement of JEV strains in a region with newer genotypes over last two decades. Genotype III (GIII) has been a predominant serotype in Asian countries. However, during the past decade, JEV GI has been introduced into South Korea, Thailand and China and has replaced the GIII strains that had been circulating in Japan and Vietnam during the mid 1990s. Until 2007, all known strains isolated in India belonged to GIII, however, GI has been reported along with GIII from several areas, including Uttar Pradesh and Assam.

There are still no specific drugs available to treat JEV infection. JE is managed only with supportive therapies and preventive measures. Prevention of JEV is based on mosquito control, avoiding mosquito bites, pig immunization, and human immunization. Vector control is one of the most important aspects of the prevention of JE spread. Larvicides and insecticides help in controlling mosquitoes in paddy fields. Vaccination is the most effective means of preventing JEV infection and should be practiced routinely in regions where the virus is responsible for human and veterinary disease. Multiple types of JE vaccines available for humans including mouse brain-derived inactivated, cell culture-derived live-attenuated, cell culture-derived killed-inactivated, and genetically engineered live-attenuated chimeric vaccines. An inactivated JE vaccine (JE-VAX; Biken Institute) produced from infected mouse brain was developed in Japan and licensed for use internationally. The mouse brain-grown JE vaccine had several drawbacks and its production was halted in 2006 and has been phased out from most countries, including India since 2011. A live-attenuated cell culture-derived JE vaccine was developed in China based on SA14-14-2 strain raised in primary kidney hamster cells gives good protection and is the most widely used JE vaccine in JE-endemic areas. More than 300 million doses of this vaccine have been used with excellent record of safety and efficacy in a two-dose regimen. Vero cell-derived, inactivated JE vaccine (designated IC51) has been established using the attenuated SA14-14-2 strain under different trade names (IXIARO, JESPECT, and JEEV) in US, Europe, Canada, Australia, Hong Kong, Switzerland, and India. A live recombinant vaccine (ChimeriVax-JE, engineered by replacement of the immunogenic virion surface proteins, prM and E, in a yellow fever virus vaccine with those of JEV) has shown promising results. This Vero cell-derived ChimeriVax-JE vaccine is now commercially available (IMOJEV, JE-CV, THAIJEV). Other experimental vaccines, including recombinant poxvirus vaccine, DNA-based vaccine are also under various stages of development for human use.

JE in livestock is a notifiable OIE-listed disease that could affect trade between countries. Vaccination is recommended for the control of disease in animals for reducing abortions in pigs or protecting high value race horses. In South Korea, a live attenuated JE vaccine (Anyang 300 strain, G3) has been used in swines for more than 30 years. This has proven highly effective at reducing incidence of disease in pigs. A formalin-inactivated vaccine derived from porcine kidney cells or chick embryo cells and live-attenuated vaccines derived from porcine kidney cells are used in swines or horses in several Asian countries. Vaccination of horses is effective in protecting against disease, particularly high value animals such as racehorses that travel to JE endemic regions. In India, no vaccination has been practiced for animals, including horses and swine. Since pigs are the amplifying host for the JEV, vaccination in pigs in the JEV-endemic areas will help in curtailing the source of JEV infection. Vaccinations of pigs will break the mosquito-pig-human transmission cycle and thus helping in control of JEV.

The environmental and ecological factors responsible for the spread of JE need further investigations, with a view to control the spread of this devastating disease. Intensification and expansion of irrigated rice production systems over the past few years have contributed in increased JE incidence. Effective JE control is possible by means of effective surveillance systems and timely vaccination of persons or animals at risk. The animal diagnostic facilities for JE in India need to be strengthened for timely diagnosis and forecasting of the impending outbreaks. Newer diagnostic methods are needed for monitoring the spread of the disease in human, animal and vector population. There is no specific treatment for the disease. Vaccination is the most effective means of preventing JEV infection and should be practiced routinely in regions where the virus is responsible for human and veterinary disease. There is need for development of safe and effective vaccines for use in animals. Strategies for the reduction of virus burden in ecology by controlling vector population and relocating pig populations away from human habitation may be considered for effective global control of JE.

### **T7-03**

#### **JOHNE'S DISEASE IN ANIMALS AND ITS CONTROL**

S. Manoharan, Dr.B.Samuel Ronald Masillomony\* and K.Kumanan

Vaccine Research Centre- Bacterial Vaccine, Centre for Animal Health Studies, TANUVAS \*Dept. Of Veterinary Microbiology, Madras Veterinary College, Chennai-7

Johne's disease or Paratuberculosis, caused by *Mycobacterium avium subsp. paratuberculosis* is an emerging disease affecting the livestock industry. Johne's disease is prevalent in domestic animals worldwide and has significant impact on the global economy. On infected premises where clinical cases have been observed, as many as 70 percent of the animals may be in various stages of infection and disease progression. Thus clinical disease may only represent the "tip of the iceberg". The economic

losses from Johne's disease are primarily due to premature culling of animals, mortality and reduced milk production. Therefore, control of paratuberculosis is a complex issue. Since Johne's disease is so insidious within an animal population, management is considered to be the most useful tool for controlling paratuberculosis within domestic livestock herds. However, management fails due to the prevailing unique circumstances in individual farms.

In India, Johne's disease is endemic and significantly high prevalence has been reported in many organized herds ranging from 15 to 50% in goats. Increasing incidences of JD in sheep and goats (10 – 12%) has been recorded in organized and unorganized farms in Tamil Nadu.

A vaccine that prevents animals from becoming infected would be an ideal goal. Johne's disease vaccines appear to provide partial protection, by modifying the immunopathogenesis and stop progression of paratuberculosis from early subclinical infection to clinical disease in cattle, sheep and goats. Vaccination of replacements appeared to be the most cost effective strategy for controlling paratuberculosis in sheep flock. In India, vaccine for Johne's disease is not available at present. Hence this Johne's disease vaccine was developed mainly for the control of the disease and not for eradication.

The control of paratuberculosis is a complex issue. It involves both management and vaccination. Based on the cost/benefit analysis and epidemiological simulation model developed by Juste and Casal (1993), vaccination of replacements appeared to be the most cost effective strategy for controlling paratuberculosis in sheep flock. This holds good in the Indian scenario too, since majority of the sheep and goat owners come under the small farmer category.

*Mycobacterium avium subsp. paratuberculosis* was isolated from Johne's disease suspected sheep and goat in Herrold's egg yolk medium with Mycobactin J and confirmed by IS900 PCR and IS1311 PCR RFLP. The isolates were confirmed to be Bison type. The isolates were adapted in Reid's synthetic medium followed by heat inactivation and adjuvanted with mineral oil as per OIE with slight modification.

Extensive field trial has been done in the TANUVAS as well as some private farms and the efficacy was assessed in terms of cell mediated immune response. The developed vaccine provides prolonged gamma interferon response, reduced mortality due to Johne's disease, delayed exhibition of clinical signs due to Johne's disease, reduced fecal shedding, safe and cost effective vaccine.

**AB-21**

**Identification of subclinical mastitis pathogens in milk by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and antibiogram study**

Ashok V Bhosle, Mahesh B Kulkarni, Avinash G Karpe, Shivaji Mehatre\*, Shaunak Deshmukh

Department of Veterinary Microbiology,

College of Veterinary & Animal Sciences, Udgir;

\*Vaccine R&D, Ventri Biologicals Venkateshwara hatcheries Pvt. Ltd., Urawade, Pune, Maharashtra

**Abstract**

Subclinical mastitis is a common and widely disseminated disease in cattle and buffaloes. The routine diagnosis via bacterial identification through traditional tests is a time-consuming process. In the present work, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows rapid bacterial identification with high confidence and speed (6 Hrs for bacterial growth and analysis). With the use of MALDI-TOF MS, 204 bacterial culture isolates from milk of different dairy cows and buffaloes from several farms were analyzed. The results were compared with those obtained by classical biochemical methods. MALDI-TOF MS bacterial identification and its increased sensitivity was illustrated by the additional identification of coagulase-negative *Staphylococcus* species and mixed bacterial cultures. Matrix-assisted laser desorption-ionization mass spectrometry considerably accelerates the diagnosis of mastitis pathogens, especially in cases of subclinical mastitis. Fast reliable and accurate animal management strategies for mastitis control and treatment can therefore be applied.

The major bacterial isolates identified were *Staphylococcus aureus*, *Arcanobacterium pyogenes*, *Klebsiella pneumoniae* & *Escherichia coli*. The sensitivity towards amoxicillin, ciprofloxacin, gentamicin, enrofloxacin and resistance towards penicillin, streptomycin has been recorded.

**AB-22** **Diagnosis of *Mycobacterium bovis* based on Fluorescence polarization Assay.**

<sup>1</sup> Rabyia javed, <sup>1</sup> Deepti Narang, <sup>1</sup> Mudit Chandra, <sup>2</sup> S.T.Singh, <sup>3</sup> G.Filia.

<sup>1</sup> Department of Veterinary Microbiology, <sup>2</sup> Directorate of Livestock Farms, <sup>3</sup> ADRC, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences, Ludhiana.

Email: rabiajavedkhan@gmail.com

A Fluorescence polarization assay (FPA) utilizing the *Mycobacterium bovis* antibody test kit (TB\*FPA ellie), was used to detect antibodies to *Mycobacterium bovis* in cattle sera. The diagnostic test (qualitative) uses a fluorescein – labeled peptide with the same amino acid sequence of an epitopic region of the *Mycobacterium bovis* –bacterium MPB70 protein. A Fluorescence polarization instrument is used to measure the polarization state of the light emitted by the peptide conjugate. Polarization is low when no antibodies are present: polarization increases when antibodies bind to the conjugate. The study included sera from (n=192) cattle, which were screened for the CMI response by Comparative Intradermal tests and Gamma interferon test (IFN-Y) as per the standard protocol of OIE 2009. A total of 33 samples were found positive from the above performed screening tests. These 33 samples were further subjected to fluorescence polarization assay, out of which 7 came out to be positive from confirmatory protocol given in the kit. The analysis of the results was done on the basis of the screening and confirmatory protocol given in the kit. The kit was standardized to a gain factor of 1.122 and the incubation time varied according to the sample size. The negative control must be read between 70 and 90 mP and the positive control must read between 120 and 250 mP. The FPA results were compared to the results of the comparative intradermal tests (CID) for 192 cattle. Out of a total of 192 animals screened by CID and IFN-Y, 14 and 23 animals were positive, respectively. A total of 53 animals were selected for the FPA. The FPA detected 12/53 (22.64%). Out of the 53 animals tested for FPA, 33 were positive by either of the CID and IFN tests while 20 were negative by either of the CID and IFN-Y tests.

**AB-23**

**Expression and characterization of A4L gene of capripoxvirus for its diagnostic potential in ELISA**

Aparna Madhavan, Gnanavel Venkatesan, Amit Kumar, Sargam Arya and A.B. Pandey  
Pox virus laboratory, Division of Virology, IVRI, Mukteswar, Uttarakhand

Capripoxviruses of sheep and goats code for many structural immunogenic proteins in their genome. ORF 095, a 39 kDa structural core protein of capripox virus synthesized at late stage of infection is a homolog of Vaccinia virus A4L protein and plays role assembly and disassembly of virion. Sequence analysis of ORF 095 revealed them highly conserved (96.3- 97.7% and 92- 96.3% identity at nucleotide

and amino acid levels) among capripox viruses. The predicted protein characteristics namely high degree of antigenic index, hydrophilicity and surface probability have given positive signal to select ORF 095 gene as candidate for expression in E.coli BL21-CodonPlus cells and its characterization as diagnostic antigen in ELISA format for detection of GTPV/SPPV specific antibodies in infected/vaccinated sheep and goats. The amplified full length ORF 095 of GTPV was cloned in to pET32a vector and over-expressed as His-tagged fusion protein in BL21-CodonPlus bacterial cells using 1mM IPTG (24g/ml) at 30p C. The optimum level of expression was found at 4h post induction as characterized by SDS-PAGE followed by Western blotting using Ni-NTA HRP conjugate. Recombinant proteins were purified by Ni-NTA affinity chromatography under denaturing 8M urea buffer and eluted at 300mM imidazole at pH 8. The purified and concentrated proteins were used as diagnostic antigen in Indirect ELISA to evaluate its diagnostic potential after optimizing antigen and antibody dilution by checkerboard titration. The mean ELISA reactivity of rORF095 protein with anti-GTPV serum was 0.778 while that of anti-SPPV serum was 0.63 whereas it reacted at negligible level with healthy goat and sheep serum. It has no cross reactivity with other related viruses like Orf, bluetongue, *peste-des-petits ruminants* and Foot and Mouth disease. The rORF095 ELISA showed a progressive immune response in vaccinated and challenged goats. This study showed the potential of ORF 095 protein as safe antigen in ELISA for sero-epidemiological study of capripox infection in sheep and goats.

**AB-24**

**Isolation and molecular characterization of *Pasteurella multocida* type B causing Hemorrhagic Septicemia in Buffaloes**

K. Manimaran\*, R. Bharathi, K. Karthik, S. Arunkumar, K. Shoba and K. Kumanan  
Central University Laboratory, Centre for Animal Health Studies,  
Tamil Nadu Veterinary and Animal Sciences University,  
Madhavaram Milk Colony, Chennai-600 051, Tamil Nadu.  
\*E-mail:maranvet@yahoo.com

Hemorrhagic septicemia is an important livestock disease in the Asian continent. An investigation was carried out to know the reason for the sudden death of buffaloes in a farm containing 45 animals in Vadaperumpakkam village, Tiruvallur district, Tamil Nadu. Animals were reported with the clinical signs of drooling of saliva, respiratory distress, fever and sudden death. Postmortem examination revealed severe congestion in trachea and lung with fibrinous pneumonia, fibrinous pericarditis with endocardial haemorrhages, amphistomes in rumen and liver. Heart blood, lung and tracheal swabs were collected along with heart blood smear, peripheral blood smear and lung impression smear. On examination of the

smears by leishmans staining method, there was presence of bipolar organisms which was characteristic of Pasteurella organism. All the swabs and organs collected were cultured on blood agar, Mac Conkey agar and Brain heart infusion agar plates and incubated at 37 °C for 24 hours. After 24 hours of incubation there was small grayish glistening colonies on brain heart infusion agar and non hemolytic colonies on blood agar and no growth on Mac Conkey agar. On gram staining of the isolated colonies showed gram negative cocco-bacilli and biochemical parameters were oxidase, catalase and indole positive. On triple sugar iron slant, there was yellow colour slant and butt. DNA was extracted from the isolated colonies and was subjected to genus identification employing kmt gene specific primers. There was amplification at 460 bp which was corresponding to Pasteurella genus. Further the DNA was subjected to multiplex capsular typing PCR using already available published primers. There was amplification at 760 bp which was corresponding to capsular type B. Thus results revealed that the cause of death of the buffaloes was due to Hemorrhagic septicemia caused by Pasteurella multocida type B.

**AB-25 Expression of recombinant chimeric BP26-BLS protein in *E.coli* system and assessing its potential use as diagnostic antigen for bovine brucellosis**

*M. Nagalingam*<sup>1</sup>, *Thaslim J. Basheer*<sup>1</sup>, *V. Balamurugan*<sup>1</sup>, *Rajeswari Shome*<sup>1</sup>, *S. Sowjanya Kumari*<sup>1</sup>, *G. B. Manjunatha Reddy*<sup>1</sup>, *B. R. Shome*<sup>1</sup>, *H. Rahman*<sup>2</sup>, *Parimal Roy*<sup>1</sup> and *RK Gandham*<sup>3</sup>

<sup>1</sup>ICAR-National Institute for Veterinary Epidemiology and Disease Informatics, Bengaluru, Karnataka 560 064 India

<sup>2</sup>International Livestock Research Institute, New Delhi 110 012 India

<sup>3</sup>ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122 India

**Abstract**

Brucellosis is one of the major zoonotic disease with serious economic implications in animals and human beings caused by *Brucella* spp. Recombinant protein based diagnostics have the advantage of avoiding cross reactivity and no necessity of handling pathogenic *Brucella* spp. cultures in the laboratory. In this study, widely documented BP26 and Brucella lumazine synthase (BLS) proteins' coding sequences were amplified from *Brucella abortus* S99 DNA by individual PCRs and gene products were linked through the DNA sequence coding for the protein linker by another PCR. The final amplified chimeric *bp26-bls* gene sequence was cloned in pET vector and transformed into competent *E.coli* cells. After verifying sequence, chimeric *bp26-bls* recombinant clones were subsequently transformed in to *E.coli* BL21 cells for expression. The chimeric BP26-BLS protein expression was optimized with IPTG concentration and duration of induction. Then the protein was purified by His-tag affinity purification method using Ni-NTA and characterized by SDS-PAGE and Western blot. Indirect ELISA was standardized

using this protein for the diagnosis of bovine brucellosis. Cut off was determined using Receiver Operating Characteristics (ROC) curve and diagnostic sensitivity and specificity were calculated. Recombinant chimeric BP26-BLS protein based ELISA has greater specificity and has a potential to use it as a complement test in diagnosing bovine brucellosis.

**Key words:** Brucellosis, BP26, *Brucella* Lumazine Synthase (BLS), recombinant protein

**AB-26**

**Comparative evaluation of serodiagnostic efficacy of recombinant LLO and PI-PLC-based ELISAs for detection of listeriosis in animals and field Veterinarians**

Rahul D. Suryawanshi\*, S.V.S. Malik, Bhushan Jayarao, S. P. Chaudhari, Emily Savage, Nitin V.

Kurkure, S. B. Barbuddhe, D. B. Rawool

Division of Veterinary Public Health,

Indian Veterinary Research Institute, Izatnagar, Bareilly (UP), India- 243122

Email: [rahulvph@gmail.com](mailto:rahulvph@gmail.com)

The present study for the first time evaluates the serodiagnostic efficacy of two recombinant antigens namely, listeriolysin O (rLLO) and phosphatidyl-inositol phospholipase C (rPI-PLC). Indirect ELISA with the above recombinant antigens was used on samples collected from bovines (n=106), goats (n= 138) and pigs (n=92) having either a history of abortion, emaciation and/or apparently healthy animals. Isolation of *Listeria* was attempted from the blood samples using USDA-FSIS method. Sera samples collected from 221 field veterinarians were also tested employing these recombinant antigens. On screening of test sera by rLLO-based ELISA, antibodies against anti-listeriolysin O (ALLO) were observed in goats (22.46%), bovines (15.10%) and pigs (16.31%). As advocated, after adsorption of positive serum samples with streptolysin O (SLO), the seropositivity for ALLO was marginally reduced ( $p > 0.05$ ) in goats (21.73%) and bovines (10.38%), whereas, in pigs the reduction (5.43%) was significant ( $p < 0.05$ ). Similarly, on screening human sera by rLLO-based ELISA showed significant ALLO titer in 37.56%, which on SLO adsorption reduced significantly ( $p < 0.05$ ) to 13.57%. On the contrary, rPI-PLC-based ELISA revealed higher nonspecific seropositivity for antilisterial antibodies in goats (45.65%), bovines (31.13%) and pigs (8.69%). Further, on comparing the seropositivity with isolation rate, of the 16 animals that were culturally-positive for *L. monocytogenes*, 15 showed ALLO positivity in unadsorbed as well as SLO-adsorbed sera by rLLO-based ELISA, however, rPI-PLC-based ELISA could detect seropositivity in only 5 animals. Moreover, rPI-PLC-based ELISA also showed seropositivity in those animals (7/30)

that were culturally positive for other *Listeria* spp. Likewise, in human sera samples also the higher nonspecific seropositivity (51.58%) was observed. In conclusion, rLLO can serve as a better antigen than rPI-PLC in ELISA for the serodiagnosis of listeriosis in animals and humans; however, prior adsorption of test sera with SLO is required to avoid false positive results.

**Keywords:** ELISA, *Listeria*, rLLO, PI-PLC

**AB-27**

**Pro-inflammatory cytokine and apoptotic gene mRNA levels against lentogenic and velogenic Newcastle disease virus pathotypes in *in-vivo* and *in-vitro* biological systems**

Ranjani Rajasekaran, J. John Kirubakaran, P. Shilpa, M. Vidhya, N. Daniel Joy Chandran

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

Knowledge on the influence of pro-inflammatory cytokine and apoptotic gene mRNA levels in the pathogenesis of Indian field isolates of Newcastle disease virus (NDV) is little. In this study, cytokine mRNA levels were elucidated in spleen of chickens (*in-vivo*) and chicken embryo fibroblast cells (*in-vitro*) infected with lentogenic D58 strain and viscerotropic velogenic D165 isolate respectively until five days post infection (dpi). In spleen of chickens infected with D165, maximum upregulation of pro-inflammatory cytokines (IL-1 $\alpha$ , IL-6, TNF- $\alpha$ ), chemokine (IL-8) and apoptotic gene (Caspase-8) at 3dpi correlated with the onset of severe clinical signs and necrotic histopathological lesions in spleen, proventriculus, intestine and caecal tonsil of chickens. Similarly, in CEF cells infected with D165, upregulation of pro-inflammatory cytokine and apoptotic gene mRNA levels correlated with the appearance of CPE. In spleen of chickens and CEF cells infected with D58, there was comparatively minimal upregulation of pro-inflammatory cytokine and apoptotic gene mRNA levels which did not cause histopathological changes in tissues and CPE formation in CEF cells. In both *in-vivo* and *in-vitro* systems, upregulation of anti-inflammatory cytokine IL-10 showed inhibitory effects on the mRNA levels of pro-inflammatory cytokines. Thus, this study reports variation in the cytokine mRNA levels elucidated in response to two different pathotypes isolated from India and associates the same with the clinical signs and pathological lesions produced during the course of ND.

**AB-28**

**EVALUATING THE DIAGNOSTIC POTENTIALS OF  
RECOMBINANT IMMUNODOMINANT PROTEIN LIPL32  
OF PATHOGENIC LEPTOSPIRA IN BOVINES**

T.V.Meenambigai, Sworna Kumari, Gautami Sadekar, G.Balakrishnan, and K.Kumanan  
Central University Laboratory  
Centre for Animal Health Studies  
Tamil Nadu Veterinary and Animal Sciences University  
Madhavaram Milk Colony, Chennai, 600 051, Tamil Nadu.

There is a need for developing novel diagnostics for Leptospirosis, an emerging infectious disease that imparts a large disease burden in domestic animals. LipL32 is the most abundant immunodominant protein of pathogenic leptospira. In the present study the full length gene of LipL32 of *Leptospira interrogans* serogroup autumnalis was cloned and expressed in pRSET A vector and confirmed by western blotting. The developed recombinant LipL32 protein was utilized to evaluate its serodiagnostic potentials in detection of bovine Leptospirosis by indirect ELISA. The efficacy of the assay was compared with the gold standard Microscopic agglutination test (MAT). A total of 112 bovine serum samples collected from different districts of Tamil Nadu were screened for the presence of leptospiral antibodies. The sensitivity and specificity of recombinant LipL32 based ELISA as compared to MAT was 89.62 and 93.54 per cent. These findings indicate that the antibody response to this immunodominant protein is efficacious and could be used as a specific marker for serodiagnosis of bovine Leptospirosis.

**AB-29**

**Application of Recent Diagnostic Techniques for Detection of  
Cysticercosis in Pig and Taeniasis in Human Population**

Vilas M. Vaidya\*, Ravindra J. Zende, Ashish M. Paturkar, Rupesh N. Waghmare  
and Sujata S. Bhave

Department of Veterinary Public Health, Bombay Veterinary College, Maharashtra Animal and Fishery  
Sciences University, Mumbai, India.

\*Presenting author: [vilasmvaidya@gmail.com](mailto:vilasmvaidya@gmail.com), Mobile No: +91 9967280577

Cysticercosis/taeniasis is one of the neglected parasitic zoonoses in the developing countries like India. In the present study, the recent diagnostic methods like Polymerase Chain Reaction (PCR) assay and

sequencing, Enzyme Linked Immunosorbent Assay (ELISA), Flow Through Assay (FTA) and Western Blotting (WB) were used to detect cysticercosis/taeniasis in clinical samples of pigs and humans. These samples were procured from the different states such as Maharashtra, Goa and Assam. The study revealed that out of 16,354 pigs screened by Post Mortem examination, 129 pigs (0.79%) were found to be harboring cysticercosis. By ELISA, FTA, WB and PCR, the prevalence of cysticercosis in pigs was found to be 5.70% (20/351), 13.39% (47/351), 5.31% (12/226) and 0.9% (20/2228), respectively. In humans, the prevalence of taeniasis was 3.15% (39/1238) by ELISA and 2.04% (2/98) by PCR assay. The PCR products of 7 positive pig cyst samples were sent for sequencing to Bio Innovations, Mumbai, India. The resulting nucleotide data was submitted to gene bank and got the accession number for one cyst sample of pig from NCBI as KR701908. It is recommended that the postmortem inspection is useful for the diagnosis of cysticercosis in slaughtered pigs but the serodiagnosis is an important tool for the identification of disease status at ante-mortem level. The application of recent diagnostic tests will help for proper diagnosis of the disease so that effective preventive and control measures can be implemented to minimize the incidence of disease.

**T8**

**Bioinformatics And Molecular  
Epidemiology**

Prasad Minakshi<sup>1\*</sup>, Upendera P Lambe<sup>1</sup>, Basanti Brar<sup>1</sup>, Ikbali<sup>1</sup>, Koushlesh Ranjan<sup>2</sup> and Jyoti Misri<sup>3</sup>

<sup>1</sup>Department of Animal Biotechnology, LUVAS, Hisar, Haryana, India-125004

<sup>2</sup>Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India, 250110

<sup>3</sup>Division of Animal Science, Krishi Bhavan, New Delhi - 110 114

The infectious diseases are one of the greatest threats to the animal and human population living in the developing world. Some infectious agents can also be used as a part of biological warfare agent (MacKenzie et al., 2015). These diseases have capacity to initiate in a small area and the spread very fast to the rest of the world and cause a heavy pandemic situation for example, avian influenza pandemic. Such diseases infect large masses of population and may lead to loss of lives and also cause huge economic losses (Casco et al., 2011). Therefore, the best way to control these diseases is by diagnosing it at a very primary level and taking necessary precautionary measures so as to avoid the spread.

Since last few years, the diagnostic approach has changed from tedious molecular biological techniques, to easy and rapid diagnostic techniques. These techniques do not require sophisticated laboratories, expert personnel, resulting in very cheap diagnostic approach. Thus, it can also be performed at the field level where the patient is present and get the results then and there itself. Hence they are also called as pen side test or lab on chip diagnostic assays. These techniques have greatly influenced the diagnostic approach in the veterinary as well as medical field. Especially in the developing countries such as India, where the laboratory services are not available at the village level, these techniques have facilitated the disease diagnosis the most. Nanotechnology has played a crucial role in the designing of diagnostic assays. The nano materials have many versatile properties such as optical properties, piezo-electric properties, thermal properties, electro-chemical properties, enzyme mimicking properties etc. By exploiting these properties, the workers have designed different approaches for diagnosis.

A biosensor is a compact analytical device with a ligand-specific bio-recognition element, e.g. antibody, enzyme, receptor, nucleic acid, aptamers, peptide/protein, lectin, cells, tissue or whole organisms, immobilized on a sensor surface integrated directly or indirectly with a signal conversion unit called transducer. The nanotechnology is a new emerging field which has contributed the most for the development of the biosensor technological approach. The nano materials exhibit the physico-chemical properties such as electrochemical (Rathee et al., 2016) chemical luminescence (Roda et al., 2016) Optical (Tereshchenko et al., 2016) which are completely different than the actual material. These properties are generally exploited for the designing of biosensors. There are many reports being said that the nanoparticles have property of mimicking the properties of certain enzymes, thus these particles can be used to design the immuno assays. These days even smartphone based biosensors are being developed (Diming et al., 2016; Cevenini et al.,

2016; Roda et al., 2016). In this review, the Nano-diagnostic biosensors for the detection of pathogens which are human and veterinary importance are discussed. There are various approaches being used for the development of nano-diagnostic assays. The nano diagnostic can be classified into two categories, in-vitro and in-vivo. In-vivo is the diagnostic imaging techniques in case of live animals. On the other hand, the in-vitro techniques which include, different antibody based immune assays and different nucleic acid based hybridization assays coupled to the nanoparticles.

**Nanotechnology based immune assays:** These are the type of assays which can detect the substrate without labelling the biomolecule with any enzyme. The Ag-Ab reaction is detected by exploiting diverse properties of nanoparticles. In olden days, immuno sensors utilized the very specific binding affinity of antibodies for a specific compound or antigen. The specific nature of the antibody-antigen interaction is similar to a lock and key hypothesis in that the antigen will only bind to the antibody if it has the correct conformation. Binding events result in a change that in combination with a tracer, such as a fluorescent molecules, enzymes, or radioisotopes, that can generate a signal for detection of agent (Marazuela and Moreno, 2002). But due to advancements in nanotechnology, the need of labelling the biomolecule with enzyme or radioisotope is not required. These are replaced by Nano-particles (Tianshu et al., 2015) there are several types of antibody/antigen interactions detection systems are available which are currently used for detection disease as well as biological molecules.

<b>Agent</b>	<b>Nanoparticle</b>	<b>Detection Limit</b>	<b>Reference</b>
HIV-1	GNP	0.24pg/ml	Xiuli et al., 2016
HIV MYO	GNP	1.56ng/ml	Ruihua et al., 2016
Mycoplasma pneumoniae	AF-647	0.3830pg/ml	Liming et al., 2016
TB	GNP	100pg/ml	Corstjens et al., 2016
Prostate specific Ag	Photon up-converting NPs	41ng/liter	Juntunen et al., 2016

**Surface plasmon resonance (SPR) based sensors:** It was first demonstrated for biosensing in 1983 by (Liedberget al.1983). Nanoparticles display unique physical properties due to their nano-size. Metallic nanoparticles have intense absorbance and scattering properties due to Surface Plasmon Resonance (SPR). When an oscillating electric field interacts with the free conductive band of electrons at the surface of the AuNP, collective dipolar oscillation of the electrons occurs. This is called Surface Plasmon<sup>15</sup> (Radwan et al.,2009) Since then it has been extensively explored and has gradually become a very powerful label-free tool to detect the pathogens (Pattnaik, 2005; Van, 2001 and Homola, 2003). In this system a surface plasmon wave (SPW) is a charge density oscillation that occurs at the interface of two media with dielectric

constants of opposite signs, such as a metal (gold or silver) and a dielectric. This technique was successfully used for the detection of viruses and bacteria (Boltovets et al., 2004).

<b>Organism</b>	<b>Nano Particle</b>	<b>Type of detection</b>	<b>Ag/Antibody</b>	<b>Reference</b>
Adeno virus	Triangular AuNPs	Raman Scattering	Polyclonal	Chia et al., 2011
H1N1	Silver NPs	Fluorescence OPDA	Monoclonal	Yanxia et al., 2014
Encephalomyocarditis virus	Triangular AuNPs	Raman Scattering	Polyclonal	Chia et al., 2011
Salmonella	AuNPs	Microfluidic	Polyclonal	Giyoung et al., 2015
Duck Hepatitis virus	Silicon wafers	Ellipsometry Imaging	Polyclonal	Cheng et al., 2011
HIV	Fe-Au shell	Amperometric	Glycoprotein 160	Ning et al., 2009
Salmonella pullorum	Blue Silica & MNPs	Sandwich assay	Polyclonal	Qian et al., 2016
Salmenella	Quantum dots	Magnetic sensor	Polyclonal	Giyoung, 2015
Human T lymphotropic virus	GNPs	Immunoaffinity assay	Monoclonal	Randol et al., 2016
Hepatitis B Virus	GNPs	Immunoaffinity assay	Monoclonal	Randolph et al., 2016
Orchid Virus	Gold Nano rods	SPR	Label free	Hsing et al., 2014
General Virus	GNP Chip	Fluorescence	Fluorescence Microscopy	Yen et al., 2016
H1N1, H5N1, H7N9	ZnO Nano rods	PDMS	Electrochemical	Ji-Hoon et al., 2016
H1N1	GNPs	Micro fluidic system	Aptamers	Tseng et al., 2016

**Nanozymes:** Enzymes are very popular bioreceptors due to their specific binding capabilities and catalytic activity. Analyte recognition is enabled through several possible mechanisms such as: enzyme converting the analyte into a product that is sensor-detectable, detecting enzyme inhibition or activation by the analyte

(Pohanka, 2013) or monitoring modification of enzyme properties resulting from interaction with the analyte. The main reasons for the common use of enzymes in biosensors are: its ability to catalyze a large number of reactions, potential to detect a group of analytes (substrates, products, inhibitors, and modulators of the catalytic activity) and suitability with several different transduction methods for detecting the analyte. It is important that enzymes are not consumed in reactions; the biosensor can easily be used continuously. The catalytic activity of every enzyme also allows lower limits of detection compared to common binding techniques. However, the sensor's lifetime is limited by the stability of the enzyme. (Lucie et al., 2011). Recently there are few advancement in these types of biosensors. The aggregation of insulin causes type 2 diabetes. To detect this condition a biosensor Nano-cage-mediated refolding of insulin by PEG-PE micelle is developed (Xiaocui et al., 2016). Cholin a breast cancer marker is detected form serum by nano interface technology (Thiagarajan et al., 2016) similar way, blood glucose level is monitored by a noninvasive saliva biosensor (Wenjun Zhang et al., 2015). Aptamer based GnRH biosensor in equine urine is demonstrated by (Richards et al., 2016). There are several types of enzymatic interactions detection systems are available which are currently used for detection of agent associated with health concern.

Compound	Nanoparticle	Type of sensor	Detection	References
Norepinephrine	FeMoO <sub>4</sub> rods	Cyclic voltammetry	Withoug modification	Kunda et al., 2016
IFN Gamma	AuNP	ITO	HPR-Ab	Yaru, 2016
Protein estimation	MNPs	Colorimetric	Punctates	Gero et al., 2016
IL-3	AuNP	iPCR	Polyclonal Ab	Lucie et al., 2011
Stem cell factor SCF	GNP	iPCR	Polyclonal AB	Lucie et al., 2011
Nano Mass	Graphene films	Ultrasound frequency shift	Piezoelectric	Li & Wang, 2016

**Hybridization based detection:** Biosensors employ nucleic acid interactions can be referred as genosensors. The recognition process is based on the principle of complementary base pairing, such as A:T and C:G in DNA. If the target nucleic acid sequence is known, complementary base sequences can be synthesized, labeled, and then immobilized on the sensor. The hybridization probes then base pair with the target gene, generating an optical signal. The favored transduction principle employed in this type of sensor has been optical detection (Marazuela and Moreno, 2002). There are several types of Nucleic acid (DNA/RNA) interactionsdetection systems are available which are used for detection of different viruses or other agents are associated with the disease.

<b>Organism</b>	<b>Nanoparticle</b>	<b>Sesnsor type</b>	<b>Nucleic acid</b>	<b>Reference</b>
Arabis Mosaic Virus	SMP	Magnetic	RNA	Ning et al., 2014
Lily symptomless Virus	SMP	Magnetic	RNA	Ning et al., 2014
HSV	SMP	Magnetic	RNA	Ning et al., 2014
GYSVD	SMP	Magnetic	RNA	Ning et al., 2014
HBV	AuNP	Barcode amplification	DNA oligos	Wang et al., 2010
HBV	MNPs	Hybridization	DNA oligo	Wang et al., 2010
Dengu	3D Graphene	Impedimetric sensor	RNA	Seon et al., 2016
Canine adeno	GNPs	Microarray chip	DNA	Yadav et al., 2015
Salmonella	GNPs	LFICA	16s rRNA	Cheng et al., 2013
HBV	Cu Nano cluster	Colorimetry	DNA	Xiaoxia et al., 2016
Influenza virus	CdZnTeS Quantum dots	Fluorescence	Molecular beacons	Oluwasesan et al., 2016
White spot syndrome virus	GNPs	LAMP	DNA oligo	Yortyot et al., 2013
Porcine epidemic diarrhea	GNPs	Nano RT-PCR	RNA	Wanzhe et al., 2015
Influcnza	Sugar chain GNP	RT qPCR	SYBR green	Yasuo et al., 2015
HCV	GNPs	Hybridization	5'UTR DNA	Sherif et al., 2010

## **Conclusion**

Nanomaterials offer a vast number of breakthroughs such as cost effective, lower risk to consumers and faster approach that will further enhance the clinical aspect of veterinary sciences in future and conceived that bacterial infections can be eliminated in the patient within minutes, instead of using treatment with antibiotics over a period of weeks. Nanotechnology has found its way into the food industry to improve food shelf life, safety and quality control. In coming years it can be expected that nanotechnology

may practically apply in artificial creation of cells, tissues and organs. The artificial cells can be used in replacement of defective cells and organs, especially in metabolic disorders. Nanotechnologies have power to extent the modern molecular diagnostics to personalized medicine and therapeutics. Such techniques have been used in the field of pathogen detection, DNA detection assay, biomarker discovery and cancer diagnosis. Nano medicine also has important role in future therapeutics as well as diagnostic assays. Although nanotechnologies have several applications and benefits, it is still in the early stages of its development and yet to apply throughout the world for routine diagnostics and therapeutics approaches.

### References:

- Giyoung et al., 2015 Giyoung K, Ji HM, Chang YM, Jongguk L (2015) A microfluidic nano-biosensor for the detection of pathogenic *Salmonella*. *Biosensors and Bioelectronics*. 67:243–247.
- Jiang X, Chen K, Han H (2011) Ultrasensitive electrochemical detection of *Bacillus thuringiensis* transgenic sequence based on in situ Ag nanoparticles aggregates induced by biotin-streptavidin system. *Biosens. Bioelectron*. 28: 464-468.
- Randolph et al., 2016 Randolph DG, Nancy JM, Dickson K, Saher M, Robert JT, Chad AO (2016) Enhancement of pathogen detection and therapy with laser-activated, functionalized gold nanoparticles. *Applications of Nanobiomaterials*. 6:229–260.
- Roda A, Mirasoli M, Micheline E, Massimo D F, Zangheri M, Cevenini L, Barbara , Patrizia S (2016) Progress in chemical luminescence-based biosensors: A critical review. *Biosensors and Bioelectronics*. 76:164–179.
- Tseng et al., 2016 Tereshchenko A, Bechelany M, Roman V, Volodymyr K, Valentyn, Nikolay S, Yakimova R (2016) 677 Optical biosensors based on ZnO nanostructures: advantages and perspectives. A review. *Sensors and Actuators B: Chemical*. 229: 664–671.
- Yanxia L, Mei H, Bin Q, Zhenyu L, Yiting C, Zongwei C, Guonan C (2014) Highly sensitive fluorescent immunosensor for detection of influenza virus based on Ag autocatalysis. *Biosensors and Bioelectronics*. 54:358–364

### **T8-02**    **Role of National Animal Disease Referral Expert System (NADRES) as animal health support system in controlling livestock diseases in India**

Dr. Parimal Roy

Director

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI),  
Post Box No.6450, Ramagondanahalli, Yelahanka, Bengaluru - 560 064, Karnataka, India

Human population and urbanization are increasing and demand of food is also increasing. The need for efficient food production has never been saturated. Livestock production and agriculture are

intrinsically linked, each being dependent on the other, and both crucial for overall food security. Half the world's food comes from farms that raise both. The reducing availability of land and water, soil degradation and climate change greatly effecting the harvest and thereby demand for animal protein is increasing. The vast Indian livestock resource has immense potential to provide livelihood to two third of rural population and contribute in generating income to strengthen economy of India, specially the rural India. Livestock sustainability and constant maintenance of good health will largely contribute in increasing farmers' income and supply of good quality animal protein to reduce malnourishments among humans. Farmers' income can be increased by maintaining healthy livestock in several ways:

- (i) Healthy livestock will increase the availability of food with quality nutrients from animal origin to reduce malnourishments
- (ii) Healthy livestock have a higher productivity and less variable production than diseased livestock to meet the market demands and increase the earnings of the farmers.
- (iii) Healthy animals do not transmit infections (for zoonotic diseases) to humans and expenditure towards treatment can be saved.
- (iv) Sick animals do not fetch good price on sale.

Livestock sector also offering employment opportunities to unemployed youths. Animal husbandry can easily be adopted by tribal and rural women to augment their income and it acts as a supplementary and complementary enterprise significantly supporting the socio-economic growth of country and contributing 4.1% in National Gross Domestic Product (GDP).

Different state governments are trying to augment production in this sector by introducing a wide range of schemes to improve the quality of livestock through cross-breeding programmes, resulting in increased susceptibility of these livestock to various diseases including exotic diseases. With increases in globalization, animal movements and trade coupled with climate change has potential to cause diseases. Disease outbreaks or even rumors of an outbreak can result in widespread consumer alarm, food safety concerns, disruption of trade, high economic losses and severe effects on incomes, not to mention the human cost of illnesses and deaths arising from animal disease.

ICAR -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) has an interactive, dynamic online animal disease forecasting and forewarning system called NADRES (National Animal Disease Referral Expert System) with overall aim to improve animal health and alert for taking preventive measures against the diseases for which forewarning is given. This early warning

creates preparedness to control important livestock diseases and improve veterinary health care system of the country for the benefit of farmers. Early warning of disease incidence or outbreaks and the capacity of prediction of risk of spreading disease to new areas is an essential pre-requisite for the effective containment of epidemic of animal diseases, including zoonosis. Disease information data are collected from different states and UTs through AICRP on ADMAS centres, analyzed by considering base line data and environmental parameters and possible risk of livestock diseases are forecasted two months in advance for the benefit of the farmers and other stakeholders to assist in prevention and control of livestock disease threats, including zoonosis. For Zoonotic disease events, alerts of animal outbreaks or incidence can provide warning so that human surveillance could be enhanced and preventive action can be taken. Sharing assessments of an outbreak will enable a joint and comprehensive analysis of the disease events and its possible consequences. Joint dissemination of information will furthermore allow harmonized action by the central and state animal health departments, ICAR-NIVEDI, regarding disease control strategies.

**Disease Outbreak data base:** Database on livestock disease outbreaks are collected regularly through the networks of AICRP on ADMAS with 31 centers across the country, once data received they are entered into NADRES database in a double data entry validation mode to achieve to zero error entry.

**Risk factors database :** Risk factors such as weather parameters from different sources includes the monthly precipitation(mm), sea level pressure (millibar), minimum temperature ( $^{\circ}$ C), maximum temperature( $^{\circ}$ C) , wind speed (m/s), vapour pressure (millibar), soil moisture(%), perceptible water(mm), potential evaporation transpiration (mm), cloud cover(okta) etc., extracted from National Centre for environmental prediction (NCEP), Indian Meteorological Department(IMD), National Innovations Climate Resilient Agriculture (NICRA) and other sources. The remote sensing variables like Normalized Difference Vegetative Index (NDVI) and Land Surface temperature were extracted from MODIS/LANDSAT/LISS III or IV satellite images. The livestock population and densities were extracted from Livestock census 2012.

**Statistical Model:** A multivariate logistic regression model is used to predict the probability of disease risk by considering weather parameters, remote sensing variables and livestock population or densities. The goal of logistic regression is to find the best fitting (yet biologically reasonable) model to describe the relationship between dichotomous characteristic of interest (disease outbreak) and a set of predictors (weather parameters, RS variables and demographics).

#### **Livestock disease status:**

In India, the occurrence of Foot and mouth diseases were found more frequent over the last 10 years with the total outbreak of 7313 and Meghalaya state has reported highest number of outbreaks (n= 1572). But the incidences have reduced compared to earlier years. Prevalence of Hemorrhagic septicemia

is observed to have reduced over the years with total outbreak of 3561 and Karnataka has reported more number of outbreaks. The events of Black quarter was observed through the years. Incidence of Peste des petits ruminants was noted to be consistent and highest reporting of this disease was done from West Bengal. The disease burden for BT was reduced but increased again, needs more attention. The occurrence of Anthrax disease is continued to be recorded over the years. Frequency of Sheep and goat pox remains same whereas the Incidence of Swine fever has reduced over last 5 years. The reporting system is very much useful for preparedness to control the outbreaks, study of strain variation and taking up further work to combat emerging threats.

Among zoonotic diseases anthrax was found to be predominating, control measures for all the zoonotic diseases need to be strengthened. Good quality anthrax vaccines are available but awareness and effective control strategy need to be formulated. Disease surveillance and health monitoring are essential to maintain healthy livestock and improve farmers wealth.

**T8-03**

**Immunobiotechnological contributions to our understanding of epidemiology and evolution of animal origin enteric viral pathogens**

Yashpal S. Malik

ICAR-National Fellow

Indian Veterinary Research Institute,  
Izatnagar 243122, Uttar Pradesh (India)

Infectious diarrhea is a widespread problem and associated with colossal losses worldwide. Its impact is much higher among < 5 years children and is reported to cause ~800,000 (~10.5%) of global deaths in this age group during 2015. As per the estimates, approximately two-third of these deaths are documented from South Asia and sub-Saharan Africa. Amongst various causes of infectious diarrhea the viral aetiology is of much more significance. Amid enteric viruses, rotavirus is the most common etiological agent affecting neonates of all species including humans. Nonetheless, list is being expanded with the discovery of several other viruses which can easily traverse through human and animal population with inferior immune status by virtue of malnutrition and pose serious challenge to mankind. High mutation rates in some viruses like picobirnavirus and rotavirus enable enteric viruses to overpower the outdated preventive measures and emerge as a new type with entirely new genetic makeup. Researchers are also highlighting extra-intestinal detection of enteric viruses for example, rotavirus causing encephalitis, astrovirus associated with meningitis and disseminated infections etc. Moreover, to stop airborne spread of enteric virus such as Noro- and Rotavirus is another new type of challenge being experienced in developed countries and is closely related to urban lifestyle. To better understand and tackle such existing and emerging enteric viruses there is an urgent need to acquire more scientific knowledge of these pathogens

including their reservoir hosts, immunopathobiology, genetic diversity and the development of state-of-art diagnostic tools. As animal hosts are known to play important role in maintenance and possible transmissibility of enteric viruses to humans, there is always a need felt to assess enteric infections in animals. According to the last livestock census pig population of country is 10.29 million. Pigs are known to harbour number of viruses which causes diarrhea.

Since 2011, we are working on screening porcine population for enteric viruses, specifically rotavirus, picobirnavirus, astrovirus, porcine calicivirus, kobuvirus. Worldwide, rotavirus (RV) is considered as one of the main causes of diarrhea affecting young animals of different host species. Classification of rotaviruses into nine distinct serologic groups designated A to I is based on the capsid VP6 protein. Group A rotaviruses (RVA) are most often responsible for diarrhea in piglets. Due to the difficulty in diagnosis of diarrhea caused by other rotavirus groups, non-group A rotaviruses are rarely reported. Since the discovery of first group A rotaviruses in the faeces of diarrheic piglets in the 1970s, several serologically distinct non-group A rotaviruses, such as RVB, RVC, RVE and RVH, have been encountered around the world. Still, little attention has been paid on the circulation of rotaviruses of different groups in Indian swine population. In contrast, prevalence studies in many countries around India reported relatively frequent occurrence of RVs infections. At this moment it remains unclear to which extent RVA, RVB, RVC, RVE and RVH are circulating in the Indian swine population. During our survey of enteric viruses in porcine population from various NE states for RVA, RVB, RVC and RVH, we found high prevalence of RVA as well as RVC but couldn't see RVB and RVH in these states. Recently, Picobirnavirus (PBV) has emerged as a potent cause of neonatal diarrhea. It is the single genus identified under newly identified family *Picobirnaviridae* and carries a dsRNA genome. Based on RNA polymerase gene, PBVs have been further classified into genogroups. The rearing of pigs in the organized farms or as a backyard farming in the close vicinity to human population also possess a possible chances of viral zoonosis. According to earlier reports pigs are considered to be the potent carriers of PBV infection. We have plotted a comprehensive picture of the PBV strains circulating in the porcine population of different North Eastern States of India. State-wise highest prevalence and distribution of the PBV showed variability in susceptibility of the population, ranging from 48.05% to 9.09%. The multiplex PCR based genogrouping confirmed genogroups I (GG-I) as predominant PBVs in comparison to genogroups II (GG-II). Taking into account the outcome of the present study, a strict national surveillance program for PBV and other enteric viruses in the pig population is necessitated. The sequenced RdRp gene have been further analysed for the molecular characterization of these PBV strains circulating in porcine population in India. Furthermore, in-depth assessment of the impact of these viral infections on the animal health and production becomes indispensable and thus necessitates the development of precise diagnostics for apprising the diagnostic algorithms of enteric pathological conditions. During the presentation, the research leads of our laboratory, important differentials of diarrhea in the young piglets and the impact of co-infections of rotavirus and other viruses will be presented.

**T8-04**

## **An overview of Avian Mycoplasmosis**

K.Manimaran

Assistant Professor,

Central University Laboratory,

Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony,

Chennai-51, Tamil Nadu.

E-mail:maranvet@yahoo.com

### **INTRODUCTION**

Poultry farming has emerged as one of the fully viable self-sufficient and sophisticated industry contributing significantly to the Indian economy in terms of value of the products, employment generation and it is improving the living standards of people. This tremendous achievement in the poultry production has been brought mainly by the adoption of improved methods of breeding, nutrition, management and health coverage programmes. However, with the evolution of superior genetic stock of poultry, intensive rearing and selective breeding policies for higher production, the birds are put to all sorts of stress beyond the limit of their normal physiological tolerance, making them more vulnerable to many diseases. Among the various prevailing diseases, Avian Mycoplasmosis is one of the most serious diseases affecting poultry industry in the world. Avian Mycoplasmosis is an economically important and highly contagious disease of chickens, turkeys, ducks and geese, caused by the bacterium *M. gallisepticum* (MG), *M. synoviae* (MS), a Gram negative, pleomorphic organism. Avian mycoplasmosis mainly causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. Though there are many *Mycoplasma spp.* affecting the poultry, only *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae* have been established as pathogens. They are very small prokaryotes (0.25 – 0.5  $\mu$ m). One of their major characteristics is the lack of cell wall; they are limited only by a plasma membrane. Pathogenic mycoplasma species such as MG and MS tend to grow slowly (after 3-10 days at 37°C).

### **ECONOMIC LOSSES DUE TO AVIAN MYCOPLASMOSIS**

Avian mycoplasmosis causes substantial economic losses directly through embryo mortality (5-20%), chick mortality (5-10%), slow growth (8-25%), condemnation of carcass (5-10%), and production drop (8-20%). Indirect losses due to Avian mycoplasma is increase of sensitivity to *E.coli* infection, *Avibacterium paragallinarum*, Infectious bronchitis, Newcastle disease, vaccine viruses, faulty management and depression of immune system.

### **ETIOLOGY**

Four species of Avian Mycoplasmas are known to cause disease in domestic poultry. They are

1. *Mycoplasma gallisepticum*
2. *Mycoplasma synoviae*
3. *Mycoplasma meleagridis*
4. *Mycoplasma iowae*

### SUSCEPTIBLE HOST AND AGE

Avian Mycoplasma affects chickens and turkeys worldwide, causing the most significant economic losses. Infection also occurs in pheasants, partridges, peafowl, pigeons, quail, ducks, geese, and psittacine birds. Avian mycoplasmosis has been reported in younger birds of 4-8 weeks age and the incidence is 26% in India.

### PREVALENCE

Avian Mycoplasmosis was recorded in USA (37%), Nigeria (32.4%), Pakistan (20%), Serbia (19%), Lebanon (7%), Turkey (5%) and Israel (4%). In India, Avian Mycoplasmosis has been recorded in all the states such as Jammu and Kashmir (61%), Himachal Pradesh (71%), Manipur (78.8%), Tripura (59%), Nagaland (83%), Assam and Meghalaya (77%), Punjab (76%), Haryana (72%), Bihar (57%), Uttar Pradesh (75%), Madhya Pradesh (64%), Gujarat (87.5%), Maharashtra (14%), Goa (78%), Kerala (37%), Karnataka (17%), Andhra Pradesh (30%), Tamil Nadu (30%) and Rajasthan (39%). However, the highest prevalence has been reported in Gujarat, Nagaland, Assam and Punjab.

### HABITAT

Mycoplasmas are very fragile organisms, lacking the protective cell wall of other bacteria; therefore they are highly susceptible to heat, cold, dehydration and disinfectants. As a result, mycoplasmas survive very poorly outside of their host. In a hot and dry environment, the survival period may extend.

### SURVIVAL OF AVIAN MYCOPLASMA OFF THE HOST

	<b><i>Mycoplasma gallisepticum</i></b>	<b><i>Mycoplasma synoviae</i></b>
Feathers	2-4 days	2-3 days
Cotton	12 hours to 4 days	-
Hair	8 hours to 3 days	-
Rubber, Straw	2 days	-
Ear, Nose	4-24 hours	12 hours
Other Materials	-	8-12 hours

## MODE OF TRANSMISSION

1. MG is transmitted vertically within some eggs from infected breeders to progeny.
2. MG is transmitted horizontally via infectious aerosols and through contamination of feed, water, environment, and by human activity on fomites.
3. Infection may be latent in some birds for days to months, but when birds are stressed horizontal transmission may occur rapidly.
4. Stressors such as viral infections, vaccinations with live viruses, cold weather and crowding also facilitate infection, disease and transmission.
5. With MG, egg transmission occurs only during the stage of active infection of the abdominal air sac which is in direct contact with the ovules. Thus, if this lesion heals before egg production starts, the egg transmission rate of MG is minimal.
6. With respect to MM, the infection comes primarily from the vagina with contaminated semen and thus there is a constant insult of the hen during the egg laying period.
7. The egg transmission rate of MG is from 1-5% and with MM, around 25% for the entire production cycle. It is because of these differences in the infection and transmission patterns that MM is considered to be much more difficult to eradicate than MG. The mechanism of egg transmission of MS is not known.

## PATHOGENICITY

Mycoplasma escapes from the host defensive immune mechanisms using the high frequency variation of the surface proteins. It also “hides” from host defences by invading cells despite the strong immune reactions. MG colonises the host cells after a successful attachment which is considered the most important virulence factor. Epithelium of the conjunctiva, nasal passages, sinuses, and trachea are most susceptible to initial colonization and infection. However, in severe acute disease, infection may also involve the bronchi, air sacs, and occasionally lungs. Once infected, birds may remain carriers for life. There is a marked interaction (polymicrobial disease) between respiratory viruses, *Escherichia coli*, and *M. gallisepticum* in the pathogenesis and severity of chronic respiratory disease.

## DISEASE MANIFESTATION

1. *Mycoplasma gallisepticum* mainly causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys

2. *Mycoplasma synoviae* causes synovitis in chicken and turkeys which may be of acute or chronic in nature. Moreover, it also causes airsacculitis concurrently with Newcastle disease and Infectious bronchitis.
3. However, *M. iowae* has also been reported to have pathogenic effect on fox chicken and turkeys.

## CLINICAL SIGNS

MG infections vary from asymptomatic to severe, depending upon the infecting strain and other factors such as the infection with other respiratory pathogens like IBV, NDV and *E.coli*. Infected birds usually develop symptoms like

Depression

Rales

Coughing and Sneezing

Nasal discharges

Swelling of infra orbital sinuses

Conjunctivitis

Dyspnoea

Decreased weight gain, feed efficiency and egg production

*Mycoplasma synoviae* usually causes subclinical upper respiratory tract infection in multiage layer flock leading to mortality ranging from 1 to 10% and morbidity ranges from 2 to 75% with 5 to 15% being usual.

*Mycoplasma synoviae* causes synovitis in chicken and turkeys which may be of acute or chronic in nature. Moreover, it also causes airsacculitis concurrently with Newcastle disease and Infectious bronchitis.

However, *M. iowae* has also been reported to have pathogenic effect on chicken and turkeys.

## GROSS LESIONS

The most consistent gross lesions associated with *M. gallisepticum* (MG) infection in chicken are the presence of mucoid to mucopurulent exudates in the trachea, bronchi, air sacs and nasal passages.

The most important pathological lesion is cloudy appearance of one or more air sacs. Cheesy material is present in the air sacs in turkeys.

*M. synoviae* causes airsacculitis and synovial lesions at hock region, footpads and keel bursa.

## SAMPLES TO BE COLLECTED

**From live birds**, swabs may be taken from choanal cleft, oropharynx, oesophagus, trachea, eyes, cloaca and phallus in PPLO broth

**In the case of dead birds**, samples may be taken from the nasal cavity, infraorbital sinus, trachea or air sacs. Exudates can be aspirated from the infraorbital sinuses and joint cavities in PPLO broth.

## DIAGNOSIS

1. Diagnosis of Avian Mycoplasmosis is based on **History** like Poor hatchability, Embryo mortality, Decreased egg production and Decreased growth rate.

2. Based on the **Pathological** lesions like airsacculitis, synovitis and exudates in the trachea, air sacs and lungs.

3. **Isolation and Identification** of avian mycoplasma following inoculation in PPLO medium: *M gallisepticum* should be confirmed by isolation from swab samples of infraorbital sinuses, nasal turbinates, choanal cleft, trachea, air sacs, lungs, or conjunctiva. Primary isolation is made in mycoplasma medium containing 10%–15% serum (PPLO medium)

### 4. Identification

- i) Fried egg colonies can be seen on PPLO agar medium.
- ii) Cultural character like change of PPLO medium colour from pink to yellow colour.
- iii) On Giemsa staining, the avian mycoplasma appears to be pleomorphic.
- iv) Based on the Growth inhibition test and Digitonin inhibition test.

### 5. Serological tests

Serological tests have also been used for the detection of antibodies against avian mycoplasma and antigenic identification.

The serum plate agglutination test (SPA) gives the best results with fresh and clear serum. It has the advantage of rapidity, simplicity, high sensitivity, low specificity, reproducibility and it can be used as a screening test. For doing this test, sera are collected from a flock. If not tested immediately, sera are stored

at 40 C and not frozen. The test should be carried out at room temperature. This test detects primarily IgM, the first antibody produced in MG or MS infection. It can detect infection within 5-7 days (after infection). Also, it is very cheap and easy to perform. The most serious disadvantage is that the test may have non-specific or false-positive reactions. Also it is common for a flock to show positive SPA reactions for MG when it is infected with MS or vice-versa. This is because MS and MG share common antigens. This makes it difficult to determine if a flock is infected with MS or MG, or both, if only the SPA is used.

For all these reasons, another test must be done to confirm the avian mycoplasma infection by using HI or ELISA. The HI titres showing more than 1:80 or above are considered to be positive for avian mycoplasma and titres of 1:40 are strongly suspicious. Sera giving non-specific reactions to the RSA test do not usually give a positive reaction in the HI test on using live HA antigen. Positive SPA reactions can be confirmed by the HI test with sera taken after the first 2-3 weeks of infection. However, the HI test tends to be strain specific and therefore may lack sensitivity. The **Haemagglutination Inhibition test (HI)** is more time consuming and expensive than the SPA test but it is more specific and accurate.

Samples of serum should not be frozen before use in SPA tests. They should be free from haemolysis and contamination to avoid nonspecific reactions. The use of inactivated vaccines for other diseases may result in nonspecific reactions. Samples should be tested as soon as possible (within 72 hours) because mycoplasma antibodies

may deteriorate on storage. Sera may be inactivated in a water bath at 56°C for 30 minutes.

**6.** Based on the **Molecular techniques**, **PCR** can also be used for detection of *M. gallisepticum* DNA using swabs taken directly from infected sites or after growth in culture.

## **DIFFERENTIAL DIAGNOSIS**

*Mycoplasma gallisepticum* infection must be ruled out whenever sinusitis or respiratory disease is present in turkeys, although MG infection may be confused with **Avian influenza, Infectious bronchitis, Newcastle disease, Fowl cholera, E.coli and Infectious coryza**.

*Mycoplasma synoviae* infection should be considered whenever synovitis lesions are present. Differentiation from infectious tenosynovitis, infection with *Staphylococcus aureus*, or other bacterial infection is essential.

*Mycoplasma meleagridis* infection in turkeys should be considered when air sac lesions are found in young poults or when stunting and/or skeletal problems are present.

*Mycoplasma iowae* infection should be ruled out in cases of reduced hatchability with late embryo mortality in turkeys.

## PREVENTION AND CONTROL

### ERADICATION

Very strict hygienic system is essential to keep a flock free of infection.

1. Limit visitors and movement of vehicles between farms.
2. People entering the farm should shower and change into clothing provided by farm.
3. Equipments should be disinfected or fumigated.
4. Proper disposal of carcasses and infected materials.
5. Recommended disinfectants for buildings and equipment include phenolic or cresylic acid disinfectants, hypochlorite, and 0.1% glutaraldehyde. Birds should not be reintroduced for 2 weeks.\

#### **Killing of *M. gallisepticum* in hatching eggs:**

i) Eggs are heated for 2-3 hours by keeping them in egg incubator. After this, the eggs are dipped in a watery solution of 0.04 to 0.1% tylosin tartarate, the temperature of which should be 2 to 5°C (refrigerator temperature). Eggs are dipped in the solution for 10 to 30 min. This method causes expansion of egg contents by heating to 37°C to 38°C (incubator temperature) and dipping in cold antibiotic solution causes shrinkage and entry of the solution, sufficient to kill the organisms.

ii) *M. gallisepticum* in eggs can also be killed by heat treatment, which kills the organisms but not the chick embryo. The eggs at temperature (25-26°C) are kept in an incubator having a temperature of 46°C for a period of 12 to 14 hours. Then, these eggs are incubated for normal hatching in incubator. This method has a disadvantage of lowering the hatchability by 8 to 12 percent.

#### **Killing of *M. gallisepticum* in hatched chicks:**

Tylosin can be given to newly hatched chicks by mixing in drinking water at a concentration of 0.05% on 1st day of hatching. Similar treatment is repeated on 4th or 5th day after hatching.

## TREATMENT

Most strains of *M. gallisepticum* are sensitive to a number of broad-spectrum antibiotics, including tylosin, tetracyclines, and others but not to penicillins or those that act on the cell wall.

Tylosin - 0.05 to 0.1 percent can be given in drinking water for 3 to 5 days continuously.

Oxytetracycline or Chlortetracycline can be given at the rate of 200 g per tonne of feed.

Tiamutin 45 percent solution should be administered in concentration of 0.025% for 3 consecutive days in drinking water.

## VACCINATION

### Avian Mycoplasma Live Vaccines

Sr. No.	TradeName & Company	Composition	Indications	Route of Administration	Age
1	VAXSAFE MG TS 11 VACCINE (Fort Dodge)	Live <i>Mycoplasma gallisepticum</i> (Strain TS-11)	Prophylaxis of <i>Mycoplasma gallisepticum</i> infection	Eye drop or intranasal or intraocular	At 9 weeks of age
2	NOBILIS MG 6/85 (Intervet)	It is a live vaccine, prepared from 6/85 strain of <i>Mycoplasma gallisepticum</i> in a freeze dried preparation sealed under vacuum	Prophylaxis of <i>Mycoplasma gallisepticum</i> infection	Fine spray	At 6 weeks of age or older
3	MG-F strain	It is a natural low pathogenic strain	Prophylaxis of <i>Mycoplasma gallisepticum</i> infection	Spray or drinking water	Between 9 to 20 weeks of age

**Avian Mycoplasma Killed Vaccines**

Sr. No.	Trade Name & Company	Composition	Indications	Route of Administration	Regimen
1	MYCVAC (Stallen)	1 dose of vaccine contains: Inactivated <i>Mycoplasma gallisepticum</i> : not less than 3.1010 CFU	Prophylaxis of <i>Mycoplasma gallisepticum</i> infection in chickens	Layers/ Breeders: Inject 0.5 ml by S/C in the back of the neck	First Vaccination: At 10-12 weeks Booster dose: 4 weeks before laying
2	NOBIVAC MG (Intervet)	Inactivated virus strain S6 in an emulsion developed for the enhancement of immune response	For the protection of layers and breeders against <i>Mycoplasma gallisepticum</i> infection	Inject 0.5 ml / bird by S/C route into the lower back part of the neck or I/M route into the breast muscle	Chickens can be vaccinated from 5 weeks of age onwards and healthy birds at least 3-4 weeks before production starts

Vaccination	F strain	6/85	Ts-11
Virulence	Moderate	No	No
Persistency	Excellent	-	Good
Transmission	Moderate	Poor	Poor
Form	Lyophilized	Lyophilized	Lyophilized
Antibody	Moderate	None	Weak

**T8-05**

## **Antibody and cellular response in Brucellosis affected vaccinated and unvaccinated cattle**

*Hari Mohan Saxena and Sugandha Raj*

Department of Veterinary Microbiology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India 141004.

### **Abstract:**

Brucellosis is an economically important re-emerging zoonotic disease. In the absence of a DIVA assay, it is difficult to assess whether the anti-*Brucella* immune response of animal is due to vaccination or infection. We compared the anti-*Brucella* antibody titers and levels of total leukocytes, neutrophils and lymphocytes in blood of naturally Brucellosis affected unvaccinated cows, previously vaccinated infected cows, normal healthy vaccinated cows and healthy unvaccinated calves. The titers of antibodies were estimated by indirect ELISA. Interestingly, the titers in naturally affected cattle which had never been vaccinated were very significantly ( $p < 0.01$ ) higher than those of Brucellosis affected cows which had been vaccinated during calf hood. The latter being very significantly ( $p < 0.01$ ) higher than that of uninfected vaccinated cows.

The Leukocyte levels were very significantly ( $p < 0.01$ ) higher in naturally infected unvaccinated cattle (IU) compared to Brucellosis affected cattle which had been vaccinated during calf hood (IV) and healthy vaccinated (HV, uninfected by Brucellosis) cattle. In contrast, Neutrophil levels were significantly ( $p < 0.05$ ) higher in IV compared to IU cattle and very significantly ( $p < 0.01$ ) higher than HV cattle. Lymphocyte levels of IU cattle were significantly ( $p < 0.05$ ) higher than IV cattle and very significantly ( $p < 0.01$ ) higher than HV cattle.

### **Introduction:**

Brucellosis is endemic in India and is prevalent in all parts of the country. It causes heavy economic losses to the industry through delayed conception, late-term abortions, and retention of placenta (Kollannur et al., 2007) in females and orchitis and epididymitis in males. The organisms are excreted in semen, uterine discharges, and milk (Godfroid et al., 2013). The occurrence of the disease varies from 10% in marginal herds to 50% in organized farms.

The *Brucella abortus* strain 19 vaccine against bovine Brucellosis has been found to be very useful under most conditions but has some undesirable traits also (Moriyon et al., 2004). Although vaccination with S19 is effective in preventing Brucellosis in cattle and buffaloes, it is not uncommon to

find adult cattle vaccinated in calf hood with S19 vaccine, getting naturally infected with Brucellosis (Mohan et al., 2016). Although there is vast information on various aspects of bovine Brucellosis in the available literature, there is hardly any systematic study on the comparison of antibody levels between infected and vaccinated animals. We therefore compared the antibody titers of different categories of animals by ELISA to investigate if titers alone could indicate whether the animal is infected or vaccinated. ELISA has been claimed to be more sensitive followed by RBPT and STAT when applied to cattle sera.

The immune response induced by *Brucella* is complex, involving both humoral and cellular effectors (Poester et al., 2013). Mediators of humoral immunity such as antibody, complement and lysozyme can remove microorganisms by opsonization and bactericidal actions. Cellular components of the immune system, including macrophages, dendritic cells, neutrophils and innate T cells, have major roles in innate immunity. However, little is known about the immune cell profile after vaccination and during disease in cattle.

Although it is well known that cell mediated immunity plays an important role in elimination of intracellular pathogens like *Brucella*, the S19 vaccine currently in use for bovine Brucellosis is the one which promotes antibody rather than cellular response. It is no wonder that a substantial portion of vaccinated animals succumb to Brucellosis and keep on spreading infection to other healthy animals also on becoming carrier.

It would be useful to compare the status of cell mediated immunity in naturally infected animals with those of vaccinated animals. We have therefore undertaken the present study to assess the levels of cells involved in mediating immunity against *Brucella* organisms. We studied the total blood leukocytes and neutrophils mediating innate immunity and lymphocytes responsible for adaptive immunity in Brucellosis affected vaccinated or unvaccinated cattle as well as healthy vaccinated cattle. The present study offers valuable insight which could help in devising appropriate control strategies for this dreaded zoonosis.

#### **Materials and methods:**

Permission of the Institutional Animal Ethics Committee (IAEC) was obtained and IAEC guidelines were followed throughout the study.

**Collection of blood:** Blood samples were collected from naturally infected clinical cases of Brucellosis in cattle which had never been vaccinated (6) identified in villages in and around Ludhiana district, from naturally infected animals vaccinated during calthood (21), normal healthy (uninfected) vaccinated cattle (6) and healthy unvaccinated calves (6) of a dairy farm in Haibowal area, Ludhiana. Sera were separated from clotted blood and stored at -20°C till further use for studying the antibody response of the animals.

Blood smears were made from anticoagulated blood and peripheral blood leukocytes were separated on Ficoll Hypaque.

**Rose Bengal Plate Test (RBPT):** RBPT was carried out on sera as per the standard method (Morgan et al., 1978). Equal volumes (25 $\mu$ l each) of RBPT colored antigen (Punjab Veterinary Vaccine Institute, Ludhiana) and test serum were mixed on a clean glass slide. The slide was observed till 3 min. for formation of clumps. Formation of clumps (agglutinate) indicated a positive reaction while the absence of clear clumps was considered as a negative reaction.

### **Enzyme Linked Immunosorbant Assay (ELISA)**

Serum samples of cattle were tested by ELISA (Falconi et al., 2009) using Ingezim *Brucella* Bovina 2.0 Brucellosis serum ELISA test kit (Ingenasa). ELISA was carried out as per the manufacturer's instructions accompanying the kit described below.

- a) 90 $\mu$ l of diluent was added in each well of the plate except the ones destined as controls. 10 $\mu$ l of diluted individual sample (1/10) was added to the remainder wells of the plate.
- b) 100 $\mu$ l of the controls provided in the kit were added. Plate was incubated for 1 hour at room temperature (RT, 20-25°C).
- c) The plate was washed three times with 300 $\mu$ l of washing solution.
- d) 100 $\mu$ l of the conjugate was added to each well and plate was incubated for 30 minutes at room temperature. The plate was washed three times with 300 $\mu$ l washing solution.
- e) 100 $\mu$ l substrate was added to each well and plate was kept in dark for 10 min at RT
- f) 100 $\mu$ l of the stop solution was added to each well.
- g) Optical density (O.D.) of each well with spectrophotometer was done at 450 nm within 5 minutes after the addition of stop solution.

The test was valid when: O.D. value for positive control serum was e<sup>+</sup> 1.0, and was considered negative when the OD of the control serum was d<sup>+</sup> 0.2.

Cut off = OD<sub>450 nm</sub> positive Control x 0.4 = 40% positivity

### **Total Leukocyte Count and Differential Leukocyte Count in blood:**

The Total Leukocyte Count (TLC) of the blood samples collected from cattle was determined by automatic Hematology Analyzer. Differential Leukocyte Count (DLC) was carried out manually by making blood smears on glass slides, Leishman's staining and examining under microscope.

### **Statistical analysis of data**

The data was statistically analyzed by ANOVA.

### **Results and discussion**

The antibody titers estimated by ELISA are presented in tables 1 to 4. The mean antibody titer ( $\log_{10}$ ) in case of Brucellosis affected cattle which had been vaccinated during calf hood was found to be  $1.518 \pm 0.005$  (Table 1). The mean titer in case of naturally infected cattle which had never been vaccinated was  $1.5441 \pm 0.005$  (Table 2). In case of healthy unaffected cattle vaccinated during calf hood the mean titer was  $1.504 \pm 0.002$  (Table 3) and in unvaccinated healthy calves it was  $0.560 \pm 0.016$  (Table 4). It was interesting to find that in naturally affected cattle which had never been vaccinated previously the antibody titers were very significantly ( $p < 0.01$ ) higher than those of Brucellosis affected cattle which had been vaccinated during calf hood and normal healthy vaccinated cattle unaffected by the disease. The mean titer in Brucellosis affected cattle which had been vaccinated during calf hood was very significantly ( $p < 0.01$ ) higher than that of normal healthy vaccinated cattle unaffected by the disease.

The differences between mean titers of vaccinated infected and unvaccinated infected, vaccinated infected versus vaccinated healthy, vaccinated infected versus unvaccinated healthy, unvaccinated infected versus vaccinated healthy, unvaccinated infected versus unvaccinated healthy and vaccinated healthy versus unvaccinated healthy were very significant ( $p < 0.01$ ).

The titers in unvaccinated infected cattle were very significantly ( $P < 0.01$ ) higher than those of infected vaccinated, healthy vaccinated and healthy unvaccinated animals. The titers in vaccinated infected cattle were very significantly ( $P < 0.01$ ) higher than those of healthy vaccinated and healthy unvaccinated animals.

In a study by Saz et al (1987), the performance of an ELISA for detection of total antibodies to *Brucella* spp. was compared with that of the Rose Bengal Plate Test, standard tube agglutination test and Coombs test in the diagnosis of Brucellosis. ELISA was the most sensitive test (97%), showing greater specificity (96%) and good predictive positive and negative values (98% and 94% respectively). ELISA

was the only positive test in 6% of patients in whom Brucellosis had been confirmed by culture. ELISA has been claimed to be more sensitive followed by RBPT and STAT when applied to cattle sera (Ganesan and Anuradha, 2006).

Assessment of immunological data from *Brucella* infected cattle can be helpful in charting the disease process, diagnosis and prognosis and may help in understanding the pathophysiology of disease. It was reported (Mohan et al., 2016) that naturally affected animals have very high titers of agglutinating antibodies than the vaccinated animals.

Numerous outer and inner membrane, cytoplasmic, and periplasmic protein antigens of *Brucella* have been characterized, yet the antigen that dominates the antibody response is Lipopolysaccharide (LPS). Some antigens are recognized by the immune system during infection and are potentially useful in diagnostic tests.

The role of humoral immunity against intracellular bacterial infections is limited and may not be protective. Antibody mediated opsonization by immunoglobulins (IgM, IgG1, IgG2a and IgG3) enhances phagocytic uptake of bacteria, limiting the level of initial infection with *Brucella* but has little effect on intracellular course of infection (Bellaire et al., 2005; Baldwin and Goenka, 2006). The L7/L12 ribosomal proteins are important in stimulating cell-mediated responses (Oliveira and Splitter, 1994).

Since the infected animals in the first group were the ones who had already been vaccinated during calf hood, the infection in these animals may suggest that the vaccine was unable to induce adequate protective levels of antibody. Secondly, the heightened antibody response after infection in vaccinated animals may indicate a secondary immune response to *Brucella* antigens against which the lymphocytes were primed by calf hood vaccination.

#### **Total leukocyte levels:**

The mean leukocyte count ( $\times 10^3/\mu\text{L}$ ) in Brucellosis affected cattle which had been vaccinated during calf hood was 8.137 (Table 5). In case of naturally infected cattle that had never been vaccinated, the leukocyte count was 11.76 (Table 6). In case of healthy vaccinated cattle uninfected by Brucellosis, leukocyte count was 6.84 (Table 7) whereas in case of normal healthy unvaccinated calves, leukocyte count was 11.10 (Table 8).

The leukocyte count of Brucellosis infected unvaccinated cattle was very significantly ( $p < 0.01$ ) higher than that of Brucellosis affected vaccinated cattle. The leukocyte count of healthy unvaccinated calves was very significantly ( $p < 0.01$ ) higher than that of Brucellosis affected vaccinated cattle. The

leukocyte count of unvaccinated infected cattle was very significantly ( $p < 0.01$ ) higher than that of healthy vaccinated cattle. The leukocyte count of healthy unvaccinated calves was very significantly ( $p < 0.01$ ) higher than that of healthy vaccinated cattle. The difference between leukocyte counts of Brucellosis affected vaccinated cattle and healthy vaccinated cattle as well as between unvaccinated infected cattle and healthy unvaccinated calves was non-significant.

### **Neutrophil levels:**

The mean neutrophil count (percent) in Brucellosis affected cattle which had been vaccinated during calf hood was  $38.25 \pm 6.50$  (Table 5). In case of naturally infected cattle that had never been vaccinated, the neutrophil count was  $30 \pm 5.36$  (Table 6). In case of healthy vaccinated cattle uninfected by Brucellosis the neutrophil count was  $17 \pm 3.22$  (Table 7) whereas in case of healthy unvaccinated calves, neutrophil count was  $15.5 \pm 4.9$  (Table 8).

The Neutrophil count of Brucellosis affected vaccinated cattle was significantly ( $p < 0.05$ ) higher than that of Brucellosis infected unvaccinated cattle and very significantly ( $p < 0.01$ ) higher than that of healthy vaccinated cattle and healthy unvaccinated calves. The Neutrophil count of unvaccinated infected cattle was very significantly ( $p < 0.01$ ) higher than that of healthy vaccinated cattle and healthy unvaccinated calves. The difference between the mean Neutrophil counts of healthy vaccinated cattle and healthy unvaccinated calves was nonsignificant.

### **Lymphocyte levels:**

The mean lymphocyte count (percent) in Brucellosis affected cattle which had been vaccinated during calf hood was  $39.625 \pm 9.14$  (Table 5). In case of naturally infected cattle that had never been vaccinated, the lymphocyte count was  $51 \pm 5.58$  (Table 6). In case of healthy (uninfected by Brucellosis) vaccinated cattle, lymphocyte count was  $27 \pm 4.56$  (Table 7) whereas in case of healthy unvaccinated calves, lymphocyte count was  $26.166 \pm 4.87$  (Table 8).

The Lymphocyte count of Brucellosis affected vaccinated cattle was significantly ( $p < 0.05$ ) lower than that of unvaccinated infected cattle, but higher than that of healthy vaccinated cattle and healthy unvaccinated calves. The Lymphocyte count of unvaccinated infected cattle was very significantly ( $p < 0.01$ ) higher than that of healthy vaccinated cattle and healthy unvaccinated calves. The difference between mean values of healthy vaccinated cattle and healthy unvaccinated calves was nonsignificant.

It was interesting to find that the leukocyte counts were very significantly higher in naturally infected cattle that had never been vaccinated compared to Brucellosis affected cattle vaccinated during calthood,

as well as in healthy vaccinated cattle. Conspicuously, leukocyte count in healthy unvaccinated calves was comparable to the unvaccinated infected cattle and the difference between the two was non-significant. A possible reason for the decreased leukocyte count in vaccinated cattle affected with Brucellosis could be the mitigation of pathogenesis of infection due to the earlier priming of immune system by vaccination. Higher levels in healthy unvaccinated calves may suggest a suppressive role of vaccination on leukocyte proliferation.

Immunohematological data is helpful in charting the disease process, diagnosis and prognosis and may help in understanding the pathophysiology of infection in cattle.

Sikder *et al.* (2012) carried out a hematological study to determine the variations in blood parameters of Brucellosis seropositive commercial dairy cattle. No significant ( $p < 0.05$ ) variations were found in TLC and DLC between the Brucellosis positive and negative group. However, slightly increased values of TLC and monocytes were recorded in the positive group. In addition, a little decline in the values of neutrophil was found in the same group. The values of lymphocytes remained unchanged. They concluded that *Brucella* organisms are not responsible for a significant change in the hematological values. However, as evident from our results, overall suppression of cellular immune responses prevailed in Brucellosis infected animals.

Cell mediated immunity involves activation of the bactericidal mechanisms of antigen presenting cells (e.g., macrophages and dendritic cells) and the subsequent expansion of antigen specific CD4 and CD8 T cells. *Brucella* antigens induce the production of type 1 helper T cell ( $T_H1$ ) cytokines and an adequate  $T_H1$  immune response is critical for the clearance of *Brucella* infection (Baldwin and Goenka, 2006). The L7/L12 ribosomal proteins are important in stimulating cell-mediated responses (Oliveira and Splitter, 1994).

Since Brucellosis is a disease where cell mediated immunity is vital and vaccination with S19 is known to induce antibody response rather than cellular immune response, the finding of our study may have implications for devising a suitable strategy for control of Brucellosis.

### **Conclusion:**

The anti-*Brucella* antibody titers by ELISA in naturally affected unvaccinated cattle were very significantly higher than those of Brucellosis affected vaccinated cattle and normal healthy vaccinated cattle unaffected by the disease. Leukocyte levels were very significantly ( $p < 0.01$ ) higher in IU compared to IV and HV cattle. Neutrophil levels were significantly ( $p < 0.05$ ) higher in IV compared to IU cattle and

very significantly ( $p < 0.01$ ) higher than HV cattle. Lymphocyte levels of IU cattle were significantly ( $p < 0.05$ ) higher than IV and very significantly ( $p < 0.01$ ) higher than HV cattle.

#### Acknowledgement:

The study was funded by a grant under RKVY scheme (PI: Prof. H M Saxena).

**Table 1. Titers of anti-*Brucella* antibody by ELISA in infected cattle vaccinated in calthood**

<b>Animal number</b>	<b>Titer</b>
B1	1.5221
B2	1.5167
B3	1.5133
B4	1.5126
B5	1.5199
B6	1.5169
B7	1.5133
B8	1.5134
B9	1.5267
B10	1.5187
B11	1.5154
B12	1.5253
B13	1.5169
B14	1.5157
B15	1.5139
B16	1.5139
B17	1.5135
B18	1.5335
B19	1.5148
B20	1.5226
B21	1.5209
<b>Mean <math>\pm</math> SD</b>	<b>1.518 <math>\pm</math> 0.005</b>

**Table 2. Anti-*Brucella* antibody titers by ELISA in unvaccinated infected cattle**

<b>Animal no.</b>	<b>Titer</b>
I	1.5467
II	1.5387
III	1.5481
IV	1.5469
V	1.5361
VI	1.5481
<b>Mean <math>\pm</math> SD</b>	<b>1.5441<math>\pm</math> 0.005</b>

**Table 3. Anti-*Brucella* antibody titers by ELISA in healthy vaccinated cattle**

<b>Animal no.</b>	<b>Titer</b>
1303	1.5027
1431	1.5076
1468	1.5045
1378	1.5032
1470	1.5034
1374	1.5075
<b>Mean <math>\pm</math> SD</b>	<b>1.504<math>\pm</math> 0.002</b>

**Table 4. Anti-*Brucella* antibody titers by ELISA in unvaccinated healthy calves**

<b>Animal no.</b>	<b>Titer</b>
1632	0.5671
1634	0.5589
1638	0.5847
1577	0.5334
1575	0.5564
1581	0.5634
<b>Mean <math>\pm</math> SD</b>	<b>0.560<math>\pm</math>0.016</b>

**Table 5: Levels of leukocyte subsets in Brucellosis affected vaccinated cattle**

No	Animal number	Cell counts		
		Total Leukocytes (x10 <sup>3</sup> /μl)	Neutrophils (%)	Lymphocytes (%)
1	B1	9.3	36	54
2	B2	9.8	48	38
3	B3	8.10	37	33
4	B4	9.50	20	50
5	B5	8.00	42	67
6	B6	10.1	45	37
7	B7	8.36	38	40
8	B8	8.41	47	52
9	B9	7.6	30	31
10	B10	7.1	44	41
11	B11	7.6	37	24
12	B12	7.92	43	35
13	B13	8.23	38	37
14	B14	8.26	36	39
15	B15	7.8	32	30
16	B16	9.8	46	40
17	B17	7.61	32	30
18	B18	9.1	41	31
19	B19	7.12	44	45
20	B20	5.99	35	40
21	B21	7.02	35	37
22	B22	7.04	44	38
23	B23	8.32	35	42
24	B24	7.21	33	40
<b>Mean ± SD</b>		<b>8.137 ± 1.03</b>	<b>38.25 ± 6.50</b>	<b>39.625 ± 9.14</b>

**Table 6 : Total Leukocyte Count and Differential Leukocyte Count in Brucellosis affected unvaccinated cattle**

Animal no.	TLC (x10 <sup>3</sup> /μl)	Neutrophils (%)	Lymphocytes (%)
A I	12.73	36	58
A II	11.90	22	54
A III	11.46	28	49
A IV	11.28	36	42
A V	11.17	28	49
A VI	12.04	30	54
<b>Mean + SD</b>	<b>11.76 ± 0.58</b>	<b>30 ± 5.36</b>	<b>51 ± 5.58</b>

**Table 7: Total Leukocyte Count and Differential Leukocyte Count in healthy vaccinated cattle**

Animal number	TLC ( $\times 10^3/\mu\text{l}$ )	Neutrophils (%)	Lymphocytes (%)
C1	7.93	18	30
C2	6.14	16	28
C3	7.01	12	33
C4	6.89	17	27
C5	6.04	17	24
C6	7.08	22	20
<b>Mean <math>\pm</math> SD</b>	<b>6.84 <math>\pm</math> 0.69</b>	<b>17 <math>\pm</math> 3.22</b>	<b>27 <math>\pm</math> 4.56</b>

**Table 8: Total Leukocyte Count and Differential Leukocyte Count in healthy unvaccinated calves**

Animal no.	Total Leukocytes ( $\times 10^3/\mu\text{l}$ )	Neutrophils (%)	Lymphocytes (%)
D1	10.70	14	27
D2	9.87	18	27
D3	11.98	12	32
D4	12.01	15	30
D5	10.27	10	22
D6	11.82	24	19
<b>Mean <math>\pm</math> SD</b>	<b>11.108 <math>\pm</math> 0.94</b>	<b>15.5 <math>\pm</math> 4.9</b>	<b>26.166 <math>\pm</math> 4.87</b>

## References

- Baldwin C L, and Goenka R (2006) Host immune responses to the intracellular bacterium *Brucella*: does the bacterium instruct the host to facilitate chronic infection? *Critical Reviews in Immunology* 26: 407-442.
- Bellaire B H, Roop R M 2<sup>nd</sup> and Cardelli J A (2005) Opsonized virulent *Brucella abortus* replicates within non acidic, endoplasmic reticulum – negative, LAMP-1 positive phagosomes in human monocytes. *Infection and Immunity* 73: 3702-3713.
- Falconi, C., Oleaga, A., Lopez-Olvera, J. R., Casais, R., Prieto, M. et al. (2009) Prevalence of antibodies against selected agents shared between Cantabrian chamois and domestic goats. *European Journal of Wildlife Research* 56 (3): 319-325.

- Ganesan P I and Anuradha P. 2006. Rose Bengal Test and dot- ELISA in diagnosis of bovine Brucellosis. *Indian Veterinary Journal* **83**: 907.
- Godfroid J, Garin-Bastuji B, Saegerman C and Blasco J M (2013). Brucellosis in Terrestrial Wildlife. *Revue Scientifique et Technique de l' Office International des Epizooties* **32**(1): 27-42.
- Jain, U., Bisht, B., Sahzad, P. and Dwivedi, K. (2013) Outbreak of Brucellosis in buffaloes aborted in a village Mahuan, district Mainpuri, UP, India - A case report. *Vet. World*, 6(1): 51-52.
- Kollannur J D, Rathore R and Chauhan R S (2007). Epidemiology and economics of Brucellosis in animals and its zoonotic significance. *Proceedings of XIII International Congress in Animal Hygiene* pp 466-68.
- Mohan A, Saxena H M and Malhotra P (2016). A comparison of titers of anti-*Brucella* antibodies of naturally infected and healthy vaccinated cattle by standard tube agglutination test, microtiter plate agglutination test, indirect hemagglutination assay, and indirect enzyme-linked immunosorbent assay. *Veterinary World* 9(7): 717-722.
- Morgan W J, Mackinnon D T, Gill K P W, Gower S G M and Norris P I W. 1978. Brucellosis diagnosis: Standard Laboratory Techniques Report Series no. 1, Weybridge, England.
- Moriyon, I., Grillo, M.J., Monreal, D., Gonzalez, D., Marin, C., Lopez-Goni, I., Mainar-Jaime, R.C., Moreno, E. and Blasco, J.M. (2004) Rough vaccines in animal Brucellosis: Structural and genetic basis and present status. *Veterinary Research* 35(1): 1-38.
- Oliveira, S.C. and Splitter, G.A. (1994) Subcloning and expression of *Brucella abortus* L7/L12 ribosomal gene and T-lymphocyte recognition of the recombinant protein. *Infection and Immunity* 62: 5201-5204.
- Pandeya, Y.R., Joshi, D.D., Dhakal, S., Ghimire, L., Mahato, B.R., Chaulagain, S., Satyal, R.C. and Sah, S.K. (2013) Seroprevalence of Brucellosis in different animal species of Kailali district Nepal. *International Journal of Infection and Microbiology* 2 (1): 22-25.
- Poester F P, Nielsen K and Samartino L E. 2010. Diagnosis of brucellosis. *The Open Veterinary Science Journal* **4**: 46-60.
- Saz J V, Beltrán M, Díaz A, Agulla A, Merino F J, Villasante P A and Velasco A C. 1987. Enzyme-linked immunosorbent assay for diagnosis of Brucellosis. *Clinical Microbiology* **6**(1): 71-74.
- Sikder S, Rahman SSM, Alim MA and Das S (2012) Hematological Variations in *Brucella abortus* Antibody Positive Cross-bred Cattle at Chittagong, Bangladesh. *YYU Veteriner Fakultesi Dergisi* 23 (3), 125 – 128.
- Splitter G, Oliveira S., Carey M., Miller C., Ko J. and Covert J. (1996) T lymphocyte mediated protection against facultative intracellular bacteria. *Vet. Immunol. Immunopathol.* **54**:309–319.

**T8-06**

## **Recent Trends in Molecular Epidemiology : Techniques and Applications**

Nitin V Kurkure

Department of Veterinary Pathology

Nagpur Veterinary College, MAFSU, Nagpur 40 006

Epidemiology aims to study the distribution, determinants, and causes of health-related problems in order to prevent and control them effectively. In cases of infectious diseases, scientists have always been curious about the answers to the cause and pathogenesis of an infectious disease. Secondly, why do people / animals respond differently to a specific pathogen, therapy, or vaccine. Using traditional epidemiological studies alone, it is difficult to gain deeper understanding of the above questions, while, molecular epidemiological (ME) studies have a great potential to address these issues. Molecular epidemiology is a branch of epidemiology developed by merging molecular biology into epidemiological studies. It was the development of high throughput laboratory techniques, applicable to large sample sizes that enabled epidemiologists to conduct large-scale epidemiological studies at molecular levels. Although it is claimed that the term “molecular epidemiology” was first introduced by Lower et al. in 1979, since then, a dramatic rise in the number of molecular epidemiological studies have been observed in various research areas. In the field of infectious disease, ME studies have provided valuable information about infectious disease causation, pathogenesis, circulation, transmission, prevention, and therapy. There have been major advances in molecular epidemiology of veterinary microbes and pathology caused by them specially in the understanding susceptibility to these infections by host.

To club epidemiology with molecular biology, help of following molecular diagnostic techniques is taken.

### **1. Polymerase chain reaction and real-time PCR**

PCR is the most frequently used molecular technique in a molecular pathology laboratory. Using a pair of priming complementary sequences (oligonucleotide primers) flanking a location of interest, together with unique heat-resistant polymerases (DNA copying enzymes), multiple copies of a targeted chimeric gene can be obtained. Each PCR cycle involves 3 basic steps: denaturing, annealing, and polymerization. During denaturing, the 2 strands of the helix of the target genetic material are unwound and separated by heating at 90° to 95°C. During annealing, or hybridization, oligonucleotide primers bind to their complementary bases on the single-stranded DNA. This step requires a much cooler temperature, 55°C. Finally, during polymerization (at 75°C), the polymerase reads the template strand and quickly matches it with the appropriate nucleotides, resulting in 2 new helices consisting of part of the original strand and

the complementary strand that was just assembled. The process is repeated 30 to 40 times, each cycle doubling the amount of the targeted genetic material. At the end of the PCR procedure, millions of identical copies of the original specific DNA sequence have been generated. Since these copies are identical in electrical charge as well as molecular weight, they are expected to migrate simultaneously, forming a single band, when applied to an electrophoretic gel.

PCR can also be used to amplify an RNA target sequence. This procedure is termed reverse transcriptase PCR (RT-PCR). The RNA sequence is first converted to a double-stranded nucleic acid sequence (cDNA) by using a reverse transcriptase enzyme borrowed from a retrovirus. The cDNA sequence can then be amplified by using the same PCR cycles already described. RT-PCR is used for detection of RNA viruses, such as CSFV, and of messenger RNA (mRNA) of originally large DNA translocations (>300–500 bp, such as *BCR-ABL*). Since RNA is not as stable as DNA, fresh samples are generally required for RNA, whereas archival paraffin-embedded samples can be used for DNA.

The recent development of “real-time” PCR (Q-PCR) added great advantages to traditional PCR. As the name indicates, this technique allows for the real-time quantitation of PCR product following each of the 40 amplification cycles. The computerized Q-PCR instrument measures after each cycle the amount of fluorescence emitted from a dye intercalated in the double-helix DNA product; the amount of fluorescence is proportional to the number of copies of the amplification target. When a certain critical copy number is reached, the amount of fluorescence increases by an exponential amount. As depicted in the fluorescence vs cycle number plot in *Figure* , the cycle in which the critical copy number is reached (16th or 36th) is directly dependent on how many target DNA copies were present in the original sample before any amplification (100 copies vs 1). Q-PCR therefore offers a great rapid quantitative advantage. It is, moreover, less prone to contamination since the entire process of amplification and quantitation of the original target DNA for each sample is done in a single sealed tube. Q-PCR is of great utility in the assessment of minimal residual disease following novel targeted therapy against specific molecular defects. Results can be obtained in 2 hours and, depending on the instrument used, as many as 96 samples can be tested in a single run.

### **Multilocus sequence typing (MLST)**

Multilocus sequence typing (MLST) is a technique in molecular biology for the typing of multiple loci. The procedure characterizes isolates of microbial species using the DNA sequences of internal fragments of multiple housekeeping genes. Approximately 450-500 bp internal fragments of each gene are used, as these can be accurately sequenced on both strands using an automated DNA sequencer. For each housekeeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the loci define the allelic profile or sequence

type. MLST directly measures the DNA sequence variations in a set of housekeeping genes and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The relatedness of isolates is displayed as a dendrogram constructed using the matrix of pairwise differences between their allelic profiles, a minimum spanning tree (MST). The dendrogram is only a convenient way of displaying those isolates that have identical or very similar allelic profiles that can be assumed to be derived from a common ancestor; the relationships between isolates that differ at more than three out of seven loci are likely to be unreliable and should not be taken to infer their phylogeny. The MST connects all samples in such a way that the summed distance of all branches of the tree is minimal. MLST appears best in population genetic study but it is expensive. Due to the sequence conservation in housekeeping genes, MLST sometimes lacks the discriminatory power to differentiate bacterial strains, which limits its use in epidemiological investigations. To improve the discriminatory power of MLST, a multi-virulence-locus sequence typing (MVLST) approach has been developed using *Listeria monocytogenes*.

#### **Multiple loci VNTR analysis (MLVA)**

Multiple loci VNTR analysis (MLVA) is a method employed for the genetic analysis of particular microorganisms, such as pathogenic bacteria, that takes advantage of the polymorphism of tandemly repeated DNA sequences. A “VNTR” is a “variable-number tandem repeat”. This method is well known in forensic science since it is the basis of DNA fingerprinting in humans. When applied to bacteria, it contributes to forensic microbiology through which the source of a particular strain might eventually be traced back, making it a useful technique for outbreak surveillance. In a typical MLVA, a number of well-selected and characterised (in terms of mutation rate and diversity) loci are amplified by polymerase chain reaction (PCR), so that the size of each locus can be measured, usually by electrophoresis of the amplification products together with reference DNA fragments (a so-called DNA size marker). Different electrophoresis equipment can be used depending on the required size estimate accuracy, and the local laboratory set-up, from basic agarose gel electrophoresis up to the more sophisticated and high-throughput capillary electrophoresis devices. From this size estimate, the number of repeat units at each locus can be deduced. The resulting information is a code which can be easily compared to reference databases once the assay has been harmonised and standardised MLVA has become a major first line typing tool in a number of pathogens where such an harmonisation could be achieved, including *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Brucella*.

### **Pulsed-field gel electrophoresis (PFGE)**

Pulsed-field gel electrophoresis (PFGE) has been considered the gold standard approach for molecular epidemiology. PFGE is among the first technologies to propel the era of molecular epidemiology. Broadly applicable to bacteria of many different genera, PFGE entails the liberation of intact genomic DNA by *in situ* lysis within an agarose plug, restriction enzyme digestion, and separation of fragments using pulsed-field gel electrophoresis. Pairwise comparisons are made among strains' banding patterns, which are interpreted according to the number of dissimilar restriction fragments that are observed: "indistinguishable" (no difference), "closely related," "possibly related," and "different" (signaling the greatest amount of strain divergence). Although alternative methods have been developed to enable molecular typing of bacteria or of particular species, none has displaced PFGE as a gold standard approach, in part because the approach provides a measurement of differences spanning the entire genome. Despite its utility, there are legitimate criticisms of PFGE. PFGE is both time-consuming and labor-intensive and is technically challenging for many multipurpose clinical laboratories to implement. Reproducibility among different laboratories can be difficult to achieve and disparities between predicted and achieved PFGE banding patterns have been reported.

### **Whole genome sequence analysis**

Whole-genome sequencing (WGS) represents a relatively new and increasingly accessible means for tracking disease outbreaks that has garnered success in multiple applied contexts). Using massively parallel (or "next-generation") DNA sequencing technologies, it is now possible to examine the complete or nearly complete genomes of bacterial isolates. WGS can theoretically distinguish strains which differ at only a single nucleotide and, in the limited number of studies where direct comparisons with PFGE have been performed, has provided greater resolution. Sequencing findings suggest that relatively large numbers of sequence variants can accumulate in bacterial genomes which are undistinguishable by PFGE. The numbers of genomic polymorphisms detectable by WGS that were able to distinguish strains deemed identical by PFGE. WGS appears to offer superior resolution of strain types and is less prone to false-positive and false-negative findings. Further, genomic differences distinguishing strains can be precisely measured and are highly reproducible, allowing high-resolution inference of phylogenomic relationships. At present barriers to the universal adoption of WGS by clinical laboratories include relatively high costs of instrumentation and a lack of bioinformatic expertise, these beneficial capabilities of WGS will potentially enable epidemiological investigation of bacterial outbreaks in real time and with an unprecedented ability to define strain relationships, significantly impacting the practice of infection control and patient outcomes and justifying the additional efforts required for its implementation.

**Molecular epidemiology has key role in understanding and control of the infectious diseases in following manner.**

**1. Identification of the cause of infectious diseases**

Molecular epidemiological studies are needed to confirm the causative role of a candidate pathogen in a given disease. In this regard, usually a case-control study is conducted at first, including cases that have and controls that do not have the disease symptoms. The aim is to see whether a given pathogen is present in cases, and absent in controls or not. It has been noted that the vast majority of pathogens are not detectable by traditional culture-based techniques, therefore, culture-based research in this area might not detect the causative agent in cases, and might provide misleading information about the exact cause of infectious disease. However, advanced molecular techniques have provided the opportunity to detect trace amounts of genetic materials of a pathogen in various specimens with sensitivity that is far beyond culture-based methods. Here, pathogens whose genetic material is detected in specimens of cases but not in specimens of controls become candidate causative agent(s) of the disease of interest. After this step, we need to confirm if the pathogen(s) do cause the disease or not. To achieve this goal, we need to see if infection with the pathogen precedes the disease onset. This hypothesis can be checked efficiently in a cohort or a nested case-control study.

**2. Identification of pathogenesis of infectious diseases**

Combination of molecular techniques with epidemiological studies can reveal more secrets about the pathogenesis process of infectious diseases. For example, by comparing the genome sequence of a particular pathogen between severe, fatal, or drug-resistant cases and mild, non-fatal or drug-sensitive controls, we can find out if there is any difference between genome sequences of microbes in both groups of patients. Genetic regions that are not identical in two groups of patients might be responsible for the observed clinical symptoms in cases, here, the virulence, case fatality, or drug-resistance, respectively. For example, a new type of methicillin-resistant *Staphylococcus aureus* (MRSA) was identified to cause community-acquired MRSA with high fatality rate. Therefore, using sequencing techniques, scientists determined whole genome sequence of this species, isolated from an infant with fatal septicemia and septic arthritis due to this species. They compared the genome sequence of this species with those obtained from two cases of hospital-acquired MRSA presenting milder clinical manifestations. They found that the pathogen responsible for community-acquired MRSA carries a range of genes that is distinct from extant hospital-acquired MRSA strains which could be attributed to medically important phenotypes of the new species, i.e. virulence and case fatality. Such findings would be applicable for developing appropriate tools to detect if there is a potential for severe or fatal conditions and prevent them appropriately. Different levels of disease severity caused by a pathogen can also be due to differences in human host response to

the infection. Such a difference might be due to differences in individuals' genetic susceptibility to or inherent immunity against the pathogen. One epidemiological method that has a great ability to assess this possibility is Genome Wide Association (GWA) study. These types of studies can be considered as a derivative of case-control studies; they compare the DNA of two groups of individuals: those who have the disease (cases) and those who are similar to cases but do not have the disease (controls).

### **3. Identification of infectious diseases' sources/reservoirs**

Molecular tools have enhanced the ability to detect trace amounts of genetic materials in the environment (such as water, soil, human, etc.) which was not traceable before by traditional culture techniques. This opportunity has helped scientists to find new reservoirs/sources of infection and design more effective preventive strategies accordingly. In this regard, they need to identify if the pathogen isolated from the infected individual is genetically similar to the pathogen obtained from suspected sources or not. If they are genetically similar in a significant manner, then the suspicious source is considered as a new source of infection for that pathogen. To check for genetic similarity between two pathogens, epidemiologists have turned to grouping/clustering methods used in evolutionary biology. Phylogenetics is one of these methods that attempts to study the similarity and evolutionary relationships among various species. An example in this area is the identification of chicken as a new reservoir for Extraintestinal Pathogenic Escherichia coli (ExPEC) causing Urinary Tract Infection (UTI) in Humans. Previously, the host's intestinal tract was considered to be the only source of ExPEC-induced UTI. Usually such endogenous diseases are not expected to produce outbreaks. Bergeron et al. conducted a series of studies; they collected samples of temporally and geographically matched E. coli isolates from animals and from humans with UTIs. With the aid of phylogenetic methods, they found that E. coli isolates from chicken are genetically similar to isolates from humans with UTIs in a significant manner. So a new reservoir was found for the pathogen which brought appropriate preventive strategies to the community accordingly.

### **4. Circulation of microbial strains**

Routine, population-based genotyping of circulating pathogens is now implemented in many settings as a tool for surveillance of existing microbial strains and drug resistance mutations in the herds. For pathogens, in which different strains have different tendency to cause the diseases, knowledge about circulating strains is essential for developing strain-specific vaccines. For this reason, monitoring local, national and regional levels of drug resistance inform treatment guidelines and provide feedback on the success of infectious disease treatment and prevention programs.

## **5. Identification of infectious diseases' transmission probability**

Molecular epidemiological studies can also provide valuable information about transmission probability of infectious diseases which cannot be provided correctly otherwise. Additionally, as molecular tools can also determine genome of the pathogen in asymptomatic partners, transmission events are ascertained more exactly this way, therefore, estimation of transmission probabilities are more accurate in ME studies rather than traditional methods.

## **6. Identification of between herd transmission patterns**

Phylogenetic methods, when combined with demographic and animal behavioral data, can provide valuable information about transmission of the pathogen between herds. Phylogeography is the study of the historical processes that probably shaped current geographic distributions of living species, such as viruses. Today, these studies are done in light of the information gained from gene genealogies. Genealogies are family trees that represent the ancestor-descendent relationships between individuals in a population, e.g., viruses in an epidemic. A number of methods have been proposed to trace dispersal patterns of pathogens. Within these methods, the family tree is first reconstructed, without including any spatial information, and the phylogeographic inference is then made from this tree. These methods, however, do not address all the uncertainties associated with spatial dispersion of a pathogen. Therefore, more rigorous and sophisticated methods have been recently developed that use the Bayes theorem to integrate and simultaneously estimate all the parameters associated with spatio-temporal dispersion of a pathogen.

The application of molecular techniques to epidemiologic studies have enhanced our understanding of infectious diseases. Molecular tools have improved our ability to sub-classify the organisms into meaningful groups, detect even trace amounts of living organisms in the human body or in the environment, and early detect diseases' symptoms. Conduction of epidemiologic studies on these data, can assist molecular biologists to identify previously-unknown aspects of infectious diseases, such as infectious diseases etiology, pathophysiology and infectious agents sources, reservoirs, circulation pattern, transmission probability, and transmission patterns. The information obtained in each of the above aspects helps practitioners and decision makers in the development of more effective prevention and control strategies at national and global levels.

**T8-07**

**Development of diagnostic systems and molecular epidemiology studies of bluetongue virus in India**

*Sushila Maan*

College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar,  
125 004, Haryana, India

Dr Sushila Maan, Principal Scientist, Department of Animal Biotechnology, COVS, LUVAS, Hisar

Email: sushilamaan105@gmail.com; sushilamaan105@luvas.edu.in

During the past 20 years, multiple strains of bluetongue virus (BTV) and other arboviruses have emerged globally, possibly linked to changes in climate and global trade. However, lack of data / incomplete data concerning global strain-diversity and distribution had limited our ability to understand and respond to control these events. These knowledge-gaps have been addressed by isolating, identifying and characterizing arboviruses from India, to study their distribution, abundance, relationships, and movements that result in disease outbreaks. The primary focus of these studies is bluetongue virus (BTV), although diagnostic samples used to detect and isolate viruses, has also provided materials and potentially isolates of other arboviruses from the region. BTV is endemic in warmer regions around the world, between ~45-53°N and 35°S, including North and South America, Africa, the Indian subcontinent, Australasia, and Asia. The severity of the disease is dependent on host species, breed/strain, and on their serological status for BTV.

The nucleotide sequences of BTV isolates reflect their geographic origins (1-3), and the majority of the BTV genome segments can clearly be divided into eastern or western groups / topotypes (4, 5). This indicates that these viruses have evolved, with little genetic exchange between regions, over a very long period of time, allowing them to acquire multiple point mutations and clear regional differences. With the identification of some novel serotypes recently, there are now 27 distinct serotypes of BTV that can be identified by the specificity of reactions between their outer capsid proteins and neutralising antibodies generated during infection of the mammalian host. The BTV genes encoding these outer-capsid proteins show nucleotide sequence variations that correlate with both virus serotype and the geographical origin of the virus isolate (6). Fifteen BTV serotypes have been isolated in India (BTV-1, -2, -3, -4, -5, -8, -9, -10, -12, -16, -17, -18, -21, -23, -24), eleven of these in the last decade, with serological evidence of presence of eight more (BTV-6, -7, -11, -13, -14, -15, -19, -20), containing a unique mixture of viruses from both geographic regions (7).

Molecular epidemiology studies can be used to compare RNA sequences from novel BTV isolates with those of existing strains from known locations and dates, identifying both virus serotype and topotype. Sequence variations can also identify individual virus lineages within the same area and the presence of

reassortant strains containing genome segments from different parental strains. BTV molecular epidemiology studies depend on development of sequence databases for the RNA segments of known virus isolates from defined locations with well documented isolation dates and passage histories. Ideally these viruses should be held in long-term reference-collections, allowing sequence data to be linked to biological characteristics and epidemiology of specific isolates. We have generated well characterised and documented virus isolates from different Indian states, as a basis for a reference collection of 'Indian arboviruses', focusing particularly on BTV. The location of each sample was recorded using the proforma devised for this purpose. This has provided materials for development of relevant vaccines (seed stocks and challenge strains) based on local stains and validation / further-development of diagnostic assays for identification, detection and surveillance of these viruses on the Indian subcontinent. Web linking of 'Indian-reference-collections' will add to the global resources and our knowledge concerning these viruses.

Full genome characterisation studies have shown that live attenuated vaccine like viruses belonging to at least BTV- 2, and 10 (western toptotype) are circulating in India (8, 9). These data suggest that live attenuated vaccine-viruses and the international trade in livestock could both play important roles in the geographical (inter-continental) movement of BTV genome segments, and the arrival of western BTV strains or genome segments in India. Indeed Rao et al (10) report that that cattle were imported into India during 2002 to 2005, from Belgium, France, Germany, Nepal, Russia, South Africa, Thailand, the UK and USA. The Central Sheep Breeding Farm (CSBF), Hisar, Haryana, India, also imported Corriedale, Merino and Dorset sheep from Australia and Rambouillet sheep from America during late the 1970s and 1980s (11, 12). Some of these countries used live attenuated vaccines during this period, it is therefore possible that introduction of BTV western and/or vaccine-strain genome-segments into India, could potentially be linked to animal movements. These activities have generated an unprecedented mix of BTV field and vaccine strains within India and have provided multiple and widespread opportunities for the exchange / reassortment of genome segments. Characterization of complete genomes of post 1982 BTV isolates have showed the presence of genome Seg-5 of western origin irrespective of serotype confirming the reassortment event within circulating eastern and western strains. Efficiency with which insect become infected depends on level of viraemia in the target host which is related to virulence. We have tested a plaque purified reassortant strain of BTV having western genome segment 5 and a non-reassortant BTV strain of BTV in BSR cells. The preliminary data indicated that the strain with western genome segment 5 grows to a higher titer in BSR cells having implications in viral transmission.

Additionally, a panel of molecular diagnostic assays have been developed and evaluated. This includes an Pan-BTV real time TaqMan probe based assay to detect all known serotypes of BTV, LAMP assay to detect and discriminate eastern and western strains of BTV that are circulating in India and real time RT-PCR assays for detection and typing of currently known 29 serotypes of Bluetongue Virus.

Additionally there are highly suitable to identify mixed serotypes in a single sample which was not possible using serological assays. The primer and probes designed in these studies are the candidates for a patent application which has been filed.

We have also established the fact that majority of times the bluetongue disease is seen in combination with other pathogens like PPRV, ORFV and *Brucella* spp. These findings explain to some extent the increased severity of disease seen in Indian local breeds of sheep and goat and the importance of developing cross reactive vaccines that could protect against multiple serotypes and pathogens.

Key words: Arboviruses, bluetongue virus, topotype, serotype, reassortant, international trade, molecular tests.

### **References:**

- Gould AR, Pritchard LI. Relationships amongst bluetongue viruses revealed by comparisons of capsid and outer coat protein nucleotide sequences. *Virus Res.* 1990;17(1):31-52.
- Pritchard LI, Gould AR. Phylogenetic comparison of the serotype-specific VP2 protein of bluetongue and related orbiviruses. *Virus Res.* 1995;39(2-3):207-20.
- Pritchard LI, Sendow I, Lunt R, Hassan SH, Kattenbelt J, Gould AR, et al. Genetic diversity of bluetongue viruses in south east Asia. *Virus Res.* 2004;101(2):193-201.
- Maan S, Maan NS, Ross-smith N, Batten CA, Shaw AE, Anthony SJ, et al. Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. *Virology.* 2008;377(2):308-18.
- Maan S, Maan NS, Nomikou K, Anthony SJ, Ross-smith N, Singh KP, et al. Molecular epidemiology studies of bluetongue virus. In: Mellor PS, Baylis M, Mertens PPC, editors. *Bluetongue*. 1st ed. London Elsevier/Academic Press 2009. p. 135-66.
- Mertens PPC, Maan S, Samuel A, Attoui H. Orbiviruses, Reoviridae. In: Fauquet CM, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball, editor. *Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier/Academic Press; 2005. p. 466-83.
- Rao PP, Hegde NR, Reddy YN, Krishnajyothi Y, Reddy YV, Susmitha B, et al. Epidemiology of Bluetongue in India. *Transbound Emerg Dis.* 2014.
- Maan NS, Maan S, Nomikou K, Guimera M, Pullinger G, Singh KP, et al. The genome sequence of bluetongue virus type 2 from India: evidence for reassortment between eastern and western-topotype field strains. *J Virol.* 2012;86(10):5967-8.
- Maan S, Maan NS, Pullinger G, Nomikou K, Morecroft E, Guimera M, et al. The Genome Sequence of Bluetongue Virus Type 10 from India: Evidence for Circulation of a Western-topotype Vaccine Strain. *J Virol.* 2012;86(10):5971-2

Rao PP, Hegde NR, Reddy YN. Intercontinental movement of bluetongue virus and potential consequences to trade. J Virol. 2012 (In Press.).

Jain NC, Sharma R, Prasad G. Isolation of bluetongue virus from sheep in India. Vet Rec. 1986;119(1):17-8.

Maan NS, Maan S, Nomikou K, Prasad G, Singh KP, Belaganahalli MN, et al. Full genome sequence of bluetongue virus serotype 1 from India. J Virol. 2012;86(8):4717-8.

**T8-08**

**Chronic Respiratory Disease in Poultry: Current scenario of the disease in India and advances in diagnosis**

Dr. (Mrs.) Rajashree Gandge Patil

Professor

Department of Microbiology, Bombay Veterinary College, Parel, Mumbai-400 012.

Email ID: rajashrigandge@yahoo.co.in

Poultry industry is one of the organized and vibrant sectors of India with current investment of Rs. 200 billion and with 8-10% annual robust and 15 to 20% annual growth rate (Anonymous, 2011). It's a source of employment for about 1.5 million people. Every family in rural areas and every fifth family in urban areas are associated with poultry production in one way or the other (Rashid *et al.*, 2009). Today India is world's 5<sup>th</sup> largest egg producer and 8<sup>th</sup> largest producer of broilers (Indian mirror, 2015) and as the world human population grows, expected to reach 9.5 billion by 2050. However, Chronic Respiratory Disease (CRD) caused by *Mycoplasma gallisepticum* (MG) is one of the most important diseases of poultry causing high economic losses poultry industry. Since recently, it appears to have emerged with renewed vigour, has become more common and a serious challenge to the commercial poultry industry due to co-infection of *M. gallisepticum* with LPAI virus ((H3N8) and other pathogens, antigenic variability and phenotypic switching of *M. gallisepticum*, persistence and vertical transmission, ineffective vaccination protection, sub-clinical form and poor diagnostic facility. The high prevalence and wide distribution of MG infection in India warrants immediate attention and preventive strategies to minimize economic impact of MG infection (Reddy, 2014).

CRD is found worldwide and has been reported from Egypt, Pakistan, Iran, Algeria, Brazil, Saudi Arabia, Korea, Nigeria (Lamyaa and Abd El-Samie, 2012; Atique *et al.*, 2012; Adel and Mehrdad, 2012; Aimeur *et al.*, 2013; Silvio *et al.*, 2015; Ayman *et al.*, 2016; Kilhan *et al.*, 2016; Ahemad *et al.*, 2015) etc. Scanty data is available on CRD from India, since sufficient research has not been carried out on

poultry mycoplasmosis. However, the disease is reported from various parts of India (Baksi *et al.*, 2016) and the prevalence of MG in different regions of India was 18.64% in Central, 01.00% in East, 1.76% in North and 11.25% in South (Reddy, 2014) . The prevalence rate of 27 % (Tomar *et al.*, 2017) from Haryana, 53.84% (Thilangwathi *et al.*, 2017) in Kerala, 53.40% from Tamilnadu (Shanmugasundaram, 2016). Incidence rate of 66.04 % and 56.67% (Kamble *et al.*, 2015; Bukte, 2015) is reported from Maharashtra. Majee and Gandge (unpublished data) observed 8.33%, 2.59%, 13.63% and 2.89% incidence from Karnataka, Gujrat, Madhya Pradesh and Telangana respectively, whereas from Delhi and Kolkata the incidence rate of 25% and 13.51% respectively is detected by Vergese (2016). The incidence of disease depends on various factors viz. management stresses and/or other respiratory pathogens, season, age, etc. Highest MG incidence is recorded at 18 weeks of age and during winter season. (Borah *et al.*, 2017; Sikder *et al.*, 2005). Thus, in India more studies on surveillance are required to be carried out in various parts to know the exact status of disease in all parts of country and application of research data for implementation of disease prevention and control strategies is the need of an hour. Available methods of MG control largely include stringent biosecurity, biosurveillance practices and vaccination. However, therapeutic means of using antimicrobial is commonly followed in India rather than using vaccines. Intra-species variability among *Mycoplasma* strains and their ability to interact with other disease-producing factors display the wide variability in clinical manifestations, difficulties in diagnosis due to different aspects of etiological agent from conventional bacteria (Kleven, 1998).

Various conventional and molecular diagnostic techniques are used for the detection of pathogenic *Mycoplasma*. The isolation and identification of *Mycoplasma* is the gold standard test for confirmation of diagnosis (Ley, 2008). Basic biochemical tests can be helpful in preliminary classification of isolates but final identification is by immunological tests, the most satisfactory being fluorescent antibody and immunoperoxidase tests. Several serological tests recommended to detect MG or MS antibodies for flock screening rather than for testing individuals. The most commonly used are the rapid serum agglutination (RSA) test, the enzyme-linked immunosorbent assay (ELISA) and the haemagglutination inhibition (HI) test (OIE, 2008). The requirements of a screening test for *Mycoplasma gallisepticum* antibodies is also fulfilled by rapid whole cell protein antigen based Latex agglutination test (Sankar *et al.*, 2013) .

DNA detection methods based on the polymerase chain reaction and other molecular techniques are used for detection of their DNA in field samples or cultures in specialised laboratories. Presently PCR has become a valuable tool in the diagnosis of mycoplasmas, not only for its sensitivity but also for its specificity (Garcia *et al.*, 2005). PCR has allowed the study of microbial genes, directly amplified from samples, without the need for cultivation (Kamble *et al.*, 2015; Bukte, 2015; Vergese, 2016) and a highly specific and sensitive PCR is developed by targeting *16S rRNA*, *mgc2*, pMGA *pvpA*, *gapA* and *mgc2*, *vlha* etc. genes. Duplex PCR is carried out for simultaneous identification of MG and MS and suggested

that duplex PCR is more rapid and inexpensive method than the single PCR for detection of MG and MS. (Malekhoseini *et al.*, 2017). It is widely used for genetic screening, disease screening and other applications. Whereas, Nested PCR is an analytical test and in this two pairs of primers (the external primers and internal primers) could amplify two regions of a gene and is thus more sensitive (Jalaladdini *et al.*, 2014). Real time quantitative polymerase chain reaction (qPCR) assay is capable of unambiguous detection of *Mycoplasma* with high sensitivity of 10 to 1000 times better sensitivity than conventional PCR and culture and specificity for differentiation of vaccine strain and field strain. The assay has the most uniform amplification efficiency over the broadest range of species and it is extremely specific towards *Mycoplasma*. (Salling, and Bang-Christensen, 2016).

The genus specific PCR-RFLP was found to be more sensitive and accurate for a range of mycoplasmal species. (Marois *et al.*, 2002). Most avian *Mycoplasma* species can be differentiated, according to their unique RFLP patterns generated with different restriction endonucleases (RE) pattern (Kempf *et al.*, 1993). Whereas, AFLP method is able to identify and differentiate both MG field strains and vaccine strains. It has higher discriminatory power and reproducibility over molecular typing techniques such as gene-targeted typing by DNA sequence analysis of the MG cytoadhesin-like protein encoding gene, *mgc2*, and random amplified polymorphic DNA assay (Yang *et al.*, 2005).

Recently, PCRs and subsequent sequencing is used for molecular typing and are regarded as the 'state of the art' typing method for MG and other mycoplasma spp. Gene targeted sequence analysis of surface-protein genes of MG is a sensitive and reproducible typing method and will allow rapid global comparisons of nucleotide variation and genetic diversity and strain detection (Ferguson, 2005; Shaker *et al.* 2015, Gandge and Majee, 2017). Whereas, Multilocus Sequence Typing (MLST) is considered the second best alternative after whole genome sequencing for genotyping. MLST is a reproducible molecular strain typing technique with sufficient discriminatory power to perform large scale epidemiological studies (Tocqueville *et al.*, 2014; Register *et al.*, 2015; Rosales *et al.*, 2015) and used to determine allelic differences and define sequence types (ST) (Glaeser and Kampfer, 2015). Whole genome sequencing is regarded as the current 'reference' in genotyping. It is more feasible and resulted in a paradigm shift in the efficiency of outbreak investigation, understanding infection dynamics and epidemiology as well as planning future prevention and control strategies. Whole-genome sequences of *M. gallisepticum* vaccine strains and field isolates are compared to the *M. gallisepticum* R<sub>low</sub> reference genome, in order to identify genome differences among vaccine revertants, vaccine strains and field isolates (Camir *et al.*, 2017). Other cost-effective, quick, and sensitive method for the detection of *M. gallisepticum* viz. LAMP assay (Zhang *et al.*, 2015), Western blot techniques, Lateral flow assays, improved serological tests with mAbs, recombinant proteins/ antigens etc. are available for diagnosis of CRD. MALDI-TOF mass spectrometry is a new technique fast and accurate but not yet achieved a significant database of MG for comparing results.

**Conclusion:** Chronic Respiratory Disease caused by *Mycoplasma gallisepticum* (MG) is prevalent in India and causing high economic losses due to overlook and poor diagnosis of disease; therefore regular screening of breeder stock and maintaining mycoplasma free breeder stock is essential for reducing future incidences. Although cultural isolation is gold standard; the recently developed advanced molecular techniques and other methods have made the diagnosis of CRD easier. An early and accurate detection of etiological agent and diagnosis of disease can help in prevention and control of the disease in order to lower mortality and spread of infectious pathogen in the flock. Moreover, surveillance and molecular epidemiological study will be useful to plan the strategies for prevention and control of CRD more effectively which in turn increase the GDP of nation.

### References:

- Adel Feizi and Mehrdad Nazeri (2012). Survey of Layer Flocks Contamination to *Mycoplasma gallisepticum* in East Azerbaijan Province by Rapid Slide Agglutination (R.S.A) Method. Current Research Journal of Biological Sciences 4(1): 48-51.
- Ahmed JS, Lawal SM, Fatihu MY, Moses DG1, Barde IJ, Kumbish PR, Oladele SB. (2015) Isolation and serological detection of mycoplasma gallisepticum and mycoplasma synoviae using a combined mg/ms enzyme-linked immunosorbent assay kit in indigenous chickens in niger state, nigeria. African Journal of Cellular Pathology 4: 70-73
- Aimeur Rachida, Bouaziz Omar , Bererhi ElHacène , KabouiaRachid , Abdeldjelil Mohamed Cherif. (2013). Serological Investigation on Avian Mycoplasmosis in Laying Hen Farms in Eastern Algeria IJAVMS. 75: 170-177.
- Atique M. A. , F. Abbas, M. A. Awan, M. M. Tariq, Z. Ahmed, Irshad Ali and M. A. (2012). Alam Identification of avian *Mycoplasma* species in commercial broilers and layers with respiratory symptoms in Balochistan. African Journal of Biotechnology 11(100):16557-16559.
- Ayman Elbehiry, Musaad Al-Dubaib , Eman Marzouk (2016). Serological, Rapid Molecular Characterization and Antibiotic Resistance for Field Isolates of Mycoplasma Gallisepticum in Chicken in Saudi Arabia. Alexandria Journal of Veterinary Sciences.49 (2): 70-79.
- Anonymous (2011). Economic Survey of Pakistan. Finance Division, Economic Advisors Wing, Ministry of Finance, Government of Pakistan, Islamabad, Chapter 2: 28-29.
- Baksi Surajit, Savaliya Bhumika F, Trivedi Bhargav and Rao Nirav (2016). Seroprevalence of *Mycoplasma Gallisepticum* in Different Parts of India Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases. 37(2): 63- 66.
- Borah MK, R Islam, M Sarma, JD Mahanta and N Kalita. (2017) Prevalence and seasonal variation of certain microbial diseases in Kamrup and Kamrup (Metro) Districts of Assam. International Journal of Chemical Studies. 5(3): 724-726.

- Bukte S.R. (2015). Detection of Mycoplasmosis in poultry by conventional and molecular techniques. M.V.Sc.thesis.submitted to MAFSU, Nagpur.
- Camir Ricketts, Larissa Pickler, John Maurer, Saravanaraj Ayyampalayam, b Maricarmen García and Naola M. Ferguson-Noela (2017) Identification of Strain-Specific Sequences That Distinguish a *Mycoplasma gallisepticum* Vaccine Strain from Field Isolates. *Journal of Clinical Microbiology*. 55: :244–252
- Fanqing Zhang, Shijun Bao, Shengqing Yu, Jinghua Cheng, Lei Tan, Xvsheng Qiu, Cuiping Song, Yabin Dai, Rongmei Fei, Chan Ding (2015). Development of a loop-mediated isothermal amplification targeting a gene within the pyruvate dehydrogenase complex, the *pdhA* gene, for rapid detection of *Mycoplasma gallisepticum*. *Journal of Veterinary Diagnostic Investigation* .27(3): 260– 267.
- [Ferguson NM](#), [Hepp D](#), [Sun S](#), [Ikuta N](#), [Levisohn S](#), [Kleven S H](#) and [García M](#). (2005). Use of molecular diversity of *Mycoplasma gallisepticum* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. *Microbiology*: 151(6):1883-93.
- García M, Ikuta N, Levisohn S and Kleven S H. (2005) Evaluation and Comparison of Various PCR Methods for Detection of *Mycoplasma gallisepticum* Infection in Chickens. *Avian Diseases* 49 (1): 125-132.
- Glaeser SP and Kampf P (2015). Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. *Syst Appl Microbiol*. 38(4):237-45.
- Jalaladdini, S.M., S.A. Pourbakhsh and B. Kheirkhah. (2014). Isolation and identification of *Mycoplasma gallisepticum* in chicken from industrial farms in Kerman province. *Int. J. Adv. Biol. Biomed. Res.*, 2 (1): 100-104
- Kamble S Y, Gandge R S and Majee S B (2015). Diagnosis of poultry mycoplasmosis by cultural isolation and PCR. *Indian Journal of Animal Sciences*. 85 (10):1073-76.
- Kempf I, Blanchard A, Gesbert F, Guittet M and Bennejean G. 1993. The polymerase chain reaction for *Mycoplasma gallisepticum* detection. *Avian Pathology* 22 (4): 739–750.
- Kilhan Kwak , Heungiae Lee, Hyunsu Yuk, Jaek Lee, Kwanho Lee, Young-Joo Yi, Sang-Myeong Lee. Seroprevalence and molecular detection of *Mycoplasma gallisepticum* and *M. synoviae* infection in broiler breeder in Jeonbuk providence, Korea *Korean Journal of Veterinary Service Korean J Vet Serv*, 2016, 39(2), 101-105.
- Kleven S H. 1998. *Mycoplasma* in the etiology of multifactorial respiratory diseases. *Poultry Science* 77: 1146-1149.
- Lamyaa M. Reda and L.K. Abd El-Samie (2012). Some Studies on the Diagnosis of *Mycoplasma Gallisepticum* in Chicken. *Nature and Science*.10(12) : 247-51.

- Ley D H. 2008. *Mycoplasma gallisepticum* infection. In: Disease of Poultry, 12th edn. (Fadly AM, Gilson JR, Mc Dougald LR, Nolan LK and Swaney DE, edn.). Iowa State University Press, Ames, Iowa. : 807- 834.
- Malekhoseini G, Pourbakhsh SA, Homayounimehr AR, Zolfeghari MR, Ashtari A and Abtin AR (2017). Simultaneous identification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by duplex PCR assay. Immune System and Disorders Journal. 1(1);1-9.
- Rashid S, Naeem K, Ahmed Z, Siddique N, Abbas M A and Malik S A. 2009. Multiplex polymerase chain reaction for the detection of and differentiation of avian influenza viruses and other poultry respiratory pathogens. *Poultry Science*. 88: 2526- 2531.
- Reddy M.R. (2014). Abstract published in 2nd International Conference on Animal & Dairy Sciences September 15-17, 2014 HICC, Hyderabad, India.
- Rosales RS, Churchward CP, Schnee C, Sachse K, Lysnyansky I, Catania S, Job L, Ayling RD, Nicholas RAJ. (2015) "Global Multilocus Sequence Typing Analysis of *Mycoplasma bovis* Isolates Reveals Two Main Population Clusters". *J Clin Microbiol.* 55(5):1596-1597. doi: 10.1128/JCM.00230-17.
- Salling H. K. And Bang-Christensen S.R. (2016). Multi-primer qPCR assay capable of highly efficient and specific detection of the vast majority of all known *Mycoplasma*. *Biologicals*, 44(3):129-138.
- Sankar S., G. Krishnan Nair, M. Mini and M. Hiron Harshan ( 2013) Development and evaluation of a latex agglutination test for the detection of mycoplasma gallisepticum antibodies in chicken sera. *Indian J. Anim. Res.*, 47 (4) : 356-359
- Shanmugasundaram Udhayavel, Tippichett ypalayam Ramasamy Gopala Krishna Murthy, Vasudevan Gowthaman\*, Kandasamy Senthilvel, Ganesan Sureshkumar (2016). Detection of Sub Clinical Infection of *Mycoplasma gallisepticum* in Commercial Chicken by Indirect ELISA. *Advances in Animal and Veterinary Sciences* 4(8): 438-440.
- Sikder A.J. , M.A. Islam , M.M. Rahman and M.B. Rahman (2005). Seroprevalence of Salmonella and *M. gallisepticum* infection in six model breeder poultry farm at Patuakhali district in Banladesh. *International J of Poultry Science*. 4(11) 905-910.
- Sílvio G. de Sá et al. 2015. Occurrence and risk factors assessment associated with *Mycoplasma gallisepticum* (MG) infection in chickens in the semiarid region of Pernambuco, Brazil . *Pesq. Vet. Bras*. 35(6):531-535.
- Thilagavathi K., S. Sivaseelan, G.A. Balasubramaniam, A. Balasubramaniam, A. Arulmozhi and R. Madheswaran. (2017). Detection of *mycoplasma gallisepticum* from field samples of laying chicken using pcr. *International Journal of Science, Environment and Technology*. 6(4) : 2594 – 2499
- Tomar Piyush, Y. Singh , N.K. Mahajan , Naresh Jindal and Mahavir Singh (2017) - Molecular Detection of Avian Mycoplasmas in Poultry Affected with Respiratory Infections in Haryana (India) *Int.J.Curr.Microbiol. App.Sci* 6 (6): 2155-2162.

- Vergese S. C. (2016). Molecular diagnosis of avian mycoplasmosis employing various pcr assays.M.V.Sc.thesis.submitted to MAFSU, Nagpur.
- Véronique Tocqueville, Séverine Ferré, Ngoc Hong Phuc Nguyen, Isabelle Kempf and Corinne Marois-Créhan (2014) Multilocus Sequence Typing of Mycoplasma hyorhinis Strains Identified by a Real-Time TaqMan PCR Assay. J. Clin. Microbiol. 52(5):1664-71.
- World Organization of Animal Health (OIE), 2008. Avian Mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*). Manual of diagnostic tests and vaccines. Paris. Chapter 2.3.5. : 482-496.
- Yang H., Maricarmen García, Sharon Levisohn, Paul Savelkoul, Victoria Leiting, Inna Lysnyansky, David H. Ley, and Stanley Kleven H. (2005). Differentiation of Mycoplasma gallisepticum Strains Using Amplified Fragment Length Polymorphism and Other DNA-Based Typing Methods. Avian Disease. 49 (1): 43-49.

**AB-30**

**Sequence and phylogenetic analysis of sheeppox and goatpox viruses by A12L gene**

Aparna Madhavan, Gnanavel Venkatesan, Sargam Arya, Amit Kumar and A.B. Pandey  
Pox virus laboratory, Division of Virology, IVRI, Mukteswar, Uttarakhand

The genus *Capripoxvirus* belongs to family *Poxviridae* comprises of three closely related viruses, namely goatpox virus (GTPV), sheeppox virus (SPPV) and lumpy skin disease virus. Since capripox viruses are serologically identical, their specific identification relies exclusively on the use of molecular tools by single or multigene analysis. ORF 103 is one of the structural proteins of the genus *Capripoxvirus* (CaPV) and a homolog of vaccinia virus A12L gene. Fourteen GTPV and thirteen SPPV isolates were used for the amplification of A12L gene with an open reading frame of 573bp in length using specific primers. The sequences were compared with other ORF 103 sequences of capripoxviruses available in the database. Between Indian SPPV and GTPV isolates, it had shown a percentage identity of 96-97% and 94.7-96.8% at nucleotide and amino acid levels, respectively. Sequence analysis revealed considerable percentage of identity among and between CaPV isolates and is conserved among the CaPV genus. Phylogenetic tree revealed the presence of three distinct clusters representing the three different members of the CaPV genera. Several unique signature residues (both nt and aa) are also observed throughout the length of 103 genes of CaPVs. Presence of three cysteine residues in GTPV and only two in case of SPPV isolates would be an interesting feature of ORF 103 gene which could be exploited as differentiating marker between these two species. *In-silico* analysis of ORF 103 gene by restriction enzyme (RE) mapping showed the potential RE sites (*Hind III* for GTPV and *Xba I* for SPPV) for use in genotyping of CaPV. Further, secondary structure prediction of ORF 103 revealed that it low hydrophobicity with good antigenic index and surface probability which are basic criteria for potential candidate antigen to use in diagnostics and prophylactics.

Key words: Sheeppox virus; goatpox virus; A12L; immunogenic protein; genotyping; diagnosis

**AB-31**

**Isolation and identification of *Microsporium canis* from dog**

C.Sannat\*, N. Rawat, R. Hazari and K. K.Kushwaha and S.D. Hirpurkar  
Department of Veterinary Microbiology

College of Veterinary Science & A.H., Chhattisgarh Kamdhenu Vishwavidyalaya, Anjora,  
Durg (CG)-491001 E mail address of presenting author\*:csannat@rediffmail.com

Present investigation was conducted to isolate *Microsporium canis* from a dog with dermatitis. A male Doberman dog aged 1.5 year having red popular lesion, circular alopecia and constant itching on

skin, was brought to Teaching Veterinary Clinical complex, College of Veterinary Science & A.H., Anjora, Durg. Skin scrapings and plucked hair samples were collected in sterile vial for mycological examination and sensitivity. Samples were initially examined by KOH wet mount and Lacto phenol cotton blue (LPCB) staining. Further, samples were subjected to mycological culture in Saboraud's dextrose agar. Direct examination revealed presence of ectothrix infection and arthrospores. On culture, isolate showed cottony spreading colony with hairy texture and yellowish pigmentation. LPCB staining of culture showed presence of hyaline hyphae and large thick walled spindle shaped macroconidia with tapering end. Staining morphology and cultural characteristics observed during present study conform to that of *Microsporum canis*.

**AB-32**

### **Swiss Modelling of cAMP Receptor protein**

Ghorpade Prabhakar B<sup>1</sup>, S.D Deshpande<sup>2</sup> and Bhaskar Sharma<sup>3</sup>

Email: dr.prabhakar08@gmail.com

1 & 2. Department of Veterinary Biochemistry

College of Veterinary & Animal Sciences, Parbhani.

Maharashtra Animal and Fishery Sciences University, Nagpur.

3. Indian Veterinary Research Institute, Izatnagar, Bareilly

In recent years the DNA sequencing technologies has been crucial in determining DNA sequences rapidly. The experimental protein sequences determination is relatively slow process. The known experimentally determined protein sequence could give clue to determination of protein sequence structure in-silico via comparative protein modelling. The Swiss Model Repository (SMR) is protein model database. The more similar sequences are likely to have similar structures. Structural similarity is more conserved than sequence similarity. The present work was undertaken with objective of determining 3D structure of cAMP Receptor protein (CRP) of the *Salmonella typhimurium* by Swiss modelling. The CRP is catabolic activator protein. CRP is sensitive to cAMP concentration in host organism. On binding with cAMP the CRP tightly bind promoter of gene. This lead to protein transcription of that gene. The current CRP model was generated using swiss prot pipeline. The model generated is based on template 1i5zB with 1.90Å resolution. The quality QMEAN score for this protein is -0.33. The Z score for C\_beta interaction energy is 1.18, for all atom pairwise energy is 1.27, solvation energy is 1.28, Torsion angle energy is -1.40. The ProSA for our protein is -7.28. In Procheck, the Ramachandran plot shows the most favoured region is 91.8%, whereas disallowed region is 0.0% indicates that model is of good quality. The in-silico determined protein 3D structure give information of protein interaction. This 3D structure could be helpful in identifying promising drug candidate targeting particular protein via docking.

AB-33

### Genetic characterization of orf virus isolate from black buck by VEGF and VLTF-4 genes

Gnanavel Venkatesan<sup>1</sup>, Sargam Arya<sup>1</sup>, Amit Kumar<sup>1</sup>, Karikalan Mathesh<sup>2</sup> and A B Pandey

<sup>1</sup>Division of Virology, Indian Veterinary Research Institute, Mukteswar-263 138

<sup>2</sup>Division Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122

Contagious ecthyma (orf) is a highly contagious, zoonotic viral disease caused by orf virus (ORFV) of the genus *parapoxvirus* (PPV) of the family *poxviridae*. It affects sheep, goats and other domesticated/wild ruminants worldwide. This study is about genetic characterization of ORFV isolate from black buck (Bareilly/2015) targeting vascular endothelial growth factor (VEGF), one of the virulence genes playing role in severity of skin lesions and viral late transcription factor-4 (VLTF-4) gene involved in virus replication. Gene specific primers for VEGF and VLTF-4 genes had showed an amplification of PCR products of ~475 and 750 bp in size respectively. Cloned PCR products of the isolate were sequenced and edited sequences revealed an open reading frame size of 450 bp and 684 bp respectively for VEGF and VLTF-4 genes as reported for other ORFV isolates of sheep and goats. Comparative sequence and phylogenetic analysis of these genes revealed variable genetic relatedness and evolutionary relationship with other circulating strains/isolates of different members of the genus PPV. Sequence analysis based on VEGF revealed high variations even among ORFV isolates (31.2-98.9% at nt and 34.1-96.7% at aa levels) with maximum identity to Chinese isolates. With other members of the genus namely PCPV (50.7-56.0%), BPSV (36-39.3%) and PVNZ (45.2-46.3%), it showed very low level of identities. Genetic analysis of black buck isolate by VLTF-4 gene revealed that it is moderately conserved among ORFV isolates (92.3-97.5%) whereas it showed a percentage homology of 71.1-71.2% with PCPV isolates and 48.5-49.6% with BPSV isolates at nucleotide level. This study provides an evidence of genetic variations among different members of the genus, evolutionary relationship and movement of circulating ORFV strains in black buck and necessitates the regular and systematic investigation on occurrence of contagious ecthyma in wild ruminants to monitor and control the disease.

**AB-34**

**EVOLUTIONARY ANALYSIS OF RABIES VIRUS IN MUMBAI  
REGION OF INDIA**

R. R. Pharande, S. B. Majee, S S.Gaikwad\*\*, A. S. Bannalikal, R. S. Gandge, D.G. Dighe,  
S. S. Moregoankar, A. Y. Doiphode, S.A.Ingle and S. Mukherjee\*

Department of Microbiology, Bombay Veterinary College, Parel, Mumbai-400012 (India)

\*\*Department of Animal Biotechnology, COVAS, Parbhani (India)

\*Department of Virology & Immunology, Haffkine Institute, Parel, Mumbai, India

Rabies is a highly fatal viral zoonotic disease of mammals caused by a neurotropic virus belonging to the *Lyssavirus* genus of family *Rhabdoviridae*. Rabies is an enzootic disease that is globally wide spread and represents a serious zoonotic risk to human population. Rabies virus glycoprotein plays a critical role in the pathogenesis of the disease. Despite the importance of rabies for animal and human health, little is known about the evolutionary dynamics of Rabies virus in dog populations and patterns of biodiversity. To address these questions on a small scale, a total of 38 dog brain samples suspected for rabies were screened by Seller's staining test (SST), Mouse Inoculation Test (MIT) and Direct Fluorescent Antibody Test (dFAT), Glycoprotein (G) gene PCR and Nucleoprotein (N) gene PCR. Out of 38 samples, 19 were positive by SST, 25 were positive by MIT, 29 by dFAT, 29 by G gene PCR and 30 by N gene PCR. A total of 6 samples of G gene PCR were sequenced and the sequences were submitted to Gene Bank. Time-scaled evolutionary analysis of sequenced samples for rabies virus revealed that the Rabies viruses circulating in Mumbai region in India are divided into three lineages: India I, II, III. Time scaled phylogeny by Bayesian coalescent analysis method estimated that the most common recent ancestor for sequences under study cluster to be 1993 and Indian clusters to be 1962. The Time to Most Recent Common Ancestor (TMRCA) of all datasets was observed around 1655 which is in accordance to previous reports. Principal component analysis (PCA) of relative synonymous codons usage (RSCU) also reveals that there was location specific codon usage in partial G gene, suggesting location dependent evolution of rabies virus.

**AB-35**

**Isolation and Molecular Characterization of *Listeria monocytogenes*  
from diarrheal cases in young animals and human infants**

Shubhangi Warke, Tumlam U.M., V.C.Ingle and Sumedha Bobade

Department of Veterinary Microbiology and Animal Biotechnology T & RC, Nagpur Veterinary  
College, MAFSU, Nagpur. Email: shubhangi\_warke@rediffmail.com

Listeriosis in general is considered to be invasive in nature exhibiting neural, visceral and reproductive disorders particularly in various species of animals and humans. *L. monocytogenes* can cause

a non invasive acute, self -limiting, febrile gastroenteritis in healthy individuals including immunocompromised hosts. In the present study sporadic febrile diarrheal cases in human infants (less than 5years of age) and young animals (0-6 months) ( bovine, ovine, canine and porcine ) were initially analyzed for isolation of *L. monocytogenes* . The isolates recovered were further characterized for their virulence potential using *in vitro* and *in vivo* pathogenicity tests and serogrouping by multiplex PCR (*inlA*, *inlB*, *actA* and *hlyA* gene). A total of 267 diarrhoeal samples (115 human infants and 152 young animals) were collected and analyzed for isolation of *L. monocytogenes* . On microbiological and biochemical analysis, four *L.monocytogenes* isolates were identified, two from human infants one from calves and one from piglets respectively. The overall isolation rate of *L. monocytogenes* was very low in human infants (1.73%) and young animals (1.31%); None of the samples from caprine and canine were found positive for *L. monocytogenes*., however all the isolates were found to be highly pathogenic when assessed using *in vitro* and *in vivo* assays. Further, all the 4 isolates revealed amplified products for all the virulence associated genes targeted in the present study. Serotyping revealed that all the four *L. monocytogenes* isolates, belonged to serogroup 4b, 4d, 4e, which is a matter of great concern as serovar 4b in particular, is generally associated with listerial foodborne outbreaks worldwide. To conclude, future human and animal health perspective, due consideration should be imposed for either isolation or detection of *L. monocytogenes* from febrile gastroenteritis cases.

Key wards: *Listeria monocytogenes* ,diarrheal cases, PCR,serogrouping

**AB-36**

**Detection of *Coxiella burnetii* in bovine bulk milk samples by nested PCR in Nagpur region**

Shubhangi Warke, V.C. Ingle and Sumedha Bobade

Department of Veterinary Microbiology and Animal Biotechnology T & RC,  
Nagpur Veterinary College, MAFSU, Nagpur. Email: shubhangi\_warke@rediffmail.com

Q fever is a zoonotic disease caused by an intracellular bacterium named *Coxiella burnetii* . This disease is observed throughout the world, except in New Zealand. The bacterium is very stable in different environments. It is also highly infectious and one to ten organisms can cause Q fever in humans. Cattle, sheep, and goats are the main sources of human infection. In order to be able to manage prevent, and have effective treatment of the disease, early detection is very useful. PCR is a useful, sensitive, and specific method for detection of *C. burnetii* in different samples. The objective of this study was to assess the prevalence rate of *C. burnetii* by nested PCR and the associated risk factors in dairy bovine herd. One hundred samples were collected randomly from dairy herds (2–10 years old cross breed cows) in Nagpur

city, Maharashtra and tested for the presence of *Coxiella burnetii* .DNA were extracted by kit , primers which were designed from the nucleotide sequence of the com1 gene, encoding a 27 kDa outer membrane protein (OMP) .Of the tested samples, 9 samples positive for *C. burnetii* . Infected animals can also shed bacteria by other routes such as feces, urine, placenta, and vaginal mucus. Therefore it is advisable to screen various types of samples in order to reach to conclusion. More studies are required to be undertaken to understand the epidemiology of *C.burnetii* infection. In Research regarding environmental surveillance is also suggested.

Key words : *C. burnetii*, milk, nested PCR

**AB-37**

**Isolation, characterization and antibacterial activity of *Malassezia pachydermatis* from dogs**

V. Dilliwar, S.D.Hirpurkar, C.Sannat\*, N. Rawat, R. Hazari and K. K.Kushwaha

Department of Veterinary Microbiology

College of Veterinary Science & A.H., Chhattisgarh Kamdhenu Vishwavidyalaya,

Anjora, Durg (CG)-491001

E mail address of presenting author\*:csannat@rediffmail.com

Present study was conducted with the aim to isolate and characterize *Malassezia pachydermatis* from dogs. Dogs (n=60) with dermatitis, wound, otitis and pyometra were investigated for *Malassezia pachydermatis* during two different seasons of the year. Apparently healthy dogs (n=10) were taken as control. *Malassezia pachydermatis* was screened by cytological examination and isolated in Sabouraud's dextrose agar and mDixon agar. Study was further extended to evaluate the antibacterial activity of whole cell protein of *M. pachydermatis* against *Staphylococcus aureus* and *E. coli*. The prevalence of *M. pachydermatis* was higher in pre monsoon (14.81%) as compared to monsoon season (6.06%), but differences were not significant (P>0.05). None of the samples from apparently healthy dog showed presence of *M. pachydermatis*. A total of six isolates were obtained from clinical cases yielding 10% prevalence. Isolates obtained were non lipid dependent lipophilic, ellipsoidal yeasts with polar budding. Morphology, dimension, cultural and biochemical characteristics of isolates conform to those of *M. pachydermatis*. Isolates were sensitive to nystatin and miconazole followed by amphotericin-B and ketoconazole, whereas resistant isolates were reported against fluconazole (33.33%) and clotrimazole (66.67%). On turbidity reduction assay, whole cell protein extract of *M. pachydermatis* reduced bacterial count of *Staph. aureus* and *E. coli* markedly (i.e. from  $3 \times 10^8$  cfu/ml to  $1.5 \times 10^8$  cfu/ml). Wide zone of inhibition (10-15 mm) was reported by protein extract against bacterial isolates by disc diffusion method.

**T9**

**Advances In Animal Husbandry  
And Allied Sciences Through  
Biotechnology**

**T9-01**

## **Doubling Indian Dairy Farmers' Income through Dairying**

P. G. Wasnik<sup>1</sup> and S. S. Chopde<sup>2</sup>

<sup>1</sup>Associate Professor and <sup>2</sup>Assistant professor,

Department of Dairy Engineering, College of Dairy Technology, Udgir

Email id: [pgwasnik@gmail.com](mailto:pgwasnik@gmail.com)

### **ABSTRACT**

Earlier strategies for progress of agriculture sector in India were focused principally on raising agricultural output and improving food security. The net result is the farmer's absolute income remained low which lead to agrarian distress. The country also witnessed a sharp increase in the number of farmers' suicides. It is apparent that income earned by farmers from agriculture is crucial to address distress and promote farmers welfare. Union budget 2016-17 emphasizes the need to go beyond food security and provide farmers a sense of income security. The agriculture allied sector such as dairying, poultry, goatry, horticulture etc. will play crucial role in achieving the government's goal to double farmer's income by 2022. Dairying and livestock sector form the backbone of agriculture and its contribution to agriculture GDP is around 28%. The progress in the sector results in balanced development of the rural economy particularly in reducing the poverty amongst the weaker sections. Dairy is a more equitable agriculture occupation. To improve the share of dairying in farmers' income, innovative measures are needed to be explored and shall be implemented on large scale.

**Keywords:** Doubling income, Indian farmers, Dairying

### **INTRODUCTION**

Agriculture is the backbone of Indian economy. Indian agriculture sector accounts for 18 per cent of India's gross domestic product (GDP) and provides employment to 50% of the countries workforce (Madhusudhan, 2015). Though, with the growth of other sectors, the overall share of agriculture on GDP of the country has decreased. Still, Agriculture continues to play a dominant part in the overall economic scenario of India. Persistent low level of farmer's income has increased the farmers' suicides. To secure future of agriculture and to improve livelihood of half of population, adequate attention needs to be given to improve the welfare of farmers and raise agricultural income. With annual growth rate of 10.41 per cent in farmers' income, their income could be doubled by the year 2022-23 (Niti Aayog website). The agriculture allied sector such as dairying, poultry, goatry, horticulture etc. will play crucial role in achieving the government's goal to double farmer's income by 2022. Dairying is an integral component of Indian agriculture supporting livelihood of more than two-thirds of the rural population. Today, the livestock

sector contribution to agriculture GDP is around 28% (FAO, India, 2005). Dairy sector contribution is around 65-70% to livestock sector. Dairy is a more equitable agriculture occupation. 85% of the small and marginal farmers in India own 45% of the land, but own 75% of the bovine (The free press journal, 2016). For a marginal, small farmers and landless person, dairying is one of the best occupations.

In India, dairying has been considered as one of the activities aimed at alleviating the poverty and unemployment especially in the rural areas in the rain-fed and drought-prone regions. Moreover, this sector is crucial for reducing income inequalities. Dairy is the best insurance against the vagaries of nature like drought, famine and other natural calamities (Ramphul, 2011). India ranks first in milk production, accounting for 18.5 % of world production, achieving an annual output of 155.5 million tonnes during 2015-16 as compared to 146.31 million tonnes during 2014-15 recording a growth of 6.28 %. Thus, the dairy sector rearing has vast scope for improving economic and in turn, the nutritional status of such people mainly coming from rural area. The progress in this sector results in a more balanced development of the rural economy (FAO, 2009). Union budget 2016-17 aims to double farmers' incomes by 2022 to tackle suicides among them (PTI, New Delhi, 2017). The budget emphasizes the need to go beyond food security and provide farmers a sense of income security. However, certain issues need to be addressed in order to affect a turnaround in the economic condition of our farmers. For this, the following innovative measures to enhance productivity and quality need to be explored and implemented.

#### **A) Improving Productivity of Milch Animal**

India has the world's largest livestock population of 512.05 million numbers. The total number of female cattle is 122.9 million while female buffalo is 92.5 million. Owing to this huge bovine stock, though India has managed to attain numero uno position in milk production, the full potential of Indian milch herd remains unattained. Over the last three decades (1982 to 2012), average productivity of Indian cattle and buffaloes has grown from 1.9 to 3.9 kg per day, and from 3.7 to 6.2 kg per day respectively. The average daily milk yield for crossbred cattle is better at 7.1 kg per day, but still significantly lesser than the best of global standards — UK, US and Israel are at 25.6, 32.8 and 38.6 kg per day, respectively (Ministry of agriculture, 2014). The major causes of low productivity in India are both intrinsic (low genetic potential) and extrinsic (poor nutrition/feed management, inferior farm management practices, ineffective veterinary and extension services and inefficient implementation of breed improvement programmes).

#### **1. Breeding initiatives**

A cattle and buffalo breeding policy needs to be framed, keeping in view the production traits, economic performance and draftability of breeds of animals in context with the agro-climatic factors, existing infrastructure and support systems. Government initiatives such as the National Project on Cattle

& Buffalo Breeding (NPCBB) have contributed significantly to strengthening artificial insemination (AI) services. At present the AI coverage in India is a mere 40% — another cause of poor animal productivity and a prime concern for the dairy sector. All efforts should be undertaken to expand AI services. But lack of focus on progeny testing due to unavailability of technical manpower, small herd size and inefficient implementation has been an impediment (Narke, 2017).

Cross-breeding with high yielding exotic breeds should be encouraged in areas with adequate facility for feed and fodder. Genetic upgradation by way of breeding non-descript cattle with defined indigenous breeds needs to be encouraged in resource deficient areas. India should emulate Brazil which successfully improved Zebu cattle and made it more heat and disease resistant. Also, efforts are on all over the world to develop stable synthetic breeds, an excellent example of which is Girlanda (Gir x Holstein). To improve breeds the Government should evolve a policy to encourage selective breeding. It should allow import of high progeny Gir semen from Brazil and also embryos from Zebu superior genetic animals for developing a stock of breeding animals (Narke, 2017). For developing sustainable breeding strategies it is also necessary to have comprehensive national level mapping and database development on number of species of livestock and their breeds, available animal genetic resources, breeding infrastructure and development facilities.

## **2. Feed management**

With rapidly shrinking land and natural resources, availability and quality of feed and fodder is increasingly becoming a challenge. The current deficit of green fodder and concentrates is 34 per cent. Further, there is a supply demand gap for quality forage seeds as well.

Imbalanced nutrition is a major factor responsible for low livestock productivity. The use of total mixed ration blocks will help reduce gap between demand and supply of fodder and would scale up the ongoing ration balancing activities like the one implemented by NDDDB. The National Dairy Development Board (NDDDB) of India has developed user-friendly computer software for advising milk producers on their doorstep to balance the ration of their lactating animals with the available feed resources and area-specific mineral mixtures. Innovative approaches to attain feed and fodder sufficiency such as the use of silage, azola and technologies including hydroponics need to be explored and implemented.

## **3. Veterinary Services:**

High quality veterinary services are required for improving animal output. But currently due to unavailability of trained manpower and lack of mobility, the services provided are not able to create desired impact.

**B) Farm Management Practices:**

On-farm practices should ensure that milk is produced by healthy cattle under sustainable economic, social and environmental conditions. It is important to note that good management of a farming system constitutes the grassroots of the system's economic, environmental and social sustainability. The value of clean milk production throughout the dairy chain needs to be inculcated. Managing cattle in a scientific manner with hygienic and clean housing conditions, deworming of cattle etc are measures that need to be expeditiously adopted.

**C) Government Subsidies:**

A subsidy is a monetary aid or support that a government organization provides to company/institutions/individual firm, with the intention of promoting economic and social policies. Presently, National Bank for Agriculture and Rural Development (NABARD) provides subsidy for establishing dairy farm/milk chilling centre/dairy plants under Dairy Entrepreneurship Development Scheme (DEDS) (NABARD website).

Governmental support by way of subsidies is also required for calf rearing programmes. Such calf rearing programme at farmers' doorstep will enhance milk productivity and reduce mortality by approximately 5% in cow calves and 15% in buffalo calves (Narke, 2017).

**D) Minimum Support Price for Milk**

Minimum Support Price (MSP) is a form of market intervention by the Government of India to ensure agricultural producers against any sharp fall in farm prices. The minimum support prices are announced by the Government of India at the beginning of the sowing season for certain crops on the basis of the recommendations of the Commission for Agricultural Costs and Prices (CACP). MSP is price fixed by Government of India to protect the producer - farmers - against excessive fall in price during bumper production years. The minimum support prices are a guarantee price for their produce from the Government.

This MSP scheme is provided only to the grains and cereals (agricultural commodities) but not for the milk. If the scheme is applied to dairy sector, dairy farmers will get fixed price for their milk irrespective of milk production.

**E) Special Budget for Dairying**

Special provision shall be made in the union budget for putting the dairy sector back on progressive track. The world's biggest dairy development program, operation flood has transformed India from milk

deficient to the world's largest milk producer. But to meet the demand of ever increasing population, country needs to plan a suitable widespread dairy development programs which will have its impact on all the parts of country.

Currently, National dairy plan (phase I) is in implementation with a total investment of about Rs. 2242.00 Crore. The objective of this scheme is to increase the productivity of milch animal and to provide rural milk producer with greater access to the organized milk processing sector (NDDB website).

In the Union Budget 2017, Rs 8,000-crore dairy processing and infrastructure development fund has been provided. This would strengthen India's numero uno position as the world's largest milk producer. The special fund will help set up processing capacity of an additional 500 lakh litres of milk per day in the country (Nabard website, 2017).

Along with this, National Programme for Dairy Development (NPDD) under the Central Sector Scheme "National Programme for Bovine Breeding and Dairy Development (NPBBDD) is approved during 2013-14 with the budget provision of Rs.600 crore for implementation during 12<sup>th</sup> Plan. The objective of the scheme is to create dairy infrastructure for improved procurement, processing and marketing of milk and milk products.

#### **F) Cold Chain Infrastructure**

The lack of sufficient and efficient cold chain infrastructure is a major contributor to food losses and waste. The basic challenge in the development of cold chain for dairy is that the producers are small and scattered. At present there is no cold chain facility for small producer of dairy products. Big producers have got their own arrangement. Creation of cold chain network at the farm level will result in a win-win arrangement for the farmers, agents and processors as well.

#### **G) Value Addition to the Milk:**

Value added dairy product fetch more profit compare to fluid milk. The profit of margin for value added dairy products is around 12-15% while for the fluid milk it is only 5-6%. In addition, value added dairy products have larger shelf life than raw milk. So, farmers should start cottage level milk product manufacturing unit using traditional manufacturing technologies. These technologies require small investment and processing equipments/ materials could be easily arranged at village level.

#### **H) Implementation of AMUL-Milk Co-operative Federation model throughout the India:**

Cooperative movement is an attractive mechanism for pooling farmer's meager resources for solving common problems relating to credit, supplies of inputs and marketing of agricultural produce. For

instance, AMUL, a dairy company that was founded by a few farmers with mission to stop the exploitation by middlemen, gradually became the biggest brand in the nation. It is managed by the Gujarat Co-operative Milk Marketing Federation Ltd. (GCMMF), which is jointly owned by more than 3.6 million milk producers of Gujarat. AMUL spurred the white revolution in India, which placed India at the top of milk producing nations. "GCMMF has successfully quadrupled the income of its dairy farmers in last seven years, demonstrating the efficacy of Amul model," (Amul website).

Financial results of the apex body of dairy cooperatives in Gujarat, declared in the 43<sup>rd</sup> annual general meeting of GCMMF held on 15<sup>th</sup> June 2017 that the income of its dairy farmers-members has quadrupled in last seven year, demonstrating the efficacy of the Amul model in exceeding the national goal of doubling farmer's income in six years.

#### **I) Animal Insurance**

Implementation of the livestock insurance scheme would encourage farmers to insure their milch cattle and buffaloes. A system should be developed to sensitize farmers to go for animal insurance. Government should launch a low premium insurance cover for milch cows and buffaloes with an objective to create a protection mechanism for dairy farmers and cattle owners against any loss of their livestock during natural disasters.

#### **J) Availability of Feed and Fodder in the Drought Affected Areas:**

Drought conditions greatly reduce the available forage for livestock. Drought is a part of the normal production cycle. Dealing with these dry periods and decreased feed supplies needs to be part of the overall management plan. Inadequate green fodder will affect the productivity of the animal. Severe drought situations force farmers to sell their milch animal.

Frequent droughts in several states have also brought out the need to develop fodder banks in vulnerable areas and strategies for improving the supply/ transportation of fodder from one region to other region in the country. Government of India should provide support to the farmers on purchase of feed and fodder in the drought affected areas.

#### **K) Establishment of Farmers Dairy Training Centre/ Dairy Vigyan Kendra:**

Training is necessary to provide a constant support of recent technological knowledge to farmers and it provides an opportunity to inculcate necessary skill along with gain in theoretical knowledge. It can be successfully bring about certain changes in the outlook and attitude of the farmers and thereby making them capable of rendering this task more effectively and efficiently.

Like krishi vigyan kendra, a specialized training centre i.e. dairy vigyan kendra providing exhaustive trainings on dairy farming, milk processing and dairy product manufacturing shall be established in each districts.

#### **L) Mobile Training Centre**

To reach out to every farmer especially those in the outskirts that due to proximity seem to be denied by opportunities to grow, to learn and be updated on the new beneficial know-how, mobile training centres should be provided with respective district agriculture extension departments.

### **CONCLUSION**

Persistent low level of farmer's income has resulted in distress among farmers and lead to increase in farmers suicide. To secure the future of agriculture and to improve livelihood of half of India's population, past strategies for development of farm sector needs to be reviewed. Doubling the farmer's income by 2022-23 from the base year 2015-16 is challenging but it is achievable. The agriculture allied sector will have to play crucial role in achieving the target. The above discussed measures would go a long way in increasing productivity and its benefits would automatically accrue to the farmers positively impacting their economic conditions.

### **REFERENCES**

- Food and agriculture organization of the United Nation- India. 2005. Livestock sector brief. <https://www.nabard.org/news-article.aspx?id=25&cid=552&NID=51> accessed on 05/11/2017.
- Madhusudan, L. 2015. Agriculture role on Indian economy. Business and economics journal, 6: 176. Doi:10.4172/2151-6219.1000176.
- Ministry of Agriculture, Government of India, Press Information Bureau. 2014. Salient Features of 19<sup>th</sup> Livestock Census.
- Narke, N. 2017. From the president's desk - Doubling dairy farmers' income. Indian dairyman. PTI, New Delhi. Aim to double farmers' income by 2022 to tackle suicides: Govt to SC. The Indian express August 28, 2017.
- The dairy industry and its relevance to India. 2016. Free Press Journal. [www.amul.com/m/43rd-annual-general-body-meeting-held-on-a5th-june-2017](http://www.amul.com/m/43rd-annual-general-body-meeting-held-on-a5th-june-2017) accessed on 7/11/2017.
- [www.nabard.org/content.aspx?id=591](http://www.nabard.org/content.aspx?id=591) accessed on 13.11.2017
- [www.nddb.org/ndpi/about/brief](http://www.nddb.org/ndpi/about/brief) accessed on 13.11.2017
- [www.niti.gov.in](http://www.niti.gov.in) . Policy paper on doubling farmer's income. Accessed on 14/11/2017

T9-02

## ENTOMOPATHOGENIC FUNGUSES AS BIOPESTICIDES: EMERGING CONCEPT FOR TICK CONTROL IN INDIA

GreeshmaRao U B and B.W Narladkar

Department of Veterinary Parasitology, College of Veterinary & Animal Sciences, MAFSU,  
Parbhani- 431 402 (MS)

**Introduction:** Ticks are obligate blood-feeders that require an animal host to survive and reproduce. They feed on a wide variety of mammals, birds, reptiles, and even amphibians. Ticks can be a nuisance; their bites can cause irritation and, in the case of some ticks, paralysis. Severe infestations on animals can cause anemia, weight loss, and even death from the consumption of large quantities of blood. Ticks can also transmit many human and animal disease pathogens, which include viruses, bacteria, rickettsiae, and protozoa.

The control of this tick parasite is based on chemical products. However, *R. microplus* has developed resistance to almost all pesticides used including organophosphates, pyrethroids, and amidines, requiring higher doses or a mixture of several products for their effective control. These practices result in increased production costs and contamination of the environment (Li et al. 2004; Miller et al. 2005).

An alternative is the use of biological control such as the use of predators, parasitoids, and entomopathogens, including fungi, bacteria, viruses, and nematodes. Within the bacterial group, the microorganism most widely used worldwide with the highest success in the control of several insect pests is the bacterium, *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Fernández-Ruvalcaba et al, 2010)

The present technology to control ticks is based mainly on the use of chemical products; however, the ability of *B. microplus* to develop resistance to different acaricides, the demands of consumers for chemical free foods and the negative environmental effects of acaricides call for the development of alternative strategies (Kay and Kemp, 1994). Although entomogenous fungi have been used widely for the control of agricultural and forest pests (Ferron, 1981; Anderson *et al.*, 1988;), little effort has been made to evaluate the applicability of biocontrol potentials of entomogenous fungi against ticks which are vectors of human and animal diseases.

Entomopathogenic fungi are known to infect different tick species and the efficacy of these interesting fungi to control ticks is highly strain-specific (Samish and Rehacek 1999). Among these fungi, *Beauveria bassiana* and *Metarhizium anisopliae* received major attention (Kalsbeek *et al.*, 1995). Different studies on the potential use of *M. anisopliae* and *B. bassiana* as entomopathogenic agents were performed in

laboratories; however there is an urgent need for checking the novel strains and even fungal species from different geographic regions to find out potent species.

**Work undertaken at Parasitology lab at College of Veterinary and Animal Sciences Parbhani: (Narladkar et al, 2015; Greesma Rao, 2017)**

The development and evaluation of herbal and biological control agents are the scientific steps in this direction. Since long herbal and fungal control agent are used against this tick. As a part of similar efforts, present research was planned to evaluate a herbal acaricide product containing 4 herbs and 3 different fungi with their Parbhani and Rahuri isolates against adult and egg stage of the same tick species and results are reported.

The eight fungal treatments namely *Metarhizium anisopliae* Rahuri Old (MRO), *Metarhizium anisopliae* Rahuri New (MRN), *Metarhizium anisopliae* Parbhani (MP), *Beauveria bassiana* Rahuri Old (BRO), *Beauveria bassiana* Rahuri New (BRN), *Beauveria bassiana* Parbhani (BP), *Verticillium lecanii* Rahuri Old (VRO) and *Verticillium lecanii* Rahuri New (VRN), when evaluated against adult female ticks resulted into the mortality around 0.29 to 0.71, if expressed in percentage it can be around 29-71% indicating that fungus holds good as tickicidal *in-vitro*. The total 8 fungal treatments showed significant differences and amongst all; treatment with conidial concentration of  $5g \times 10^8$  conidial /litre and  $6g \times 10^8$  conidia per litre gave the optimum results. The 3 fungi with their Parbhani and Rahuri isolate and recently harvested (new) or harvested before 4 years (old) combinations had shown potent activity in producing the mortality of the adult ticks.

Ticks survived with the treatments with 8 fungal treatments laid the eggs. When comparison of number of eggs deposited by such treated females with control group was made, it was found that all treatments resulted in significant reduction of egg laying capacity. The average numbers of eggs laid at the concentration of  $5g \times 10^8$  conidia/ litre were 102.85, 34.29, 29, 27.85, 47.57, 8.85, 10.71, 10.71 and 1578.55 for MRO, MRN, MP, BRO, BRN, BP, VRO, VRN and control respectively.

Another experiment undertaken with the females which have been survived and deposited the eggs, such eggs grouped into the batches of 100 and allowed to hatch in reference with the control. At the end of the experiment, it was observed that eggs which were harvested from the treated females, maximum of them failed to hatch and only few could yield the 1<sup>st</sup> stage larvae. The hatchability recorded at the concentration of  $5g \times 10^8$  conidia/litre was 3.29, 0.85, 1.43, 0.43, 1.85, 4.71, 1.85, 1 and 97.57 for MRO, MRN, MP, BRO, BRN, BP, VRO, VRN and control respectively. This experiment also demonstrated that these fungi causes damage and produces the effect in the same generation as horizontal effect and in next generation as vertical effect.

Another experiment was run by maintaining a group of blood engorged female ticks for egg laying. The eggs laid by these females were grouped into the batches of 100 and treated with the fungi. It was found that all 8 different fungal treatments resulted in substantially reducing the hatchability of eggs. The reduction in hatchability at the concentration of  $5g \times 10^8$  conidia/litre was 11.80, 38.60, 6.60, 20.40, 4.39, 8.60, 11, 1.60, 96.80 for MRO, MRN, MP, BRO, BRN, BP, VRO, VRN and control respectively. This experiment also demonstrated that funguses have efficacy as a ovicidal and can be promising tool in minimizing tick population before it takes birth on the earth.

When all parameters combined together in the form of OER (Overall Effect Rate) and Scorecard, it was found that the treatments VRN@ 6g/l, VRO@ 6g/l and BP@6g/l were having highest activity while MRN @1g/l, BRN@1g/l and MRO@1g/l were having the lowest activity. The results with concentration of  $5g \times 10^8$  conidia /litre were taken into account, on the ground that, same concentration is routinely used by farmers against crop pests and hence are well acquainted and not much difference between 5g and 6g/l in the effect rate.

An experiment involving all the three fungal species was undertaken *in-vivo*, by spraying fungal solution at 5g/l at the sites where ticks have deposited the eggs in the cattle shed. Post treatment observations of the said experiment indicated high potency of the funguses as ovicidal against the tick eggs.

**Conclusions:** Three funguses *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* as bio-pesticide against showed significant activity in terms of mortality of adult ticks, reducing egg laying capacity, less hatchability and less hatchability of treated eggs and are emerging as new tools for control of important tick pest in India.

#### References:

- Andersen M., Maga N., Mead A. and Chandler D. (2006) Development of a population-based threshold model of conidial germination for analysing the effects of physiological manipulation on the stress tolerance and infectivity of insect pathogenic fungi..Environ.
- Drummond R.O., G. Lambert, H. E. Smalley Jr. and C. E. Terrill (1981) Estimated losses of livestock to pests. In D.Pimentel (ed.), Handbook of Pest Management in Agriculture, Vol. 1. CRC Press, Inc. Boca Raton, FL.
- Fernandez-Ruvalcaba M., Romo-Martinez G.A.,Hernandez-Velazquez V., Bravo de Parra A., Perez De La Rosa D. 2010. Evaluation of *Bacillus thuringiensis* pathogenicity for a strain of the tick,*Rhipicephalus microplus*, resistant to chemical pesticides. *Journal of Insect Science* 10: 1-6.
- Ferron P. (1981) Pest control by the fungi *Beauveria* and *Metarhizium*. In: Burgess HD, editor. *MicrobialControl of Pests and Plant Diseases* 1970-1980, 24:465-482. London, Academic Press.

- Kalsbeek V., Frandsen F. and Steenberg T. (1995) Entomopathogenic fungi associated with Ixodesricinus ticks. Exp. Appl. Acarol. 19, 45–51.
- Kay, B.H. and Kemp, D.H. (1994) Vaccines against arthropods. Am. J. Trop. Med. Hyg. 50, 87–96.
- Li AY, Davey BR, Miller JR, George EJ. 2004. Detection and characterization of amitraz resistance in the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae). *Journal of Medical Entomology* 41(2): 193-200
- Miller JR, Davey BR, George EJ. 2005. First report of Organophosphate-resistant *Boophilus microplus* (Acari: Ixodidae) within the United States. *Journal of Medical Entomology* 42(5): 912-917
- Narladkar B.W, P.R. Shivpuje and P.C. Harke (2015) Fungal Bio-Control Agents for Integrated Management of *Rhipicephalus (Boophilus) microplus* Ticks. Indian Vet. J., May 2015, 92 (5): 34 – 37.
- Samish M. and Rehacek J. (1999) Pathogens and predators of ticks and their potential in biological control. Annual Review of Entomology 44, 159–182.

**T9-03 Upliftment of livelihood of tribal farmers by doubling their annual income through implementing the scientific goat farming practices in Thane district (Maharashtra).**

Dr. H.Y. Palampalle and Dr. S. D. Jagadale

Instructional Livestock Farm Complex,

Bombay Veterinary College, Parel, Mumbai – 400 012

Maharashtra Animal and Fishery Sciences University, Nagpur Email ID: [hypalle1@gmail.com](mailto:hypalle1@gmail.com)

**Introduction**

India is a rich repository and diverse genetic resource of 28 pure breeds of goat as per National Bureau of Animal Genetic Resources (NBAGR, 2017). The total goat population as per 19<sup>th</sup> Livestock Census - 2012 in the country is 135.17 million which represents 26.40% of the total livestock population. The goat population has declined by 3.82% over the previous census and 8.43 million in Maharashtra. Goat (*Capra hircus*) is the earliest ruminant and has been around man as a domestic animal since about 6000 B.C. The goats have high reproductive capability and endure with a minimum amount of input which makes them a reliable source of food and ready cash. It is the backbone of landless and marginal farmers particularly in areas where, agriculture is low and farmers have to depend upon livestock for their livelihood (Tantia and VIj, 2000).

Goatery plays a significant role in economic upliftment of rural poor and have a distinct, social, economic, managerial and biological advantage over other livestock species and significantly contributing to the agrarian economy. It plays a vital role in the livelihood security of the small and marginal farmers

and resource poor rural people through meat, milk, skin and hair production. In pastoral societies in India, goats are kept as a source of additional income and as an insurance against income shocks of crop failure. In addition, the rural poor who cannot afford to maintain a cow or a buffalo find goat as the best alternative source of supplementary income and milk. (Shalander Kumar, et al., 2010) This is one reason why poor rural households maintain a few number of goats. Unlike a cow or buffalo, a few goats can be maintained easily and can be easily liquidated in times of distress. In recent years, goat enterprise has also shown promise of its successful intensification and commercialization (Kumar, 2007, a, b).

The goattery rearing is occur unorganized sector and women force are mainly engaged in one to two goat rearing along with daily wages activities. As a part of government policy of skilled development in Animal Husbandry sector the scientific goat rearing is now full time occupation of many small and landless farmers. It provide dependable source of income throughout the year particularly to below poverty line people therefore, goat are popularly known as “poor man’s cow” in our country (Shanugusunderun, 1980).

National Commission of Farmers (NCF) strongly recommended in the report that keeping small ruminants of livelihood provision in draft porn area to curb farmer suicide amongst the farmers (Swaminathan Report, 2002). Amongst livestock species goat is more suitable species for tribal because 49% and 51% of tribals are living in TSP and OTSP area respectively and engaged in forest related activities. Majority of tribal are agriculturist with hilly area nearby forest. Their daily livelihood depends on forest related commodities. Keeping in this views of their natural habitat to nature and without affecting their tradition and culture goat rearing at hilly area is advantageous than other livestock rearing for their livelihood.

#### **Pilot Project for Tribal:**

Considering the suitability of goat farming to tribal and their skill development the department of Instructional Livestock Farm Complex & Livestock Production & Management of Bombay Veterinary College, Mumbai has been sanctioned one ICAR funded project under Tribal Sub Plan (TSP) entitled “Upliftment and provision of livelihood for Schedule Tribe (ST) population of Maharashtra state” to implement in Thane and Nashik district farmer since from 2012-13, 2013-14 and 2015-16. In Maharashtra State goat rearing will be advantageous to the tribal population as the agro climatic conditions are suitable for these enterprises. Keeping in this view the importance of goat farming in tribal areas the project was formulated with following broad objectives.

1. To develop the skill through the workshop & vocational training on Scientific Goat Farming for improvement in goat rearing and to utilize the maximum potential of the animals.

2. To provide livelihood to tribal population of Maharashtra through the animal husbandry activities.
3. To make the tribal population of Maharashtra self sufficient for their livelihood on long term basis.
4. Preparation of interactive, self explanatory study materials in vernacular language for tribal.

Under this project during the year 2012-13 the project was implemented in Vikramgad and Jawhar talukas of Thane district by conducting survey in villages for selection of the ST candidates for training at ILFC unit Goregaon. During that period 34 ST candidates were trained for residential seven days hands on scientific goat farming training at goat Unit, Instructional Livestock Farm Complex, Bombay Veterinary College, Goregaon, Mumbai-65 and among this 8 families i.e. two from each villages were selected for actual individual benefits from four villages of Vikramgad and Jawhar talukas of Thane district (Tale No. 1). Provided 100 % subsidy for all beneficiaries for their economic improvement are as follows.

1. Provided one goat unit of low cost shed material which comprises of 200 sq. ft. area at their door step made up of iron angles, tins, chain links and asbestos sheet materials for each beneficiary.
2. Distributed one unit of goat containing of 10 females and 1 male buck to the each beneficiary family in selected villages. The total 88 Good & healthy condition Osmanabadi goats were purchased from Ahilya Devi Sheli Vikas Mahamandal, Dahiwadi, district – Satara, which is a Government goat farm.
3. All the animals were dewormed and vaccinated at the time of distribution to the tribal farmers at their door step with help of project Staff members. Veterinary Technical services are provided throughout the project period.

#### **Project Implementation Location:**

Thane the northern most district of konkan is a coastal district adjoining the Arabian Sea on the west coast. It lies between 18 42' and 20 20' north latitude and 72 45' and 73 45' East longitude. Thane, being a coastal district, has high humanity throughout the year. The district receives regular, well-distributed and heavy rainfall during the south – west monsoon season, which constitutes about 94 per cent of the total annual rainfall. The average annual rainfall in the district is 2,293.4 mm.

According to the census 2011, Thane district of Maharashtra has **total population of 11,060, 148**. Scheduled Tribes people in Thane District have been living in geographical isolation as a result of which some of them that are designated as primitive tribes like Katkari, Kathodi, etc. Tribal are usually referred as backward, meaning their lack of capacity to utilize the opportunities of development offered to them. The crucial issues in their development are therefore not related only to providing infrastructure and

schemes of development but to develop their capacity to utilize them on par with other citizens of the District or state. Major tribes of thane district are *Katkari, Kokna, Mahadeo-koli, Malhar-koli, Kathodi, Warli, Thakur, Dubla, Dhor-koli, Tokara-koli* etc.,

The scheduled tribes of Jawhar and Vikramgad, talukas were cut off from mainstream for a very long time and were still having a very primitive way of living. Even today their economic life depends upon natural forces. The scheduled tribe is surrounded by forests and hillocks. The tribal are basically, hill and forest dwellers. They are isolates. Forest and forest produce have been their major source of livelihood in the past.

**Table No.1 Detail information of selected Beneficiaries under TSP Project:**

Sr. No.	Name of Beneficiaries	Caste	Education	Family Size	No. of Goat Distributed	No. of Kid Born	No. of Death of kid and goat	Total goat Available at farm
1	Mahendra Jiva Dodhade	Hindu-Varli	12 <sup>th</sup> Pass	09	10 + 1 unit	9	3	17
2	Dinesh Raghunath Lahare	Hindu-Kokana	9 <sup>th</sup> Pass	09	10 + 1 unit	19	3	27
3	Kashiram Tanya Kasat	Hindu-Malap Koli	12 <sup>th</sup> Pass	04	10 + 1 unit	11	3	19
4	Subhash Janardhan Ghatal	Hindu-Katkari	8 <sup>th</sup> Pass	06	10 + 1 unit	02	6	07
5	Vijay Laxman Bhusara	Hindu-Kokana	B. A.	06	10 + 1 unit	06	3	14
6	Ramchandra Vittal Gangorda	Hindu-Kokana	12 <sup>th</sup> Pass	07	10 + 1 unit	02	6	07
7	Devrao Bhau Dhinda	Hindu-Varli	10 <sup>th</sup> pass	07	10 + 1 unit	11	2	20
8	Sakharam Ladkya Wagh	Hindu-Katkari	illeterate	07	10 + 1 unit	11	5	16

**Important care to be taken during project implementation in tribal Community:**

1. The selection of beneficiaries is crucial work and can be done with formulating authorized competent selection Committee.
2. The ideal period for goat distribution to beneficiaries in tribal area of konkan region is in month of October to November to avoid heavy mortality during heavy rainy season of the year.
3. Along with distribution of goat to tribal community at least provide 30 days concentrate mixture/ goat pelleted feed to reduce the mortality of goat after transportation from their native place to tribal areas.
4. While giving the free benefit to beneficiaries the local political leader, rural development officer and animal husbandry officer should be informed about aims and objectives of project and try to make them involved in project work with their inputs and suggestions for success of the project.
5. While providing any benefits to tribal community for the maintenance of record is very important part of project to avoid the post implementation any complications. Video shooting and photography should be taken at actual site of project and also at a time of distribution of any material to beneficiaries in the presence of two to three witnesses with their signatures on distributed documents. Obtain the undertaking about the project material issued with the witness by local people.
6. Majority of the beneficiaries are illiterate so the thumb impression may be taken in front of local resident as witness. Documentation is very essential and must in TSP projects to reduce the future implementation complication.
7. Aware about the beneficiaries about importance of insurance of goat for insurance claim. Ask them to do post mortem of goat after death with the help of local livestock development officer and veterinary officer appointed by insurance company for claim settlement.
8. Vigilance about the repeated advantage taken by same beneficiaries for 100 % subsidies @ Adhar card, Ration card, small family certificate, Below poverty line certificate, 7/12 extract photocopies are also taken for office record.
9. Regular feedback and technical help of expert at least 15 days interval up to 3 months periods.
10. Regular interval in center in the form of free vaccination, deworming and concentrate feed supplied with home visit to know the problem of tribal's.
11. Regular presentation of progress reports to funding and controlling agencies for success of project.

**Outcome of the Scheme:**

1. Empowerment of the tribal's for self sustenance through goat training and workshop.
2. Started scientifically rearing of goat.
3. Seasonal labour problem in tribal are replace by goat farming as self employment throughout the year

4. Develop the marketing skill to sale the animal for catching high price.
5. Used the goat manures for silvi-horti-pastural farming.
6. Developed confidence among the farmers for scientific goat farming.

#### **Conclusion:**

The study has enriched our understanding on the socio-economic upliftment of ST candidates for goat production in the tribal area of Thane district and demonstrated that goat rearing in an economically viable rural enterprise. This project is useful to tribal unemployed youth. In such project the incentives in the form of monthly concentrate feed on the basis of positive growth performance will be helpful. Buyback guarantee scheme in which early age goat of above 18 kg will be purchased by funding agency and distributed to other tribal to multiply and popularize the project in tribal area is recommended. In other word contract goat farming is useful to make tribal engaged in goat farming as self employment throughout the year for doubling their income. The common facilities like Chaff cutter, silage pit, feed mixing unit and demonstration unit should be made available at their villages as per their convenience different type of technological, policy and institutional support under self help group of village.

#### **Acknowledgement:**

The Authors gratefully acknowledge the Indian Council of Agricultural Research, New Delhi for their financial support and we are also thankful to Bombay veterinary college, Parel, Mumbai and Maharashtra animal and Fishery Sciences University, Nagpur for their administrative support.

#### **References:**

- Economic survey of Maharashtra 2016-17, Directorate of Economics and statistics, planning Department, Govt. of Maharashtra, Mumbai, [https://mahades.maharashtra.gov.in/files/publication/ESM\\_Eng2016\\_17.pdf](https://mahades.maharashtra.gov.in/files/publication/ESM_Eng2016_17.pdf).
- Forest Rights Act, 2006 & rules 2008, 2012, Scheduled Tribe and other traditional Dwellers. [trti.maharashtra.gov.in](http://trti.maharashtra.gov.in)
- Kumar, Shalander (2007a): "Commercial Goat Farming in India: An Emerging Agri-Business Opportunity", Agricultural Economics Research Review, Vol. 20 (Conference issue), pp. 503-520.
- Kumar, Shalander (2007b): "Commercialization of Goat Farming and Marketing of Goat in India", Final Project Report-ICAR Ad-hoc Research Scheme, CIRG, Makhdoom, Mathura.
- Livestock policy of Maharashtra, 2010.
- National Bureau of Animal Genetic Resources, Karnal- 132001 (Haryana), India, 2017, [www.nbagr.res.in/registeredbreed.html](http://www.nbagr.res.in/registeredbreed.html).

Report of Annual Plan, Tribal Development department for the year 2016-17, published by tribal department, 2016-17.

Report Summary M. S. Swaminathan Committee on Farmers, October 2006.

Shalander Kumar, C.A. Rama Rao, K. Kareemulla and B. Venkateswarlu, (2010): Role of Goats in Livelihood Security of Rural Poor in the Less Favoured Environments Ind. Jn. of Agri. Econ. Vol.65, No.4, Oct.-Dec. 2010

Shanugusunderum, S (1980): Goat ' The poor man's cow, livestock advisor, 5 (7):11

Tantia, M.s. and P.K. Vij (2000): Population estimates of sheep and goats breeds of India. Indian J. Anim. Res. 34 (1): 60-63.

**T9-04**

### **Applications of Radiomunoassay (Rai) to Improve Animal Health and Production**

Dr. Chandrakant N. Galdhar

Ph.D. (Veterinary Medicine), DMRIT, RSO (Nuclear Medicine)

Assistant Professor and Radiological Safety Officer

Department of Veterinary Clinical Medicine, Veterinary Nuclear Medicine, including Radio Isotope

Laboratory, Bombay Veterinary College, Parel-Mumbai-12

Email:chgaldhar@gmail.com, Mobile:-9420888536

Bombay Veterinary College is the only institute of the country to establish a dedicated Veterinary Nuclear Medicine facility comprising *In vivo* diagnostic facility (Gamma Scintigraphy) and *in vitro* diagnostic facility (Radio Isotope laboratory). The Nuclear Medicine facility including Radio Isotope Laboratory is approved facility from Atomic Energy Regulatory Board (AERB) and issued No Objection Certificate (NOC) for procurement and import of RIA kits. The Department having AERB nominated Radiological Safety Officer (RSO-Level-II). Currently Department is engaged in optimization and standardization of human based RIA kits for veterinary purpose.

#### **Clinical And Research Application To Improve Animal Health And Production**

Estimation of bovine and bubaline progesterone by RIA has found immense importance and application in veterinary clinical practice and research. Apart from these applications, this technique can be used for improvement in dairy animal production. Measurement of Progesterone can be used for early diagnosis of non-pregnancy and establishment of reproductive performance, appraisal of infertility and

improvement in artificial insemination services. Generally plasma and milk samples are collected from animals for estimation of progesterone. We optimized and validated progesterone hormone profile in domestic and pet animals. The hormonal values which we recorded in our laboratory are in agreement with national and international standards and showed 95% correlation in clinical, physiological and pathological situations.

Analysis of thyroid profile by RIA, proved promising and have key role in diagnosis and therapeutic management of thyroid disorders in companion animal practice. To justify the need of accurate diagnosis of thyroidal illness, in initial phase, department undertook extensive work in field of optimization and standardization of human based thyroid kits ( $T_4$ ,  $T_3$  and  $FT_4$ ) for veterinary applications and standardized the RIA procedure. Radio Isotope Laboratory of Bombay Veterinary College optimized and validated thyroid hormone status ( $T_3$ ,  $T_4$  and  $FT_4$ ) in cats by using human based RIA kits. The values of thyroid profile, which we recorded for normal cats, are in range with reported national and international standards. Similar work in concern to canines is in progress.

Department also undertook research work on estimation of Cortisol in bovines, caprines and poultry. For this work we used ready to use human based imported RIA kits. Further, the samples were analyzed as per recommended procedure. But, in the case of cortisol, we recorded subnormal to undetectable levels with poor clinical correlation. This need to study in-depth, where scope is available for modification in the assay procedure.

### **RIA and Radiation Safety**

If ready-to-use kits are employed, the handlings are simple and do not result in any significant radiation exposure to the working personnel. Normally no personnel monitoring is required for persons working in RIA laboratories, if using ready to use RIA kits. However, precautions should be taken to safeguard against spread of contamination to the counting tubes and the gamma counter itself. This can be achieved to a large extent by good work practice. Any liquid waste arising from the RIA procedure can be disposed off in the sink provided in the storage area and the used RIA vials should be disposed off in such a way as to avoid reuse. One of the suggested methods is to crush them before disposal. Further, solid waste may be kept in waste room for 10 half lives for decay. The personnel engaged in the actual work should have adequate knowledge of the basic procedures of counting and should be aware of some simple precautions to be taken in handling of radioactivity. If labeling with radio iodination, involving use of a few millicuries of  $^{125}\text{I}$ , is contemplated, extra precautions and facilities will be required. The details of guidelines to setup a radio immune assay laboratory are available on AERB web (<http://www.aerb.gov.in/>).

### **RIA kits and availability**

RIA ready to use kits may be procured from Board of Radiation and Isotope technology (BRIT), Mumbai. However, kits may be imported from importing agencies like Anand Brothers, New Delhi.

### **Acknowledgements**

Author is thankful to the Associate Dean, Bombay Veterinary College and Dr. R.V.Gaikwad (In charge, Department of Veterinary Nuclear Medicine including Radio Isotope Laboratory) for providing needful facility to undertake RIA work. Author is also thankful to Board of Radiation and Isotope technology (BRIT), Mumbai for providing technical guidance to optimize and validate the RIA.

**T9-05**

### **Advances in Animal Husbandry & Animal health Biotechnology.**

Dr.S.D.Deshpande

Professor & University Head

Department of Veterinary Biochemistry

College of Veterinary & Animal Sciences, Parbhani.

Email Id: drsdd@rediffmail.com.

Animal Biotechnology has a number of applications in livestock production. It is being used to hasten animal growth, enhance reproductive capacity, improve animal health and develop new animal products. Use of these biotechniques will boost the farmers income.

### **Feed, Growth and Production**

Biotechnology can increase the digestibility of low-quality roughage, and genetically modify plants to improve their feed value, such as the amino acid balance. It can also provide hormones and other substances that enhance animal size, productivity and growth rates.

Synthetic hormone bST (bovine somatotropin) was among the first innovations available commercially. It can increase milk yield by as much as 10 to 15 per cent in lactating cows. Current development efforts are looking at a whole spectrum of genes that affect growth and production within the animal. Ways to genetically engineer cattle to increase their own natural hormone production are being considered, thus eliminating the need for synthetic bST. Locally produced recombinant bovine somatotropin (BST) is being used in many countries as a growth stimulant and for increased milk production in cattle. Other important technologies related to feed and digestion include the following:

## Feed Additives

High-protein yeast cell products are being used as a feed additive for cattle, pigs and poultry. Highly palatable and nutritious, these products also help create a healthy balance of bacteria in the digestive tract, and prevent bacterial diarrhea.

A bacterial phytase formula, TRANSPHOS, is being used to replace the costly mineral phosphate used as an additive in the feed of monogastric animals in Korea. In the Philippines, a bacteriocin is being produced which has antibacterial properties against *Listeria monocytogenes*, *Staphylococcus aureus*, and other pathogens found in livestock feed and human food.

L-lysine monohydrochloride, a safe and stable form of lysine, being produced by the fermentation of a special strain of bacteria in raw molasses. Lysine is one of the most essential amino acids. Livestock requirements for it are hardly met by the amount present in natural feeds. Lysine supplementation improves the nutrient balance of feed, and feed conversion rates by livestock.

## Probiotics and prebiotics

**Probiotics** are defined as cultures of live microorganisms that lodge in the intestinal tract of humans and/or animals where they exert a beneficial action on the host's health. The most important genera of probiotic bacteria are *Lactobacillus* and *Bifidobacterium*. Some yeasts also exert a probiotic effect.

**Prebiotics** are food ingredients that improve the host's health by selectively stimulating the growth and/or activity of probiotic bacteria in the intestinal tract. The leading prebiotics are non-digestible oligosaccharides, including: fructo-oligosaccharides (FOS), galacto oligosaccharides (GOS), maltooligosaccharides (MOS) and xylo-oligosaccharides (XOS). The most typical prebiotics are FOS and inulin and they are sold widely for use in humans and monogastric animals.

**Symbiotics** are a combination of probiotics and prebiotics that have a beneficial effect on the host's health by improving the survival and implantation of probiotics in the gastrointestinal tract by selectively stimulating their activity and/or growth.

Scientific literature documents that the consumption of probiotics, prebiotics or symbiotics improves the intestinal flora, prevents and rehabilitates diarrhoeal diseases, improves the composition of blood lipids, reduces cholesterol, improves blood pressure, increases calcium absorption and retention, modulates immune functions and reduces the risk of colon cancer.

In livestock, the use of probiotics, prebiotics or symbiotics provides the additional benefit of reducing or eliminating the prophylactic use of antibiotics in feed or water.

### **Animal nutrition**

Nutrition represents one of the most serious limitations to livestock production in developing countries, especially in the tropics. Feed resources are inadequate in both quality and quantity, particularly during the dry seasons. Biotechnological options for improving rumen fermentation and enhancing the nutritive value and utilisation of agro-industrial by-products and other forages are: balancing of nutrients for the growth of rumen microflora thereby facilitating efficient fermentative digestion and providing small quantities of by-pass nutrients to balance the products of fermentative digestion, enhancing digestibility of fibrous feeds through treatment with alkali or by manipulating the balance of organisms in the rumen and genetic manipulation of rumen micro-organisms, currently acknowledged as potentially the most powerful tool for enhancing the rate and extent of digestion of low quality feeds. Rumen micro-organisms can also be manipulated by adding antibiotics as feed additives, fats to eliminate or reduce rumen ciliate protozoa (defaunation), protein degradation protectors, methane inhibitors, buffer substances, bacteria or rumen content and/or branched chain volatile fatty acids.

#### **Increasing digestibility of low-quality forages.**

Low-quality forages are a major component of ruminant diets in the tropics. The lignification of the cell walls prevents degradation by cellulase or hemicellulase enzymes. Fortunately, it is possible to use lignase enzyme produced by the soft-rot fungus (*Phanerochaete chrysosporium*) which causes a high degree of depolymerisation of lignin (Tien and Kirk 1983). The enzyme acts like a peroxidase and causes cleavage of carbon-carbon bonds. At present the levels of the lignase enzyme produced by the basidiomycete fungi are insufficient for the treatment of straw on a commercial scale. However, it is conceivable that the use of recombinant DNA engineering techniques will allow the modification of the lignase genes and associate proteins to increase their efficiency and stability. The lignin gene has to date been cloned and sequenced from *P. chrysosporium* (Tien and Tu 1987).

#### **Improving nutritive value of cereals**

Moderate protein content and low amounts of specific amino acids limit the nutritive value of cereals and cereal by-products (e.g. barley is low in Lysine and threonine). This is a major limitation in the ration formulation for non-ruminant livestock which necessitates addition of expensive protein supplements. There are on-going studies to enhance the low level of Lysine in barley by genetically

engineering the grain genome (Shewry and Kreis 1987). Genetic modification through insertion of genes into rice protoplasts and generation of transformed plants has already been achieved.

### **Removing anti-nutritive factors from feeds**

Anti-nutritive factors in plant tissues include protease inhibitors, tannins, phytohaemagglutinins and cyanogens in legumes, and glucosinolates, tannins and sanapine in oilseed rape (*Brassica napus*) and other compounds in feeds belonging to the *Brassica* group. Transgenic rumen microbes could also play a role in the detoxification of plant poisons (Gregg 1989) or inactivation of antinutritional factors.

### **Improving nutritive value of conserved feed**

Throughout this century, research workers have investigated ways through which the fermentation process in silage making can be controlled in order to improve the feeding quality of the resulting silage. Use of additives, to restrict the activity of the microorganisms, to stimulate the fermentation by the lactic acid bacteria or simply as nutrients has been one of the approaches.

A group of compounds classified as fermentation stimulants which include sugar sources (e.g. molasses and whey), enzymes and inocula of lactic acid bacteria. Enzymes are essential for the breakdown of cell-wall carbohydrates to release the sugars necessary for the growth of the lactic acid bacteria. Although resident plant-enzymes and acid hydrolysis produce simple sugars from these carbohydrates, addition of enzymes derived from certain bacteria, e.g. *Aspergillus niger* or *Trichoderma viridi* (Henderson et al 1982) increases the amount of available sugars.

There are two forms of indigenous lactic acid bacteria: the homofermentative type which converts hexose sugars to lactic acid with no loss of dry matter and the heterofermentative type which produces a range of compounds accompanied by loss of dry matter as carbon dioxide.

### **Improving rumen function**

The major areas of rumen function improvement include development of transgenic bacteria with enhanced cellulotic activity, capability to cleave lignohemicellulose complexes, reduced methane production capability decreased proteolytic and/or deaminase activities, increased capability for nitrogen “fixation” and increased ability for microbial production of specific amino acids. The first successful transfer of foreign genes into rumen bacteria (*Bacteriodes rumenicola*) was reported by Thomson and Flint (1989).

It can be concluded that there are several potential opportunities for improving the efficiency of ruminant digestion and possibilities for utilising a wider range of feeds than is currently possible.

Modification of rumen microbial population is one such opportunity.

### **Reproductive physiology**

Biotechnology can greatly accelerate the speed at which desirable characteristics (e.g. better growth rates, or increased milk production) can be introduced into animals. While classical breeding to enhance animal traits works well, it takes decades to produce major changes. Through biotechnology, an organism can be modified directly in a very short time if the appropriate gene has been identified.

A recent breakthrough in animal reproduction is the combined application of the existing in vitro fertilization, and the state-of-the-art ultrasound-guided transvaginal oocyte pick-up (OPU) technique in cattle. When heifers reach puberty at 11-12 months of age, their oocytes may be retrieved weekly or even twice a week for embryo production and embryo transfer. There is even the possibility of applying this technology to juveniles. In this way, high-value female calves can be used for breeding long before they reach their normal breeding age.

### **Improving the Reproductive Rate**

Number of hormone implants and treatments are being used to increase the production of mammalian oocytes and embryos. Various chilling and freezing techniques have been developed for preservation of oocytes and/or embryos, including ultra-rapid freezing by electron microscope grid.

Embryo transfer is being used on valuable animals, so that oocytes and embryos from high-value animals are transferred into the uterus of surrogate mothers. The PCR (polymerase chain reaction) test has been developed to establish the sex of cattle embryos. This is very important, particularly when combined with embryo transfer, since it gives control over the sex of the offspring. Similar tests are being developed for other types of livestock. The hypoosmotic swelling test has been developed to evaluate the quality of frozen and thawed bovine sperm.

### **Artificial insemination**

No other technology in agriculture, except hybrid seed and fertiliser use, has been so widely adopted globally as AI. Progress in semen collection and dilution, and cryopreservation techniques now enables a single bull to be used simultaneously in several countries for up to 100,000 inseminations a year (Gibson and Smith 1989). The high intensity and accuracy of selection arising from AI can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating (Van Vleck 1981).

A wider and rapid use of selected males through AI will accelerate the rate of gender improvement.

### **Embryo transfer (ET)**

Although not economically feasible for commercial use on small farms at present, embryo technology can greatly contribute to research and genetic improvement in local breeds. There are two procedures presently available for production of embryos from donor females. One consists of superovulation, followed by AI and then flushing of the uterus to gather the embryos. The other, called *in vitro* fertilisation (IVF), consists of recovery of eggs from the ovaries of the female then maturing and fertilising them outside the body until they are ready for implantation into foster females. IVF facilitates recovery of a large number of embryos from a single female at a reduced cost thus making ET techniques economically feasible on a larger scale. Additionally, IVF makes available embryos suitable for cloning.

The principal benefit of embryo transfer is the possibility to produce several progeny from a female, just as AI can produce many offspring from one male. For example the average lifetime production of a cow can be increased from 4 to 25 calves. Increasing the reproductive rate of selected females has the following benefits: genetically outstanding animals can contribute more to the breeding programme, particularly if their sons are being selected for use in AI; the rate of genetic change can be enhanced with specially designed breeding schemes which take advantage of increased intensity of female selection combined with increased generation turnover; transport of embryos is much cheaper than that of live animals; risk of importing diseases is avoided; facilitates rapid expansion of rare but economically important genetic stocks; and the stress to exotic genotypes can be avoided by having them born to dams of local breeds rather than importing them as live animals.

### **Embryo sexing and cloning**

Taylor et al (1985) concluded from a study that an all-female heifer system using ET was 50% more efficient than the highest achievable in a traditional system. It has been suggested that, if multiple sexed-embryo transfer became as routine an operation as AI is, beef operations based on this system could become competitive with pig and poultry production in terms of efficiency of food utilisation.

Clones may be produced by embryo splitting and nuclear transfer (Macmillan and Tervit 1990). These offer the possibility for creating large clone families (Woolliams and Wilmut 1989) from selected superior genotypes which, in turn, can be used to produce commercial clone lines (Smith 1989). Other potential applications of cloning include efficient evaluation of genotype x environment interactions and testing and/or dissemination of transgenics. From a research standpoint, production of identical siblings should, by eliminating variability among animals, greatly reduce the size and hence the cost of experiments.

### **Hormone use**

Use of hormonal assays to *monitor* reproductive function can be rewarding for both research purposes and commercial livestock operations. Reproduction can also be *manipulated* using hormonal treatments. Progesterone and PMSG treatment and immunisation against androstenedione increased ovulation rate in Ossimi sheep. Also, exogenous melatonin treatment of barren Rahmani ewes resulted in increased proportion of ovulating ewes and a higher ovulation rate (Aboul-Naga et al 1992).

Reproduction can also be manipulated without application of exogenous hormones. Aboul-Ela et al (1988) reported that exposing ewes to rams one week prior to mating (“the ram effect”) increased the percentage of ewes in oestrus (and hence the per cent mated) by 27%. “Accelerated lambing” - increasing the number of lambings per year - can also be used to increase annual productivity.

### **Animal genetics and breeding**

Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production on a range of environments.

### **Multiple ovulation embryo transfer and open nucleus breeding system**

Multiple ovulation embryo transfer (MOET) is a composite technology which includes superovulation, fertilization, embryo recovery, short-term *in vitro* culture of embryos, embryo freezing and embryo transfer. Benefits from MOET include increasing the number of offspring produced by valuable females, increasing the population base of rare or endangered breeds or species, *ex situ* preservation of endangered populations, progeny testing of females and increasing rates of genetic improvement in breeding programmes.

The ONBS concept is based on a scheme with a nucleus herd/flock established under controlled conditions to facilitate selection. The nucleus is established from the “best” animals obtained by screening the base (farmers’) population for outstanding females. These are then recorded individually and the best individuals chosen to form the elite herd/flock of the nucleus. The ONBS can be used for the improvement of an indigenous or exotic breed.

### **Genetic markers and marker-assisted selection**

A genetic marker for a trait is a DNA segment which is associated with, and hence segregates in a predictable pattern as, the trait. Genetic markers facilitate the “tagging” of individual genes or small

chromosome segments containing genes which influence the trait of interest. The method involves screening the genome for genes with a large effect on traits of economic importance through a procedure known as linkage analysis (Paterson et al 1988).

Marker identification and use should enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases. Already, identification of carriers of genes for resistance and introduction of such genes into a population seems feasible for resistance against *Trichostrongylus colubriformis* and *Haemonchus contortus* (Gogolin-Ewens et al 1990). It should also be possible to eliminate factors predisposing sheep to Listeriosis or Salmonellosis (Blancou 1990). There is also evidence for a major gene for resistance to the cattle tick (*Boophilus microplus*) in a Hereford x Shorthorn cattle line called Belmont Adaptaur (Kerr et al 1994).

### **Transgenic animals**

A transgenic animal is an animal whose hereditary DNA has been augmented by addition of DNA from a source other than parental germplasm through recombinant DNA techniques. Transfer of genes or gene constructs allows for the manipulation of individual genes rather than entire genomes.

Successful production of transgenic livestock has been reported for pigs, sheep, rabbits and cattle. The transgenic pigs have been bred with a porcine lactoferrin transgene. Lactoferrin is a milk protein which promotes gut growth and prevents diarrhea. Sows with this gene maintain a high lactoferrin level in their milk throughout lactation. Transgenic boars can transmit this gene to their offspring.

Transgenesis offers considerable opportunity for advances in medicine and agriculture. In livestock, the ability to insert new genes for such economically important characteristics as fecundity, resistance to or tolerance of other environmental stresses, production of medically important proteins such as insulin and clotting factors in the milk of domestic livestock.

Recent advances in *in vitro* technology (*in vitro* fertilisation and maturation) will increase the number of zygotes available for gene transfer purposes.

### **Genetic characterization of animal genetic resources**

Developing countries are endowed with the majority of the global domestic animal diversity - landraces, strains or breeds. Some livestock breeds in these countries are in immediate danger of loss through indiscriminate crossbreeding with exotic breeds. The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stresses and to traditional husbandry systems.

Genetic uniqueness of populations is measured by the relative genetic distances of such populations from each other. Polymorphism in gene products such as enzymes, blood group systems and leukocyte antigens which have traditionally been used for measuring genetic distance are being rapidly replaced by polymorphism at the level of DNA, both nuclear (Jeffreys and Morton 1987) and mitochondrial (Loftus et al 1994) as a source of information for the estimation of genetic distances. The first DNA polymorphism to be used widely for genome characterisation and analysis were the restriction fragment length polymorphism (RFLP) (Southern 1975) which detect variations ranging from gross rearrangements to single base changes. Minisatellites sequences of 60 or so bases repeated many hundreds or thousands of times at one unique locus within the genome have been used to generate DNA fingerprints typical of individuals within species (Jeffreys and Morton 1987). Microsatellites (Weber and May 1989) repeats of simple sequences, the commonest being dinucleotide repeats are abundant in genomes of all higher organisms, including livestock. Polymorphism of microsatellites takes the form of variation in the number of repeats at any given locus and is generally revealed as fragment length variation in the products of polymerase chain reaction (PCR) amplification of genomic DNA using primers flanking the chosen repeat sequence and specific for a given locus (Kemp and Teale 1991).

Randomly amplified polymorphic DNA (RAPD) (Williams et al 1990) has been extensively used for genetic characterisation of a wide range of organisms. The technique uses short (up to 10 bases) primers to amplify nuclear DNA in the PCR. The procedure does not require knowledge of the sequence of DNA under study; primers are designed randomly. Complete sequencing of the genome is the ultimate form of genetic characterization.

### **Conservation of animal genetic resources**

There are several ways, differing in efficiency, technical feasibility and costs, to conserve animal genetic resources. Developing and utilising a genetic resource is considered the most rational conservation strategy. However, there are cases where ex-situ approaches are the only alternatives. Ex-situ approaches include: maintenance of small populations in domestic animal zoos; cryopreservation of semen (and ova); cryopreservation of embryos; and some combinations of these. Cryopreservation of gametes, embryos or DNA segments can be quite an effective and safe approach for breeds or strains whose populations are too small to be conserved by any other means.

### **Animal Health**

One important benefit from biotechnology is the diagnosis of livestock diseases, and genetically transmitted conditions which damage health and productivity. Biological techniques can also produce cheaper and more efficient drugs. In cases where a natural source material is prohibitively expensive,

genetic engineering (in microbial or tissue culture systems) can be used to produce drugs of high value for humans or animals. Examples are insulin, human growth hormone and tissue plasminogen activator (used in treating heart disease).

## **Vaccines**

Vaccines are used to stimulate an animal's immune system to produce the antibodies needed to prevent infection. Recombinant DNA technology has provided the means to produce large quantities of inexpensive vaccines, while a better understanding of the immune system has helped produce vaccines that do a better job of boosting the body's immune system. These engineered products are safer than traditional vaccines. Whereas conventional vaccines sometimes revert to virulent (disease causing) forms, the new vaccines can be engineered to eliminate this threat.

Some recent developments include the following: The DNA vaccine with an encoded target gene is being used to produce new vaccines. Tests have shown that the DNA vaccines consistently induced antibody response, and were resistant to toxin challenge.

They also have other advantages. They offer protection against diseases for which no vaccine is currently available. Their production does not need dangerous infectious agents. With mass production, they will not cost much to produce. Since they are stable at room temperature, storage costs will also be low.

The combined vaccine against pleuropneumonia, pneumonic pasteurellosis and enzootic pneumonia in swine. Molecular biology has been used to produce an improved vaccine to protect pigs from swine fever. An improved vaccine to protect cattle and water buffalo against hemorrhagic septicemia is also available. This disease is the leading cause of death among these animals. The new vaccine gives improved protection at a very low cost. A number of improved vaccines for poultry have also been developed to protect birds from Newcastle disease, fowl cholera and infectious coryza.

## **Veterinary diagnostic systems**

Various methods have been used to detect and identify diseases and diagnose their causal agents, ranging from the most conventional methods using morphological and biochemical techniques to an increasing number of immunological and molecular techniques from modern biotechnology.

- a) These immuno-enzymatic techniques are being continually improved and incorporate technologies such as rapid diagnostic strip tests, which are very easy to use, even at production-unit level, and yield an immediate result.

b) Molecular diagnostic techniques which are fast becoming widespread in veterinary diagnostic laboratories. Single DNA sequences provide a high degree of specificity in the diagnosis and control of pathogenic microorganism species and subspecies, and polymerase chain reaction (PCR) techniques permit extremely high specificity levels.

Not only have molecular methods increased the sensitivity and specificity of diagnostic methods, they have also significantly reduced the subjectivity inherent in the interpretation of morphological and biological data.

c) Real-time polymerase chain reaction (RT-PCR) is one of the latest improvements in PCR techniques to have been incorporated by veterinary diagnostic laboratories. The entire process takes place in an individual sealed tube, which greatly reduces the problem of cross-contamination, and the ability to electronically process the detected fluorescence in real time obviates the need for subsequent reaction and the electrophoretic run required in traditional PCR. In addition, RT-PCR permits the use of quantitative methods.

d) The sequencing of complete genomes of pathogens yields major elements for biological studies and for improving parasite diagnosis and control.

e) The new microarray techniques allow screening for the genotypes of specific parasites and provide major support for epidemiological surveys of veterinary parasites.

f) Proteomic techniques make it possible to identify and characterise the proteins produced by pathogens and are of enormous interest to veterinary diagnosis, enabling the protein expression pattern of viruses, bacteria and other pathogens to be studied. Proteomics also allows the study of proteins that are expressed or repressed differentially as a result of being attacked by pathogens, which is extremely important for identifying new methods for using vaccines, medicinal products or other means to control pathogens.

g) Other technologies such as biosensors, fluorescent in situ hybridization (FISH) and nanotechnologies are being incorporated as new veterinary diagnostic tools.

### **Animal Products**

Biotechnology can lead to new and improved animal products. For example, it can modify the composition of milk, or the fat content of meat. Genetically transformed cows can produce designer milks with superior properties for use in various milk products. Added caseins in milk, for instance, can enhance cheese making. Increasing the phosphate group in casein can enhance the level of calcium. Removal of the

source of lactose intolerance in milk can have a significant impact on the market for dairy products, especially among the 90 % of people with an Asian or African background who are lactose-intolerant.

Some new products made possible by biotechnology include: microbial rennet which can be used in place of animal rennet to coagulate milk during the making of cheese; new lactic acid bacterium produced by cell fusion technique; safe anti-microbial agent, made from skim milk and glucose, which prolongs the shelf life of fresh milk;

Biologically active peptides extracted from animal blood at slaughterhouses. These can be used as an additive in functional foods to improve human health; Colorant extracted from animal blood, to partially replace nitrite in meat products.

## **Conclusion**

Therefore it is concluded that, biotechnology can bring about improvements in animal growth and production, nutrition, reproduction, health and animal products with due consideration of conserving natural resources and the environment.

## **References**

- Aboul-Ela M.B., El-Nakhla S.M., Gabr M.G., Hassan F.A., Aboul-Naga A.M. and Hanrahan J.P. 1988. Effect of active immunization against androstenedione on ovulation rate and fecundity in fat-tailed rahmania and Finnish landrace x Rahmani crossbred ewes. In: *Proceedings of the 11th International Congress on Animal Reproduction and Artificial Insemination, University College Dublin, Ireland, 26-30 June 1988*. Volume 4. University College Dublin, Dublin, Irish Republic. p. 487.
- Aboul-Naga A.M., Aboul-Ela M.B. and Hassan F. 1992. Manipulation of reproductive activity in subtropical sheep. *Small Ruminant Research* 7:151-160.
- Blancou J. 1990. Utilization and control of biotechnical procedures in veterinary science. *Revue Scientifique et Technique de l'Office International des Epizooties* 9:641-659.
- Gibson J.P. and Smith C. 1989. The incorporation of biotechnologies into animal breeding strategies. In: Babiuk L.A., Phillips J.P. and Moo-Young M. (eds), *Animal Biotechnology. Comprehensive Biotechnology First Supplement*. Pergamon Press, Oxford, UK. pp. 203-231.
- Gogolin-Ewens K.J., Meeusen E.N.T., Scott P.C., Adams T.E. and Brandon M.R. 1990. Genetic selection for disease resistance and traits of economic importance in animal production. *Revue Scientifique et Technique de l'Office International des Epizooties* 9:865-896.
- Gregg K. 1989. Enhancement of microbial detoxification by gene transfer. In: *Proceedings of the 7th International Symposium on Ruminant Physiology, Sendai, Japan*. Academic Press, San Diego, USA. p. 58.

- Henderson A.R., McDonald P. and Anderson D. 1982. The effect of cellulase preparation derived from *Trichoderma vivide* on the chemical changes during the ensilage of grass, lucerne and clover. *Journal of the Science of Food and Agriculture* 33:16-20.
- Jeffreys A.J. and Morton D.B. 1987. DNA fingerprints of dogs and cats. *Animal Genetics* 18:1-15.
- Kemp S.J. and Teale A.J. 1991. Dinucleotide repeat polymorphism at the bovine locus FSHB. *Animal Genetics* 22:435.
- Kerr R.J., Frisch J.E. and Kinghorn B.P. 1994. Evidence for a major gene for tick resistance in cattle. In: *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada, 7-12 August 1994. Volume 20*. International Committee for World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada. pp. 265-268.
- Mackinnon M.J. 1992. Use of genetic markers in genetic improvement programmes. *Proceedings of the Australian Association of Animal Breeding and Genetics* 10:245-251.
- Macmillan K.L. and Tervit H.R. 1990. Available technologies and prospective developments in reproduction & embryology. *Proceedings of the Australian Association of Animal Breeding & Genetics* 8:9-18.
- Shewry P.R. and Kreis M. 1987. Biotechnology and improvement of feeds. *Proceedings of the Nutrition Society* 46:379-385.
- Smith C. 1989. Cloning and genetic improvement of beef cattle. *Animal Production* 49: 49-62.
- Southern E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98:503-517.
- Taylor St. C.S., Moore A.J., Thiessen R.B. and Bailey C.M. 1985. Efficiency of food utilization in traditional and sex-controlled systems of beef production. *Animal Production* 40:401-440.
- Thomson A.M. and Flint H.J. 1989. Electroporation induced transformation of *Bacteroides ruminicola* and *Bacteroides uniformis* by plasmid DNA. *FEMS Microbiology Letters* 61:101-104.
- Tien M. and Kirk K.T. 1983. Lignin degrading enzyme from the Hymenomycete *Phanerochaete chrysosporium* burds. *Science* 221:661-663.
- Tien M. and Tu C.-P.D. 1987. Cloning and sequencing of a cDNA for a ligninase from *Phanerochaete chrysosporium*. *Nature (London)* 326:520-523.
- Van Vleck L.D. 1981. Potential genetic impact of artificial insemination, sex selection, embryo transfer, cloning and selfing in dairy cattle. In: Brackett B.G., Seidel Jr G.E. and Seidel S.M. (eds), *New Technologies in Animal Breeding*. Academic Press, New York, USA. pp. 221-242.
- Weber J.L. and May P.E. 1989. Abundant class of DNA polymorphisms which can be typed using polymerase chain reaction. *American Journal of Human Genetics* 44:388-396.
- Williams J.G., Kubelik A.R., Livak K.J.C., Rafalski J.A. and Tingey M.S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18(22):6531-6535.

**T9-06**

## **Platelet-rich plasma (PRP): A new vision for tissue repair under regenerative therapy**

Dr. V. D. Aher

Professor and Head, Department of Veterinary Surgery and Radiology,  
College of Veterinary and Animal Science, Parbhani  
MAFSU, Nagpur (Maharashtra)

### **INTRODUCTION**

From a historical point of view, the first PRP preparations began during the 1960s and become routine preparations through the 1970s. In recent years, the use of platelet-rich plasma (PRP) has increased notably in a range of diseases and conditions, such as wound healing. Healing of wound is a physiological phenomenon that initiates from the loss of integrity of the skin, generating a solution of continuity that reaches the underlying layers in diverse degrees and depends on a series of chemical reactions classically divided in four phases: inflammation, debridation, reparation and maturation. PRP therapy involves the injection of a small volume of plasma or the application of PRP gel foam directly to the site of injury. Platelets were found to release wound healing substances ( growth factors) that initiated the repair of injured tissues and vessels in cutaneous ulcers (Margolis et al., 2001). Later in the 1990s, platelets were introduced into maxofacial surgery as autologous modifications of potent adhesives known as fibrin glues. The use of platelets was particularly fortuitous given that the main initial interest was to take advantage of the adhesive and haemostatic properties of the homologous fibrin during bone surgery. A realization of the clinical potential of PRP therapies followed the positive clinical observations, such as enhanced bone formation and anti-inflammatory functions, during wound and fracture healing. Muscle healing, like tendon healing, occurs in a series of overlapping phases, including inflammation, proliferation and remodeling. These events are also coordinated by growth factors and cell-to-cell interactions. Healing is dependent on local vascularity and regeneration of intramuscular nerve branches, both of which may be enhanced by PRP.

### **PLATELET RICH PLASMA**

Platelet-rich plasma (also known as platelet-enriched plasma, platelet-rich concentrate, autogenous platelet gel, or platelet releasate) is being evaluated as an enhancement for soft-tissue healing by placing supraphysiologic concentrations of autologous platelets at the site of tissue damage. A rich source of the complex group of growth factors (GF's) essential to natural wound repair is the platelet. The platelets act in the hemostasis, wound healing and reepithelialization liberating diverse GF's that stimulate the angiogenesis, promoting growth and vascular fibroblast proliferation that in turn provide an increase in

the collagen synthesis. Degranulation of platelets by proteins such as thrombin causes them to release these factors which include transforming growth factor- $\beta$  (TGF- $\beta$ ), fibrinogen, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), vascular endothelial growth factor (VEGF), platelet thrombo-plastin, thrombospondin, coagulation factors, calcium, serotonin, histamine and hydrolytic enzymes. Platelet-rich plasma (PRP) is 100% biocompatible and safe. It poses absolutely no infectious risk to the patient because it is made from the patient's own plasma. Auto Gel and Safe Blood are autologous preparations in which blood is drawn from the patient and centrifuged to create platelet-rich plasma that is applied to the wound, fractured site and tendons.

PRP, in general, has a higher concentration of platelets compared with baseline blood. Clinically valuable PRP, however typically contain 1 million platelets or more per microliter. Procuren® an autologous product that has been used as treatment in the past for chronic wound healing, but it is no longer manufactured or commercially available. Until now, the use of PRP use has been restricted to the hospital setting. This was mainly due to the cost of separating the platelets from the blood (thousands) and the large amount of blood needed to produce a suitable quantity of platelets. An easy, cost-effective way to obtain high concentrations of growth factors for tissue healing and regeneration is autologous platelet storage via PRP. This new technology permits to safely harvest and produce a sufficient quantity of platelets from only 20 ml of blood drawn from animals in veterinary hospitals. Ease of procurement along with application of PRP in the wounds, fracture and its beneficial outcomes has popularized it among the clinicians.

## **PREPARATION OF PRP AND GEL**

Platelet rich plasma (PRP) is essentially an increased concentration of autologous platelets suspended in a small amount of plasma after centrifugation. Centrifugation process accelerates the sedimentation of the heavier cells such as white blood cells and red blood cells, while platelets (which sediment to a lower rate) remain floating within the plasma fraction at the top side of the tubes. PRP can be prepared in a laboratory or veterinary clinic from blood collected in the immediate preoperative period. Basically, animal's blood is collected (approx. 20 ml) and centrifuged at varying speeds until it separates into 3 layers:

platelet poor plasma (PPP) on topmost layer, PRP in middle, and red blood cells at the bottom. Usually 2 spins are used. The first spin (Hard spin) separates the platelet poor plasma (PPP) from the red fraction and platelet rich plasma (PRP).

The second spin (Soft spin) separates the red fraction from the PRP. This is achieved by simple bench centrifugation at high gravitational force. After centrifugation, material with the highest specific gravity (PRP) will be deposited at the bottom of the tube. Immediately prior to application, a platelet

activator/agonist (topical bovine thrombin and 10% calcium chloride) is added to activate the clotting cascade, producing a platelet gel. The whole process takes approximately 12 minutes and produces a platelet concentration that of native plasma which is used fracture healing where PRP is injected at the fracture site and if the prepare PRP kept undisturbed then it will form PRP gel which is used on wound healing. However, in larger wound and compound fracture cases, 80 to 100 ml of whole blood should be drawn, so as to obtain the larger amounts of PRP needed for larger surgical defects. The preparation and processing of PRP is similar in most of commercial systems available, except anticoagulant used, speed and duration of centrifugation may vary with different systems.

### **CLINICAL APPLICATION OF PRP IN WOUND & FRACTURE HEALING**

At present, different PRP cell-separation systems are commercially available. The most commonly used technique is to obtain a blood sample from the patients themselves (autologous), but homologous techniques are also a valid option. When treating a wound with PRP, the amount of bioavailable growth factors depends on both the platelet storage and the release into the microenvironment. PRP injection should take place within the first 10 minutes after PRP activation with the procoagulant substance. PRP will be injected in wound edges in the wound bed and at the fracture sites. Topical application may be combined with intralesional use. As happens with wound dressings and the different alternatives and advanced treatments, efficacy will mainly depend on the presence of a properly prepared wound bed. Prior to PRP application, the wound should be cleaned and adequately debrided. If there is a high amount of necrotic or unviable tissue, it should be removed and PRP postponed until necessary. The choice of the secondary dressing depends on the amount of wound exudate. Before topical application if PRP is mixed with bioactive polymers viz hydrogels, shows profound effect on the inflammatory, proliferative, and remodeling phases of wound healing. These polymers enhance the action of the fibrin mesh that is naturally obtained in the clot after PRP activation. Additionally, the use of these polymers for gelification enables an immediate production of PRP gel, which may be helpful in veterinary practice.

### **OTHER APPLICATIONS OF PRP**

In ulceration of any part, the application of platelet rich- derived therapies gives ground for optimism. The therapeutic use of PRP has also been extended to other fields, including facial plastic surgery, eye surgery and cosmetic surgery. Because of its hemostatic properties, it has been demonstrated that the platelet-rich preparation is effective in sealing capillary beds during surgery and thereby controlling bleeding. In addition, it decreases postoperative complications and avoids the re-exploration of the patients, which is cost effective. This approach opens a new and emerging field of wound pharmacology that represents an exciting advance in plastic surgery therapeutics.

In case of humans, PRP finds its profound use in corneal ulcers, dealing of non healing ulcers in diabetic patients, plastic surgeries and grafting.

## **BENEFITS OF PRP**

PRP acting as a vehicle for growth factors offer several advantages like: (1) PRP is a by-product of the patient's own blood; therefore, chances of infectious disease transmission is rare,(2) PRP can be easily generated in the veterinary clinics while the patient is undergoing an outpatient surgical procedure, like fracture fixation, large wound, variety of grafting and soft tissue procedures, oral surgery etc.,(3) as there is super saturation of the wound with PRP, and thus growth factors, fasten tissue regeneration,(4) since PRP harvesting is done with only 8-10 ml of blood, the patient need not bear the expense of the harvesting procedure in hospital or at the blood bank,(5) it is easy to handle and actually improves the ease of application of bone substitute materials by making them more gel-like,(6) preparation time is relatively short and concentrate can be obtained within 12 min.

## **CONCLUSION**

PRP preparation provides a fairly simple method to deliver a variety of natural growth factors to the patient. High concentrations of proteins acting in concert through different pathways can be achieved by commercially available systems that can be used in the operating room. The risks of contamination and immunogenic response are considerably low. The remaining risk of coagulopathies could be minimized by using alternative activation methods to standard bovine thrombin. The PRP and its gel can be obtained in a more economical form and used in places where no resources but that usually make use of a centrifuge and common materials in hospital environments as syringes, needles and tubes for blood collection. The use of PRP gel as an improved therapy for surgical or non-healing wounds and fracture in animals and in immune compromised, emaciated animals could provide quality healing of acute wounds that would be of significant personal, economic and social advantage. It is the responsibility of the clinician to gain a thorough understanding of this biotechnology and to use it correctly and wisely for the benefit and wellbeing of our animals, who trust our judgment.

## **REFERENCES**

- Bertone AL. Management of exuberant granulation tissue. *Vet Clin North Am Equine Pract.* 1989;5:551-62.
- Carter AC, Jolly DG, Worden CE, Hendren DG, Kane CJM. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. *Exp Mol Pathol.* 2003;74:244-55.

- Dugrillon A, Kluter H. Current use of platelet concentrates for topical application in tissue repair. *TherTransfus Med.* 2002;29:67-70.
- Dyson M. Advances in wound healing physiology: the comparative perspective. *Vet Dermatol.* 1997;8:227-33.
- Fitch RB, Swaim SF. The role of epithelization in wound healing. *CompContEduPract Vet.* 1995;17:167-77.
- Harrison P, Cramer EM. Platelet  $\alpha$ -granules. *Blood Rev.* 1996;7:52-62.
- Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofacial Surg.* 2004;62:489-96.
- Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. (2001). Effectiveness of platelet releasate for the treatment at diabetic neuropathic foot ulcers. *Diabetes Care* 24(3):483-88
- Robson MC. The role of growth factors in the healing of chronic wounds. *Wound Rep Regenerat.* 1997; 5:12-7.
- Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol.*2004;36:28-35
- Vendramin FS, Franco D, Nogueira CM, Pereira MS, Franco TR. Platelet-rich plasma and growth factors: processing technique and application in plastic surgery. *Rev Col Bras Cir.* 2006;33:24-8.
- Strukova SM, Dugina TN, Chistov IV, Lange M, Markvicheva EA, Kuptsovas S, Zubov VP, Glusa E. Immobilized thrombin receptor agonispeptide accelerates wound healing in mice. *ClinApplThrombHemost.* 2001;7:325-9.
- Kliche S, Waltenberg J. VEGF receptor signaling and endothelial function. *IUBMB Life.* 2001;52,61-6.
- Adelmann-Grill BC, Wach F, Cully Z, Hein R, Kreig T. Chemotactic migration of normal dermal fibroblasts towards epidermal growth factor and its modulation by platelet-derived growth factor and transfer

**AB-38**

**Socio-economic profile of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method.

After collection and analysis of data, it was concluded that a majority of respondents were of middle age group, educated up to higher secondary school level, possessing medium herd size, annual income, social participation, information sources, market orientation with medium level of knowledge and adoption of buffalo management practices.

The probable reason for medium level of knowledge could be medium sized landholding, school level education and recurring drought conditions. Whereas, the reason for medium level of adoption might be that the respondent had adopted scientific management practices for making an enterprise sustainable, techno-economically feasible and judicious use of resources.

**AB-39**

**Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method.

After collection and analysis of data, it was concluded that, in relation to the information needs, respondents expressed marketing and break-even analysis, in particular as the most important information need; followed by demand and supply, traditional practices and government guidelines. The respondents expressed animal health as the second most important information need. After marketing and health, respondents expressed housing as most important information need with waste management been the most important followed by management during different seasons, construction of shed, farm layout, selection of site and pre-requisites for starting buffalo enterprise in descending order. In relation to information needs, information about breeding and nutrition were responded as the most important in the descending

**AB-40**

**Advance biotechnological tools for increasing fish production and biodiversity conservation**

Swapnil S. Ghatge\* and Milind M. Girkar\*

\*Assistant Professor, College of Fishery Science, Udgir, Dist:- Latur

The field of biotechnology has been developing at a very fast rate and its tools have gained importance in development of all biological fields including fisheries and aquaculture research. Application of biotechnological tools in fisheries and aquaculture in Indian context has been relatively a recent practice. Doubling of farmers income through increasing fish production by application of biotechnological tools is possible along with biodiversity documentation and conservation. The present paper reports the advance biotechnological tools available for increasing fish production and biodiversity conservation

**Key word:** *Biotechnological tools, biodiversity, fisheries*

**AB-41**

**Culture of *Pangasius sutchi* in polythene lined farm ponds in Vidarbha and Marathwada region of Maharashtra**

Umesh suryawanshi<sup>1</sup>, Shamkant Shelke<sup>2</sup> and Somnath Yadav<sup>3</sup>

<sup>1,2</sup> Assistant Professor, College of Fishery Science, Nagpur, Maharashtra.

<sup>3</sup> Assistant Professor, College of Fishery Science, Udgir, Maharashtra.

*Pangasius sutchi* possess different culture characteristics like good growth in crowded condition, less culture period, acceptance of wide variety food etc. Therefore, present study is carried out to examine productivity of *Pangasius* in polythene lined ponds of Vidarbha and Marathwada region of Maharashtra. Polythene lined farm ponds having water depth in the range of 5 to 7 m have been constructed for agriculture irrigation purposes. *Pangasius sutchi* seed size of 3-5 cm is stocked in different polythene lined agriculture tank of Vidarbha and Marathwada region of Maharashtra at the rate of 10 numbers per m<sup>2</sup>. Stocked fishes were fed with commercial floating feed according to observation of daily feed consumption, total biomass by sampling every 15 days interval. Water quality parameters managed by employing appropriate method whenever required. Results revealed that fish growth to size range of 750 to 1200 gm within 8 to 10 months culture period with production level of 20 to 25 Kg per meter square. Hence *Pangasius sutchi* is found suitable species to culture in polythene lined farm pond in Vidharbha and Marathwada region of Maharashtra.

**Key words:** *Pangasius sutchi*, Polythene line farm ponds, culture, Vidarbha, Marathwada  
Shamkant Shelke email address:- [shamshelke@gmail.com](mailto:shamshelke@gmail.com)

**PS**

**Poster session**

## POSTER SESION

XXIVth Annual Convention and National Conference of Indian Society of Veterinary Immunology and Biotechnology & One Day Satellite Seminar for Veterinary Officers On Recent Trends in Veterinary Immunology and Biotechnology for Doubling Farmers'Income through Livestock Health and Production (5-7<sup>th</sup> December 2017)

**PO-01**

### **Identification of Methicillin resistant *Staphylococcus pseudintermedius* in dogs of Chennai with skin infection**

Hridya Susan Varughese<sup>1\*</sup>, M. AnandaChitra<sup>1</sup>, S.Rajalakshmi<sup>1</sup>, G.Dhinakar Raj<sup>2</sup> and J. John Kirubaharan<sup>1</sup>

<sup>1</sup>Department of Veterinary Microbiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600007. <sup>2</sup>Director, TRPVB, MMC, Chennai-600051

\*Presenting author: drhridyasv@gmail.com

*Staphylococcus pseudintermedius* is a skin commensal of dog and cat colonizing the oral, nares, groin and perianal area. Phenotypic identification methods are inaccurate and hence require molecular methods. Methicillin resistant *Staphylococcus pseudintermedius* (MRSP) is a diagnostic as well as therapeutic concern in veterinary science. The present study was aimed to identify MRSP isolates from routine clinical cases in dogs of Chennai. The cases screened for MRSP include pyoderma, otitis and other dermatitis of dogs. A total of 200 swab samples from skin infections were collected and cultured on mannitol salt agar and individual colony was initially identified for *S. pseudintermedius*. Confirmed *S. pseudintermedius* isolates were screened for methicillin resistance for *mecA* gene by PCR. Among the 200 isolates, 88 were *S. pseudintermedius* positive and 23 isolates were identified as MRSP as they were positive for *mecA* gene. Incidence of 26% of methicillin resistance was noticed in *S. pseudintermedius* isolates in Chennai, India.

**PO-02**

### **A systematic review and Meta-analysis on prevalence of Methicillin-Resistant *Staphylococcus aureus* in Animals**

Hamsapriya S, Rashmi R. Kurli, Krishnamoorthy P Suresh K.P and Parimal Roy

National Institute of Veterinary Epidemiology & Disease Informatics, Bengaluru-560064

Antimicrobial resistance is one of the most serious concern world-wide. The Methicillin Resistant *Staphylococcus aureus* is a Super Bug, pathogenic to both humans and animals, and is one among the various pathogens causing Mastitis as LA-MRSA in India. It is present in wide range of animals, as a pathogen and as healthy carriers. The Prevalence of this super bug in animals and its products, and the spread of the same is an alarming issue to be studied.

The present study aims at employing Meta-analytical tools to analyze and determine MRSA prevalence in data extracted from different studies. The information on incidence of the MRSA strains, were retrieved from various Indian journals, classified based on the type/source of samples (milk samples/ others) and meta-analytical tools such as fixed effect models and random effect models were utilized to synthesize the results.

The electronic database of search yielded 22 articles from 2014-17 on MRSA prevalence / proportions with 2921 animals. The results viz. number of samples tested and number of positives were extracted. The results were subjected to meta-analysis, with 2921 samples provided the heterogeneity co-efficient ( $\tau^2$ ) as significant and hence random effect model was considered. The prevalence of MRSA was obtained as 24.0% (95%: 14.0-37%),  $\tau^2=2.011$ ;  $P<0.001$ . Further samples were stratified on the source of samples (milk samples/others) and meta-analysis of random effect model yielded 26% (95%: 16.0-39%),  $\tau^2=0.8468$ ;  $P<0.001$  for milk samples and 21% (95%: 7.0-49%),  $\tau^2=3.69$ ;  $P<0.001$  for other samples. Use of meta-analysis to aggregate individual studies enabled in determination of robust values of prevalence of MRSA in India and this information is useful for researchers, veterinarians and policy makers in developing appropriate intervention strategies.

**PO-03**

**Phenotypic Characterization and 16S rRNA sequence Analysis of Equine Isolates of *Streptococcus pluranimalium***

R. Javed, A. K. Taku, M.A.Bhat, R. K. Sharma and G.A.Badroo

Department of Microbiology, Faculty of Veterinary Sciences & Animal Husbandry, R.S. Pura, Jammu, Jammu and Kashmir, India. Email: [rabiajavedkhan@gmail.com](mailto:rabiajavedkhan@gmail.com).

*Streptococcus pluranimalium* has been associated with meningoventriculitis in calf, probably as a sequel to septicæmia, and endocarditis. *Streptococcus pluranimalium* isolates have been recovered from several species of domestic animals, including cattle (genital tract, tonsils, subclinical mastitis), canaries (crop, respiratory tract), a goat (tonsil) and a cat (tonsil). In the present study, the Hi Strep Identification kit (Himedia, India) and 16S rRNA sequence analysis were used to identify *S. pluranimalium* among the emerging isolates. Ninety-six Nasal swab samples were collected from Katra region of J & K, India and further were analyzed at Division of Microbiology SKAUST-J. These were inoculated on 5% Sheep Blood Agar plates (Himedia, India). After 24 hours incubation,  $\alpha$ -haemolytic colonies with a diameter of approximately 1 mm were subcultured on Columbia Nalidixic Acid Agar (Himedia, India) and Edward's agar (Himedia, India). Isolates that were  $\alpha$ -haemolytic, Gram positive and catalase negative were selected. Single colonies were harvested for biochemical characterisation using the HiStrep identification kit (Himedia, India). Four isolates of *Streptococcus pluranimalium* were identified. The isolates were confirmed as *S. pluranimalium* by 16S rRNA sequence analysis. A 1,481 base pair 16S rRNA product from each isolate was sequenced following amplification by PCR using oligonucleotide primers. BLAST1 analysis against the Gen Bank database identified a closest match to *S. pluranimalium* (>99% sequence

identity). The nucleotide sequence was submitted in ncbi genbank with (Accession No. for nucleotide sequence: SUB 2029862 Seq 1 KY020401).

**Key words:** *16S rRNA, S.pluranimalium, Polymerase Chain Reaction*

**PO-04**

### **Efficacy of a Polyherbal Drug against Indigestion in Bovine**

A.M.Syed, V.D.Aher, P.M.Mane and G.R.Gangane

Teaching Veterinary Clinical Complex,

College of Veterinary and Animals Sciences,

MAFSU, Parbhani, M.S. (India)

Corresponding author : [mujeebvet@rediffmail.com](mailto:mujeebvet@rediffmail.com)

#### **Abstract**

The study aims to evaluate efficacy of polyherbal drug Powder Rumec ES for the treatment of simple indigestion in bovine. Twenty (n=20) clinical cases presented with history of loss of appetite, dullness, depression, absence of ruminal contraction and suspended rumination were treated with powder Rumec ES @ 15gm orally twice daily till complete cure. Powder Rumec ES, is a polyherbal stomachic, digestive and tonic drug manufactured by Rakesh Pharmaceuticals, Gandhinagar, Gujarat, containing 24 different herbs. The significant change in temperature and non-significant changes in heart rate and respiratory rate after treatment of affected cattle and buffaloes were noticed. After treatment of affected cattle and buffaloes no significant changes in rumen fluid pH and significant changes in protozoal density and protozoal motility were recorded. In present investigation, non-significant changes in hemoglobin, packed cell volume and total leukocyte count before and after treatment of affected cattle and buffaloes were noticed. Out of twenty animals treated, eighteen were completely recovered. One animal recovered partial and one animal died during treatment might be due to complete anorexia and severity of condition. The results indicated that Powder Rumec ES could be effective in amelioration of simple indigestion in ruminants as a co-therapy with sodium bicarbonate, B complex injections, antibiotics and supportive therapy.

**PO-05**

### **Efficacy of Polyherbal Drug against Tympany in Bovine**

A.M.Syed, V.D.Aher, P.M.Mane and G.R.Gangane

Teaching Veterinary Clinical Complex,

College of Veterinary and Animals Sciences, MAFSU, Parbhani, M.S. (India)

Corresponding author : [mujeebvet@rediffmail.com](mailto:mujeebvet@rediffmail.com)

#### **Abstract**

The study aims to evaluate efficacy of polyherbal drug Liq. Bloatosafe for the treatment of tympany in bovine. A total twenty (n=20) clinical cases were presented with history of loss of appetite, dullness, depression, absence of ruminal contraction, suspended rumination and enlargement of left paralumbar fossa. Affected animals were treated with Liq. Bloatosafe @ 50ml orally twice daily till complete cure. Liq. Bloatosafe, a polyherbal antibloat drug contains 15 different herbs. Therapeutic efficacy of the polyherbal drug (Liq. Bloatosafe) was determined on the basis of relief from tympany and normal functioning of rumen. In present investigation, non significant change in temperature and significant changes in heart rate and respiratory rate was observed. There was a statistically significant improvement in post-treatment ruminal motility in Liq. Bloatosafe treated animals. Non significant improvement in rumen fluid pH and significant changes in protozoal density and protozoal motility were recorded. In present investigation, non-significant changes in haemoglobin, packed cell volume and significant changes in total leukocyte count before and after treatment of affected cattle and buffaloes were recorded. Out of twenty animals treated, 19 were completely recovered and one animal not recovered and died during treatment might be due to severity of condition. The results indicate Liq. Bloatosafe could be effective in amelioration of primary tympany in ruminants, as evident through improvement in tympany, ruminal motility and normal functioning of rumen. The treatment with Liq. Bloatosafe proved to be effective, safe and did not show any untoward effect at recommended therapeutic doses as co-therapy with sodium bicarbonate, B complex injections, rumenototics, antibiotics and supportive therapy in treatment of primary tympany.

**PO-06**

### **Therapeutic Efficacy of Herbal Drug Against Post-parturient Reproductive Disorders in Bovine**

P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade

Teaching Veterinary Clinical Complex,

College of Veterinary and Animals Sciences, MAFSU, Parbhani, M.S. (India)

Corresponding author : [drprashantmafsu@gmail.com](mailto:drprashantmafsu@gmail.com)

#### **Abstract**

This study was conducted to evaluate efficacy of polyherbal drug for the treatment of various post parturient disorders in bovine. The trial was conducted at Teaching Veterinary Clinical Complex, College

of Veterinary and Animal Sciences, Parbhani and some of the local farms in and around Parbhani district. Total twenty bovine reported with history and clinical signs of post parturient disorders was treated with Utrovet forte bolus given orally two boli twice daily for five days. In the present study, out of twenty animals presented with history of post partum disorders, sixteen animals (80.00 %) exhibited cyclicity after treatment, followed by fourteen (70.00 %) animals with clear nature of oestral discharge after initiation of treatment. The result of the study reveals that the ecobolic polyherbal drug is an effective treatment regimen for post parturient disorders as a co-therapy. It also helps to reduce intercalving period as well as restoration of production and fertility.

**PO-07**

**Induction of Oestrus in Anoestrus Bovine by Using Herbal and Mineral Preparations**

P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade

Teaching Veterinary Clinical Complex,

College of Veterinary and Animals Sciences, MAFSU, Parbhani, M.S. (India)

Corresponding author : [drprashantmafsu@gmail.com](mailto:drprashantmafsu@gmail.com)

**Abstract**

This study was conducted to evaluate efficacy of polyherbal and mineral drug for the treatment of anoestrus in bovine. The trial was conducted at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Parbhani and some of the local farms in and around Parbhani district. Twenty anoestrus affected animals were treated with Estrona Forte Bolus and Minarex Bolus. In the present study, out of twenty animals treated, sixteen were exhibiting proper oestrus i.e. 80.00 per cent efficacy and the time interval for onset of oestrus was recorded from the initiation of treatment up to the onset of first behavioral sign of oestrus was  $12.06 \pm 0.75$  days. It may be concluded that from the present study, this combined therapy of polyherbal heat inducer (Estrona Forte Bolus) and mineral supplementation (Minarex Bolus) proved effective for the treatment of anoestrus in bovine.

**PO-08**

**Comparative Studies on Haemato- Biochemical Constituents in Anoestrus Bovine**

P. M. Mane, V. D. Aher, A. M. Syed and P. B. Ghorpade

Teaching Veterinary Clinical Complex,

College of Veterinary and Animals Sciences, MAFSU, Parbhani, M.S. (India)

Corresponding author : [drprashantmafsu@gmail.com](mailto:drprashantmafsu@gmail.com)

**Abstract**

This study was conducted to evaluate efficacy of polyherbal and mineral drug for the treatment of anoestrus in bovine. The trial was conducted at Teaching Veterinary Clinical Complex, College of Veterinary

---

*VIBCON - 2017 (PARBHANI)* [257]

and Animal Sciences, Parbhani and some of the local farms in and around Parbhani district. Twenty anoestrus affected animals were treated with Estrona Forte Bolus and Minarex Bolus. Blood samples were analysed before and after treatment for haemoglobin, Calcium, Phosphorus and Glucose. Highly significant variations ( $P < 0.05$ ) in haemoglobin ( $11.78 \pm 0.21$  vs  $12.39 \pm 0.19$  g/dl) and significant variations ( $P < 0.01$ ) in calcium ( $9.90 \pm 0.27$  vs  $10.44 \pm 0.25$  mg/dl) before and after treatment of anoestrus bovine were observed, respectively. However, nonsignificant variations in phosphorus ( $5.08 \pm 0.14$  vs  $5.21 \pm 0.14$  mg/dl) and glucose ( $49.76 \pm 1.23$  vs  $49.91 \pm 1.15$  mg/dl) before and after treatment of anoestrus bovine were observed, respectively. It can be concluded that from the present study, this combined therapy of polyherbal heat inducer (Estrona Forte Bolus) and mineral supplementation (Minarex Bolus) proved effective for the treatment of anoestrus in bovine.\

#### **PO-09 EFFECT OF *MORINDA CITRIFOLIA* JUICE ON DIABETIC RATS**

Dr. N. D. Jadhav, Dr. S. R. Rajurkar and Abhinna Keshari Behera,

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary and Animal Sciences, MAFSU, Parbhani. Maharashtra

#### **Abstract**

The present investigation was carried out to study the effect of *Morinda citrifolia* in diabetic rats for a period of 28 days with *Morinda citrifolia* fruit juice. The *Morinda citrifolia* fruit juice was made to evaluate anti-diabetic activity for the treatment of group IV animals.

Twenty four rats were divided into four groups comprising of six rats of either sex in each group. Group I served as healthy control and was given normal pelleted feed and drinking water for 28 days. The group II, III, and IV were given alloxan @100 mg/kg BW intra-peritoneal for induction of diabetes. After confirmation of diabetes the experiment was initiated and that day was considered as day 0 of experiment. During experiment rats from control group did not show any apparent behavioral changes, group II rats showed symptoms of dullness, depression polydipsia and polyurea.

In group III animals were treated with metformin @ 100 mg/kg BW, in group IV they were treated with *Morinda citrifolia* fruit extract @ 2ml per rat twice in a day.

Significant increase in serum glucose was observed in group II, III & IV on 0 day i.e 422.30, 443.42 and 396.72 and subsequently decrease on 28<sup>th</sup> day of experiment i.e. 412.65, 129.68 and 155.88 respectively. The haemoglobin concentration did not change significantly throughout the experiment period.

Grossly liver and pancreas did not showed any noticeable visual change in any of the experimental rats. No significant change in weights of liver and pancreas in treatment groups. The histopathological changes like moderate fatty degeneration, congestion and cytoplasmic rarefication were predominantly noted in liver and pancreas suggesting induction of diabetes in group II animals. In treatment group there was partial restoration of histoarchitecture of these organs after treatment in all groups.

**PO-10**

**Development and characterization of nanoparticle antimicrobial agent loaded SPRAYON type polymeric in-situ gelling bandage system for wound healing in animals.**

Authors: Isha V. Naik, Kaveri R. Kalola, Dr. Mala D. Menon

Affiliation: Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai.

Wounds are physical, chemical or thermal injuries, breaking the integrity of skin. Untreated wounds desiccate, leading to healing delays and higher incidence of infection and scarring. Clinically, wounds exposed to air are more inflamed, painful, and pruritic, have thicker crusts, and are more likely to scar. Veterinary wounds are prone to licking and biting by self, surrounded by thick and hairy skin, exposed to sun, likely to be contaminated with barn environment pathogens, prone to maggot formation, thus making veterinary wound bandaging more challenging. An ideal dressing should achieve rapid healing at reasonable cost with minimal inconvenience to the patient. Traditional bandages are of fixed size and cannot cover the entire wound area; also there is difficulty in application of these due to lack of cooperation in animals. Commonly used dressings, viz. gauze, films, hydrogels, may not contain antimicrobial agent and animals need to be stabilized for their application. Thus, a SPRAY-ON type polymeric in-situ gelling (ISG) bandage system which gels in presence of wound fluids, containing metal nanoparticles (MNPs) as anti-microbial agent was developed. The film would form on the wound surface, leaving no area of nonadherence to create fluid-filled pockets for the proliferation of bacteria. The MNPs prepared by chemical reduction method had a mean diameter ranging between 30-45nm. These MNPs were incorporated into an ISG solution made up of naturally obtained biodegradable polymers such as alginate salts and gums.

The ISG solutions were found to be odourless, opaque, greyish green in colour and free of any bubbles or fibres having pH 7.4 and viscosity of 740cps. The time for film formation was 1- 2 mins. SEM images showed homogenous distribution of MNPs in the film formed. The film formed had tensile strength, folding endurance, active content assay, percent swelling of  $2.309 \pm 0.050$  g/cm<sup>2</sup>,  $294 \pm 38$ ,  $88.1 \pm 1.64\%$  and  $112.37\%$  respectively; the film in both, wet and dry condition, retained about 85% of moisture for 3 days. The films showed good antimicrobial activity with zone of inhibition of more than 3mm for 3 days. Thus, this system will serve as a very good alternative for the conventional bandages for facilitating veterinary wound healing.

**PO-11** **Effect of lower doses of the cloprostenol on oestrus synchronization, physio-chemical properties of cervico-vaginal mucus and fertility in repeat breeder cross bred cows**

M.B. Amle, K.P.Khillare, M.N.Rangnekar and A.B Korade

Department of Animal Reproduction, Gynaecology & Obstetrics

KNP College of Veterinary Science, Shirval Dist Satara Maharashtra

Total one hundred repeat breeder cows were randomly allotted into four groups, treated (estrus synchronized) with 500 µg PGF<sub>2</sub>α (cloprostenol) intramuscularly (group-I), 250 µg PGF<sub>2</sub>α through IVSM (intra-vulvo submucosal) route ipsilateral to CL in the ovary (group-II), 250 µg PGF<sub>2</sub>α through IVSM route contralateral to CL in the ovary (group-III). The 4<sup>th</sup> group was control, which was kept untreated.

The percentage of clear, mild flakes/turbid and dirty cervical mucus discharge after estrus synchronization including all groups of cows in the present study were observed as 90 (36/40), 10 (4/40) and 0 (0/40) % in fertile (conceived) cows and 58.33 (28/48), 33.33 (13/48) and 8.33 (4/48) % in non-fertile (not conceived) cows, respectively. Overall percentage of all fertile (conceived) and non fertile (not conceived) cows showing clear, turbid/cloudy or dirty cervical mucus discharge including all groups were recorded as 72.72, 18.18 and 4.5 %, respectively. The frequencies of typical, atypical and nil fern pattern of cervical mucus in cows of group-I synchronised using Cloprostenol by i/m route were found to be 65.21 (15/23), 26.08 (6/23) and 8.69 % (2/23), respectively. Overall percentage of typical, atypical and nil cervical mucus fern pattern in all twenty one synchronized cows in group-II were found to be 61.90 (13/21), 28.57 (6/21), and 9.52 (2/21) %, respectively. In group-III these values were recorded as 57.89 (11/19), 26.31 (5/19) and 15.78 (3/19) %, respectively. In group-IV (untreated animals) all the 25 repeat breeding cows, the frequencies of typical, atypical and nil fern pattern during natural estrus recorded was 60 (15/25), 28 (7/25), and 12 (3/25) %, respectively. The mean pH of cervical mucus discharge of fertile cows (n=40) at induced and natural estrus including all groups was recorded as 7.79±0.07 (7.5 - 8.0). Whereas in non-fertile cows (n=48) it was found to be 8.04±0.047 (7.5 - 9.0). The overall pH of estrus mucus including all fertile and non-fertile cows (n=88) at induced and natural estrus was found to be 7.92±0.12. The mean SBK (spinbarkeit) values of cervical mucus of fertile (n=40) and non-fertile (n=48) cows at induced and natural estrus including all groups were found to be 17.12 cm and 12 cm, respectively. The overall mean SBK value of cervical mucus including fertile and non-fertile cows (n=88) at induced estrus including all groups was recorded as 15.03±2.85 cm with the range of 6 cm to 24 cm.

The results of WST (White side test) of cervical mucus revealed no infection in 66.69 % animals with induced estrus and 56 % in untreated animals. The percentage of cows reacting to WST in groups I to IV were reported as: in normal condition 65.21, 71.42, 63.15, 56 %, mild infection 26.08, 19.04, 26.31,

36.0 %, moderate infection 8.6, 9.52, 5.26, 0.0 % and severe infection 0.0, 0.0, 5.26, 4.0 %, respectively. Overall mean percentage of WST including cows of all groups in normal condition, mild infection, moderate infection and severe infection were 63.63, 27.27, 5.68, and 2.27 %, respectively. The mean vaginal cervical mucus electric resistance (W) in groups I to IV of fertile and non-fertile cows was recorded as 195.83±9.248 and 227.27±9.731, 185±9.88 and 245.55±11.317, 211.25±11.56 and 227.27±13.62, 176.25±6.797 and 220±12.366 (W), respectively. Decreased vaginal electric resistance during estrus could be due to increased mucus secretion in vagina during estrus.

The cows exhibited estrus within 40-96 h in the treatment and control groups were bred by artificial insemination. Those cows failed to conceive with first insemination were inseminated in the succeeding natural estrus. Thereafter pregnancy was confirmed by per rectal examination of the genitalia on day 50-60 post artificial insemination. The percentage of repeat breeding crossbred cows that became conceived in 1<sup>st</sup> and 2<sup>nd</sup> service in the treatment group I were 43.47 and 40 % (av. 52.17 %), group II: 52.38 and 25 % (av. 57.14 %), group III: 31.57 and 66.66 % (av. 42.10 %) and group IV: 28 and 25 % (32 %), respectively. The overall conception rate of cows including three treatment groups was 50.47 % and in untreated cows it was 32 %.

**PO-12 Studies on improvement of conception rate in normal cyclic cows and buffaloes with two different doses of GnRH.**

**S.R. Chirfare, N.M. Markandeya, B.L. Kumawat and A. Wankar**

Department of Animal Reproduction, Gynecology & Obstetrics, COVAS-Parbhani-431402

**Abstract**

Study was carried out to evaluate the effects of two different doses of GnRH at the time of insemination in normal cyclic, 36 cows and 36 buffaloes equally divided into three experimental groups. Group I was administered GnRH @ 10 mcg at the time of AI, Group II was given GnRH @ 20 mcg at the time AI, and Group III was untreated control. Follow-up was carried out for three months and it was observed that the conception rate was 83.33, 75.00 and 50.00 per cent in cows, and 83.33, 83.33, and 41.67 per cent in buffaloes, respectively in the groups. Greater proration of cows and buffaloes that received GnRH became pregnant compared to that of untreated, but the difference was non-significant amongst the groups. The results of present study revealed that the administration of GnRH with low dose at the time AI improves the conception rate in normal cyclic cows and buffaloes.

**PO-13**

**Studies on improvement of conception rate in non infectious repeat breeder cows and buffaloes with two different doses of GnRH.**

S.R. Chirfare, N.M. Markandeya, A.B. Mali and A.G. Sawale

Department of Animal Reproduction, Gynecology & Obstetrics, COVAS-Parbhani-431402

**Abstract**

This study was carried out to evaluate the effects of two different doses of GnRH at the time of insemination in non infectious repeat breeder 18 cows and 18 buffaloes divided equal into three experimental groups. Group I injected GnRH @ 10 mcg at the time of AI, Group II used GnRH @ 20 mcg at the time AI, Group III untreated control. On follow up was carried out for three months and it was observed that the conception rate was 83.33, 66.67 and 33.33 per cent in cows, and 83.33, 83.33, and 16.67 per cent in buffaloes, respectively in the groups. Greater proration of cows and buffaloes that received GnRH became pregnant compared to that of untreated, but the difference was non-significant amongst the groups. The results study revealed that the administration of GnRH at the time artificial insemination improves the conception rate in non infectious repeat breeder cows and buffaloes.

**PO-14**

**Efficacy of Neem extract and *E.coli* LPS for treatment of endometritis in cows and buffaloes**

G. A. Thombre, M. N. Markandeya, B. L. Kumawat and V. B. Thombre

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

COLLEGE OF VETERINARY & ANIMAL SCIENCES

PARBHANI-431402

**Abstract**

Present study was conducted on endometritic cows (n=24) and buffaloes (n=24). Objective of study was to diagnose endometritis at field level and to evaluate the efficacy of herbal extract and immunomodulator in terms of recovery and pregnancy rate in endometritic bovines. Endometritis was diagnosed based on alkaline pH, Metrichick score and positive White side test of cervico-vaginal mucus. Selected cows and buffaloes were randomly divided into two treatment groups containing 09 cows and 09 buffaloes in each viz. Group-I was treated with 20 per cent Neem seed extract @ 20 ml by intrauterine route whereas Group-II was treated with 100 mcg of *E.coli* LPS in 30 ml PBS solution by intrauterine route. Group-III (n=6+6) was kept as untreated control. The pH value of cervico-vaginal mucus reduced significantly after treatment. Overall recovery of endometritis following herbal therapy was found as 66.67% and 55.55% in cows and buffaloes, respectively. Whereas, the same was observed as 88.89% in both cows and buffaloes treated with *E.coli* LPS therapy. The pregnancy rate in group-I was recorded as

55.55% and 44.44% in cows and buffaloes, respectively. On the other hand in Group-II the pregnancy rate was 77.78% for both cows and buffaloes. In conclusion, *E.coli* LPS therapy proved more efficacious for the treatment of endometritis in cows and buffaloes.

**PO-15** **Does efficacy of *E.coli* LPS improves with immunostimulant in the treatment of endometritis in dairy animals?**

Puro, N.A., N. M.Markandeya, A. B. Mali, and S. G. Swami,

*Department of Veterinary Gynaecology and Obstetrics,*

*Collage of Veterinary and Animal Sciences, Parbhani: 431402 (MAFSU)*

E-mail- nitish31.puro@gmail.com

**ABSTRACT**

A study was undertaken to evaluate the effect of administration of intrauterine immunomodulator and its combination with immunostimulant on the recovery and conception rate in endometritic cows and buffaloes. Total 24 cows and 23 buffaloes were diagnosed positive for endometritis by assessment of pH, metricheck score and white side test of CVM. The cases were categorised in three different groups to undertake treatment on the day of estrus viz. Group 1 was treated *E.coli* LPS @ 100µg in 30 ml PBS intrauterine in 8 cows and 7 buffaloes ; Group 2 was treated with *E.coli* LPS as per group 1 along with inj. levamisole @ 2.5 mg/Kg BW subcutaneously in 6 cows and 6 buffaloes. Both the groups were tested against equal number of cases with control group and 30 ml PBS was administered intrauterine in each animal from control group. No animal was inseminated during treatment estrus. Recovery rate was assessed by evaluating the CVM of treated animals in next estrus and its was found to be 87.50 per cent in cows and 85.70 per cent in buffaloes from group 1 and 100.00 per cent in cows and 83.33 per cent in buffaloes from group 2. The recovered animals were inseminated and followed to record conception rate and the same was found to be 71.42 per cent in cows and 83.33 per cent in buffaloes from group 1 and 83.33 per cent and 80.00 per cent in cows and buffaloes from group 2. Thus, it was concluded that *E.coli* LPS can be preferred on the basis of its course of action through improvement in body defense mechanism and its efficacy can be improved with immunostimulation in cows, but the same needs to be further explored in buffaloes.

**PO-16**

**Influence of different levels of Xylanase enzyme on performance, litter quality and economics of broiler chicken**

D S Gade<sup>1</sup>, M.V.Dhumal<sup>2#</sup>, [dhumalmv@gmail.com](mailto:dhumalmv@gmail.com), and M G Nikam<sup>3</sup>

<sup>1</sup>Post-graduate scholar, Department of Poultry Science, Department of Animal Husbandry and Dairy, Govt. of Maharashtra, Solapur (Maharashtra ) INDIA

<sup>2</sup>Professor, Department of Poultry Science, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fishery University Nagpur (Maharashtra) INDIA

<sup>3</sup>Assistant Professor, Department of Poultry Science, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fishery University Nagpur (Maharashtra) INDIA

**ABSTRACT**

An experiment was carried out on 300 one day old straight run commercial broiler chicks to assess the effect of different levels of xylanase enzyme on performance parameters. The experimental design consists of 4 treatment groups with three replicates consisting of 25 birds per replicate. Treatment group A served as control diet. The ration was prepared with reduced energy (-100 kcal/kg) for treatment group B without addition of xylanase as negative control. The treatment groups C and D rations were prepared with reduced energy by 100 kcal/kg and supplementation of xylanase enzyme @ 60 and 100 g/ton of feed respectively. The results revealed significantly higher cumulative weight gain for xylanase supplemented groups compared with non supplemented groups. Significantly higher cumulative feed consumption was observed for the birds reared on negative control ration (-100 Kcal/kg) treatment group B. Xylanase supplemented groups revealed significantly better FCR compared to negative control group. Significantly higher mortality was noted in negative control group. Highly significant differences were observed for moisture content and pH of litter among various treatment groups. The net profit per Kg live weight was highest for treatment group D supplemented with xylanase at 100g/ton of feed. Overall results in the present study concluded that supplementation of xylanase enzyme @ 100 g/ton of feed in broiler ration is more beneficial in higher fiber containing diets for improvement of performance, livability, litter quality and profitability in commercial broiler chicken.

**PO-17**

**Isolation of *proteus* from poultry faecal sample**

Pawara V.P., Acharya P.S., Bramhapurkar P.P., Bhonsle A.V.

Department of Veterinary Microbiology,

College of Veterinary and Animal sciences, Udgir-413517. MAFSU, Nagpur, Maharashtra, India

There was high mortality at the broiler poultry farm in Udgir taluka. Foul smelling watery diarrhoea was observed. 10 fecal samples were collected. For isolation of proteus species from we used nutrient VIBCON - 2017 (PARBHANI)

broth for overnight incubation and streaking of growth was done on nutrient agar and XLD agar. Swarming growth on nutrient agar and non lactose fermenting colonies on Mac conkeys agar was observed and Diene's phenomenon was observed to identify colonies of proteus. Biochemical tests were performed and its Indole negative among enterobacteriaceae family.

Key words: Poultry Birds, fecal samples, selective media , biochemical tests

**PO-18**

**In vitro efficacy of bacteriophages against mastitis caused by antibiotic resistant bacteria**

(Dr. A.R. Deshpande, Dr. P.R. Suryawanshi, Dr. S.S. Gaikwad, Dr. O.S. Kashikar\*)

Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani

[kashikaronkar@gmail.com](mailto:kashikaronkar@gmail.com)

Abstract

Bovine mastitis is an economically inflammatory disease condition of the udder affecting cattle, buffaloes and other domesticated animals and having a serious impact on the economy of dairy enterprise. The losses incurred to the dairy farmers are in the form of reduction in milk yield, discarded spoiled milk, premature culling of animals and replacements. Mastitis is usually caused by multiple etiological agents and it is a big challenge for Veterinarians to control the infection against antibiotic resistant bacterial infections. Antibiotic resistance is one of the serious challenges to animal health and therefore policy changes will be required to mitigate the consequences of antibiotic resistance. Resistant bacterial infections are difficult to treat, and there are very few new antibiotics in the drug development pipeline that merely useful in treatment. The bacteriophages instead can be a more effectively utilized as an alternative for the effective treatment of antibiotic resistant cases of mastitis.

In the present study bacteriophages were isolated from the faeces of Red Kandhari cattle which were utilized for the *in vitro* testing of its efficacy against mastitis causing antibiotic resistant bacteria. The bacterial isolations were made and *E.coli*, *Staphylococcus aureus* and *pseudomonas* organisms were identified by cultural characteristics on EMB agar, Mannitol salt agar and Pseudomonas isolation agar respectively & confirmed by biochemical tests. The specificity of bacteriophages against the bacteria was assessed using soft and hard agar plates. The agar plates spreaded with individual bacteria after incubation with bacteriophages resulted in to formation of irregularly circular clear zones (plaque formation) which clearly indicated the lytic action of bacteriophages on bacteria. This preliminary work proved that the *in vitro* use of bacteriophage against mastitis causing *E.coli*, *Staphylococcus aureus* and *pseudomonas* organisms can be possible and therefore may be useful in future prospective as an alternative to the antibiotic in curing antibiotic resistant cases of mastitis.

**PO-19** **Isolation and Identification of *Erysipelothrix rhusiopathiae* from an outbreak of Swine Erysipelas**

A.R. Deshpande, P.R. Suryawanshi, S.S. Gaikwad, K.N. Kadam, O.S. Kashikar, Neha Singh  
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani  
[kadamk112@gmail.com](mailto:kadamk112@gmail.com)

**Abstract**

Swine erysipelas is an infectious disease of pigs caused by *Erysipelothrix rhusiopathiae*. Organisms of the genus *Erysipelothrix* are gram positive, facultative anaerobic small, slender, rod shaped, non-spore forming, non-motile, nonacid fast and catalase negative which are responsible for causing high mortalities in pigs of adult age. In the present study two pigs of 3-4 months age were presented to the Teaching Veterinary Clinical Complex from local piggery. The pigs were recorded with high temperature (104<sup>o</sup>F), joint pain and diamond skin like vesicular patches all over the body. There were circling movement and severe dehydration. Both the pigs died during treatment and there was history of 10 pigs dead already with similar clinical signs and symptoms. Post mortem of the pigs was performed and the samples were collected from spleen, liver and heart blood swabs from the dead carcass. The collected samples were incubated in Brain Heart Infusion broth and after 24 hrs. of incubation they were streaked on Brain Heart Infusion agar plates. Smooth, convex and circular with even edges colonies were isolated and gram staining of these colonies were confirmed to be gram positive slender rods. Biochemical tests were performed which results catalase negative, coagulase positive and H<sub>2</sub>S production. Further infection was prevented by implementing necessary hygienic measures.

**PO-20** **Identification of phytobiotic potential of certain herbal extracts against mastitis causing bacteria**

S.S. Vyawahare\*, Dr. A.R. Deshpande, Dr. P.R. Suryawanshi, Dr. S.S. Gaikwad and Dr. O.S. Kashikar  
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani  
[Shubhangivyawahare08@gmail.com](mailto:Shubhangivyawahare08@gmail.com)

**Abstract**

Mastitis is a major concern to the dairy farmers because of its multi-etiological nature. Inappropriate and irrational use of antimicrobial agents against mastitis has led to increase in development of antimicrobial

resistance. Antimicrobial resistance has been resulted in difficulty in controlling the cases of mastitis in the domesticated dairy animals. Herbal plants have been utilized essentially by human being for the treatment of both human and animal disease effectively. Affirmation of the quality, adequacy and security of the herbal medicinal plants and their products has now turned into a key issue in developing dairy sector as well as solution for the antimicrobial resistance. The phyto-biotic potential of *Turmeric, Garlic, Clove, Cinnamon, Carom seeds, Black pepper, mint* has been depicted in Great Indian materia medica i.e. Ayurveda.

Present study claims the principle role of herbal plants against the mastitis causing organisms on the basis of culture sensitivity test pattern. In this study mastitis causing bacteria viz. *Staphylococcus aureus* and *E. coli* were isolated from twenty clinical cases of mastitic milk samples. Instead of using the antibiotic discs, filter paper discs dipped and dried in extracts of mentioned herbal plants were used on the nutrient agar plates for testing the efficacy against the bacteria which were resistant to some of the commonly used antibiotics. It was observed that *E.coli* was found susceptible to clove, ginger and cinnamon whereas *Staphylococcus spp.* were found susceptible to garlic, cinnamon carom seed black pepper and mint.

**PO-21**

**Nutritional technology in livestock products: Eubiotic's role in broiler feed**

Acharya P.S., Bramhapurkar P.P., Telange N.P., Dadke A.R.

College of Veterinary and Animal sciences, .Udgir-413517. MAFSU, Nagpur, Maharashtra, India

**ABSTRACT**

The study was conducted to see the effects of using eubiotics in poultry feed. Eubiotics mainly used for gut health and for regulation of microbial flora. Also helps in manipulating balanced eubiosis in GI tract. In the area of feed additives, today eubiotics is on highest position as there is peculiar characteristic of eubiotic is, it inhibits bacterial activities in intestinal mucosa, inhibits adhesion. Study was conducted in 100 broiler birds flock and studied its advantages like body weight gain, good digestive systems as their feed consumption ratio was good, essential fatty acids are responsible to improve broiler performance. EFA and different essential oils, microbes are used as eubiotics. It also maximizes energy and protein utilization. Along with regular diet of broilers eubiotics were added and during early 20 days and after that alternate days.

Key words: Eubiotics, Poultry feed, EFA, Gut health, energy.

**PO-22**

**Antimicrobial activity of ginger extract on the bacteria isolated from pyometra case**

Neha Singh\*, A.R. Deshpande, P.R. Suryawanshi, S.S. Gaikwad, K.N. Kadam, S.R. Lawange  
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Parbhani.

The study investigated the antimicrobial activity of ginger (*Zingiber officinale*) extract on the bacteria isolated from the pus sample of cow suffering from pyometra infection. The extract was obtained from the rhizomes of ginger by methanol soluble extract method. Three bacteria were isolated *Staphylococcus aureus*, *E.coli* and *Pseudomonas spp.* was found in the predominance number. Initially the antibiotic sensitivity test was performed by disc diffusion method using the commonly used antibiotics but the bacteria were found to be resistant to most of the antibiotics tested. These antibiotic resistant bacteria were then selected for the efficacy testing of ginger extract which showed clear antibacterial activity against the *Staphylococcus aureus* at the concentration of 0.2mg/ml and 0.1mg/ml. *E.coli* and *Pseudomonas spp.* were found to be resistant to Ginger extract.

Keywords –Antimicrobial activity, *Zingiber officinale*

**PO-23**

**Assessment of Shelf life of Wheat Flour Based Quail Meat Enriched Noodles at (35±2°C) Room Temperature Storage**

D.M. More, S.V. Londhe, D.P Patil, C.K.Choudhary, P.A.Shinde and P.N.Bhumre  
Department of Livestock Products Technology,  
College of Veterinary and Animal Sciences, Parbhani (M.S)

The present study was aimed to develop noodles with incorporation of different levels of quail mince meat (0%, 40%, 50% and 60%). Quails were slaughter and dressed in the department of Livestock Products Technology. The skin, subcutaneous fat, tendon, separable connective tissue were trimmed off from quail meat and packed in low density poly ethylene (LDPE) and stored overnight at 4±1°C for ageing, thawed meat which was subsequently used for product formulation. The results indicated that, during storage at room temperature (35±2°C), all the sensory scores of the product declined with the increase in storage period. Contrarily moisture, fat and protein decline with the increase in storage but the pH, TBA number, tyrosine value and total plate counts increased considerably, but were within the spoilage limit up to 60 days of storage period. In fresh product the moisture content was 13.66 per cent (0 day) which reduce considerably at the end of storage period upto 60<sup>th</sup> day storage was 12.07 per cent. The storage mean of fat % and protein% ranges between 2.01% to 1.89% and 20.40% to 17.50% respectively during the storage of QMM noodles at room temperature (35±2°C). The storage mean values were recorded

for pH, TBA and Tyrosine value ranges between 5.81 to 6.14., 0.20 to 0.31 and 0.41 to 0.90 respectively. The TPC storage mean (Total Plate Count) was recorded for QMM noodles during storage of 0 to 60<sup>th</sup> day at room temperature (35±2°C) was 1.45 log cfu/g to 3.76 log cfu/g Coliform counts in all the samples were not detected throughout the storage period. Based on the above findings, it is concluded that the noodles made with incorporation of 40% quail mince meat in wheat flour based noodles were acceptable for a period of 60 days when packed aerobically in LDPE bags and stored at room temperature at (35±2°C).

**Keywords:** Quail meat, wheat flour, noodle extruder machine, spices

**PO-24**

### **Evaluation of Cost Structure of Quail Meat Enriched Noodles**

D.M. More, S.V. Londhe D.P Patil, P.N.Bhumre and A.S.Nemade

Department of Livestock Products Technology,

College of Veterinary and Animal Sciences, Parbhani (M.S)

India ranks sixth in the world poultry meat production. The poultry industry is one of the largest and fastest growing agro based industry in the world. Growing urbanization, increasing trend of working women, changing socio-economic status and increasing interest of school going children in snack foods and ease in carrying to long distant and remote area have also contributed to the enhanced consumption of processed and convenience meat products. Today's major challenge is to develop inexpensive foods that are nutritionally superior and highly acceptable to consumers. The present study was concluded on the basis of cost of production for preparation of quail mince meat enriched noodles. The overall cost for the production of 1 kg 0%, 40%, 50% and 60% of quail mince meat enriched wheat flour based noodles was Rs.59.24/-, Rs. 176.74/-, Rs.206.74/- and Rs.236.74/- whereas cost of production of 100 g quail mince meat enriched noodles was Rs.5.92/-, Rs.17.67/-, Rs.20.67 and Rs.23.67 respectively. The cost of production revealed that, the cost effective noodles were prepared with incorporation of 40% quail mince meat in wheat flour based noodles as compared to 50% and 60% quail mince meat enriched wheat flour based noodles.

**Keywords:** Quail meat, wheat flour, noodle extruder machine, LDPE bags

**PO-25**

### **Development and Quality Evaluation of the Wheat Flour Based Quail Meat Enriched Noodles**

D.M. More, S.V. Londhe, D.P.Patil, P.A.Shinde, C.K.Choudhary and A.S.Nemade

Department of Livestock Products Technology,

College of Veterinary and Animal Sciences, Parbhani (M.S)

The present study was aimed to develop noodles with incorporation of different levels of quail mince meat (0%, 40%, 50% and 60%). Quails were slaughter and dressed in the department of Livestock

---

*VIBCON - 2017 (PARBHANI)* [269]

Products Technology. The skin, subcutaneous fat, tendon, separable connective tissue were trimmed off from quail meat and packed in low density poly ethylene (LDPE) and stored overnight at  $4\pm 1^{\circ}\text{C}$  for ageing, thawed meat which was subsequently used for product formulation. The results indicated that noodles made with addition of 40% quail mince meat incorporated wheat flour exhibited higher scores for all sensory attributes. The sensory score for the selected 40% level were 7.44 for appearance, 7.55 for flavour, 7.55 for juiciness, 7.33 for texture, 7.66 for overall palatability. The results showed non-significant ( $P < 0.05$ ) decrease in pH of noodles made with 40% quail mince meat in wheat flour based noodles. The pH for 0%, 40%, 50% and 60% level were recorded during 0 to 60<sup>th</sup> day of storage at room temperature is 5.92, 5.86, 5.85 and 5.43 respectively. There was non-significant ( $P < 0.05$ ) decrease in bulk density (0%-0.35g/ml and 40% 0.34g/ml) and significant ( $p > 0.05$ ) increase in true density with addition of quail mince meat in wheat flour based noodles. Among the cooking parameter, the cooking time increased significantly i.e. for 0% -8 min, 40%-11 min, 50%-12.33 min and 60%- 15 min. The water uptake for 0%, 40%, 50% and 60% was 2.73 ml/g 2.37 ml/g, 1.85 ml/g to 1.56 ml/g (decreased significantly) respectively while the swelling index for 0%, 40%, 50% and 60% was 1.67, 1.62, 1.53 and 1.52 respectively and differed non-significantly within treatments.

**Keywords:** Quail meat, wheat flour, noodle extruder machine, spices, digital pH meter

**PO-26**

### **Microscopic observations of small intestine in probiotic feed Japanese quail**

Mamde C. S., Waghaye J. Y., Thakur P. N. , Karad N. M. and S. D. Kadam

Department of Veterinary Anatomy and Histology

College of Veterinary and Animal Science, Parbhani

#### **Abstract**

The present study was conducted on twenty four female Japanese quail birds divided in to two groups as group A (Normal / Control) and Group B (Probiotic Feed). Twelve birds of each group were further divided into two subgroups of three and six weeks of age. Birds from group B provided with probiotic (derived from lactobacillus acidophilus, pediococcus acidilactii and saccharomyces boulardii) @ 1gm/lit along with water and feed. Histologically small intestine showed three different tunics i.e. tunica mucosa, tunica muscularis and tunica serosa. Tunica submucosa was absent. Throughout the length of small intestine , tunica mucosa was modified into slender like projections known as villi which were finger shaped in duodenum, tongue shaped in jejunum and spatulate in case of ilium. The use of probiotic caused significant increase in the intestinal length and body weight of probiotic feed birds. Similarly height of villi also increased. The histological parameters like thickness of tunica muscularis , height of villi showed a significant increase with advancement of age . The feeding of probiotic led to a significant increase in the weight and length of small intestine.

**PO-27 Cultural and Molecular detection of viruses from clinical samples of poultry**

Shilpi S. Singh, R.R. Pharande, Sonal Ingle, V. D. Thorat, R.S. Gandage, S.B. Majee

Department of Microbiology, Bombay Veterinary College, Parel, Mumbai 12

The present study was undertaken to screen poultry flocks for Avian Adenovirus (AAV), Avian Reovirus (ARV) and Infectious Bursal Disease Virus (IBDV) by isolation in specific pathogen free (SPF) eggs, Agar Gel Precipitation Test (AGPT), Reverse Transcriptase – Polymerase chain Reaction (RT-PCR), Polymerase chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP). The bursal samples were collected from 13 different farms of the birds showing gross lesions and transported to laboratory. Out of a total of 20 bursa samples that were subjected to RT-PCR and AGPT only 3 showed amplification of the VP2 gene of IBDV at 643 bp, however none were detected by AGPT. Out of 76 bursae processed and inoculated in by chorioallantoic membrane (CAM) route and allantoic route in the SPF eggs 23 isolates showed lesions specific for IBDV in the embryos. The gross lesions observed were oedematous distension of the abdomen, petechial hemorrhages and congestion of the skin, stunted growth, swollen head, and haemorrhages on the CAM. AGPT of the isolates was carried out against IBD specific antiserum and keeping known positive antigen of intermediate strain vaccine as control. It was observed that 08 out of the 23 isolates showed a line of precipitation. RNA and DNA were extracted from the above isolates by TRIZOL method and subjected to RT-PCR and PCR for IBDV, ARV and AAV respectively. The cDNA synthesized by RT-PCR was used for VP2 gene amplification of IBDV and S3 gene amplification of ARV. Out of the 23 isolates 09 samples amplified to yield a 643 bp product specific for IBDV. On RE digestion of the 643 bp product of the VP2 gene with enzyme Hha I, product digests of ~283 to ~360bp were obtained in all of the 9 isolates. The isolates were found to be negative for the S3 gene of the ARV as compared to the control strain which amplified at 548 bp. The DNA extracted from the isolates was subjected to PCR amplification of the L1 region hexon gene of AAV all the 23 isolates were found to be negative as compared to the standard isolate which amplified at 897bp. From the above study we can conclude that classical strain IBDV could be directly detected from the samples as well as from the isolates by RT-PCR and PCR-RFLP confirming that the IBDV was of classical type. However none of the samples contained either ARV or AAV indicating absence of any multiple infections with IBDV, AAV and ARV.

**PO-28 Effect of *Andrographis paniculata* on experimentally induced *E. coli* pathology in broilers**

Suvarna R. Sonwane, R. S. Ingole\*, Madhuri S. Hedau, P. R. Rathod, S. W. Hajare and M. V. Ingawale

Department of Veterinary Pathology,

Post Graduate Institute of Veterinary and Animal Sciences, Akola- 444 104 (MS), India

The effect of *Andrographis paniculata* on experimentally induced *E. coli* pathology in broilers was evaluated. One hundred a day old broiler chicks were randomly divided into four equal groups.

Group T1 was treated as control while group T3 and T4 were fed with dried leaves powder of *Andrographis paniculata* @ 5 gm/kg of feed from day old to the end of experiment. On day 8, each bird from group T2 and T4 were challenged with single oral dose of *E. coli* broth culture @ 0.2 ml ( $1 \times 10^9$  cfu/mL). On 14<sup>th</sup>, 28<sup>th</sup> and 35<sup>th</sup> day of experiment, six birds from each group were randomly sacrificed and observed for gross and histopathological observations. Necropsy examinations of control and T3 group birds did not reveal any gross pathological alteration; while T2 group birds showed hydropericardium distended abdomen and yellowish cheesy material on duodenal mucosa 28<sup>th</sup> and 35<sup>th</sup> day. The severities of lesions were mild in T4 group compared to T2 group birds. On histopathological examination, T2 group showed extensive hemorrhages, congestion, edema and peribronchial lymphoid aggregation in lung, extensive degenerative changes with prominent lymphoid aggregations in liver, myocardial degenerative changes with fibrinous exudates on epicardium in heart, extensive vacuolar changes in tubules with glomerular degeneration in kidney, lymphoid depletion and necrosis on in spleen and necrosis at the tip of villi and sloughing of mucosa in duodenum on 28<sup>th</sup> and 35<sup>th</sup> day of experiment. The lesions in all organs were well appreciated on 28<sup>th</sup> and 35<sup>th</sup> day of infection compared to 14<sup>th</sup> day of infection. *Andrographis paniculata* in *E. coli* challenged group showed comparatively normal architecture with mild degenerative changes in all organs indicated putative protective effect of plant during *E. coli* inducted pathology in broilers.

**Key words:** *Andrographis paniculata*, *E. coli*, pathology

**PO-29**

### **MUTATIONAL ANALYSIS OF BRCA2 GENE IN CANINE MAMMARY TUMOR**

Vaidya A.B<sup>1</sup>, Sawane M.P<sup>1</sup>, Chaudhari P.R<sup>2</sup>, Umrikar U.D<sup>1</sup>, Pawar V.D<sup>1</sup>, Deshmukh R.S<sup>1</sup>,  
Komatwar S.J<sup>1</sup>, Chopade M.M<sup>1</sup>

1. Bombay Veterinary College, Parel, Mumbai.

2. Comparative Oncology and Genomic Facility, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC)

#### Abstract

Mammary tumor is the most common type of cancer in women as well as in female dogs. There are number of common tumor suppressor gene reported in human and canines such as P53, BRCA1, BRCA2 and CHEK2. The mutations in these genes often results in to development of cancer in both. Therefore, the present study was undertaken with the aim to find out mutation in BRCA2 gene of dogs suffering from mammary tumor. BRCA2 gene plays important role in maintaining genomic stability by double strand break repair by interacting with RAD51. Germline mutation in BRCA2 gene accounts for the 5 per cent of the individual risk of developing breast cancer and increases risk up to 84 per cent of individual. Numerous studies have been done on human BRCA2 gene which shows 68 per cent homology with canine BRCA2.

BRCA2 gene contains 27 exons out of which exon 11 is the largest exon contains eight BRC repeats. The genomic DNA was extracted from 20 samples (blood and tissue) and 2.5 kb region of exon 11 of BRCA2 gene was amplified and sequenced using automated sequencer. After sequence analysis total fifteen nucleotide changes were observed. Out of these mutations three mutations (AAAAGA, AAAàCAA, AGTàAGG) in three different cases were reported earlier, while twelve were novel mutations. Out of these mutations, eleven mutations were missense mutations (73 per cent) and four mutations were silent mutation (27 per cent). The nucleotide change (AAAàAGA) changes amino acid from Lysine to Arginine which is located on BRC repeat 3. Mutation in this repeat affects the normal function of BRCA2 which causes lethal effect and increases the probability of the cancer. Present findings may be useful for early detection and thereby prevention of mammary tumor in canines and humans.

**PO-30**

**Importance and Prevalence of Sexed Semen In Dairy Industry- A Review.**

S. N. Solanke<sup>1</sup>, M. F. Siddiqui<sup>2</sup>, M. D. Kharwadkar<sup>3</sup>, V. S. Ingle<sup>4</sup>, A. S. Rathod<sup>5</sup>,  
M. B. Wakchaure<sup>6</sup> and N. P. Telange<sup>7</sup>  
e-mail:solankesonali3@gmail.com

Department of Livestock Production and Management  
College of Veterinary and Animal Sciences, Parbhani-431402  
Maharashtra Animal and Fishery Sciences University, Nagpur (MS)

**Abstract**

Breeders have tried to manipulate the sex of offspring at conception for centuries (Garner and Seidel, 2008). Sexed semen Technology is now commercially available in many countries around the world and is primarily used in dairy cattle breeding. Sperms are sorted by Flow Cytometry on the basis of a 4% differences in DNA content between sperm containing X and Y chromosome. Despite reliably producing 90% gender bias, the facility of sexed semen produced is compromised compared with conventional semen (Butler et al, 2014). Use of sexed semen for artificial insemination of cows in Dairy Herd Improvement was characterized by breeding year, parity, service number, region, herd size and herd milk yield (Norman et al, 2010). The principle of using sexed semen is increased numbers of heifer calves born, with approximately 90% of successful pregnancies resulting in a heifer calf. The subsequently increased availability of replacement heifers may be utilised to expand herd size and production. Alternatively these calves may be sold as heifers, which would increase revenues compared with the sale of lower value dairy bull calves. Using female sexed semen may also allow farmers to reduce the incidences of difficult calving and improve bio security by increasing herd size while maintaining a closed herd. In seasonal calving systems, the use of male sexed semen from beef sires on later calving cows may also be

considered to increase the value of beef output from the dairy herd, as male beef calves traditionally command a premium over females (Butler et al, 2014). Thus it can be concluded that use of sexed semen in dairy industry can increase number of heifer calves born ultimately intensifying the production.

Keywords:- Sexed Semen, Flow Cytometry, Heifer calves Born, Dairy Industry.

**PO-31**

**Precision Dairy Farming (PDF) Technologies- Milk Yield and Milk Electrical Conductivity- A Review**

A. S. Rathod<sup>1</sup>, M. F. Siddiqui<sup>2</sup>, M. D. Kharwadkar<sup>3</sup>, V. S. Ingle<sup>4</sup>, S. N. Solanke<sup>5</sup>, K. S. Mane.

e-mail:dr.amolrathod.vet@gmail.com.

Department of Livestock Production and Management

College of Veterinary and Animal Sciences, Parbhani-431402

Maharashtra Animal and Fishery Sciences University, Nagpur (MS)

**ABSTRACT**

Dairy farming from being traditional family runway of life has grown to an organised dairy industry with technological innovations in feeding, housing, breeding and health care management (Kamboj *et al*, 2017). Precision Dairy Farming (PDF) is defined as “the use of information and communication technologies for improved control of fine-scale animal and physical resources variability to optimize economic, social and environmental dairy farm performance” (Eastwood *et al*, 2012). Dairy milk yield and Electrical Conductivity (EC) are the most important components that are required to be recorded and monitored. Manual recording of the yields is very time and labour consuming. Most of the mechanised and automated milking parlours, now being set up in India, has the provision of on-line milk yield and EC recording provision and computerised data storage and retrieval with or without a herd management software. Milk yield and electrical conductivity are sensitive to changes in animal health status and the machine milking systems that provide data on milk yield and milk electrical conductivity at every milking provide very detailed information on each cow or buffalo (Kamboj *et al*, 2017). Significant changes in milk yield and electrical conductivity can be observed as early as 10 days before diagnosis of an adverse health event (Lukas *et al*, 2009). Change in milk yield can be observed 10 days before the day of diagnosis (Tozer *et al*, 2004). This may alert the dairy farmer at an earlier stage of the disease. These changes do not indicate what type of disease is emerging, but they give the farmer a time advantage that can be used to identify and eliminate the environmental stress factors any diseases condition such as Mastitis (Khatun *et al*, 2017), Physiological, Hormonal Imbalance (Fan *et al*, 2017) Thus, it can be concluded that PDF- Milk Yield and Milk Electrical Conductivity provide data that measures cow comfort, which can then be used for forecasting of any future disease condition well in advance. Cow comfort can lead to better overall health, which

lowers the cost of animal care and treatment and can increase animal production, health and welfare and profit too.

**Keyword: - PDF, Milk yield, Milk electrical conductivity**

**PO-32 Karyological analysis of Boer breeding bucks using QFQ banding**

Kokani S.C., Pawar V. D., Sawane M. P., Chopade M. M., Khade S. B., Komatwar S.J.,  
Deshmukh R.S. and C.B. Nimbkar  
*Dept. of Animal Genetics and Breeding,*  
*Bombay Veterinary College, Parel, Mumbai-12. (dr.s.c.kokani@gmail.com).*

Cytogenetic screening of breeding animals helps to detect major chromosomal abnormalities and prevent further dissemination of these abnormalities in the population. In the present study 15 breeding bucks of Boer breed maintained at Nimbkar Agriculture Research Institute, Phaltan, Dist-Satara were screened for cytogenetic defects using QFQ- banding technique. Short term lymphocyte culture technique was used for display of metaphase chromosomes. For optimisation of QFQ banding different concentrations of quinacrine dihydrochloride and exposure time were tried and best bands were obtained at 0.50 per cent of Quinacrine dihydrochloride treatment for 20 minutes. The modal diploid chromosome number was  $2n=60$  for breeding bucks of Boer goat. There were 29 pairs of autosomes and one pair of sex chromosome in complement. The X chromosome was the largest acrocentric chromosome whereas the Y chromosome was the smallest dot like in majority of the metaphase examined however in few metaphases it was found to be submetacentric in morphology. The chromosomes were arranged according to their band intensity. First 14 pairs of the chromosome showed characteristic Q- band patterns. Whereas, from chromosome 15 to 29, all the pairs presented pale intensity of fluorescence on the telomere whereas the proximal and central regions presented varied degree of fluorescence. The X chromosome was the longest with more intense band, arms identified and proximally intense band and centrally medium band, distal end pale. The Y chromosome showed intense band on long arm and the intensity of band on proximal pale band identified. The QFQ banding technique was found to be simplest banding technique and helped in identifying almost all the chromosome. However, it was noted that the goat chromosome do not present the brilliant fluorescence. Present investigation did not reveal any marked chromosomal abnormality in the screened Boer breeding bucks.

PO-33

**Prolactin (bPRL) and Growth Hormone (bGH) genes polymorphism  
in Red Kandhari and Deoni cattle**

SHAIKH GM, MAHENDRA KUMAR, SS DESHPANDE, SA DHAWARE, MS VAIDYA AND PV  
NANDEDKAR

College of Veterinary and Animal Sciences, Parbhani-431402 (M.S.) INDIA

Prolactin (bPRL) and Growth Hormone (bGH) genes are the two important genes of quantitative trait loci regulating production traits in cattle. So present study was undertaken to detect polymorphism in Prolactin and Growth Hormone genes in Red Kandhari and Deoni cattle breeds of Marathwada region.

A total of hundred blood samples from Red Kandhari (n=50) and Deoni (n=50) cows were collected from the various organized farms and the field. Polymorphism in bovine Prolactin and Growth Hormone genes was detected by PCR-RFLP technique. A 156 bp fragment of bPRL(exon III) and 329 bp fragment of bGH (intron III) were amplified by PCR. Amplification of gene was confirmed by agarose gel electrophoresis and PCR products of bPRL and bGH genes were digested with RsaI and MspI restriction enzymes, respectively. Restriction enzyme digested PCR products were analysed by 2.5% agarose gel electrophoresis and 6% polyacrylamide gel electrophoresis. PCR-RFLP analysis of bPRL and bGH genes of Red Kandhari and Deoni cattle revealed two alleles and three genotypes.

Genotypic and allelic frequencies obtained for bPRL gene were 0.31-AA, 0.51-AB and 0.18-BB and 0.57-A and 0.43-B in Red Kandhari cattle; and 0.26-AA, 0.42-AB and 0.32-BB and 0.47-A and 0.53-B, in Deoni cattle. Genotypic and allelic frequencies obtained for bGH gene in the present study were 0.71 – MspI (-/-), 0.27 – MspI (+/-) and 0.020 – MspI(+/+) and 0.84 – MspI(-) and 0.16 – MspI(+), respectively in Red Kandhari cattle; and 0.86 – MspI (-/-) and 0.14 – MspI(+/-), and 0.93 – MspI (-) and 0.07 – MspI(+), respectively in Deoni cattle. One animal with MspI (+/+) genotype was detected in Red Kandhari animal, which was not reported earlier among Indian cattle breeds.

In bPRL, the heterozygous AB genotype (0.51 in Red Kandhari and 0.42 in Deoni) was present in the highest frequency and the frequencies for the alleles A and B were almost of similar magnitude. In case of bGH gene, frequency distribution of MspI (-) allele obtained were strikingly high for Deoni (0.93) and for Red Kandhari (0.84) cattle.

Frequency distribution pattern of these genes indicates that Red Kandhari and Deoni cattle breeds are genetically distinct from taurine cattle breeds and have maintained the Indian characteristics at bPRL and bGH gene loci.

The detailed investigation needs to be carried out to find variation in prolactin and growth hormone genes in Red Kandhari and Deoni cattle by sequencing the PCR product and by studying large samples.

**PO-34**

**SLAUGHTER WASTE: A SOURCE OF REGENERATIVE MEDICINE**

NANDEDKAR P.V., MOHITE N.R., MAHENDRA KUMAR, SS DESHPANDE,  
SADHAWARE AND MS VAIDYA

College of Veterinary and Animal Sciences, Parbhani-431402 (M.S.) INDIA

Stem cells are believed to revolutionize the way medicine is practiced in the near future. Antibiotics and drugs may soon be replaced by cell-based therapies. Extensive research is ongoing to realize their clinical potential. Embryonic stem cells are believed to be highly versatile and possess maximum therapeutic potential. These embryonic stem cells are isolated from the inner cell mass of blastocyst which are a precious commodity and are source material used in regenerative medicine.

In vitro maturation and in vitro fertilization of oocytes obtained slaughtered buffalo ovaries have recently provided a practical means for producing large number of bovine zygotes at low cost for research and commercial settings. Application of this assisted reproduction technology in buffalo will not only improve productive and reproductive potential but will also help rescue the precious germ plasm going to waste by indiscriminate slaughter of this animal.

In the present study we have used buffalo ovaries and testis obtained from the abattoir to establish sophisticated procedures of in vitro maturation, in vitro fertilization of eggs, and embryo culture. To optimize in vitro embryo production of buffalo, study was designed with an objectives (1) to compare the oocyte maturation rates in media containing buffalo estrus serum and foetal bovine serum; (2) to compare the embryonic development of presumed zygotes by co-culturing with and without cumulus cells in the media.

Immature oocytes were graded on the basis of cumulus expansion and grade A & B oocytes were matured in TCM 199 supplemented with hormones and growth factors divided in two groups. Twenty-four hours post in vitro oocyte maturation in the medium (TCM-199+ 5µg FSH+ 1µg 17β-estradiol + Gentamicin, 50µg/ml + 0.8mM sodium pyruvate + 5 per cent follicular fluid) supplemented with 10% FBS and 10% EBS, MII stage oocytes were 55.91 and 62.11 per cent, respectively.

Total 108 in vitro matured oocytes were fertilized with capacitated spermatozoa and were divided into two groups 1) 54 zygotes cultured in mCR2 medium with cumulus cells and 2) 54 zygotes cultured in mCR2 medium without cumulus cells. In 1st group a total of 29 (53.70 per cent) zygotes were cleaved, 9 (16.67 per cent) zygotes were reached upto morula stage of embryo and 16 (29.63 per cent) zygotes were

arrested at different stages of embryonic development, where as in IInd group total 31 (57.41 per cent) zygotes were cleaved, 10 (18.52 per cent) were reached upto morula stage and 13 (24.07 per cent) zygotes were arrested at different embryonic stages of development. Results in the present study upto morula stage of embryonic development are satisfactory and showing marked differences in the both the groups.

In-vitro maturation, fertilization and embryo culture procedures on oocytes obtained from abattoir ovaries (inedible part of carcass) has created resource material to develop blastocyst as source of embryonic stem cells used in regenerative medicine in near future.

**PO-35** **Socio-economic profile of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method.

After collection and analysis of data, it was concluded that a majority of respondents were of middle age group, educated up to higher secondary school level, possessing medium herd size, annual income, social participation, information sources, market orientation with medium level of knowledge and adoption of buffalo management practices.

The probable reason for medium level of knowledge could be medium sized landholding, school level education and recurring drought conditions. Whereas, the reason for medium level of adoption might be that the respondent had adopted scientific management practices for making an enterprise sustainable, techno-economically feasible and judicious use of resources.

**PO-36** **Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P., and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method.

After collection and analysis of data, it was concluded that, in relation to the information needs, respondents expressed marketing and break-even analysis, in particular as the most important information need; followed by demand and supply, traditional practices and government guidelines. The respondents expressed animal health as the second most important information need. After marketing and health, respondents expressed housing as most important information need with waste management been the most important followed by management during different seasons, construction of shed, farm layout, selection of site and pre-requisites for starting buffalo enterprise in descending order. In relation to information needs, information about breeding and nutrition were responded as the most important in the descending

**PO-37**

**Constraints faced by buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P. and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method. After collection and analysis of data, it was concluded that, in relation to constraints faced by buffalo dairy entrepreneurs, I rank was given to two constraints viz. cost of high yielding buffaloes and costly animal treatment. The II, III, IV and V rank was given to low milk prices, high cost of veterinary medicine, costly concentrate and buffaloes require more ration respectively. No availability of loan, long dry period in buffaloes, high cost of construction of shed, insufficient knowledge about vaccination and diseases and no veterinary service nearby was given VI, VII, VIII, IX and X rank respectively. The ranking of XI, XII, XIII, XIV and XV were given to no education facilities for dairy farming locally, no grasslands, shortage of clean water, shortage of green fodder and no timely veterinary services. Inability to detect heat, unavailability of skilled labour and no breeding bull available was given XVI, XVII and XVIII rank respectively.

**PO-38**

**PATHOLOGICAL STUDIES OF *Haemonchus contortus* (Barber Pole Worm) IN GOAT**

Utkarsh.B. Wankhede Deepika.D.Ghodke, Roshni Padole, Priyanka Tikore, A.S.Rathod, P.R. Rathod

*Department of veterinary pathology,*

*Post Graduate Institute of Veterinary and animal Sciences,*

*Krishinagar Akola 444104,MAFSU(MH) India*

Berari breed of goat having age of 9th month from Borgaon Manju farm of District. Akola was presented for postmortem examination in the Department of Veterinary Pathology, PGIVAS, Akola, having

history of diarrhea, dehydration, anorexia and anemia. Symptomatically treatment of animal done by local veterinarian and animal died within 24 hrs after treatment. On necropsy examination it revealed that haemonchus contortus which were found in the abomasum attaches to the wall of abomasum, gall bladder was distended, ulceration found in the rectum part of the large intestine. Histopathological study show abomasum showed desquamation of branched tubular gastric glands and mononuclear cell infiltration in submucosa and degenerative changes in tunica muscularis. Submucosa showed increased collagen fibers, adipose tissue and reticular fibers. Liver showed centrilobular necrosis, spleen shows Decrease in lymphoid population and degeneration and necrosis in lymphoid follicle necrosis. Ulceration in large intestine. It shows severe damage to vital organs. The presented case was died due to severe infestation of haemonchus contortus in goat and causes severe anemia and causes heavy losses in goat meat industries.

**PO-39**

**Multiple organ failure in Labrador dog – A case report**

Roshani M. Padole, A. S. Rathod, Priyanka Tikore, Deepika Ghodke, Madhuri Hedau, P.R. Rathod, N. M. Bhojane and R. S. Ingole

*Department of Veterinary Pathology*

*Post Graduation Institution of Veterinary and Animal Sciences, Akola- 444104 (MS) India.*

A 4 year old female Labrador dog was brought to Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola with history of pus discharge from vagina since 1 month revealed that open pyometra. Ovariohysterectomy was done. Recovery of animal. After 1 month bitch showed the symptoms of melena, anorexia, vomiting and anemia. During treatment patient was died. Postmortem was conducted in the Department of Veterinary Pathology and representative tissue samples were collected and fixed in 10% formalin for histopathological examination. On gross examination, animal showed pale conjunctiva. Lungs showed black spots of carbon particles revealed that anthracosis. Trachea showed froth. Liver showed yellow discoloration with pin point haemorrhages and hepatomegaly with distended gall bladder. Gastric and intestinal mucosa was thickened with many petechial to echymotic haemorrhages and ulcers measuring about 0.5 to 1.5 cm diameter. And contain blood mixed with faecal material. Spleen was slightly swollen. Histopathological examination revealed, lung showed the pigment seen as black granules in the alveolar walls and within phagocytes. Congestion and infiltration of erythrocytes and leucocytes in gastric and intestinal mucosa. On basis of clinical history, post mortem examination and histopathological examination, it is concluded that animal might have died due to multiple organ failure.

Keyword : Anthracosis, Dog, Gastric ulcers, Liver.

**PO-40**

### **Cardiac Cirrhosis In Horse -A Case Report**

Priyanka Tikore, Deepika Ghodke, Roshani Padole, Atul Rathod,  
Utkarsh Wankhede, N. M. Bhojane, R. S. Ingole and M.S. Headu

*Department of Veterinary pathology*

*Postgraduate Institute of Veterinary and Animal Sciences, Krishinagar, Akola 444 104 (MS)*

A carcass of 9 years old non descript breed horse of was presented for the department of veterinary pathology for postmortem examination from Borgaon manju Tq. Akola with the a history of weakness, anorexia, colic and lack of defecation was treated by local veterinarian symptomatically. During necropsy examination revealed there was presence of congestion, emphysema of lung. Pinpoint hemorrhages on heart and constrictive pericarditis is observed. Mucosa of intestine pinpoint hemorrhages was present and froth in colon. Liver show presence of swollen, severe hepatic congestion, uneven surface, and due to chronic proliferation leads to hardness which characterized by cirrhosis. There was presence of pin point hemorrhages on surface of kidney and Spleen show presence of wrinkles on the surface. On the basis of postmortem examination, the present case of horse was diagnosed as the cardiac Cirrhosis of liver. and representative tissue samples were collected and fixed in 10% formalin for histopathological examination.

**Keywords :** liver, horse, cirrhosis.

**PO-41**

### **ASPIRATION PNUMONIA IN GOAT KID – A CASE REPORT**

Deepika D. Ghodke\*, Roshani Padole, A. S. Rathod, Priyanka Tikore, U.B.Wankhede,  
P.R. Rathod, R. S. Ingole & M.S. Hedau

*Department of Veterinary Pathology*

*Post Graduation Institution of Veterinary and Animal Sciences, Akola- 444104 (MS) India.*

Aspiration pneumonia is a pulmonary infection characterized by inflammation and necrosis due to inhalation of foreign material. The severity of the inflammatory response depends on the material aspirated, the type of bacteria aspirated, and the distribution of aspirated material in the lungs. A four month old male goat kid was presented to the Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola with a history of clinical signs showed anorexia, nasal discharge, diarrhoea, abdominal distension from last 3 days. During treatment patient was died. Postmortem was conducted in the Department of Veterinary Pathology, Grossely there was presence of froath in trachea, the lungs are markedly congested with focal areas of interlobular edema. Bronchi are hyperemic and full of froth. The pneumonic areas tend to be cone shaped the base toward the pleura which further leads to suppuration and

necrosis followed. The foci become soft, liquefied, reddish brown in color and foul smelling. There was usually an acute fibrinous pleuritis, often with pleural exudates, intestinal congestion, haemorrhages on intestinal wall. On basis of clinical history and post mortem examination, it was concluded that animal might have died due to aspiration pneumonia.

Key words: Aspiration Pneumonia, Goat kid

**PO-42 TAPEWORM INFESTATION IN PEACOCK – A CASE REPORT**

Roshani Padole, Atul S. Rathod, Priyanka Tikore, Deepika Ghodke, U. B. Wankhede,  
P. R. Rathod, Madhuri Hedau, N. M. Bhojne & R.S. Ingole

*Department Of Veterinary Pathology*

*Post Graduate Institute Of Veterinary And Animal Sciences, Akola – 444104 (MS) India*

A one to one and half year old male peacock was presented for postmortem examination from Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary & Animal Sciences, Akola with a history of anorexia, fever, diarrhea & emaciated. On post mortem examination birds showed that there was emaciation, debilitation soiled vent with faeces and conjunctiva congested. Gelatinization of subcutaneous fat which was indicative of anorexia. Granular surface of oesophagus due to deficiency of vitamin A. Trachea and larynx showed slightly congested. Lungs showed severe congested and air sac was cloudy. Both the ventricles of heart were empty. Liver was swollen with rounded borders and showed focal areas of haemorrhages. Spleen was congested with irregular borders. Haemorrhages present on mucosa of gizzard. In intestine tapeworm infestation occurs. Testicles were congested. Form the post mortem examination it was concluded that birds might have been died due to parasitic infestation leads to anaemia.

Keywords : Tape worm, Peacock, Post mortem

**PO-43 Blood picture and immune response of broilers fed altered level of crude protein with supplementation of limiting amino acids**

P.A. Sutar, K.Y. Deshpande\*, R.S. Awhad, P.V. Nandedkar and P.R. Suryavanshi

*Department of Animal Nutrition, COVAS, Parbhani*

Corresponding author: [kuldeepydeshpande@gmail.com](mailto:kuldeepydeshpande@gmail.com)

An experiment was conducted to study the blood picture and immune response of broilers as influenced by alteration in dietary crude protein levels along with supplementation of limiting amino acids. Three hundred, day-old (Vencobb-430) straight run commercial broiler chicks were distributed

randomly on equal body weight basis into four treatment groups viz, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and one control group T<sub>0</sub> with four replicates of 15 chicks in each. The control group (T<sub>0</sub>) was fed standard broiler diets as per BIS (2007); groups T<sub>1</sub> and T<sub>2</sub> were fed with broiler diets containing 0.5% and 1% reduction in CP and groups T<sub>3</sub> and T<sub>4</sub> were fed with broiler diets with 0.5% and 1% increase in CP over BIS (2007) requirements with supplementation of 5% and 10% limiting amino acids. The Hb (g/dl) and PCV (%) significantly (P<0.05) increased in T<sub>2</sub> (1% reduction in CP) and T<sub>4</sub> (1% increase in CP) over control (T<sub>0</sub>); whereas, T<sub>1</sub> and T<sub>3</sub> remained comparable. The TLC (10<sup>3</sup>/μl) increased significantly (P<0.05) in low protein groups when compared with high protein groups; whereas the TEC (10<sup>6</sup>/μl) was significantly higher in T<sub>2</sub> and T<sub>3</sub> as compared with T<sub>1</sub> and control. The total protein, albumin, globulin (g/dl) and albumin: globulin ratio (A: G ratio) remained comparable (P>0.05) amongst all treatment groups. The uric acid (mg/dl) was significantly higher (P<0.05) in high protein group (T<sub>2</sub>); whereas, the value for T<sub>1</sub> and control were intermediate. The serum antibody titer prior to vaccination in high protein groups (T<sub>3</sub> and T<sub>4</sub>) was significantly lower (P<0.05) than low protein groups (T<sub>1</sub> and T<sub>2</sub>) and control. However the antibody titer 21 days post vaccination did not differ significantly (P>0.05) amongst the treatment groups. It may be concluded that up to 1% reduction in CP in diet with addition of limiting amino acids is possible without any adverse effect on blood picture and immune response in broilers.

**PO-44**

**Effect of dietary inclusion of guar (*Cyamopsis tetragonoloba*) korma meal on gut health and immune response in broiler chicken**

R.S. Awahad, K. Y. Deshpande, P.A. Sutar and P.R. Suryavanshi

*Department of Animal Nutrition, COVAS, Parbhani*

Corresponding author: [kuldeepydeshpande@gmail.com](mailto:kuldeepydeshpande@gmail.com)

**Abstract**

A study was conducted to assess the effect of dietary inclusion of guar (*Cyamopsis tetragonoloba*) korma meal on gut health and immune response in broiler chicken for 42 days. A total of 280, day-old (Vencobb 430) straight run commercial broiler chicks were distributed into seven treatment groups with four replicates of 10 chicks in each. The control group (T<sub>0</sub>) was fed with standard broiler diet as per BIS (2007). Groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> were fed with broiler diets with guar korma meal @ 5% (50 kg/ton), 10% (100 Kg/ton) and 15% (150 Kg/ton) without and with supplementation of *α*-mannanase enzyme @ 10 million IU/ton and probiotic @ 250g/ton, respectively. The total viable count of *E-coli* (log cfu/g) ranged from 3.95 (T<sub>0</sub>) to 5.68 (T<sub>2</sub>) in the cloacal swabs of experimental birds. The weight of spleen was significantly higher (p<0.05) in T<sub>4</sub>, T<sub>1</sub> and T<sub>2</sub> and the weight of bursa (g) was found to be comparable (p>0.05) amongst the treatment groups. The immune response against Ranikhet disease virus antigen prior

to and post vaccination did not differ significantly ( $p>0.05$ ) amongst the treatment groups. It is concluded that GKM can be included in broiler diet up to 10% with or without supplementation of enzyme and probiotic to replace protein source without any adverse effect on gut health and immune response in broilers.

**PO-45**

**Molecular characterization and phylogenetic analysis of  
*Contagious ecthyma Virus* in Goats**

Gosavi, P.D., P.R. Suryawanshi, A.R. Deshpande, S.S. Gaikwad,  
S.B. Majee and P.V. Nandedkar

Department of Veterinary Microbiology, COVAS, Parbhani. MAFSU, Nagpur.

**Abstract:** In the present study, outbreaks of contagious ecthyma in goats were investigated in and around Parbhani and Aurangabad districts between August 2016 to March 2017. Out of 25 scabs samples collected from contagious ecthyma suspected goats, 10 were confirmed positive for contagious ecthyma using PCR. Based on DNA sequencing and further analysis the DNA sequences of the samples examined in this study were confirmed to be contagious ecthyma virus sequences. It was evident that the nucleotide divergence within Parbhani contagious ecthyma virus sequences was between 0.00 to 0.04 per cent. It was observed that all the five contagious ecthyma virus sequences were phylogenetically closely related with each other and with sequences of North-eastern states of Tripura and Assam than to other contagious ecthyma virus sequences of world. The evolutionary rates and time to the most recent common ancestors (tMRCA) for contagious ecthyma virus estimated to originate during the middle of 2001 with High Posterior Density (HPD) of 95% (1995.5-2007.1 95%CI).

**PO-46**

**EXPERIMENTALLY INDUCED *E.coli* INFECTION IN QUAILS AND  
ITS AMELIORATION BY *Aegle marmelos*: IMMUNOPATHOLOGICAL STUDIES**

R.A. Kokare, G.R. Gangane, G.B. Kulkarni, B.M. Kondre and B.G. Shingumare

Department of Veterinary Pathology

College of Veterinary and Animal Sciences, Parbhani

In the present experimental trail, One hundred, one week old Japanese quail birds were randomly divided into five equal groups, each containing twenty birds. Group I quail birds served as control, group II quail birds were infected with *E.coli* @ 0.3 ml inoculum having bacterial concentration of  $1 \times 10^8$  cfu/ml, group III quail birds were infected with *E.coli* and fed with *Aegle marmelos* @ 100 mg/kg of body weight from day of infection, group IV quail birds were infected with *E.coli* and fed with *Aegle marmelos*

@100mg/kg body weight since the onset of first sign and group V quail birds were fed with *Aegle marmelos* @ 100 mg/kg body weight respectively. The study was conducted for 45 days.

The study was assessed through immunological, gross and histopathological alterations. In group II birds at 30<sup>th</sup> day there was immunosuppression. There was marginal incline in mean values of relative weights of liver, kidneys and spleen and marginal decline in weights of Bursa of Fabricius in *E.coli* infected birds than that of control group birds at 30<sup>th</sup> and 45<sup>th</sup> day of study. On gross pathological examination of group II birds, there was mild to moderate hepatomegaly with pale and fragile consistency. Also, the kidneys of infected non treated birds were congested and enlarged.

On histopathological studied, liver sections showed minimal to mild, focal to multifocal, congestion, cellular swelling, vacuolar degeneration, thickening of liver capsule and necrobiotic changes. The kidney sections showed congestion, cellular swelling, vacuolar degeneration, hydropic degeneration, necrobiotic to necrotic tubular epithelium, haemorrhagic cystic degeneration and focal mononuclear cell infiltration and the histoarchitecture of spleen of birds showed focal congestion and occasional lymphoid depletion. Heart sections showed moderate mononuclear cell infiltration in heart wall and within muscles in birds of group II. The changes noted in lung were thickening of alveolar septa, inflammatory cell infiltration, congestion and occasional emphysematous changes in group II birds.

The intestines on histoarchitectural study showed desquamation, disorganization, exfoliation of epithelial linings, mild to moderate inflammatory cell infiltration, blunt appearance of villi, mild to moderate congestion and catarrhal exudation in lumen. Similarly the birds of group III and IV showed mild degenerative changes in liver, cellular swelling in kidneys, occasional lymphoid depletion in spleen and MNC infiltration in heart muscles with low intensity and extent as compared to group II.

**PO-47**

**Haemato – biochemical alterations in *Ipomoea carnea* induced toxicity in Wistar rats**

V.G. Khandare, G.R. Gangane, S.D. Moregaonkar, B.M. Kondre, S. A. Londhe and S.C. Bhatane

Department of Veterinary Pathology,

College of Veterinary and Animal Sciences, MAFSU Parbhani (M.S.) 431402.

An experimental study was carried out to assess the toxic effects of *Ipomoea carnea* in Wistar rats. For the period of 28 days of experiment, 36 rats were divided into 4 Groups. Group I rats fed with commercial diet and served as control group while Group II and Group III were fed with *Ipomoea carnea* leaves @ 4% and 8% of diet respectively. Haematological and biochemical alterations were studied on 14<sup>th</sup> and 28<sup>th</sup> day of study period. On haematological assessment, haemoglobin conc. of Group II and Group III rats

found to be decreased significantly, however, the total erythrocyte count declined non-significantly at 28<sup>th</sup> day. The blood sample when subjected to total leukocyte count revealed significant incline in mean values of Group II and III in male and female rats at both the intervals of study. The mean values of DLC in male and female rats showed non-significant decrease in lymphocyte, increase in neutrophil and eosinophil, whereas, monocytes did not showed significant alteration.

Biochemically, there was significant increase in alanine transaminase (ALT) and aspartate transaminase (AST) mean values in male and female rats of both the treatment groups at 28<sup>th</sup> day. At 14<sup>th</sup> day of study period, these mean values remained comparable, except, the female rats of group III found to be elevated significantly. The mean values of blood urea nitrogen at both the intervals of study period in male and female rats were increased significantly. however, these values at 14<sup>th</sup> day in male rats of group II remained comparable. In male of both treatment group showed significant rise in serum creatinine level at 14<sup>th</sup> day. However, at 28<sup>th</sup> day, these values showed non-significant variation. In female rats of both treatment groups at both intervals, the serum creatinine value found to be increased significantly except the female animals of group II. There was significant decline in mean values of serum total protein in male and female rats of group II and III at both intervals of study period. The mean values of serum calcium in male as well as female rats of both treatment groups declined significantly and non-significantly at 14<sup>th</sup> day of study respectively. There was significant reduction in mean values serum calcium in male and female rats of group II and III at 28<sup>th</sup> day. The mean values of Serum phosphorus in male and female rats of group II and III at both intervals of study were inclined significantly than the control group values.

**PO-48**

**Ameliorative effect of *Picrorhiza kurroa* against *Lantana camera* induced toxicity in Wistar rats: Haematological studies**

Birajdar B.W, Gangane G.R, Moregoankar S.D, Kondre B.M, Aulwar B.S and Shingumare B. G.

The present experiment was conducted by grouping 48 rats into four groups each comprising of twelve rats (6 male & 6 female). For a period of 28 days, the rats of group I were given normal feed with drinking water *ad libitum*. The rats of group II were given *Lantana camera* leaves powder @ 0.25%/kg body weight through feed and group III rats were fed with *Lantana camera* leaves powder @ 0.25%/ kg Bwt + *Picrorhiza kurroa* powder @ 50mg daily through feed. Similarly, the rats of group IV were fed powder of *Picrorhiza kurroa* @ 50mg daily through feed. Blood was collected at 0, 14<sup>th</sup> and 28<sup>th</sup> day of experiment for haematological study.

The haematological parameters such as Haemoglobin (Hb) and Total erythrocyte count (TEC) were found to be non-significantly decreased in *Lantana camera* intoxicated rats at 14<sup>th</sup> and 28<sup>th</sup> day of

study intervals. However, there was marginal incline in mean values of total leucocyte count and non significant reduction in mean values of lymphocyte count when compared to the control group. There was significant increase in mean values of clotting time in *Lantana camera* toxicated group than the values of control group. The mean values of all studied haematological parameters did not show any significant variation in rats of group IV when compared with group I values.

**PO-49**

### **Prevalence of respiratory affections in goats**

Aulwar B. S., G.R. Gangane, S.D. Moregaonkar, P.R. Suryawanshi and B.W. Birajdar

DEPARTMENT OF VETERINARY PATHOLOGY

COLLEGE OF VETERINARY AND ANIMAL SCIENCES MAFSU, PARBHANI

The study on respiratory affections with special reference to bacterial infections in goats was conducted. In toto 60 died goats presented to Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani for conduct of post mortem examination were screened. During current study, all the carcasses were systematically examined for noting gross lesions in the organs of respiratory tract. Amongst 60 goats, which were died and presented to Department of Veterinary Pathology, COVAS, Parbhani, 35 carcasses were of goats aging up to 1 year and 25 were of more than 1 year age. These carcasses were when screened for noting the gross respiratory affections revealed 97.14% prevalence in goats aging up to 1 year and this percent prevalence of respiratory affections in goats aging more than 1 year was 92.00%. The goats died of respiratory affections when subjected for studying its prevalence revealed 81.39% prevalence of fibrinous pneumonia occupying first position followed by marbling (9.30%), hemorrhage (4.65%) and consolidation (4.65%). However, in case of trachea, presence of froth was observed in 71.42% goats followed by congestion to an extent of 28.57%.

**PO-50**

### **Ameliorative effect of *Swertia chirata* during experimental Aflatoxicosis in quails (*COTURNIX COTURNIX JAPONICA*): Biochemical evaluation**

Babar D. T., Gangane G.R., Kulkarni G.B., Moregaonkar S.D., Shingumare B. G. and Chavan P.A.

Department of Veterinary Pathology

College of Veterinary and Animal Sciences, Parbhani

The experimental study was conducted on One hundred and twenty one week old Japanese quails were divided into six equal groups. Group I served as control without any treatment with normal feed. The group II and III were given aflatoxin @ 200 ppb and 400 ppb respectively. The group IV was fed with *Swertia chirata* @ 70mg/kg BW of birds and group V quails were given both aflatoxin and *Swertia*

*chirata* powder @ 200ppb and @ 70 mg/kg of BW respectively through feed, while group VI quail birds were given both aflatoxin and *Swertia chirata* powder @ 400 ppb and 100 mg/kg of BW respectively daily for 60 days. Blood was collected in clot activator vial for biochemical estimation.

The study was assessed through estimating biochemical parameters such as Serum Total Protein, Blood glucose, Serum creatinine, AST and ALT at 30<sup>th</sup> and 60<sup>th</sup> day of study period. There was significant decrease in mean values of serum total protein at 30<sup>th</sup> and 60<sup>th</sup> day of study. Also, there was significant increase in mean values of AST, ALT, blood glucose and serum creatinine at both the intervals, except, the glucose level at 60<sup>th</sup> day in birds of group II was numerically comparable with the healthy treated group. In birds of group V and VI, the mean values of Serum Total Protein showed significant reduction, however, the mean values of ALT, AST and glucose showed significant increase at 30<sup>th</sup> and 60<sup>th</sup> day of study. The mean values of serum creatinine in the birds of group II and III increased significantly at both the intervals, however, these mean values in birds of group V and VI were comparable with control.

**PO-51** **Histoarchitectural alterations in Mercuric chloride induced toxicity and its amelioration by *Allium sativum* in male Wistar rats**

A.U. Ranveer, G.R. Gangane, B.M. Kondre, S.C.Bhatane, S.A. Londhe and R.U.Tandale

Department of Veterinary Pathology,

College of Veterinary and Animal Sciences, MAFSU Parbhani (M.S.) 431402.

An experimental trial was conducted for assessment of mercuric chloride induced toxicity and its amelioration by *Allium sativum* in male wistar rats. The study was conducted for 28 days. The 24 Wistar rats were divided into four groups. Group I rats were fed with *ad libitum* water and normal feed, Group II rats with Mercuric chloride @ 200 ppm through drinking water, Group III rats were given Mercuric chloride @ 200 ppm and *Allium sativum* bulb powder @ 2% through feed and Group IV were fed with *Allium sativum* @ 2% through feed.

The histopathological examination of experimental rats was undertaken at the end of trial. Kidneys of Mercuric chloride intoxicated rats showed severe congestion at corticomedullary junction and tubules. Moreover, the tubules showed necrobiotic changes, vacuolar degeneration and cellular swelling with proteinous hyaline casts in the lumen of tubules. In Group II rats, liver parenchyma showed congestion, granular degeneration, focal fatty change along with cytoplasmic rarefaction and infiltration of mononuclear cells. Dilation of central vein and increase in sinusoidal space was also noted in affected liver tissues. The multifocal mononuclear cell infiltration was noticed in liver parenchyma of rats of Group III. However, no noticeable histopathological changes were seen in the rats of Control group and Group IV.

In Group II rats administered with mercuric chloride, there was vacuolation in seminiferous tubules in testes, mononuclear cell infiltration, mild congestion, vacuolation in brain, mild and focal congestion in spleen. There were no appreciable histopathological changes in epididymis and pancreas of Group I, II, III and IV at studied intervals. Mercuric chloride in male Wistar rats at given dose for 28 days daily could elicit histomorphological changes in vital organs as evidenced by these observations.

**PO-52**

### **CHARACTERIZATION OF FecG LOCI OF GDF-9 GENE IN THE OSMANABADI GOATS**

Ukale P.S., Wagh P.T., Zunjarrao K.A., Bhawe K.G., Sawane M.P., Pawar V.D. and Doiphode A.Y.  
Bombay Veterinary College, Mumbai-12

The Osmanabadi breed of goat is one of the most prolific breed of goat in Maharashtra. Considering the variation in the fecundity of Osmanabadi goats, we have carried out genotyping Osmanabadi goats at FecG loci (G1, G4, G6, G7 and G8) as observed in ovine GDF-9 gene using T-ARMS-PCR technique. In the present study 165 blood samples of Osmanabadi breed of goat were collected from the Sheep and Goat farm maintained at Mahud, Dist. Solapur (M.S.) by Punyashlok Aahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune, and were genotyped following DNA isolation. The primers used in the present study successfully amplified PCR products of 247 bp and 396 bp at G1 locus, similarly, at G4 locus, 261bp and 417bp, at G6 locus 223bp and 362bp and at G8 locus 108bp and 198bp PCR products representing wild type and control fragment corresponding to normal non-carrier animals at respective loci. The G7, FecG<sup>E</sup>, FecTT and G1189A loci were studied on the basis of nucleotide sequence obtained by direct sequencing of G7 control fragment. The sequenced Osmanabadi goats revealed wild type allele at G7, FecG<sup>E</sup>, FecTT and G1189A loci representing non-carrier goats. In the present investigation, Osmanabadi goats are observed monomorphic carrying wild type allele at the G1, G4, G6, G7, G8 (FecG<sup>H</sup>), FecG<sup>E</sup>, FecTT and G1189A loci in the GDF-9 gene. It is concluded from the present findings that the fecundity of Osmanabadi goats is independent of FecG mutations.

**PO-53**

### **GENOTYPING OF OSMANABADI GOATS AT IGFBP-3 AND BMP-4 LOCI ASSOCIATED WITH GROWTH RATE**

Tep G., Vairagade R.S., Wagh P.T., Zunjarrao K.A., Sawane M.P., Pawar V.D. and Doiphode A.Y.  
Bombay Veterinary College, Parel, Mumbai-12

Considering the economic importance of growth rate in Osmanabadi goat and close association of IGFBP-3 and BMP-4 gene on the growth of mammals, we have carried out genotyping of IGFBP-3 and

BMP-4 gene loci, to identify polymorphisms associated with growth rate in Osmanabadi goat using PCR-RFLP and PCR-SSCP techniques, respectively. The blood samples of Osmanabadi goat (100 samples for IGFBP-3 and 200 samples for BMP-4 gene) collected from the Sheep and Goat farm maintained at various farms by Punyashlok Aahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd., Pune, were genotyped following DNA isolation. A fragment of IGFBP-3 gene, comprising of a part of exon 2, complete intron 2, exon 3, and a part of intron 3, and BMP-4 gene comprising of intron 2 was successfully amplified. The amplified fragment was found to be 654 bp for IGFBP-3 and 381 bp for BMP-4 gene. On digestion of 654 bp of IGFBP-3 gene with Hae III restriction enzyme yielded single restriction pattern of two fragments of sizes 201 bp and 201 bp in all the animals under study. On analysis of 381 bp of BMP-4 by SSCP on 12% polyacrylamide gel, similar pattern of bands were observed in all the 200 DNA samples under investigation. We could not detect any mutation in the IGFBP-3 (HaeIII) and intron 2 region of BMP4 genes of Osmanabadi goats. All Osmanabadi goats genotyped for IGFBP-3 and BMP-4 genes were found to be wild type monomorphic. It is concluded from the present findings that the growth rate of Osmanabadi goats is independent of IGFBP-3 (Hae III) and BMP-4 intron 2 mutations.

**PO-54**

**Therapeutic efficacy of tolfenamic acid 8% in clinical mastitis of bovines**

M. K. Patil, P.V.Patil and A. P. Somkuwar

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences,  
Udgir Dist:Latur (Maharashtra) 413517 (India)

**ABSTRACT**

The present study was conducted on 10 bovine animals suffering from mastitis. In mastitis cases, Tolfenamic acid 8% was administered intravenously @ 4 mg/kg body weight at the interval of 48 hours in repeated doses or single dose along with suitable antibiotics. The recovery was estimated based on various parameters such as swelling, redness, pain, hotness of udder, milk production in cases of mastitis. The observations were recorded for continuous three days and on 7<sup>th</sup> day of initiation of treatment.

Tolfenamic acid 8% was found to be effective in the treatment of clinical mastitis and subsiding swelling, pain, redness upto 7<sup>th</sup> day of initiation of treatment. In 8 cases 92% milk production was regained after 7<sup>th</sup> day of initiation of treatment without any adverse drug reaction.

**Key words:** Tolfenamic acid 8%, mastitis, milk production

**PO-55 GENOTYPING OF OSMANABADI GOAT AT FecX and FecB LOCI**

Bhave K. G., Sawane M. P., Wagh P. T., Pawar V. D., Umrikar U. D., Majee S. B. and Doiphode A. Y.  
Bombay Veterinary College, Mumbai

**Abstract**

Prolificacy is one of the most important economical traits of goat. It is affected by various genetic and non-genetic parameters in small ruminants. We have carried out genotyping of Osmanabadi goats of either sex (n=160, 152 females and 08 males) at FecX loci (FecX<sup>L</sup>, FecX<sup>H</sup>, FecX<sup>B</sup>, FecX<sup>G</sup> and FecX<sup>L</sup>) of BMP15 gene with T-ARMS-PCR. FecB/ Booroola locus of BMPR1B gene was however genotyped using AvaII PCR-RFLP method. T-ARMS-PCR products of FecX loci (FecX<sup>L</sup>, FecX<sup>H</sup> and FecX<sup>L</sup>) and PCR-RFLP of FecB locus exhibited wild type alleles. FecX<sup>B</sup> and FecX<sup>G</sup> loci could amplify only control fragment, were subjected for direct sequencing of the control fragment. Sequencing revealed wild type alleles at nucleotide position 213(G) of FecX<sup>B</sup> and at position 86 (C) FecX<sup>G</sup> locus. Ava II PCR-RFLP of FecB PCR products revealed a single band of 190 bp representing normal non-carrier animals. It indicated lack of FecX (BMP15) and FecB/ Booroola (BMPR1B) polymorphism in tested Osmanabadi goats. The prolificacy in Osmanabadi goats is therefore found to be independent of these established ovine fecundity gene polymorphisms.

**PO-56**

**BIOMARKERS OF EXPOSURE, EFFECT AND SUSCEPTIBILITY**

D.P.PATIL<sup>1</sup>, S.V. LONDHE<sup>2</sup>, KALAKUMAR<sup>3</sup>, USHARANI<sup>4</sup> and B. ANILKUMAR<sup>5</sup>

<sup>1</sup>PhD Scholar, <sup>3,4,5</sup>Department of Veterinary Pharmacology and Toxicology,  
College of Veterinary Science, Hyderabad (Telangana)

<sup>2</sup>Department of LPT, COVAS, Parbhani (M.S.)

Biomarker is a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample. They allow measuring the internal dose by chemical analysis of the toxic compound or metabolite in body fluids or excreta such as blood, urine and exhaled air. Internal dose may also mean the amount of a chemical stored in one or in several body compartments or in the whole body. The biomarkers of exposure are again classified into two subgroups selective and non-selective, according to their selectivity test, which is based on the direct measurement of the unchanged chemicals or their metabolites in biological media. The selective exposure

tests are used as specific indicators of exposure to a particular chemical. They are the indicators of a particular sensitivity of individuals to the effect of a xenobiotic. They can be genetic markers that include alterations in chromosomal structure such as restriction fragment length polymorphism's (RFLPs), polymorphism of enzyme activities. Changes in the antioxidant systems and modified macromolecules can serve as biomarkers for a variety of xenobiotics which induce oxidative stress.

**Keywords:** Biomarker, Chemical analysis, Xenobiotic, Antioxidant

**PO-57**

### **GENOTYPING OF *TOXOPLASMA GONDII* ASSOCIATED WITH ABORTED SHEEP**

Ajay S Satbige., C.Rajendran., N.A.Patil ., Vivek R. Kasaralika and R.G. Bijurkar

**Department of Veterinary Medicine, Veterinary College, Bidar**

#### **Abstract**

Genotypes of *Toxoplasma gondii* in naturally infected tissues from 30 ovine abortions from Veterinary College Hospital, Bidar all conformed to the type II lineage determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the SAG2 gene. PCR was applied directly to tissue extracts without parasite isolation. These results demonstrate the feasibility of direct analysis of *T. gondii* lineages from samples derived either from clinical cases or asymptomatic infections.

**Keywords** – Genotyping, *T.gondii*, Sheep

**PO-58**

### **Histomorphology of Oviduct in Japanese quail**

Thakur P. N., Waghaye J. Y., Mamde C. S., Karad N. M and S . D Kadam

Department of Veterinary Anatomy and Histology  
College of Veterinary and Animal Sciences, Parbhani

#### **Abstract**

Quail has great future for development of economy of poultry industries. As well as ideal bird for laboratory purpose. The current research was designed to investigate the gross and histological changes of the oviduct of Japanese quail at pre laying and post laying (4-6 week) which was carried out at the department of

Anatomy and Histology College of veterinary and Animal Sciences, Parbhani . The experimental quails were collected from College form having apparently good health and avoid of external deformities. The oviduct was collected immediately after Scarifying bird by cervical sublaxation. For histological study.. Histological sections were prepared using paraffin embedding method and samples were stained with H & E staining for histological observations. The experiment results revealed that the In histological observations, changes of the epithelial lining were found at different stages. Tall coloumnar ciliated and pseudo stratified coloumnar epithelial cells were found in pre-laying stages were as low columnar, cuboidal and pseudo stratified columnar epithelial cells were found in laying and post-laying stages. The mucosal folds of different shape and size were found in diffrent segment at different stages. The lamina propria of magnum contained albumin secreting spherical glands. Undeveloped lamina proprial glands were found in pre-laying stages were as the well developed glands were found in laying stage the post laying stages contained regressed lamina propria with its glands. The membrane forming glands were seen in lamina propria of isthumus. The isthumus had similar changes as of magnum. The lamina propria of uterus contained dark stain pigmented shell glands specially characterized sperm host glands were seen in lamina propria of uterovaginal junction at laying and post laying stages. The tunica muscularis varies in contents and diameter were observed at different stages. The thickness of epithelium, lamina propria and tunica muscularis were measured with ocular micrometer to verify the changes in thickness of infundibulum , magnum , isthumus , uterus, uterovaginal junction and vagina among prelaying , laying and post laying stages. Analysis of variance showed a significance difference in thickness of lamina epitheia, lamina propria and tunica muscularis of different segments of oviduct at different stages except lamina propria of uterus and vagina and muscularis of isthmus which were not statistically significant.

**PO-59 Constraints faced by buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state**

Borade M.T, Deshmukh D.S., Patil A.P. and Jaybhaye N.M.

The present study entitled 'Information needs of buffalo dairy entrepreneurs of peri-urban area in Marathwada region of Maharashtra state' was conducted in peri-urban areas of four districts by selecting 40 respondents purposively from each district. The data was collected with purposively structured interview schedule by individual contact method.

After collection and analysis of data, it was concluded that, in relation to constraints faced by buffalo dairy entrepreneurs, I rank was given to two constraints viz. cost of high yielding buffaloes and costly animal treatment. The II, III, IV and V rank was given to low milk prices, high cost of veterinary

medicine, costly concentrate and buffaloes require more ration respectively. No availability of loan, long dry period in buffaloes, high cost of construction of shed, insufficient knowledge about vaccination and diseases and no veterinary service nearby was given VI, VII, VIII, IX and X rank respectively.

The ranking of XI, XII, XIII, XIV and XV were given to no education facilities for dairy farming locally, no grasslands, shortage of clean water, shortage of green fodder and no timely veterinary services. Inability to detect heat, unavailability of skilled labour and no breeding bull available was given XVI, XVII and XVIII rank respectively.

**PO-60**

**Socio-economic profile of Osmanabadi goat entrepreneurs in  
Marathwada region of Maharashtra state**

Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T

The present study entitled 'Information needs of Osmanabadi goat entrepreneurs in Marathwada region of Maharashtra state' was conducted in Parbhani, Jalna, Latur and Nanded districts of Marathwada region of Maharashtra state. From each district, 40 respondents were selected purposively. Thus a total of 160 respondents were personally interacted with a structured interview schedule.

On the collection and analysis of data, it was concluded that majority of the Osmanabadi goat entrepreneurs were of middle age category, educated up to the college level, had medium land holding, size of family, flock size, annual income, social participation, knowledge and adoption level of goat management practices and market orientation.

The probable reason for medium market orientation could be for getting higher prices and consistency in maintaining supply, for which they might be trying to keep updated market information. The reason for medium level of knowledge might be that, entrepreneurs use to collect essential information for running that enterprise in a profitable way. Medium level of adoption may be because of, adoption of scientific management practices improve efficiency in production and reproduction of goat, utilization of a resources and produce with minimum inputs and maximum output. It also improve cost benefit ratio.

**PO-61**

**Information needs of Osmanabadi goat entrepreneurs in  
Marathwada region of Maharashtra state**

Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T

The present study entitled 'Information Needs of Osmanabadi goat entrepreneur in Marathwada region of Maharashtra state' was conducted in Parbhani, Jalna, Latur and Nanded districts of Marathwada region of Maharashtra state. From each district, 40 respondents were selected purposively. Thus a total of 160 respondents constituted the sample for the study. The research worker personally interviewed all the goat farmers selected for the study. On collection and analysis of data, it was concluded that, the average information need expressed as the most needed in different housing in goat (37.00 per cent), breeding management (45.25 per cent), feeding of goat (38.44 per cent), health management (41.25 per cent), care and management (41.25 per cent) marketing and others (41.25 per cent). In the area of information needs on factors affecting design of shed respondents expressed as most needed (47.50 per cent) whereas expressed as needed in the areas space requirement of goat (41.90 per cent), shed management (40.60 per cent). Respondents expressed information as the most needed in the area of preparation of flock for breeding purpose (51.20 per cent), increasing weight gain (50.60 per cent), genetic improvement (51.20 per cent). Majority of respondents expressed information need as the most needed on management of goats during different physiological stages (35.60 per cent), feeding chaffed fodder (43.10 per cent), silage making (47.50 per cent), urea treatment (58.10 per cent) and homemade concentrate technique (39.40 per cent) and storage of fodder (35.00 per cent).

Majority of the farmers expressed information need as the most needed in the areas of infections, parasitic and other diseases (48.10 per cent), isolation of diseased animals (35.60 per cent). Majority of respondents expressed most needed information in the areas of needed care during pregnancy in goats (49.40 per cent), neonatal care in kids (43.80 per cent), management during different season in goat (33.10), record keeping (41.20), rearing buck for chevon purpose(40.00 per cent). In the area of marketing and others majority of respondents were expressed most needed information of marketing practices followed by goat entrepreneurs, break-even point (61.90 per cent) and break-even analysis (61.30 per cent).

**PO-62**

**Constraints faced by Osmanabadi goat entrepreneurs in  
Marathwada region of Maharashtra state**

Jaybhaye N.M., Deshmukh D.S., Patil A.P. and Borade M.T

The present study entitled 'Information Needs of Osmanabadi goat entrepreneur in Marathwada region of Maharashtra state' was conducted in Parbhani, Jalna, Latur and Nanded districts of Marathwada

region of Maharashtra state. From each district, 40 respondents were selected purposively. Thus, a total of 160 respondents were interacted personally with a structured interview schedule.

On collection and analysis of data, it was concluded that, constrains faced by Osmanabadi goat entrepreneurs were ranked as I, II, III, IV, V, VI, VII, VIII to lack of information about care of pregnant goat, high price concentrate, insufficient working capital and difficulties in heat detection, lack of knowledge about pregnancy diagnosis, lack of training and high mortality rate in kid, lack of information about cutting naval cord, decrease grass land, record keeping, respectively.

Constraints such as non-availability of timely vaccination, lack of information regarding selling of goat, lack of land, water and electricity, lack of information about fodder production, insufficient clean water, were ranked as IX, X, XI and XII respectively; as constraints faced by the goat entrepreneurs.

**PO-63**

**A systematic review and Meta-analysis on prevalence of  
*Methicillin-Resistant Staphylococcus aureus* in Animals**

Hamsapriya S, Rashmi R. Kurli, Krishnamoorthy P Suresh K.P and Parimal Roy

*National Institute of Veterinary Epidemiology & Disease Informatics, Bengaluru-560064*

Antimicrobial resistance is one of the most serious concern world-wide. The *Methicillin-Resistant Staphylococcus aureus* is a Super Bug, pathogenic to both humans and animals, and is one among the various pathogens causing Mastitis as LA-MRSA in India. It is present in wide range of animals, as a pathogen and as healthy carriers. The Prevalence of this super bug in animals and its products, and the spread of the same is an alarming issue to be studied. The present study aims at employing Meta-analytical tools to analyze and determine MRSA-prevalence in data extracted from different studies. The information on incidence of the MRSA strains, were retrieved from various Indian journals, classified based on the type/source of samples (milk samples/ others) and meta-analytical tools such as fixed effect models and random effect models were utilized to synthesize the results.

The electronic database of search yielded 22 articles from 2014-17 on MRSA prevalence / proportions with 2921 animals. The results viz. number of samples tested and number of positives were extracted. The results were subjected to meta-analysis, with 2921 samples provided the heterogeneity coefficient ( $\tau^2$ ) as significant and hence random effect model was considered. The prevalence of MRSA was obtained as 24.0% (95%: 14.0-37%),  $\tau^2=2.011$ ;  $P<0.001$ . Further samples were stratified on the source of samples (milk samples/others) and meta-analysis of random effect model yielded 26% (95%: 16.0-39%),  $\tau^2=0.8468$ ;  $P<0.001$  for milk samples and 21% (95%: 7.0-49%),  $\tau^2=3.69$ ;  $P<0.001$  for

other samples. Use of meta-analysis to aggregate individual studies enabled in determination of robust values of prevalence of MRSA in India and this information is useful for researchers, veterinarians and policy makers in developing appropriate intervention strategies.

**PO-64**

**Phenotypic Characterization and 16S rRNA sequence Analysis**  
of Equine isolates of *Streptococcus pluranimalium*

R. Javed, A. K. Taku, M.A.Bhat , R. K. Sharma and G.A.Badroo

Department of Microbiology, Faculty of Veterinary Sciences & Animal Husbandry, R.S. Pura, Jammu, Jammu and Kashmir, India. Email: [rabiajavedkhan@gmail.com](mailto:rabiajavedkhan@gmail.com).

*Streptococcus pluranimalium* has been associated with meningoventriculitis in calf, probably as a sequel to septicaemia, and endocarditis. *Streptococcus pluranimalium* isolates have been recovered from several species of domestic animals, including cattle (genital tract, tonsils, subclinical mastitis), canaries (crop, respiratory tract), a goat (tonsil) and a cat(tonsil).In the present study, the Hi Strep Identification kit (Himedia , India) and 16S rRNA sequence analysis were used to identify *S. pluranimalium* among the emerging isolates. Ninety-six Nasal swab samples were collected from Katra region of J & K, India and further were analyzed at Division of Microbiology SKAUST-J. These were inoculated on 5 % Sheep Blood Agar plates (Himedia, India). After 24 hours incubation, a-haemolytic colonies with a diameter of approximately 1 mm were subcultured on Columbia Nalidixic Acid Agar (Himedia, India) and Edward's agar (himedia, India). Isolates that were á-haemolytic, Gram positive and catalase negative were selected. Single colonies were harvested for biochemical characterisation using the HiStrep identification kit (Himedia, India). Four isolates of *Streptococcus pluranimalium* were identified. The isolates were confirmed as *S.pluranimalium* by 16S rRNA sequence analysis. A 1,481 base pair 16S rRNA product from each isolate was sequenced following amplification by PCR using oligonucleotide primers. BLAST1 analysis against the Gen Bank database identified a closest match to *S. pluranimalium* (>99% sequence identity). The nucleotide sequence was submitted in ncbi genbank with (Accession No. for nucleotide sequence: SUB 2029862 Seq 1 KY020401).

**Key words:** *16S rRNA, S.pluranimalium, Polymerase Chain Reaction*

**PO-65**

**GENOTYPING OF *TOXOPLASMA GONDII* ASSOCIATED WITH ABORTED SHEEP**

Ajay S Satbige., C.Rajendran., N.A.Patil ., Vivek R. Kasaralika and R.G. Bijurkar

Department of Veterinary Medicine, Veterinary College, Bidar

**Abstract**

Genotypes of *Toxoplasma gondii* in naturally infected tissues from 30 ovine abortions from Veterinary College Hospital, Bidar all conformed to the type II lineage determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the SAG2 gene. PCR was applied directly to tissue extracts without parasite isolation. These results demonstrate the feasibility of direct analysis of *T. gondii* lineages from samples derived either from clinical cases or asymptomatic infections.

**Keywords** – Genotyping, *T.gondii*, Sheep

**PO-66**

**BIOMARKERS OF EXPOSURE, EFFECT AND SUSCEPTIBILITY**

D.P.Patil<sup>1</sup>, S.V. Londhe<sup>2</sup>, Kalakumar<sup>3</sup>, Usharani<sup>4</sup> and B. Anilkumar<sup>5</sup>

<sup>1</sup>PhD Scholar, <sup>3,4,5</sup>Department of Veterinary Pharmacology and Toxicology,

College of Veterinary Science, Hyderabad (Telangana)

<sup>2</sup>Department of LPT, COVAS, Parbhani (M.S.)

**Abstract**

Biomarker is a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample. They allow measuring the internal dose by chemical analysis of the toxic compound or metabolite in body fluids or excreta such as blood, urine and exhaled air. Internal dose may also mean the amount of a chemical stored in one or in several body compartments or in the whole body. The biomarkers of exposure are again classified into two subgroups selective and non-selective, according to their selectivity test, which is based on the direct measurement of the unchanged chemicals or their metabolites in biological media. The selective exposure tests are used as specific indicators of exposure to a particular chemical. They are the indicators of a particular sensitivity of individuals to the effect of a xenobiotic. They can be genetic markers that include alterations in chromosomal structure such as restriction fragment length polymorphism's (RFLPs), polymorphism of enzyme activities. Changes in the antioxidant systems and modified macromolecules can serve as biomarkers for a variety of xenobiotics which induce oxidative stress.

**Keywords:** Biomarker, Chemical analysis, Xenobiotic, Antioxidant

**PO-67**

### **Genotyping of Osmanabadi Goat at FecX and FecB LOCI**

Bhave K. G., Sawane M. P., Wagh P. T., Pawar V. D., Umrikar U. D., Majee S. B. and Doiphode A. Y.

Bombay Veterinary College, Mumbai

#### **Abstract**

Prolificacy is one of the most important economical traits of goat. It is affected by various genetic and non-genetic parameters in small ruminants. We have carried out genotyping of Osmanabadi goats of either sex (n=160, 152 females and 08 males) at FecX loci (FecX<sup>L</sup>, FecX<sup>H</sup>, FecX<sup>B</sup>, FecX<sup>G</sup> and FecX<sup>L</sup>) of BMP15 gene with T-ARMS-PCR. FecB/ Booroola locus of BMPR1B gene was however genotyped using AvaII PCR-RFLP method. T-ARMS-PCR products of FecX loci (FecX<sup>L</sup>, FecX<sup>H</sup> and FecX<sup>L</sup>) and PCR-RFLP of FecB locus exhibited wild type alleles. FecX<sup>B</sup> and FecX<sup>G</sup> loci could amplify only control fragment, were subjected for direct sequencing of the control fragment. Sequencing revealed wild type alleles at nucleotide position 213(G) of FecX<sup>B</sup> and at position 86 (C) FecX<sup>G</sup> locus. Ava II PCR-RFLP of FecB PCR products revealed a single band of 190 bp representing normal non-carrier animals. It indicated lack of FecX (BMP15) and FecB/ Booroola (BMPR1B) polymorphism in tested Osmanabadi goats. The prolificacy in Osmanabadi goats is therefore found to be independent of these established ovine fecundity gene polymorphisms.

**PO-68**

### **ISOLATION AND CULTURE OF RABBIT EMBRYONIC STEM CELLS**

B.K. Bhadane\*, S.K.Maiti, A.K.Sharma, Naveen Kumar, Amarpal, Sadan Bag and G. K. Das

Division of Vet. Surgery & Radiology, IVRI, Izatnagar, Bareilly (UP) 243 122

\* Presently working as Assistant Professor,

Department of Surgery & Radiology, COVAS, Parbhani (MS)

#### **ABSTRACT**

Stem cells have therapeutic potential in the realm of orthopaedic surgery because of their capacity to self renew and differentiate into various types of mature cells including bone. Embryonic stem cells (ESC) are pluripotent cells derived from inner cell mass of pre-implantation embryos and represent embryonic pre-cursors that give rise to any cell type in the embryo, specifically differentiated into osteogenic cells under selective culture conditions. The present studies was conducted for isolation, derivation and characterization of embryonic stem cells in New Zealand white rabbits (06 groups) and investigate the potentiality of embryonic stem cells for osteogenesis in the repair of bone defects. Isolation of rabbit ESC was done with superovulation by injecting 50-75 IU PMSG and flushing the uterine horns and oviducts of rabbits. Flushing fluid was collected in petri dish and investigated under 60x magnification to clear the

debris attached to the embryos. Derivation of ESC was carried out by seeding the ESC on buffalo fetal fibroblast (BFF) layers. Preparation of fibroblast monolayer was done by aseptically collecting 02 cm fetus, washed thoroughly in sterile normal saline solution and swabbed with ethyl alcohol. Skin samples were collected and washed with Dulbecco's phosphate buffer saline (DPBS) and disrupted to separate fibroblast cells after centrifugation. The collected cells were placed on CR11 with Fetal bovine serum (FBS) and the mitotic growth was blocked by adding mitomycin-C. The blastocyst were attached on feeder layers following hatching of inner cell mass was allowed to grow for 15-20 days. The ICM derived outgrowths were disrupted mechanically into smaller clumps and seeded on fresh BFF layers. The colonies were further allowed to grow and expand. ESC colonies were passaged after 6-8 days and cultured on fresh feeder. The attachment and growth of ESC on daily basis was observed and medium was replaced after every 72 hr. After ESC population was established, characterization was confirmed by alkaline phosphatase staining and immunocytochemistry Oct-4. The procedure and protocol used for derivation and propagation of ESC from *in-vivo* developed blastocyst was found to be successful in the study.

**PO-69**

**Detection and characterization of extended-spectrum beta-lactamase genes ( $bla_{TEM}$  and  $bla_{SHV}$ ) conferred multidrug resistance to coliform bacteria isolated from faeces of dairy cattle in Chhattisgarh, India**

Nidhi Rawat<sup>1\*</sup>, S.D. Hirpurkar<sup>1</sup>, Fateh Singh<sup>2</sup> and Chandrasahas Sannat<sup>1</sup>

<sup>1</sup>Department of Veterinary Microbiology

College of Veterinary Sciences and Animal Husbandry, Anjora, Durg, Chhattisgarh, India

<sup>2</sup>ICAR-National Institute of High Security Animal Diseases, Bhopal, Madhya Pradesh, India

\*Correspondence: e-mail: dr\_nidhirawat@yahoo.com

**Abstract**

The aim of this study was to isolate antimicrobial resistant enterobacteria from cattle faeces and to identify the extended-spectrum beta-lactamases (ESBLs) and carbapenemases genes among them. A total of 70 cattle faecal samples were collected from Durg, Chhattisgarh, and cultured in nutrient broth. Bacterial isolation recovered 120 Gram-negative bacteria. Antimicrobial susceptibility testing showed 90 (75%) coliform isolates (66 *Escherichia coli*, 20 *Klebsiella* sp., two each of *Enterobacter* sp., *Citrobacter* sp.) resistant to either of amoxicillin, amoxicillin-clavulanate, cefixime, ceftazidime, imipenem and meropenem. Bacterial isolates showed highest resistance rate to ceftazidime (61.6%) followed by cefixime, amoxicillin-clavulanate (60%). ESBL phenotype, assessed by double disc diffusion and double disc synergy tests, was shown by 50 (41.6%) isolates. PCR amplification revealed 20% and 31.6% positivity of  $bla_{TEM}$  and

*bla*<sub>SHV</sub> genes, respectively. The *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> co-expression was recorded among 08 (12.1%) *E. coli* and two (10%) *Klebsiella* sp. isolates. Restriction endonuclease analysis indicated clonal *bla*<sub>TEM</sub> and clonal and non-clonal *bla*<sub>SHV</sub> genes. Nucleotide sequencing conferred the *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genotype. None of the isolate showed carbapenemase gene. Phenotypic and genetic detection and characterization of ESBLs among *Enterobacteriaceae* bacteria visualizes the importance for planning and execution of effective control programs on emerging antimicrobial resistant pathogens.

**PO-70**

**Detection and characterization of plasmid mediated extended-spectrum beta-lactamases and carbapenemases genes, including *bla*<sub>NDM-1</sub> among Gram-negative bacteria isolated from drainage water, poultry farm environment and human urine**

Fateh Singh <sup>a\*</sup>, Sadanand D. Hirpurkar <sup>a</sup>, Nidhi Rawat <sup>a</sup>, Sanjay Shakya <sup>b</sup>,  
Satish Kumar <sup>c</sup>, and Rajiv Kumar <sup>c</sup>

<sup>a</sup> Department of Veterinary Microbiology,

<sup>b</sup> Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh. <sup>c</sup> Animal Biotechnology Section, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Tonk, Rajasthan. \*Correspondence: ICAR-National Institute of High Security Animal Diseases, Bhopal (e-mail: fateh.ars07@gmail.com).

**Abstract**

Emerging trend of antimicrobial resistance among Gram-negative bacteria is one of the foremost problems that need utmost attention. Consistent antimicrobial exposure develops selective pressure on bacteria and induces their dynamic strategic evolution against the used antimicrobials resulting in survival and spread of resistant mutants. The objective of this study was to detect and characterize the extended-spectrum beta-lactamases (ESBLs) and carbapenemases genes conferred beta-lactam resistance among Gram-negative bacteria recovered from water, poultry farm surroundings, and human urine samples. A total of 150 non-duplicate Gram-negative bacterial isolates, resistant to either of amoxicillin-clavulanate, ceftazidime, cefixime, meropenem, and imipenem, were screened for plasmid profiling and presence of ESBLs, carbapenemase and integron 1 (*Int1*) genes. Conjugation assay was performed to know the conjugative nature of plasmids. The ESBLs and carbapenemase genes were characterised by restriction endonuclease analysis and nucleotide sequencing. Bacterial isolates showed variability in number (1 to 6) and size of plasmids (2 kb to more than 21 kb). Most of the isolates harboured plasmids of 2 kb to 20 kb. A prevalence of 34%, 12.6%, 4%, and 7.3% was recorded for *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>KPC</sub>, and *bla*<sub>NDM</sub>.

respectively. Moreover, 49 (41.1%) of coliform isolates carried either of ESBL and/ or carbapenemase gene. *Escherichia coli* (1), *Citrobacter* sp. (2), *Shigella* sp. (4), *Serratia* sp. (2), *Proteus* sp. (2) and *Acinetobacter* sp. (2) isolates co-expressed both the ESBL and carbapenemase genes. These beta-lactamase genes were located on conjugative plasmids and their transfer was mediated by integron 1. Restriction analysis and nucleotide sequencing indicated clonal similarity among  $bla_{TEM}$ ,  $bla_{KPC}$  and  $bla_{NDM}$  unlike to clonally diverse  $bla_{SHV}$ . Detection and characterization of ESBLs and carbapenemase genes substantiated the antimicrobial resistance acquisition by bacteria which reflected the prompt need of antimicrobial stewardship programme to secure the public health.

**SS**

**Satellite Seminar**

SS-01

## Current trends in diagnostic and clinico-therapeutic aspects of snake bite in animals

A.U. Bhikane and R.K. Jadhav

Department of Veterinary Clinical Medicine, Ethics & Jurisprudence

College of Veterinary and Animal Sciences, Udgir

### Introduction

Since the time immemorial snakes are known to be deadliest foes of both man and animals by virtue of being highly venomous (Murthy, 1981, Singh, 2002). They often bite whenever they crawled over by other living being either accidentally or inadvertently. Snake bites most commonly occurs in farm animals during grazing. According to an estimate around 60,000 cattle and 20,000 human beings succumb to poisonous snake bite every year in India (Do-Amaral, 1951; Bhaskaran, 1982). Cobra (*Naja naja*), Russell's viper (*Viperarusselli*), Saw scaled viper (*Echiscarinatus*) and Krait (*Bungaruscoerulens*) are the important poisonous snakes reported in India. By virtue of the composition of toxins Cobra have neurotoxic, nephrotoxic and haemolytic fractions in venom. Viperine snake venom is composed of haemotoxic, coagulin, cytolyisin and more of necrotizing fractions (Sharma, 1970) while kraits have both neurotoxic and haemotoxic fractions.

Severity of snake bite depends on both snake and animal factors. Species of snake decides the severity with cobra bite being most severe in animals. Size of snake have linear correlation with the severity of toxicity. If bite by snake is first one, ultimately quantity of poison released will be more thereby causing severe toxicity. Animal factors like species, size, age and location of bite also affect the severity of snake envenomation. Large animal species tend to be resistant because of their large size and so large dose of venom is required to cause severe toxicity and death. Small sized animals like dogs are more prone to snake bite. Severity of snake envenomation is more in young animals as compared to adults (Radostitis *et al.*, 2010). Bites on the face, muzzle and neck are more dangerous as they lead to dyspnea due to excessive swelling on facial area.

Snake bites are common in grazing cattle during monsoon and post-monsoon season (July-November) in the Marathwada region. Adult male cattle (85.51%) are more vulnerable to snakebite (Bhikane *et al.*, 2016).

Early diagnosis and prompt initiation of accurate treatment are very essential in the success of managing the cases of snake bite in animals.

## Diagnosis

Diagnosis of snake bite in farm animals could be made on the basis of

**a) History :** Sometimes snake bites may be witnessed by animals while grazing hours or sudden onset of restlessness, shaking of head or leg and jumping, bellowing, animal becoming frightened and runs away are indicative of snake bite.

**b) Epidemiological factors:** Information related to the habitat preference of different species of snake could be helpful in diagnosing the species of snake responsible for envenomation. Russel's vipers are found in century plants and other cacti. Cobras are more prevalent in rice fields. Kraits like brick piles while saw scalded vipers are associated with red laterite and sandy areas. Also information regarding climate, season, topography of grazing land etc. assists in diagnosis of snake bite. Hot and humid climate, grazing in dense grass or shrub fields especially during monsoon or postmonsoon season or from July to November months (Hudelson and Hudelson, 1995; Bhikane *et al.*, 2016).

**c) Clinical Signs:** The more prevalent type of snake envenomation reported in Marathwada region is of haemotoxic type in animals. Fang marks are either not evident or clearly evident at the site of bite in cattle. Snake bites are more common on lower legs (84.05%). Ascending swelling on limbs or asymmetrical swelling on face is characteristic sign of snake bite (Bhikane *et al.*, 2016). Ascending swelling on bitten limb or uneven swelling on face if bitten on muzzle or part of face are typical clinical signs consistently observed in cases of viper bite in cattle and buffaloes. Facial swelling is characterized by secondary respiratory distress or dyspnoea and difficulty in prehension and mastication. Clinical signs like local swelling with oozing of blood, lameness if bite on limbs due to severe ascending type of swelling. If thrombocytopenia is severe in ailing animal, bleeding tendencies will be increased with melena, epistaxis, and bleeding from site of bite (Bhikane *et al.*, 2016).

Experimental studies on Cobra envenomation in buffalo calves showed clinical signs like oedematous swelling, ecchymosis and extravasation at the site of bite. Systemic signs like respiratory embarrassment, salivation, dyspnoea, regurgitation of ruminal ingesta resulting in aspiration pneumonia due to paralysis of tongue, oesophagus and larynx were observed in calves (Singh, 2002).

**d) Haematology :** Haematological analysis of blood samples from animals with snake envenomation shows significant and consistent thrombocytopenia and neutrophilia along with increased capillary blood clotting time compared to healthy animals. Other parameters of blood count reveals no significant changes compared to healthy animals. Capillary blood clotting time and platelet count helps in diagnosis, assessing the severity and monitoring the treatment of patient. Under field conditions, capillary blood clotting time

technique could serve as a diagnostic and prognostic indicator for haemotoxic envenomation (Bhikane *et al.*, 2016).

**e. Biochemistry:** Biochemical analysis of blood samples from animals suffering from snake envenomation shows significantly increased blood urea nitrogen and serum creatinine values compared to those in healthy counterparts.

**f. ELISA:** Identification of venom in blood, urine or other body fluids is highly accurate, immediate and suitable for field or laboratory diagnosis. Use of ELISA is limited by high cost on diagnostic and limited diagnosis to the snake species for which reagents are available (Radostits *et al.*, 2010).

**g. Post mortem findings:** Local oedematous swelling at the site of bite, fang marks mostly visible undersurface of reflected skin, extensive haemorrhages at the site of bite, congestion of visceral organs, petechial haemorrhages on trachea, epicardium and coronary fat are most consistent post mortem findings observed in animals died of snake envenomation (Radostits *et al.*, 2010).

## Treatment

Since ancient days first aid methods of application of tourniquet (remove for 30 seconds after every 20 minutes), washing of wound/ fang mark with 5% potassium permanganate solution, incision at site of bite etc. are being practiced in human medicine. But these techniques are not always useful in animals as snake bite is confirmed only after laboratory investigations along with clinical assessments like ascending swellings on limb or asymmetrical swelling of face and evidence of bleeding in some of the cases. Prompt institution of therapy through early diagnosis of snake bite increases the chances of success of treatment in animals.

Use of polyvalent anti-snake venom serum (PASVS) within first 24 hours along with supportive therapy of fluids, antibiotics, steroids (non-steroidal anti-inflammatory drugs in pregnant animals) and styptics proves a successful treatment protocol for management of haemotoxic snake bite. A study conducted at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Udgirto establish effective therapeutic protocol for haemotoxic snake envenomation. Treatment of haemotoxic snake envenomation in bovine with 20-40 ml of PASVS in first 24 hours, amoxicillin-cloxacillin @ 10 mg/kg iv/im once daily for 5-9 days, dextrose 5% @ 4-6 lit. iv daily for 5-6 days, Furosemide @ 1 mg/kg iv/im and Carbazochrome salicylate @ 10 ml im daily for 3-5 days Dexamethasone (only in non-pregnant animals) in tapering doses (80 mg on day 1, 40 mg on day 2-3 while 20 mg on day 4-5 iv) followed by Meloxicam @ 0.5 mg/kg iv/im and iron with B-complex depending on the severity of anemia proved effective in the management of snake evenomation. Some cases with severe thrombocytopenia leading to development of

bleeding tendencies (melena, haematochezia, haematuria) were improved on immediate institution of compatible blood transfusion of around 1- 1.5 liter quantity along with above mentioned protocol. Similarly cases with facial swelling having severe respiratory distress showed improved prognosis on tracheotomy to establish respiration. Diuretics therapy is useful for effective reduction of swelling from the site of bite especially on limbs and face thereby alleviating secondary signs like lameness and dyspnea.

Additional treatment with Neostigmine is useful in management of cobra bite as it helps in stabilization of acetyl choline thereby reducing respiratory distress as well as 50% reduction in dose of PASVS (Singh and Meshram, 1996; Singh, 2002).

### **References**

- Bhaskaran, G. (1982). Man and the Snakes: A news report from Times of India, 9<sup>th</sup> May, 1982.
- Bhikane, A.U., Masare, P.S., Ghoke, S.S., Syed, A.M. and Awaz, K.B. (2016). Epidemiology, Diagnosis and Therapeutic Management of Snake bite in animals. Joint Animal Science Research Council Meeting, MAFSU, Nagpur held at Nagpur.
- Do-Amaral, A. (1951). Snake-venomation. In: Clinical Tropical Medicine, Gradwahol (edr), C.V. Moslyoycost, Louis.
- Hudelson, S. and Hudelson, P. (1995). Pathophysiology of snake envenomization and evaluation of treatments. Part – I. The Compendium Collection. Snake Envenomation, **17 (7)**: 168-174.
- Murthy, T.S.N. (1981). “Snakes Alive”, a news report from Times of India, 4<sup>th</sup> January, 1981.
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2010). Diseases Associated with zootoxins- Snakebite. In: Veterinary Medicine- A textbook of the diseases of cattle, sheep, goats, pigs and horses. Saunders Elsevier, 10<sup>th</sup>Edn. Pp. 1916-1918.
- Sharma, S.N. (1970). Veterinary Jurisprudence. The Kothari Book Depot. Bombay, pp. 90-92.
- Singh, B. and Mehsram, M.D. (1996). Therapeutic efficacy of antivenin and neostigmine against experimental cobra envenomation in buffalo (*Bubalus bubalis*). Ind. Vet. J., **71**: 479-488.
- Singh, B. (2002). Clinico-pathology, diagnosis and treatment of snake- bite in animals. Intas Polivet, **3(1)**: 1-9.

**SS-02**

## **Role of advances in biotechnology for animal breeding**

Dr. M. S. Vaidya

Associate Professor,

Department of animal Genetics and breeding, COVAS PARBHANI

Since Independence the population of has more than doubled and is projected to rise further in the next few decades. Our role as food receiving country has changed to food exporting country in many agricultural commodities. The livestock population has not doubled yet. The production from these livestock have two major challenges: One of productivity and the other of conservation of indigenous genetic resources. This along with uncertainty in monsoon and drought has exacerbated the problem of food production in an already difficult environment of fragile ecosystems. About 70 percent of the population of is rural and depends almost exclusively on agriculture for its consumption and income needs. Measures to address the problems of poverty and food insecurity in the states must therefore be found mainly within the agricultural sector. Role of livestock is more than an important asset a good means of food and nutritional security and income generation. Livestock development is therefore a necessity and not an option and it should be achieved in such a way that it is market-oriented and technologically driven so as to double the farmers' income.

### **Advances in animal breeding and biotechnology**

Smallholder-livestock systems which support the large majority of the poor are much more dependent on the locally available knowledge and production techniques. Therefore, to address the emerging challenges posed by the rapidly growing human population and urbanization there is a need for the adoption of techniques and develop skills to adopt the technology. This will enable smallholder systems to respond to the changing social, economic and environmental challenges. Recent advances in animal breeding, molecular biology, reproductive technologies and information and communication technologies, present unprecedented opportunities for livestock improvement in the developing countries.

### **Reproductive biotechnologies**

Artificial insemination (AI), embryo transfer (ET) and semen sexing are some examples of reproductive biotechnologies. AI is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female. AI is the first reproductive technique that had a major impact on animal breeding schemes worldwide. In combination with pedigree registration and milk recording, On this aspect we are far behind the among the developing countries also. AI offers the opportunity to obtain accurate estimates of breeding values of young bulls and results in a genetic progress that is

much higher than natural mating. This is due to the high selection intensity and accuracy arising from AI since only the top bulls are selected for use in producing numerous offspring in many herds.

The main advantages of AI include increased efficiency of bull usage. This means the use of AI enables the production of a very large number of offspring from a single elite sire. Hence, it makes the maximum use of superior sires possible. For instance, natural service would probably limit the use of one bull to less than 100 matings per year. AI usage enabled one dairy sire to provide semen for more than 60,000 services. Moreover, AI reduces the danger of spreading infectious genital diseases. Time required to establish a reliable proof on young bulls is reduced through the use of AI. Other advantages include early detection of infertile bulls, use of old or crippled bulls and elimination of the dangers of handling unruly bulls.

There are also a few disadvantages of AI, which can be overcome through proper management. A human detection of heat is required and thus the success or failure of AI depends on how well this task is performed. AI requires more labor, facilities and managerial skills than natural service. Proper implementation of AI requires special training, skill and practice. Utilization of few sires, as occurs with AI, can reduce the genetic base. Thus the AI industry and animal breeders should make every effort to sample as many young sires as possible.

Artificial insemination is recognized as the best biotechnological technique for increasing reproductive capacity and it has received widespread application in large farm animals. It is widely used in India and the demand is never ending. However, owing to a number of technical, financial, infrastructural and managerial problems its applicability in different states has not yet matched that of its success in the Livestock rich states of India.

Embryo transfer is a hormonal manipulation of the reproductive cycle of the cow, inducing multiple ovulations, coupled with AI, embryo collection, and embryo transfer to obtain multiple offspring from genetically superior females, by transferring their embryos into recipients of lesser genetic merit. The high genetic merit embryos can be frozen for later transfer or sale. Most dairy farmers who use embryo transfer simply want more heifer calves from their best cows. In most cases the bull calves are more a nuisance to merchandise than an asset. The effect of this use of embryo transfer is to increase the selection intensity of dams to produce female herd replacements.

These technologies have been commercially available since the 1980s. In ET, an increase in reproductive rate of females offers the opportunity to reduce the number of dams that need to be selected for the next generation. At the same time, it leads to an increase in the amount of information available on sibs for estimating the breeding values (BV) of male as well as female selection candidates.

Embryo transfer also allows superior females to have an effect on the genetic change. However, this technology has been only beneficial to cattle where the low reproductive rates and the long generation intervals make it economically viable. So far, ET has had some experimental and limited practical applications in most developing countries. Limitations in utilization of AI and ET in India are attributable to the absence of organized breeding schemes, poor infrastructure, and a lack of human and institutional capacity.

The use of sexed semen alters the sex ratio in favor of either sex. It is a great advantage for the dairy industry for producing replacement heifers. The availability of sexed semen in dairy cattle has been eagerly anticipated for many years, and recent developments in fluorescence-activated cell sorting have brought this technology to commercial application. For a long time, the large-scale application has been hindered by slow process of semen sorting and the lower conception rates.

Semen sexing provides the potential to increase the numbers of offspring of one sex in a closed population, thereby increasing the intensity of selection for that sex. A number of studies have shown that the effect of semen sexing on the rate of genetic gain is limited. Semen sexing, however, enhances the farmers' ability to obtain a larger number of replacement heifers from their own herds. This enables farmers to expand their herd size without the need for buying replacement heifers from other farmers.

Other advancements in reproductive biotechnologies include biotechniques like cloning, gene transfer, cryo-preservation of embryos, in vitro maturation, fertilization and culture which may have very limited application in the developing countries due to the high cost and advanced infrastructural requirements for their implementation.

### **Breeding Schemes/strategies**

Sustainable livestock genetic improvement strategies that meet the needs of farmers and take the prevailing production system into consideration can make a vital contribution to food security and rural development. This requires the implementation of efficient, sustainable breeding schemes. In most of the developing countries the lack of such schemes is one of the hindrances to the contribution of the livestock sector to food production and income generation.

Developing such a scheme for tropical environments is a challenging task constrained by small flock-size, communally shared grazing, uncontrolled mating, and the absence of pedigree and performance recording. To address these issues the advances in this area include nucleus/group breeding scheme and community-based breeding system.

Nucleus/group breeding scheme is based on the principle that in each herd there is a small number of genetically very superior animals which “if brought together” will form a nucleus whose average genetic merit is far greater than that in any of the contributing herds. The important element in this scheme is therefore for a group of farmers to agree to pool their high performing animals.

Once the nucleus herd is assembled, an efficient system of recording and selection is implemented. The best males are kept for breeding in the nucleus while the other selected males are given to the base herds for breeding. By these means the improvements are quickly spread throughout the group.

The nucleus may remain open to animals from the base herds, the best females from the latter being admitted periodically and compared with those in the nucleus. Usually, only females are transferred from the base to the nucleus since sire selection will not be practicable in base herds due to managerial reasons. The main advantage of the nucleus scheme is that the genetic superiority of sire replacements coming into the base herds from the nucleus is far greater than what is achievable in each of the base herds. It is particularly attractive in situations where within-herd selection programs are ineffective due to small population size or inadequate technical skill.

The nucleus breeding scheme shifts the responsibility of operating the breeding program from the farmer to the nucleus herd. It is therefore an attractive method for the smaller communities because of the limitations discussed earlier. However, the organization of the scheme may have to be under government control because cooperative ventures among farmers may not always be practicable. As a result, implementation of nucleus breeding schemes in low-input environments has sometimes proven to be somewhat difficult. The alternatives to centrally organized nucleus schemes are community or village-based selection schemes, which are breeding activities carried out by the communities of smallholder farmers.

Establishing a breed society for conservation and improvement of a local breed that involves local communities and institutions in the design implementation and ownership of breeding strategies. Its main objective is to improve the productivity of local breeds and thereby improve the income of participating farmers by ensuring access to improved animals that respond to improved feeding and management. Developing and implementing a community-based breeding program involves a series of interconnected activities and includes a description of the production system, definition of breeding goals, evaluating market access and policies, development and implementation of a locally adapted breeding strategy.

## **Gene-based techniques**

Gene-based techniques as applied to animal breeding and improvement include several marker-based technologies such as marker-assisted selection (MAS), gene-assisted selection (GAS), marker-assisted introgression (MAI) and genomic selection (GS).

Marker-assisted selection involves selection on markers either in linkage disequilibrium (LD) or linkage equilibrium with the quantitative trait loci (QTL) while GAS involves selection on direct markers which are the causative mutation(s). On the other hand, MAI involves the use of markers to aid introgression of QTL from a donor to a recipient line. Genomic selection is defined as the simultaneous selection for many (tens or hundreds of thousands of) markers, which cover the entire genome in a dense manner so that all genes are expected to be in linkage disequilibrium (LD) with at least some of the markers. The meta-analysis indicated a number of consensus regions, the most striking being two distinct regions affecting milk yield on chromosome 6 at 49 cM and 87 cM explaining 4.2 and 3.6 percent of the genetic variance of milk yield, respectively. The first of these regions (near marker BM143) affects five separate milk production traits (protein yield, protein percent, fat yield, fat percent, as well as milk yield)

The implementation of GS technology involves genotyping selection candidates to predict breeding values, which can be performed in the absence of phenotypic records. With the availability of high-density marker-maps and cost-effective genotyping, GS methods may provide faster genetic gain than can be achieved by traditional selection methods. In the developed countries, genomic selection is expected to double the annual rate of genetic improvement in dairy cattle and it has been implemented recently in a number of dairy cattle breeding programs.

For the past 20 years, gene-based technologies have been applied to gene detection, genetic selection and assessment of genetic diversity and genetic transformation of livestock. Most of these developments and applications have taken place in the developed countries but are not free from criticism due to prohibitive costs and environmental clearances.

The use of such techniques in most developing countries is thus far limited. Moreover, in view of the existing problems hindering livestock productivity in the region, an immediate and wide scale implementation of these techniques may not be straightforward. However, until such time, due consideration must be given to capacity, infrastructural and institutional building to adapt these technologies to meet the specific needs of developing countries in the future.

## **Communication technologies**

With the advent of mobiles and social media the assets and tracking and identification devices the sharing of information collection of data keeping of record can be enhanced to wider scale and the availability solutions can be ascertained without loss of time and opportunity, which is important for an animal breeder. Predictions about next date of heat, availability of semen doses, general body condition of the animal, Id features, Remote location/online record keeping can be made hassle free with higher degree of accuracy and transparency and reliability.

## **Bibliography**

- FAO (2006). Livestock's long shadow: Environmental issues and options. Rome: Rome.
- Mehar S. Khatkar; Peter C. Thompson; Imke Tammen and Herman W. Raadsma (2004). Quantitative trait loci mapping in dairy cattle: review and meta-analysis *Genet. Sel. Evol.* 36 (2004) 163–190
- Gizaw, Solomon & Hans Komen & Johan A.M. van Arendonk (2010). Participatory definition of breeding objectives and selection indexes for sheep breeding in traditional systems. *Livestock Science*, 67"74. <http://www.fao.org/docrep/003/x8406e/X8406e01.htm>
- Rege, J.E.O. & K. Marshall & A. Notenbaert & J. Ojango & A.O. Mwai (2011). Pro-poor animal improvement and breeding “ what can science do? *Livestock Science* 136: 15”28.
- Robinson, Tim (2004). IGAD project summary. IGAD's Livestock Policy Initiative. Rome: FAO.
- Van Arendonk J. (2011). The role of reproductive technologies in breeding schemes for livestock populations in developing countries. *Livestock Science* 136: 29–37.

**SS-03**

### **Surgical strike to combat endometritis through different therapies with special reference to immunomodulation in dairy animals**

Dr. M. N. Rangnekar<sup>1</sup> and Dr. N. M. Markandeya<sup>2</sup>

Department of Animal Reproduction, Gynaecology & Obstetrics  
<sup>1</sup>KNP College of Veterinary Science, Shirval; <sup>2</sup>COVAS, Parbhani  
Maharashtra Animal & Fishery Sciences University, Maharashtra

Post partum period is a vital period in the life of dairy animals and management of different uterine diseases at an early period is required for success in the dairy industry. Amongst the various post partum complications, endometritis is most commonly seen in high producing cows and buffaloes in organized farms as well as under field conditions. Endometritis is an inflammation of the endometrial lining of the

uterus (extends no deeper than stratum spongiosum) without systemic signs. It is characterized by disruption of surface epithelium, infiltration with inflammatory cells, vascular congestion, stromal oedema and accumulation of lymphocytes and plasma cells in the superficial layer (Lewis, 1997). Endometritis is a multifactorial disease caused by nonspecific bacteria; therefore, identification of risk factors associated with endometritis is more important than treatment. Endometritis can be classified as clinical and subclinical in bovines. Clinical endometritis is characterized by the presence of purulent uterine discharge detectable in the vagina 21 days or more post partum (Potter *et al*, 2010). Subclinical endometritis is an inflammation of the internal lining of uterus in the absence of external clinical signs. Clinical endometritis can develop rapidly or slowly but subclinical endometritis develops slowly. In absence of chronic clinical endometritis, subclinical endometritis is defined by the presence of more than 18 % PMN in uterine cytology collected during 22 to 33 days post partum and more than 10 % PMN during 34 to 47 days postpartum (Sheldon *et al*, 2008). The prevalence rate of clinical and subclinical endometritis is 18 to 37 % (Drillich *et al*, 2005) and 12 to 94 % (Barlund *et al*, 2008), respectively.

#### **Causes of endometritis:**

Endometritis is caused by different bacteria, viruses and fungi. These organisms enter the uterus during the normal parturition, coitus, handling of dystokia cases, retention of foetal membranes etc. This uterine contamination may be converted into more severe form of uterine infection if immune system of animal does not respond properly or the diagnostic and therapeutic measures are not adopted at an early stage of disease. Finally, this will lead to adherence of micro-organisms to the uterine mucosa, penetration of the epithelium and release of bacterial toxins to develop uterine disease. Lack of immune response causing invasion and colonization of micro-organisms is also responsible for the occurrence of endometritis. This may lead to embryonic mortality and poor reproductive performance. *E. Coli*, *Fusobacterium necrophorum*, *Campylobacter fetus*, *Arcanobacter Pyogenes* and *Bovine Herpes Virus-4* are few infectious agents responsible for the endometritis (Kenide *et al*, 2016). *A. pyogenes* secrete the exotoxin pyolysin and is responsible for uterine inflammation (Habtamu *et al*, 2016). *Fusobacterium necrophorum* and *Campylobacter fetus* are responsible for mild endometritis and its diagnosis is not possible through rectal palpation of genitalia. *E. Coli* also responsible for endometritis leads to poor fertility and productivity. *Bovine Herpes Virus-4* is another agent responsible for vulvo-vaginitis and endometritis. Many factors viz., dystokia, retained fetal membranes, parturition stress, season, calving conditions, animal health etc predispose the animals towards endometritis.

#### **Effect of endometritis on fertility of dairy animals:**

Endometritis affected animal shows prolonged days to first service, days to conception and reduction in conception rate. An economic loss seen in endometritis is due to poor reproductive performance and

milk yield. The infection may disrupt the endocrine signaling in the hypothalamic pituitary gonadal axis (Karsch *et al*, 2002) and may reduce oocyte quality with increased rates of meiotic arrest and germinal vesicle failure (Sheldon *et al*, 2002) Animal with endometritis shows slower growth of dominant follicle, lower peripheral plasma estrogen or progesterone and are less likely to ovulate (Williams *et al*, 2007). Cows with endometritis require more services per conception.

### **Immunology of Reproduction:**

Defence mechanism of the reproductive tract is not always sufficient to prevent entry and colonisation of microorganisms and causes temporary or permanent damage to reproductive organs. There are two types of immune responses in the body as innate immunity and acquired immunity. Innate immunity is short acting while acquired immunity takes time to develop and is long lasting. Macrophages, dendritic cell, neutrophils, NK cells are the component of innate immunity. Acquired immunity is from T-cells and B-cells. Antigen presenting cells are the macrophages, dendritic cells, B-lymphocytes. These cells engulf the bacteria and place them on MHC II receptors, which are recognised by the CD4 T cells and there is cell mediated immune response leading to the release of inflammatory cytokines. T-helper cells are also activated and promote primarily antibody mediated immunity by stimulating B-lymphocytes to produce antibodies. Once bacterial infection enters in the endometrium, immunoglobulins like IgM, IgA and IgG are secreted. In cows with abnormal postpartum period, concentration of both IgA and IgG increases rapidly.

PMNs, blood monocytes and tissue macrophages constitute cellular defence mechanism by leucocytes and its activity is increased after parturition. The initial defence of the mammalian endometrium against microbes is dependent on innate immune systems, including toll-like receptors (TLRs), antimicrobial peptides (AMPs), and acute-phase proteins (Wira & Fahey, 2004). Bacteria are detected by pattern recognition receptors on mammalian cells binding molecules specific to microbial organisms, often called as pathogen associated molecular pattern (PAMPs)(O'Neill, 2008). TLR1, TLR2, and TLR6 recognize bacterial lipids such as lipoteichoic acid, whereas TLR3, TLR7, TLR8, and TLR9 recognize nucleic acids, often from viruses. Lipopolysaccharide from Gram-negative bacteria such as *E. coli* is bound to LPS-binding protein and is recognized by TLR4 in complex with CD14 and LY96 (MD2), TLR5 binds flagellin, and TLR9 also recognizes bacterial DNA. Activation of TLRs initiates signalling cascades, resulting in the synthesis and production of proinflammatory cytokines and chemokines that mobilize and activate immune cells, which in case of bovine uterine disease is particularly associated with the influx of PMNs into the uterus (Zerbe *et al*, 2003) Progesterone and estrogen have immunomodulatory properties in animals (Lamote *et al*, 2006) Alteration in hormone concentrations during parturition may be responsible for the entry of microorganism. In addition; estradiol and especially progesterone reduce the secretion of

prostaglandins, which has stimulatory effect on phagocytic activity of uterine PMN. Mucin1 expressed by epithelial cells of bovine endometrium is responsible for microbial defence mechanism (Brayman *et al*, 2004). Lectin, galactoside-binding, soluble, 3 (LGALS3) is detected in the uterus and involved in mucosal defence ((Kim *et al*, 2008)

### **Diagnostic considerations for endometritis:**

Various methods viz., Vaginoscopy, white side test, endometrial biopsy, cytology, ultrasonography etc are used by different researchers for the diagnosis of endometritis with variable results. Diagnosis of sub clinical uterine infection is slightly difficult under field conditions. White side test can be performed to some extent to diagnose sub clinical uterine infection. In white side test, uterine discharge should be heated with equal quantity of 5 % NaOH solution up to boiling. The appearance of yellow colour indicates infection in the uterus. Vaginoscope can be used to observe the presence of pus in the vaginal passage, which is useful for prediction of endometritis in bovines. However, the absence of pus observed through vaginoscope is not always indicative of absence of endometritis. Biopsy and histopathology method is expensive and time consuming compared to cytology method. However, this method is considered as gold standard to diagnose endometrial alterations because it allows to directly visualize both acute and chronic changes of the endometrium (Bonnett *et al.*, 1991). Endometrial biopsy for cytology is obtained by biopsy instrument. The instrument with the cutting tip is inserted in to the cervix and uterus to collect the sample. The uterine sample for cytology can also be collected by guarded cotton swabs technique, lavage technique and cytobrush technique. In lavage method, 0.9 % sodium chloride solution should be infused in the uterus. After uterine massage, fluid should be recovered from uterus and centrifuged. A slide is prepared with this sediments, air dried and fixed with methanol. More than 5 % Neutrophils in the sample is indicative of subclinical endometritis. The cytobrush can also be used for collection of the uterine sample. After collecting the uterine sample, a slide is prepared by rolling the brush on a clean glass slide and observed under microscope for % PMN. The cytobrush technique is more reliable than the lavage method. Guarded swab method is similar to cytobrush technique. The difference is only replacement of cytobrush with cotton swab. Ultrasonography is also used for diagnosis of endometritis by many scientists (Barlund *et al.*, 2008; Meira *et al.*, 2012). Endometrial thickness observed through ultrasonography is indicative of endometritis. Although USG is an easy and rapid technique, it should be accompanied with endometrial cytology for better diagnosis of endometritis.

**Treatment of endometritis:** Due to multi factorial etiology involved, a set treatment protocol is not possible for endometritis. However, an appropriate antibiotic can be used after identification of organism and drug sensitivity. Various antibiotics like cephalixin, enrofloxacin, oxytetracycline, gentamicin are used by different workers to treat endometritis at an early stage. However, the inadequate and indiscriminate

use of antibiotics is responsible for the emergence of partial or complete microbial resistance (Korudzhiiski *et al*, 1988). Subclinical endometritis can also be treated with PGF<sub>2α</sub>. PGF<sub>2α</sub> is responsible for the lysis of corpus luteum, which leads to the induction of estrus, myometrial contractions and removal of exudates from the uterus. PGF<sub>2α</sub> is ineffective in dairy animals with no palpable CL. PGF<sub>2α</sub> is not only luteolytic but also enhancer of neutrophil function (Lewis, 2004). Because there is increased concern about antibiotic resistance, PGF<sub>2α</sub> treatment in endometritis is a reliable method. Oxytocin was also administered to reduce the incidence of placenta retention and subsequent endometritis by Mollo *et al*, 1997). The intrauterine iodine (0.5 %) can be used for the treatment of endometritis. Chymotrypsin, trypsin and papain have proteolytic and fibrinolytic activity in inflamed tissue, which can also be used in endometritic cows (Marc Drillich *et al*, 2004). More recently, Ozone was also used intrauterine for the treatment of endometritis in HF cows with promising results (Drazen *et al*, 2014). Besides antibiotics and PGF<sub>2α</sub> treatment, biological active immunomodulators like E. coli LPS, Oyster glycogen, Autologous plasma have been used by many workers for treatment of endometritis (Williamson *et al*, 1987). Some scientists observed an effective increase in the influx of PMN into the uterus after intrauterine infusion of LPS (Saini, 1993; Anderson *et al*, 1985). Klucinski *et al* (1990) and Saini *et al* (1995) reported that one infusion of E. coli LPS into the uterine horn initiated an inflammatory process in the uterus. Saini *et al* (1995) reported that 80 % cows with endometritis conceived after treatment with single intrauterine infusion of 100 µg of E. coli LPS. Anderson (1985) reported influx of PMNs after administration of oyster glycogen. Levamisole is known to stimulate cell mediated immunity; however reports on its administration in endometritic animals are limited. A single dose of Levamisole @ 2.5 mg/kg body wt is also useful for immunomodulatory effect in dairy animals. Leukotriene B<sub>4</sub> is another agent which stimulates migration of PMNs in the uterine lumen of bovine uterus (Zerbe *et al*, 1996). A single intrauterine infusion of 30 mmol/l solution increases leukocyte count 5-10 times within 24 h. Inmodulen is a combination of inactivated cells of *Propionibacterium granulosum* 25.0 mg and E. coli LPS 2.0 mg/100 ml; Calier S.A., Barcelona, Spain). IgG concentration is increased following Inmodulen treatment. The conception can be improved in animals suffering from endometritis after treatment of Inmodulen (Nanda, 2006). Puneet Kumar (2004) reported high pregnancy rate in buffaloes treated with intrauterine infusion of 50 ml of sterile aqueous extract of *Tinosporacordifolia* (20 mg/ml). Neem oil extract increases phagocytosis or eliminates pathogens due to stimulation of uterine defense mechanism. Talwar *et al*. (1997) reported that herbal extract of Neem contains nonspecific immunostimulant, antimicrobial, antiviral and antifungal properties. It has been reported that Neem oil contains 1.1 per cent nimbidine, which is tested against gram-positive and gram-negative organisms. Microassay indicated that one mg of nimbidine was equivalent to 800 units of penicillin and 0.5 mg streptomycin sulphate (Singh and Sastry, 1981). Upadhyay *et al*. (1990) observed that infiltration of neutrophils and macrophages occurs following intrauterine infusion of a volatile fraction of Neem oil in rats. Rangnekar (2005) reported 37.5 per cent conception rate in repeat breeder infectious cows treated with neem oil intrauterine. Thus, variable results are obtained in the endometritis affected animals after treatment with different agents.

Considering all the above aspects of multi factorial etiology, endometritis needs to be tackled with multipronged diagnostic and therapeutic approaches to minimize its economic impact. Furthermore, proper cleanliness and parturition hygiene should be adopted to keep animal away from uterine contamination especially during dystokia as a preventive measure to nip endometritis in the bud.

**SS-04**

### **Recent Advances in control of Parasitism**

B. W. Narladkar

Associate Professor, Department of Veterinary Parasitology,  
College of Veterinary & Animal Sciences, Maharashtra Animal & Fishery Sciences University,  
Parbhani- MS (India) -431 402; Email: [babanar47@gmail.com](mailto:babanar47@gmail.com),

#### **Introduction**

Strategy without tactics is the slowest route to victory, tactics without strategy is noise before defeat, but strategy with tactics is a definite way of success. In livestock sector, for definite successful dairy venture “calf a year” shall be the strategy, to achieve this , one of the key factor is management of Parasitism and for effective management of parasitism principle of strategy with tactics is the way of success. Livestock systems occupy about 30 per cent of the planet’s ice-free terrestrial surface area [1] and are a significant global asset with a value of at least \$1.4 trillion. The livestock sector is increasingly organized in long market chains that employ at least 1.3 billion people globally and directly support the livelihoods of 600 million poor smallholder farmers in the developing world [2]. Currently, livestock is one of the fastest growing agricultural subsectors in developing countries. Its share of agricultural GDP is already 33 per cent and is quickly increasing. This growth is driven by the rapidly increasing demand for livestock products, this demand being driven by population growth, urbanization and increasing incomes in developing countries [3]. From this fact, it is understood that, livestock is raised for profit. Anything that negates that equation results in a loss to the producer and to the livestock economy example is parasitism. Worldwide, parasitism in cattle is often observed or determined to be subclinical or economical. Recently, Computerized programs for analysis of seasonality of the epidemiology of gastrointestinal parasites and of herd performance could predict appropriate timing and cost benefit for control measures. Therefore successful producers shall be more cost-conscious, consumer-oriented, sensitive to the environment and attuned to the economics of parasitism [4].

In the livestock sector, India too, is leading with large quantum of production of milk, meat, wool etc. Presently the country is producing 132.43 million tonnes of milk, 59.8 billion eggs and 6.3 million tonnes of meat. In 2010 India’s milk production crossed the 110 million tonnes mark, meaning that India

had more than trebled its production in the last three decades. Therefore, the livestock sector has emerged as one of the key components of agricultural growth in India and its role is crucial to fulfil growing food demand expected to increase by 40% by 2030 and may increase two fold in 2050. The increased demand of livestock products can be met by enhancing the numbers of animals, improving feed utilization efficiency, adopting better reproductive strategies and improving health coverage based on newer generation biotechnological vaccines and drugs [5]. Qualitative and quantitative improvement in the performance of livestock sector is the urgent need in India for many reasons, a) being livestock sector is an integrated component of agricultural activities in the country and there is rising demand for animal products because of changing food preferences of the next generation consumers, b) in the scenario of day by day shrinking land under cultivation( from 12.5 crore hectare in 1997 reduced to 10.7 crore hectare in 2003 due to industrialisation and urbanisation), livestock sector has to play greater role to fulfil food security legal right of the Indian people, c) to explore the fullest utility of available land, crop husks and crop residues, and d) to provide large quantum of organic manure, which is having increasing demand due to increasing concept of organic farming. It is always thought about nutritional deficiencies, hormonal imbalances, concurrent diseases (bacterial, viral) and genetic makeup of the animals for the lowered reproductive performance of cattle, buffalo, sheep and goats. But it is surprising to note that one of the real culprits behind affecting the productive and reproductive ability of animals (directly by producing pathological conditions of reproductive tract like protozoan diseases and indirectly by producing deficiency of nutrients, vitamins and minerals) is PARASITISM which is never taken in to account in real sense. Present review is a small effort in this direction.

**What is Parasitism?:** It is defined as “An intimate and obligatory relationship between two heterospecific organisms in which parasite is metabolically dependent on the host and causes harm to host”. It is a state of balance (subclinical) and on occasion of imbalance (clinical) between infection of the parasites and the livestock. During the infection animals may not exhibit signs (subclinical parasitism) and may exhibit signs (clinical parasitism). In both situation animal tries to cope up the situation and survives. But survival of such animals is at the cost of heavy losses in terms of blood, minerals, proteins affecting on weight loss, production, reproduction and health status. Parasitism may be a resultant of either internal parasitic infections caused by helminths (nematodes, trematodes, cestodes), protozoans (enteroprotezoans, haemoprotezoans, tissue dwelling) or caused by external parasites (biting and non biting flies, lice, fleas, ticks, mites). It has been proved beyond doubt that Gastro-Intestinal tract (GIT) of animals harbours variety of parasites particularly helminthes, coccidian and ciliates which results in clinical and subclinical parasitism [6], which affects on overall health, production and reproduction, weight loss, digestive disturbances and emaciation of prolonged period [7] and this has great impact on reproductive performance in terms of calving rate and calf mortality [8]. The anorexia, reduced feed intake, loss of blood and plasma proteins into GI tract, alterations in protein metabolism, depressed levels of minerals, depressed activity

of some intestinal enzymes, reduced skeletal growth brought about by mineral deficiency [9] for which the parasitism is directly responsible. Subclinical parasitism is the problem always ignored but it has great impact on economic losses which are quite higher than clinical infections [10].

#### **Review on few facts about parasitism [11]: Parasitism results in...**

- 1. Reduced appetite:** GI parasites, particularly those in the abomasum, because pathological changes that disrupt digestion and reduce appetite [12, 13, 14]. In sheep and cattle such reduced appetite affects host resilience (host maintenance during infection) and, to a lesser extent, host resistance (host's ability to prevent parasite establishment and development including immunity) [12, 14]. Further reduced appetite (anorexia—by up to 30%) that contributes to lower live-weight gain and reduced milk and wool production in domestic stock [14, 15]. It has been estimated that the cumulative food intake over 8 hrs for lambs infected with gastrointestinal parasites results into intake of 20 g/0.75 kg Live Weight compared to 55–75 g/0.75 kg Live Weight for treated and uninfected lambs [16].
- 2. Relaxation in immunity:** Periparturient relaxation in immunity encourages the maturity of hypobiotic larvae and thereby increases in faecal egg output responsible for contaminating the pasture [17].
- 3. Subclinical parasitism:** Influences bone metabolism through changes in phosphorus and calcium absorption, leading to reduced bone growth [14]. Other changes in micronutrients occur as part of the suite of changes associated with subclinical infections, including decreases in copper uptake and changes in sulfate metabolism [12]. According to Strickland [18] Subclinical production losses caused by internal parasites include reduced milk production, reduced weaning weights, delayed puberty and decreased fertility in replacement heifers, reduced pregnancy rates in mature cows, reduced feed intake, reduced feed efficiency and immune suppression in all classes of cattle.

#### **Strategic plan for control of Parasitism resulting from ecto and endo parasites:**

- 1. IPM (Integrated Pest Management):** Recent development in the area of IPM for pests can form a boon in combating the Parasitism caused by ecto-parasites. IPM modules involving physical, biological and if an essential chemical pesticide for control of biting and non-biting flies, lice, fleas, mites and ticks are the best practices. IPM practices against any pest are considered as eco-friendly, user-friendly and cost-effective. However, IPM modules designed for a particular pest shall suit to the local geography and demographic conditions. Scrupulous and Strategic implementation of IPM practices keep the animals away from these pests, pest transmitted diseases, stress of chemicals and above all free from annoyance, worries and psychological disturbances resulting from bite of ecto-parasites. Recent research undertaken on IPM of *Culicoides* midges indicated that overall milk production hike to the tune of 18.97% [19].

### IPM for flies:-

1) **Physical:** Provision of Net shed as physical barrier [20-21], mechanical control through habitat modification [22], animal shelter management [23], solar powered electrocuting trap [24].

2) **Managemental:** Hygiene / Sanitation can be done by frequent manure removal in the shed for control of house flies and stable flies [25]. Defining the feeding and biting sites, biting times and resting sites of the locally prevalent species and accordingly application of biopesticide, at the specific site and time only. Larval habit management of *Culicoides* such as making water level fluctuation, adjusting edges slopes, drainage [26] helps in reducing the larval growth of *Culicoides*. Use of woodchips, ground corncobs or saw dust for bedding of calf [27]. Animal husbandry is key to prevent wool maggots. The operations such as Lambing and shearing early in the season before onset of fly activity. Care in shearing the sheep will minimize wound sites. Prompt treating or clipping of soiled fleece [28]. Cleaning of calf pens on weekly basis [29] significantly suppresses the development of House & Stable flies.

3) **Cultural:** Use of mineral fertilizer to change grass land biotypes [30] which helps in reducing breeding sites of many fly species.

4) **Biological:** Use of microbial pesticides and Bio-control agents (BCAs) such as

a) *Metarhizium anisopliae* [31-32], *Metarhizium* is not toxic to mammals and environmentally friendly biological control agent for *C. brevitarsis*[33]

b) Use of *B. cereus*( CWBI-B1082) [30,34], *B. thuringiensis* var *israelensis* , *B. sphaericus*, *Bacillus weihenstephanensis* WSBC and *Bacillus weihenstephanensis*KBAB4[35]

c) Predacious guppy fishes: *Poecilia reticulata* [36], d) Herbal control with Neem and Karanj oil [37], e) Integrated management of *Culicoides* spp [38]

5) Early warning system and prediction models: Baylis *et al* [39,40] used climate data and Satellite imagery model to know the abundance of *Culicoides*. In which they estimated NDVI (Normalized difference vegetation index) measure of photosynthetic activity and LST land surface temperature a measure of temperature at earth surface. NDVI and LST as predictors help in population peaks of flies. Forecasting of density and time of peak fly population are important steps for undertaking the control measures, hence shall be given due consideration [41-44].

**Chemicals:** as a last resort judicious use of chemical pesticides. Use of baits, Fogging/ misting in barns [45] which control house and stable fly populations[46].

### IPM for ticks :-

**a) Physical :** Caulking process involving burning of tick eggs in the cattle shed for annihilation of breeding places, to be undertaken three consecutive times at weekly interval in a season [47], by slow burning over a period of one or two days [48]. Manual removal of ticks such as grooming, is widely used in the developing world, although it is rare in extensive systems.

**b) Managemental:** Pasture spelling (depopulating pastures while free-living ticks die because of a shortage of available hosts).

**c) Biological:**

**Herbal:** Use of three herbal oils Neem oil (*Azadirachta indica*), Karanj oil (*Pongamia pinnata*) and Nilgiri oil (*Eucalyptus globulus*) [34]. Alcoholic extracts of sitaphal (*Annonasquamosa*) and neem (*Azadirachta indica*) against different life stages [49]. Crude extracts of *A. sativum* cloves and *C. papaya* seeds [50], use of herbal preparations used in ethno-veterinary and as green-fabricated nanoparticles as novel approach [51].

**Fungal:** *Metarhizium anisopliae* and *Beauveria bassiana* [52-56]; *M. anisopliae* and *B. bassiana* gains high value in biological control of ticks because both the fungi exhibited the strongest anti-tick pathogenicity [57-59]. Control of tick populations by spraying *Metarhizium anisopliae* conidia on cattle under field conditions [60].

**Bacterial/Biological control agents/Environmental:** It includes the candidates such as predatory mites [61], chickens [62-63], parasitoid wasps [64], *Bacillus thuringiensis* [65], entomopathogenic nematodes [66].

Use of oil formulation of fungi: may increase the conidia stability; extend their persistence in the field protecting fungi against heat stress, desiccation, and particularly ultraviolet irradiation [67-71].

Pheromones used by ticks for aggregation and mating can be artificially used in combination with acaricides [72]

Tick Vaccine: Subole-sin as a candidate vaccine antigen for the control of cattle tick infestations in Indian situation [73]. Vaccines so far commercially available were developed to be effective against *B. microplus* and are based on the antigen prepared from tick midgut protein Bm86, basis of two commercial vaccines, TickGARD Plus and Gavac Plus. However, current research suggests that the target might be

conserved in a number of tick species, resulting in some successes against *B. annulatus* [74], *Hyal. anaticum anaticum*, and *Hyal. dromedarii* [75].

**Forecasting:** Early warning system using GIS information is the recent tool. Meteorological data for making the prediction model is available at district (612 centers) in India [76].

**Push pull:** Hassanali [77] taken detailed review about push-pull mechanism. Combinations of repellent and attractant semiochemistry may also find use in push-pull tactics for controlling livestock pests and disease vectors. Several possibilities are currently being explored. The adults of the brown ear tick, *Rhipicephalus appendiculatus*, the vector of the cattle disease East Coast Fever (*Theileria*), have been shown to use push-pull semiochemistry to locate bovid ears [78], Tsetse flies [79]. Push-pull may also find a useful application in controlling malaria vectors, particularly zoophilic species like *Anopheles arabiensis*. The use of animals to divert (pull) mosquitoes from feeding on and transmitting disease to human beings (zooprophyllaxis) has been considered as a possible tool in reducing mosquito numbers and levels of malaria [80].

**d) Chemical:** As a last resort and their judicious use to avoid development of resistance.

**2. IPC ( Integrated Parasite Control ) :** Term is used to describe all about the efforts in the form of biological , managemental (Pasture) and chemical (timely deworming) control of internal worm infection or endo-parasites. However, to be cost-effective, deworming programs must be strategic but flexible [81]. Views expressed about Strategic control of parasites through tactics by Russell [82] as: “It would seem that a new approach is being made in the control of nematode parasites. The emphasis is now on pasture hygiene, achieved by combining the intelligent use of anthelmintics with controlled grazing in such a way as to protect young animals from heavy infestations and at the same time to build up their resistance to parasitic diseases”.

### **Factors for deciding the deworming schedule**

- **For Cattle, Buffaloes :** Guidelines for strategic deworming as described by [83]:

**Concept and Aim of deworming:** Deworming dairy cattle is a venture beyond treating clinical disease such that the treatment of parasitism should be aimed first at the elimination of the threat of economic loss and secondly to reduce or elimination of the parasites and parasite contamination. According to Williams and Loyacano [84] “The primary purpose of parasite control is not to cure sick animals...but to reduce levels of pasture contamination and consequently prevent such episodes of illness or reduced productivity.”

1. The dairy practitioner and nutritionist along with parasitologist can play a vital role by using science to determine where, when and which cattle need deworming, and make sure that cattle are not being treated unnecessarily or those cattle which need treatment received the necessary treatment. Profile each herd identifying where parasites exist throughout grazing land starting from new born calves to mature cows and then determine the deworming strategy for each phase based on faecal worm egg counts and the type of parasites found in a geographic area.
2. Check early fresh cows to determine whether the cows were becoming infected during the dry period. The time necessary for the development of a patent infection to occur in the animal after infective stage of parasite is consumed is usually between three and six weeks depending upon the specific parasite and the age and immune status of the animal being infected.
3. For conducting a faecal examination, make sure representative (5 to 10%) individual samples are taken from the various age groups in each operation and from cows in different stages of lactation including dry cows.
4. Since each herd is different, determining how much exposure the animals have or have had to a parasite-contaminated environment and then focusing on this part of the grazing land /pasture is the best way to start the parasite control
5. After identifying the areas of parasitic infection such as grazing land/pasture where the greatest chance for parasite contamination to develop and then confirming the presence of parasite through conducting faecal worm egg counts, a control strategy can be developed.
6. Several key points are that : animals which have spent time in confinement for longer than six months have the least chance of being parasitized.
  - a) If the lactating herd is in total confinement, treatment is probably unnecessary but should be confirmed by a faecal exam.
  - b) If a herd in is total confinement but the dry cows are on pasture, the cows should receive treatment when they are moved off pasture
  - c) If a herd is held in total confinement, dry cows are in confinement, but replacement heifers are raised on pasture. Deworm these heifers strategically during the pasture phase and then make sure all first calf heifers are dewormed prior to arrival into the lactating herd.

d) If all cattle are held in confinement from birth until reaching the lactating herd; conduct faecal checks throughout different age groups looking for barnyard infections in the different management groups and then deworm all animals coming into the herd either as replacement animals or newly purchased replacement cows to make sure parasites are not introduced .

e) Grazing herds can be treated on an individual basis, herd basis or a combination by deworming all animals. Deworming individual cows at the time of calving during the year (especially those animals on pasture during the grazing season).

7. Parasite contamination on concrete is usually very low except where bedding and manure build-up occur. Parasite transmission in dairy herds predominantly occurs on pasture, exercise lots, and dirt lots, therefore, identifying these areas or operations where parasites are most likely to exist on an operation and then concentrating on these areas for conducting initial worm egg counts and setting up treatment programs will save a lot of time and money.

### **For Sheep and Goats**

Sheep and goats are considered as museum of parasites and are prone to the infections of parasites frequently, particularly to GI nematodes. Hence strategic deworming schedule round the year shall be followed for best performance in terms of production and reproduction. Deworming schedule designed for cattle and buffaloes shall not be for sheep and goats.

**Concluding remarks about deworming :** Thus for minimising the effect of parasitism, addition to deworming, intensive efforts are also needed which includes practices of IPC. The major component of IPC is pasture management and grazing Strategies.

**Grazing Management:** Approximately 80 percent of the worm larvae can be found in the first two inches of grass. Therefore, sheep grazing taller forages will have fewer parasite problems. Sheep should not be allowed to graze forages shorter than 2 inches in height. Sheep that browse also have fewer parasite problems. Another grazing strategy is to wait until the dew has lifted from the grass or grass has dried after a rain. Dry conditions force parasites to stay at base of pasture where they are less likely to be consumed by livestock. Efficient grazing practices can make the farm more profitable in several ways [85]. It is well known that parasites can largely enhance trace mineral deficiencies [86] and on the other hand, some trace minerals, including Co (used to synthesize vitamin B12) and Fe, can play a key role in affecting ruminant susceptibility [87]. At present, the non chemotherapeutic control of parasites is based mainly on grazing management strategies [88]. The inclusion of grass and herb species with high concentrations of trace minerals in pasture, proposed as an efficient tool to increase the intake of trace minerals in livestock [89].

Similarly multi-species grazing can be equally effective. Sheep (and goats) are generally not affected by the same internal parasites as cattle and horses.

### **General Control measures /steps to be taken for minimising the effect of parasitism:**

**Avoid dog/cat access to animal feed :** It will avoid the spread of protozoan parasites responsible for abortions like *Neospora caninum*, *Toxoplasma gondii* etc

**Manure disposal:** Is the key factor for dispersal of parasitic eggs on pasture. It is always advised the dung from livestock shall be dump in manure pits for considerable period before spreading over the land as fertiliser.

Snail Control : Essential for minimising the fluke infections

Proper care and timely treatment of haemoprotozoan diseases with effective tick control

**Good Management:** Internal parasite control starts with good management. Feeders which cannot easily be contaminated with faeces should be utilized for grain, hay, and minerals. Water should be clean and free from faecal matter. Pastures and pens should not be overstocked.

**Use of Clean or Safe Pastures:** Pastures which are not contaminated with the worm larvae

**Healthy Soil:** Earthworms have been shown to ingest worm eggs and larvae, either killing them or carrying them below the soil surface. Dung beetles ingest and disperse manure, thus keeping eggs and larvae from developing. Anything that is done to maintain soil health and promote these types of organisms will aid in parasite control.

**Nutritional Management:** Better nutrient supply with balanced diet is the key for developing immune response to internal parasites. Animals on low protein diets are more susceptible to infection because they produce less IgA (immunoglobulin).

### **References:**

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. & de Haan, C. 2006 Livestock's long shadow: environmental issues and options. Rome, Italy: FAO.

Thornton, P. K. et al. 2006 Mapping climate vulnerability and poverty in Africa. Nairobi, Kenya: ILRI.  
See <http://www.dfid.gov.uk/research/mapping-climate.pdf>.

- Delgado, C. 2005 Rising demand for meat and milk in developing countries: implications for grasslands-based livestock production. In Grassland: a global resource (ed.D. A. McGilloway), pp. 29–39. The Netherlands: Wageningen Academic Publishers
- Corwin RM (1997) Economics of gastrointestinal parasitism of cattle. *Vet Parasitol.* 72(3-4): 451-457  
<https://www.ncbi.nlm.nih.gov/pubmed/9460211> 2/2
- Vision 2030 (2011) Published by M C Sharma director Indian Veterinary Research Institute Izatnagar Printed by M/S Bytes and Bytes Bareilly
- Aktaruzzaman M, Rony SA, Islam MA, Yasin MG and Rahman AKMA (2013) Concurrent infection and seasonal distribution of gastrointestinal parasites in cross-bred cattle of Sirajganj district in Bangladesh, *Veterinary World* 6(10): 720-724.( Available at [www.veterinaryworld.org/Vol.6/Oct-2013/5.pdf](http://www.veterinaryworld.org/Vol.6/Oct-2013/5.pdf))
- Radostits, O. M., Gay, G. C., Blood, D. C. and Hinchkiliff, K. W. (2000) *Veterinary Medicine* 9th ed. EIBS and Bailliere, Tindall.
- Gross, S.J., Ryan, W.G., Ploeger, H.W. (1999) Anthelmintic treatment of dairy cows and its effect on milk production. *Vet Rec.* 144: 581-587.
- Soulsby, E J L (1982) *Helminths, Arthropodes and Protozoa of domesticated animals.* Seventh Edn, ELBS and Baillere Tindall London pp 239
- Martin, S.W., Meek, A.H. & Willeberg, P. (1994) *Veterinary Epidemiology: Principles and Methods.* Iowa State University press, Ames, Iowa.
- Gunn A and Irvine J R (2003) Subclinical parasitism and ruminant foraging strategies—a review *Wildlife Society Bulletin* 2003, 31(1):117–126
- Sykes, A. R. (1987). Endoparasites and herbivore nutrition. Pages 211–232 in J. B. Hacker and J. H. Ternouth, editors. *The nutrition of herbivores. Proceedings of the 2nd International Symposium on the Nutrition of Herbivores, 6–10 July 1987, Brisbane, Australia.*
- Fox, M. T., Gerrelli D. Pitt S. R., Jacobs D. E. Gill M and Gale D. (1989). *Ostertagia ostertagi* infection in the calf-effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. *Research Veterinary Science* 47:294–298.
- Van Houtert M.F.J., Sykes A.R. (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *Int. J. Parasitol.* 26:1151–1167.
- Forbes, A. B., Huckle C. A., Gibb M. J. Rook, A. J. and Nuthall R. (.2000). Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. *Veterinary Parasitology* 90:111–118.
- Kyriazakis, I., Tokamp B. J. And Hutchings M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour* 56:265–274.
- Coop, R. L., and Kyriazakis I. (1999). Nutrition-parasite interaction. *Veterinary Parasitology* 84:187–204.

- Strickland J E ( Revised by Lee Jones) (2012). Internal Parasite Control in Beef Cattle *Georgia (UGA) Cooperative Extension Bulletin 1086*.( available at [www.caes.uga.edu/publications](http://www.caes.uga.edu/publications))
- Narladkar B W ( 2012) Research project report of Development of Integrated Pest Management module against important Dipteran pests of livestock with reference to *Culicoides* midges (Diptera : Ceratopogonidae) financed and accepted by Department of Biotechnology, Govt of India New Delhi
- Calvete C, [Estrada R](#), [Miranda MA](#), [Del Rio R](#), Borrás D, [Beldron F J](#), Martínez A, [Calvo A J](#) and Lucientes J. 2010. Protection of livestock against bluetongue virus vector *Culicoides imicola* using insecticide-treated netting in open areas. *Medical and Veterinary Entomology* **24**:169-75.
- Narladkar , B. W. and Shivpuje, P. R. ( 2014) Fly Proof Net shed for Livestock : A novel concept of physical barrier for integrated Management of *Culicoides* spp ( Diptera: Ceratopogonidae). *Vet. World*, 7:899-908
- Harrup, L.E., Miguel, A., Miranda, M. A. and Carpenter, S. (2016) Advances in control techniques for *Culicoides* and future prospects. *Vet Ital*, 52 :247-264. doi: 10.12834/VetIt.741.3602.3
- Bandeira, M.D.C.A, Brito, G.A., da Penha, A. and Rebêlo, J.M.M. (2017) The influence of environmental management and animal shelters in vector control of *Culicoides* (Diptera, Ceratopogonidae) in northeastern Brazil. *Journal of Vector Ecology*, 42:113-119 .
- Pickens L G and Mills J G D. 1993. Solar-powered electrocuting trap for controlling house flies and stable flies (Diptera: Muscidae). *Journal of Medical Entomology* **30**: 872- 877.
- Pickens L G, Morgan N O, Hartsock J G and Smith J W. 1967. Dispersal patterns and populations of house fly affected by sanitation and weather in rural Maryland. *J. Econ. Entomology* **60**: 1250- 1255
- Mullens B A. 1991. Integrated management of *Culicoides varlipennis*: a problem of applied ecology. In Blue tongue, African Horse sickness and related orbiviruses. *Proceedings of the second International Symposium*, 17-21 June 1991, Paris. Eds Walton T E and Osburn B I 1992. CRC Press, Boca Raton, Florida, USA, Pp 896- 905.
- Schmidtman E T. 1991. Suppressing immature house and stable flies in outdoor calf hutches with sand, gravel, and sawdust bedding. *Journal of Dairy Sciences* **74**: 3956- 3960.
- Anonymous. 1994. In proceedings of workshop on Research and extension needs for Integrated Pest Management for arthropods of Veterinary importance. Eds C J Geden and J A Hogsette, Lincoln, Nebraska, 12-14 April 1994. Available on <http://www.Cmave.usda.ufl.edu/Lincoln.html>
- Geden C J, Rutz D A, Miller R W and Steinkraus D C. 1992. Suppression of house flies on New York and Maryland dairies using *Muscidifurax raptor* in an integrated management program. *Environment and Entomology* **21**: 1419-1426
- Dylko N I. 1979. Biological control of blood sucking flies. *Veterinary Bulletin* **50**: 4966.

- Ansari, M.A., Pope, E.C., Carpenter, S., Scholte, E-J. and Butt, T.M. (2011) Entomopathogenic Fungus as a Biological Control for an Important Vector of Livestock Disease: The *Culicoides* Biting Midge. *PLoS ONE* 6(1): e16108. doi:10.1371/journal.pone.0016108
- Narladkar, B. W., Shivpuje, P. R. and Harke, P. C. (2015a) Fungal Agents for Biological Control of *Culicoides* spp (Diptera: Ceratopogonidae) of livestock. *Vet. World*, 8:156-163.
- Nicholas, A.H. and McCorkell, B. (2014) Evaluation of *Metarhizium anisopliae* for the control of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae), the principal vector of bluetongue virus in Australia. *Journal of Vector Ecology*, 39:213-218
- Narladkar, B. W. and Shivpuje, P.R. (2012) Research project report of Development of Integrated Pest Management module against important Dipteran pests of livestock with reference to *Culicoides* midges (Diptera : Ceratopogonidae) financed and accepted by Department of Biotechnology, Govt of India New Delhi. pp 1-167
- Narladkar, B. W., Shivpuje, P. R. and Harke, P. C. (2015b) Bacterial Agents for Biological Control of *Culicoides* spp (Diptera: Ceratopogonidae). *Indian Vet J.*, 92:24-28.
- Narladkar, B.W. (2003) Studies on bio-ecological aspects, vector potentiality and integrated management of *Culicoides* spp (Diptera: Ceratopogonidae) of Marathwada region. Ph.D. thesis approved by Maharashtra Animal and Fishery Sciences University Nagpur (Maharashtra state).
- Narladkar, B. W. and Shivpuje, P. R. (2015c) Herbal Agents for Biological Control of *Culicoides* spp (Diptera: Ceratopogonidae). *Indian Vet J.*, 92(5):20-23.
- Narladkar, B.W.; Deshpande, P. D., Vadlamudi, V.P., Shivpuje, P.R. and Deshpande A.R. (2006) Integrated management of *Culicoides* sp of domesticated animals. *J Vet Parasitol*, 20:125-128.
- Baylis M, Bouayoune H, Touti J and el Hasnaoui H. 1998. Use of climate data and satellite imagery to model the abundance of *Culicoides imicola*, the vector of African horse sickness virus in Morocco. *Medical and Veterinary Entomology* 12 : 255- 266.
- Baylis M and Rawlings P. 1998. In: African Horse sickness. Edt. Mellor P S, Baylis M, Hablin C and Calisher C HPp 137-153.
- Narladkar, B.W.; Deshpande P D, Shivpuje, P.R. and Sondge, V .D. (2006) Population dynamics of *Culicoides* sp of domesticated animals of Marathwada region. *J. Vet. Parasitol.*, 20:171-174
- O'Farrell, H. and Gourley, S.A. (2014) Modelling the dynamics of bluetongue disease and the effect of seasonality. *Bull. Math Biol.* 76:1981-2009.
- Nayduch, D., Cohnstaedt, L.W., Sasaki, C., Lawson, D., Kersey, P. and Fife, M. (2014) Studying *Culicoides* vectors of BTV in the post-genomic era: Resources, bottlenecks to progress and future directions. *Virus Res.*, 182:43-49.
- White, S.M., Christopher, J. Sanders, C.J., Christopher, R., Shortall, C.R., Bethan, V. and Purse, B.V. (2017) Mechanistic model for predicting the seasonal abundance of *Culicoides* biting midges and the impacts of insecticide control. *Parasites & Vectors*, 10:162

- Harrington E P, Weingart D W, Kaufman P E, Waldron J K, Smith W G and Rutz D A. 1997. Pest and pesticide use assessment and personal protective equipment use for dairy cattle production system in New York State for 1997. NAPIAP Report. Cornell University. Pp-61
- De Keyser, R., Cassidy, C., Laban, S., Gopal, P., Pickett, J.A., Reddy, Y.K., Prasad, M., Prasad, G., Sreekumar, C., Senthilven, K., Carpenter, S. and Logan, J.G. (2017) Insecticidal effects of deltamethrin in laboratory and field populations of *Culicoides* species: how effective are host contact reduction methods in India?. *Parasites & Vectors*, 10:54. DOI 10.1186/s13071-017-1992-0
- Narladkar, B. W. and Shivpuje, P. R. (2015d) Integrated Approach for *Rhipicephalus microplus* Tick Management through Physical Measures and Herbal Bio-control agents. *Indian Vet J.*, 92:29-33.
- Muhammad, G.; Naureen, S.; Firyal, S. and Saqib, M. (2008) Tick control strategies in dairy production medicine. *Pakistan Vet J*, 28: 43-50.
- Ghosh, S., Azhahianambi, P. and Yadav, M.P. (2007) Upcoming and future strategies of tick control: a review. *Journal of Vector Borne Diseases* 44: 79–89.
- Shyma, K.P., Gupta, J.P., Ghosh, S., Patel, K.K. and Singh, V. (2014) Acaricidal effect of herbal extracts against cattle tick *Rhipicephalus (Boophilus) microplus* using in vitro studies. *Parasitol Res.*, 113:1919-1926
- Balan, B., Baskaralingam, V., Periyannan, R., Narayanan, M.P., Palaniappan, R., Kadarkarai, M., Angelo, C. and Giovanni, B. (2017) Exploitation of chemical, herbal and nanoformulated acaricides to control the cattle tick, *Rhipicephalus (Boophilus) microplus* – A review. *Vet. Parasitol.*, 244:102-110.
- Narladkar, B. W., Shivpuje, P. R. and Harke, P. C. (2015e) Fungal Bio-control agents for Integrated Management of *Rhipicephalus microplus* Ticks. *Indian Vet J.*, 92: 34-37.
- Samish, M., Ginsberg, H. and Glazer, I. (2004) Biological control of ticks. *Parasitol*, 129(S): 389-403.
- Ren, Q.Y., Liu, Z.J., Guan, G.Q., Sun, M., Ma, M.L., Niu, Q.L., Li, Y.Q., Liu, A.H., Liu, J.L., Yang, J.F., Yin, H. and Luo, J.X. (2012) Laboratory evaluation of virulence of Chinese *Beauveria bassiana* and *Metarhizium anisopliae* isolates to engorged female *Rhipicephalus (Boophilus) microplus* ticks. *Biol Contr*, 63: 98-101.
- Sun, M., Ren, Q.Y., Guan, G.Q., Li, Y.F., Han, X.Q., Ma, C., Yin, H. and Luo, J.X. (2013) Effectiveness of *Beauveria bassiana* sensu lato strains for biological control against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in China. *Parasitol Int*, 62: 412-415.
- Fernandez-salas, A., Alonoso-diaz, M.A., Alonoso-morale, R.A., Lezama-gutierrez, R., Rodriguez-rodriguez, J.C. and Cervantes-chavez, J.A. (2017) Acaricidal activity of *Metarhizium anisopliae* isolated from paddocks in the Mexican tropics against two populations of the cattle tick *Rhipicephalus microplus*. *Med Vet Entomol*. 31: 36–43.
- Gindin, G., Samish, M., Alekseev, E. and Glazer, I. (2001) The susceptibility of *Boophilus annulatus* (Ixodidae) ticks to entomopathogenic fungi. *Ann Bioeth*, 11:111–118.

- Samish, M., Gindin, G., Alekseev, E. and Glazer, I. (2001) Pathogenicity of entomopathogenic fungi to different developmental stages of *Rhipicephalus sanguineus*. *J Parasitol.*, 87:1355–1359.
- Kaaya, G. P. and Hassan, S. (2000) Entomogenous fungi as promising biopesticides for tick control *Exp Appl Acaro*, **24**: 913–926, 2000.
- [Kaaya,G.P.](#), [Samish M](#), [Hedimbi,M](#), [Gindin G](#), and [Glazer I](#). (2011)Control of tick populations by spraying *Metarhizium anisopliae* conidia on cattle under field conditions. [Exp Appl Acarol](#). 2011 Nov;55(3):273-81. doi: 10.1007/s10493-011-9471-3. Epub 2011 Jul 3.
- Holm, E., Wallace, M.M.H., 1989. Distribution of some anystid mites (Acari: Anystidae) in Australia and Indonesia and their role as possible predators of the cattle tick *Boophilus microplus* (Acari: Ixodidae). *Exp. Appl. Acarol.* 6, 77–83.
- Kohn, W.K., Norval, R.A.I., 1994. Predation of ticks by chickens in Zimbabwe. *Zimbabwe Vet. J.* 25, 33–37.
- Dreyer, K., Fourie, L.J., Kok, D.J., 1997. Predation of livestock ticks by chickens as a tick control method in a resource-poor urban environment. *Onderstepoort J. Vet. Res.* 64, 273–276.
- Hu, R.J., Hyland, K.E., Oliver, J.H., 1998. A review on the use of ixodiphagous wasps (Hymenoptera: Encyrtidae) as natural enemies for the control of ticks (Acari:Ixodidae). *Syst. Appl. Acarol.* 3, 19–27.
- Zhioua, E., Heyer, K., Browning, M., Ginsberg, H.S., LeBrun, R.A., 1999. Pathogenicity of *Bacillus thuringiensis* variety *Kurstaki* to *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol.* 36, 900–902.
- Samish, M. and Glazer, I. (2001). Entomopathogenic nematodes for the biocontrol of ticks. *Trends in Parasitology* 17, 368–371.
- Perinotto, W. M.S., Angelo,C.I., Golo,P.C. Camargo,M.G. , Quinelato, S, Sá, F.A., Caio J. B. Coutinho Rodrigues, C.J.B., Marciano, A.F., Monteiro,C.M.O. and Bittencourt , V.R.E.P.(2017) In vitro pathogenicity of different *Metarhizium anisopliae* s.l. isolates in oil formulations against *Rhipicephalus microplus*. *Ann Bioeth* , 27:338-347.DOI: 10.1080/09583157.2017.1289151
- Barreto, L. P., Luz, C., Mascarin, G. M., Roberts, D. W., Arruda, W. and Fernandes, E. K. K. (2016). Effect of heat stress and oil formulation on conidial germination of *Metarhizium anisopliae* s.s.on tick cuticle and artificial medium. *J Invertebr Pathol* , 138: 94–103.
- Camargo, M. G., Golo, P. S., Angelo, I. C., Perinotto, W. M. S., Sa, F. A., Quinelato, S. and Bittencourt, V. R. E. P. (2012) Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions. *Vet Parasitol.*, 188: 140–147.
- Camargo, M. G., Marciano, A. F., Sá, F. A., Perinotto, W. M. S., Quinelato, S., Golo, P. S. and Bittencourt, V. R. E. P. (2014) Commercial formulation of *Metarhizium anisopliae* for the control of *Rhipicephalus microplus* in a pen study. *Vet Parasitol*, 205,:271–276.

- Camargo, M. G., Nogueira, M. R., Marciano, A. F., Perinotto, W. M. S., Coutinho-Rodrigues, C. J. B., Scott, F. B. and Bittencourt, V. R. E. P. (2016). *Metarhizium anisopliae* for controlling *Rhipicephalus microplus* ticks under field conditions. *Vet Parasitol*, 223: 38–42.
- Sonenshine, D.E. (1993). Vol. II. Oxford Univ. Press. N.Y. 465. pp.
- Shakya, M., Kumar, B., Nagar, G., de la Fuente, J. and Ghosh, S. (2014) Subole-sin: a candidate vaccine antigen for the control of cattle tick infestations in Indian situation. *Vaccine*, 32: 3488-3494.
- Pipano, E., Alekceev, E., Galker, F., Fish, L., Samish, M., Shkap, V., 2003. Immunity against *Boophilus annulatus* induced by the Bm86 (Tick-GARD) vaccine. *Exp. Appl. Acarol.* 29, 141–149z
- De Vos, S., Zeinstra, L., Taoufik, O., Willadsen, P., Jongejan, F., 2001. Evidence for the utility from *Boophilus microplus* in vaccination against other tick species. *Exp. Appl. Acarol.* 25, 245–261.
- Kshirsagar DP, Savalia CV, Kalyani IH, Kumar R and Nayak DN (2013) Disease alerts and forecasting of zoonotic diseases: an overview, *Veterinary World* 6(11): 889-896.
- Hassanali, A, Hans Herren, H; Zeyaur R Khan, Z.R., John A Pickett, J A. and Christine M Woodcock, C M (2007) Integrated pest management: the push-pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. *Phil. Trans. R. Soc. B* 2008 **363**, 611-621
- Wanzala, W., Sika, N. F. K., Gule, S. & Hassanali, A. 2004 Attractive and repellent host odours guide ticks to their respective feeding sites. *Chemoecology* 14, 229–232. (doi:10.1007/s00049-004-0280-6)
- Gikonyo, N. K., Hassanali, A., Njagi, P. G. N. & Saini, R. K. 2000 Behaviour of *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) on waterbuck *Kobus defassa* Ruppel and feeding membranes smeared with waterbuck sebum indicates the presence of allomones. *Acta Trop.* 77, 295–303. (doi:10.1016/S0001-706X(00) 00153-4)
- WHO 1982 Manual on environmental management for mosquito control with special emphasis on malaria vectors. Geneva, Switzerland: World Health Organisation.
- Corwin R M. (1997). Economics of gastrointestinal parasitism of cattle. *Vet Parasitol.* 72(3-4):451-7; discussion 457-60
- Russell, A. (1949) *Vet Rec* 61:238-239.
- Bliss, D. H. and W. G. Kvasnicka. (1997). The fecal examination: a missing link in food animal practice. *The Compendium – Food Animal Parasitology.* (April): 104-108.
- Williams J C and Loyacano A F. (2001). Internal Parasites of Cattle in Louisiana and Other Southern States. LSU Research Information Sheet #104:13.
- Marta López-Alonso (2012) Trace Minerals and Livestock: Not Too Much Not Too Little ISRN Veterinary Science Volume 2012 (2012), Article ID 704825, 18 pages Hindawi Publishing Corporation <http://dx.doi.org/10.5402/2012/704825>
- Suttle, N. (2010). Mineral Nutrition of Livestock. 4th Ed. Commonwealth Agricultural Bureaux International, Oxfordshire, UK.

Singh A (2010) "Managing internal parasites in organic Livestock," <http://ebookbrowse.com/managing-internal-parasites-in-organiclivestock-pdf-d17044196>.

Niezen, J H, Charleston, W A G , Hodgson, Mackay, A D and Leathwick, D M (1996) Controlling internal parasites in grazing ruminants without recourse to anthelmintics: approaches, experiences and prospects," *International Journal for Parasitology*, vol. 26,no. 8-9, pp. 983–992, 1996.

Tame, M J (2008) "Management of trace elements and vitamins in organic ruminant livestock nutrition in the context of the whole farm system," in *Research Review: Management of Trace Elements and Vitamins*, Institute of Organic Training & Advice, 2008.

**SS-05**

### **Herbal therapy in Veterinary Practice : Recent Advances**

Sudhir R Rajurkar

Professor,

Department of Vet. Pharmacology & Toxicology, COVAS, Parbhani

Ayurveda the oldest system of medicine includes the use of natural resources (Herbs, rock and animal / human sources) in treatment of diseases.

As the advancement in the study 'Ayurveda' then diversified in to three as

Ayurveda : for prevention and treatment of Human diseases

Pashuayurveda : for prevention and treatment of Animal diseases and

Vrukshayurveda : for prevention and treatment of plant diseases.

History of use of medicinal plants in animal diseases dates back to ancient period. The first evidence on use of medicinal plants in treatment of animal diseases is from the period of King "Janaka" i.e. "HASTYAYURVED" a samhita by 'Palkapya muni' includes information on diseases of elephant and its treatment with medicinal plants.

"ASHWAYURVED" also called as "NAKUL SAMHITA" by 'Nakula' one of the pandava gives the information on use of medicinal plants in diseases of Horse.

In those days the meaning of DRUG means "DRY HERB" and whole plant was used as treatment tool.

With the advancement of science study was concentrated on use of particular plant part viz. root, stem, leaves, fruits and seed.

Herbs useful in treatment of human diseases are equally useful in treatment of animal diseases with some exceptions in mode of action and effects.

Use of locally available herbs play very important role in minimising the treatment cost and side effects of therapy.

Use of raw herbs as Liver tonics, Galactagogues, Estrous stimulants, Anti-infective, Anti-inflammatory, Anthelmintic, Carminative, Astringents are common in veterinary practice. These plants are in use since long. But many times we wont get the same results by the same plants and hence standardisation of these treatment tools is must.

So, these herbs were subjected to extraction process and scientists observed that extracted plant shows higher efficacy.

Then after instead of using raw herb focus was given to extraction procedure. This way scientist not only succeeded in improving the efficacy of product but reduced the dose of drug. This success lead to a new concept in Ayurveda i.e. "Sukshaushadhi".

Broadly the extracts of plant means the active group in plant viz. Alkaloids, Glycosides, Saponins, Resins, Tannins, Oils etc. However the "Sukshaushadhi" is use of active component only.

So then after, focus was given on extraction and isolation of active group from the plant. "Sukshaushadhi" is use of active part of the herb in treatment and so new era of isolation of active compound emerged.

Specific procedures for identification, standardisation and isolations of Alkaloids, Glycosides, Saponins, Resins, Tannins etc. are then developed.

Not only this. but, separation of active compound e.g. Withanin, somniferin from *Withania somnifera*, Curcumin from *Curcuma longa* etc. became essential to further minimise the dose of herb and also to assure the uniform activity of herb and further isolation techniques were developed.

Now a days, isolation and use of specific isolate is an emerging technology in herbal research.

At the same time it is also essential to improve and / or use new technology for the faster drug delivery.

Drug delivery means the ability of drug to reach the target site from the site of its administration. Faster is the drug delivery faster and better will be the result of treatment.

There are many barriers in the body which prevent the entry of most of the drug e.g. Placental barrier, blood brain barrier, blood-enzyme barriers etc. these barriers allow only more polar and small sized drugs to enter.

Hence, the concept of use of nano particles as drug delivery system aroused.

Synthesis and coating of metal nanoparticles with herbal extracts and its use in faster drug delivery is the new and advanced approach in herbal research.

Dept. of Vet. Pharmacology & Toxicology, COVAS, Parbhani is working on synthesis, characterisation and coating of metal nanoparticles with herbal extracts and its use as drug delivery system.

This approach will facilitate the rapid reaching of drug molecule at target tissue.

Green synthesis, Coating and characterisation of gold nanoparticles

For the synthesis of gold nanoparticles (GNPs), two clean test tubes were taken, one containing 10ml of 1mM Hydrogen tetra chloro-aurate (Himedia Chemicals, Mumbai) solution as control and the second tube containing 9ml of 1mM Hydrogen tetra chloro-aurate solution and 1ml of *Azadirachta indica* plant leaf extract which was used in all the groups except Group - I animals for the evaluation of toxicity which were coated with *Morinda citrifolia* fruit extract for Group – V, VI, VII, VIII, IX to be used. Both the tubes were incubated at room temperature for 1-2 hours. The change in colour from light yellow to ruby red was observed. The gold nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant was discarded and the pellet then was dissolved in deionised water. The synthesis of gold nanoparticles was confirmed by colour changes and qualitatively characterized by UV-vis spectrophotometry, XRD, FTIR, zeta potential, SEM and TEM images.

The synthesised Gold nanoparticles (GNPs) were then coated with medicinal plant extract, the nanoparticle solution already formed was taken (as per dose) and mixed with the aqueous fruit extract (as per dose). The resulting solution was maintained at room temperature and stirred for 1 hour to covalently modify the surface of GNPs with the phytochemicals present in plant extract.

Gold nanoparticles formed were characterized by UV-Vis spectrometry method, x-ray diffraction method, fourier transform infrared spectroscopy, zeta potential, dynamic light scattering, scanning electron microscopy and transmission electron microscopy imaging method.

The metal nanoparticles so synthesised and coated with different herbal extracts were assessed for efficacy studies *in vitro* and *in vivo* using cell culture and laboratory animal models and were observed to be the most effective when compared with the raw herbs.

Attempts are being made to use the products prepared using this technology in the field conditions, so that sure and safe medication can be done in clinical cases.

**SS-06**

## **RECENT ADVANCES IN DIAGNOSTIC IMAGING TECHNIQUES IN VETERINARY PRACTICE**

Dr. V. D. Aher

Professor and Head, Department of Veterinary Surgery and Radiology,  
College of Veterinary and Animal Science, Parbhani  
MAFSU, Nagpur (Maharashtra)

Medical diagnostic technology has made rapid strides after the advent of computer. Many of the advances in human diagnostic medicines are translated into veterinary medicine in the developed countries. In brief, newer branches like Imaging, Radiodiagnosis, Telemedicine, Telesonography and Teleradiology have emerged. Broadly, the instrumentation/devices devised with the modern technology in the present digital age are listed below.

1. Digital Radiography
2. Image Intensifier T.V. system (I.I.T.V)
3. Ultrasound
4. Magnetic Resonance Imaging (MRI)
5. Computed tomography (CT)
6. Positron emission tomography (PET)

7. Nuclear scintigraphy
8. Laparoscopy
9. Digital Subtraction Angiography
10. Endoscopy
11. Pulse Oximetry
12. Telemedicine & Teleradiology

All these imaging modalities have brought sea change in the diagnosis of a clinical case. Precise and an instant diagnosis of an intricate case can be made with their usage. The modalities which can be used under Indian conditions are:

**1) Digital Radiography:**

Computerization in Radiology in the form of digital X- rays which can be captured on a computer chip and the digitalized image can be viewed interdepartmentally. It provides a vehicle for doctors in remote locations to interact while also enabling doctors in distant locations to share information

**2) Image Intensifier T.V. system:**

Generally used in orthopedics surgery. This facilitates fracture repair using a small incision thus achieving minimal invasive surgical maneuver. IITV helps in X-ray imaging of the intraoperative site for the intraoperative orthopedics manipulations, and the same can be stored for future reference purpose. This facilitates introduction of Steinman pin giving a small incision

**3) Ultrasound:**

In small animal and equine practice, ultrasound is routinely used as a diagnostic aid. Applications of ultrasound in ruminants have not been fully exploited, except in pregnancy. There could be numerous organs which can be scanned using an ultrasound scanner. Ultrasonography seems to have a promising future in veterinary medicine, particularly for the assessment of intra-periabdominal disease. Ultrasonography is viewed as the single most versatile addition to the noninvasive and nonsurgical armamentarium of the veterinary clinician since the advent of fiberoptic endoscope. Although other sophisticated imaging modalities like CT and nuclear imaging can provide additional information, the accessibility and cost effectiveness of these procedures do not make these as promising as Ultrasonography.

#### **4) Magnetic Resonance Imaging (MRI):**

MRI is a highly sensitive and noninvasive technique providing accurate and detailed anatomic images with good contrast and spatial resolution. However, in veterinary medicine MRI is still in its infancy and its use is infrequent. To date, MRI has been used in developed countries in clinical cases as well as a research tool especially for CNS diseases in small animals. MRI has a wide spectrum of application. It can be used for imaging all body regions in small animals, but only the extremities and the head can be imaged in large animals.

It is useful in answering many questions related to the musculoskeletal diseases in animals such as understanding the pathogenesis of navicular disease, traumatic arthritis and osteochondrosis in equines and wobbler syndrome in dogs. The newer applications of MRI are magnetic resonance angiography and magnetic resonance spectroscopy. It is especially used to differentiate an inflammatory process from a neoplastic mass, tumors from peritumoral oedema. It is more specific and sensitive in detecting localizing and differentiating osteomyelitis, cellulites and abscess. However, its use is contraindicated in pregnancy.

#### **5) Computed tomography (C.T.):**

CT has been an extremely significant development which has a unique cross sectional imaging ability useful for the diagnosis of tumors, malformations, inflammation, degenerative and vascular diseases and trauma. CT is a diagnostic modality that is fundamentally different from X-ray method in which an organ is scanned in successive layers by a narrow beam of X-rays in such a way that the transmission of X-ray photons across a particular layer can be measured and by means of a computer, used to construct a picture of the internal structure.

#### **6) Positron emission tomography (PET):**

Positron emission tomography is used to detect certain brain diseases. Similarly to nuclear medicine, a short-lived isotope is incorporated into a substance used by the body such as glucose which is observed by the tumor of interest. PET scans are often viewed alongside computed tomography scans, which can be performed on the same equipment without moving the patient. This allows the tumor detected by the CT scan.

#### **7) Nuclear scintigraphy:**

Nuclear scintigraphy is a highly sensitive advanced procedure in which radioisotopes are used to detect the functional abnormalities of the body system. The interpretation is based on the appearance of the

increased (hot spots) or decreased (cold spots) radioactivity regions viz. an active process is indicated by a hot spot while a dull process like lack of perfusion is indicated by cold spot. Nuclear scintigraphy has been used to detect functional disorders of the kidney, liver, lungs, GI tract, thyroid gland and many other organs. It is very useful in the diagnosis of occult lameness, lung perfusion and ventilation and patency of the ureter in both large and small animals. Also used for vertebral column imaging and monitoring the progress of fracture healing and in tumor detection.

#### **8) Laparoscopy:**

Laparoscopy has been a valuable diagnostic and therapeutic tool in human clinical medicine. Only in the last 15 years, its use has been extensive in various animal species for research and clinical diagnostic and therapeutic purposes. Laparoscopic surgery offers significant advantages over open surgeries in fields of cholecystotomy, appendectomy, vagotomy, hernia repair and adhesion release etc. For gynecological problems like ovarian cyst or in the case of oophorectomies and hysterectomies, laparoscopic surgery (scarless surgery) is now considered a better alternative in addition to laparoscopic sterilization. The most advantageous characteristic of laparoscopy is that it allows direct examination of abdominal cavity with only minimal and superficial surgical intervention.

Thoracoscopy has been employed in man for the diagnosis and treatment of diseases of the pleura, lung, mediastinum, great vessels, pericardium and oesophagus. Visceral inspection of the thoracic cavity by thoracoscopy has been used to provide a more accurate diagnosis and prognosis in horses affected with pleurapneumonia and other thoracic and oesophageal disorders. Thoracoscopy allows visualization and biopsy of a large surface of the lung and provides adequate specimen for histopathological diagnosis.

#### **9) Digital Subtraction Angiography:**

DSA is a radiographic modality which allows dynamic imaging of the vascular system following intravascular injection of iodinated X-ray contrast media through the use of image intensification, enhancement of the iodine signal and digital processing of the image data. Temporal subtraction of the images obtained during the first arterial phase of injection of the contrast medium from the images obtained before and after contrast medium administration yield images which are devoid of bone and soft tissue. This imaging modality plays an important role in highlighting the vascular pathologies like stenosis etc.

#### **10) Endoscopy:**

It is a minimal invasive diagnostic modality which aids in a best way to document mucosal inflammation- hyperemia, active bleeding, irregular mucosal surface, and facilitates biopsy in tubular organs like the GI tract, and respiratory and the urogenital organ systems.

#### **11) Pulse Oximetry:**

Pulse oximetry represents the greatest advance in the patient monitoring. It has the unique advantage of continuously monitoring the saturation of haemoglobin with oxygen, easily and noninvasively, providing a measure of cardiorespiratory function. The fundamental physical property that allows the pulse oximeter to measure the oxygen saturation of haemoglobin is that blood changes colour as haemoglobin absorbs varying amounts of light dependent on its saturation with oxygen. Hence, pulse oximetry remains the standard of care during anesthesia as well as in the recovery room and intensive care unit. A vital part of treating equine problems is an initial accurate diagnosis. High quality images are an important aspect of this. As well fixed and mobile X-ray machines, the Hospital has an image intensifier for intra-operative monitoring with x-rays. The Ausonics Impact ultrasound machine provides high quality images of muscles, tendons and ligaments.

## 12) Telemedicine & Teleradiology:

Veterinary surgeons have a long tradition of consulting one another about problem cases and many have unwittingly practiced telemedication when discussing case by telephone or by sending laboratory reports by telefax. Telemedication and teleradiology extend this further by use of internet.

To conclude, the advances in diagnostic technology in veterinary surgery is in infancy stage in India. An all out effort is required to introduce the basic imaging modality - ultrasound in veterinary practice at district polyclinics and city hospitals. The use of radiology needs to be strengthened by its optimum use in clinical cases.

“The little neglect may breed mischief...for want of a nail the shoe was lost; for want of a shoe the horse was lost; and want of a horse the rider was lost.” - Benjamin Franklin

SS-07

### **Recent Advances in Poultry Management with special reference to back yard poultry**

M.V. Dhumal and M G Nikam

Department of Poultry Science

College of Veterinary and Animal Sciences, Parbhani 431 402

Historically, the base and origin of poultry birds is located in India and its nearby countries in Asia. During those ancient days, they were known as Red Jungle fowl (*Gallus gallus*), Javan Jungle fowl (*Gallus varius*), Grey Jungle fowl (*Gallus sonnerati*), Ceylon Jungle fowl (*Gallus lafayetti*). Today's modern chicken (*Gallus domesticus*) is descendents of the wild species Red Jungle fowl (*Gallus gallus*) found in South East Asia.

Till beginning of 19<sup>th</sup> century, the fowls were maintained mainly for game of cock fighting and scarifying to worship God and Goddess. It was in the early 19<sup>th</sup> century that chickens were raised for their fancy appearance, attractive body forms, plumage colour and comb types. Least attention was paid to their growth and egg production potential.

Commercial poultry keeping was promoted in India's first five year plan (1951-56) with launching of pilot project in Orissa. Later that was transformed into All India Poultry Development programme during second five year programme (1956-61) which is the foundation of modern poultry farming in the country. Under the freedom from hunger campaign, poultry stocks were imported from Australia in 1965 for Government breeding farms. Intensive poultry development projects (IPDPS) were introduced to provide necessary inputs for profitable poultry keeping. All these afford led to rapid increase in the number of commercial farms across the country. The private sector played a commendable role in growth of poultry industry which has made availability of poultry meat and egg in reasonable price even in rural area.

The total poultry population which was only 73.5 million in 1951 has made tremendous growth in during the past 50 years and has reached 729.21 million during 2011. Poultry industry in India is currently growing at 8 to 12% per annum while production of agricultural crops has been growing at the rate of 1.5 to 2.5%. Within the poultry industry, broiler production is growing faster than egg production. About 66.7% of the total output from poultry is realized from poultry meat sector and 33.3% from egg production (Prabhakaran, 2014).

India is the third largest producer of egg in the world while it was ranked only 12<sup>th</sup> during 1980. India's 75% of egg production is consumed by the 25% population living in urban and semi urban areas. The per capita availability of the egg has increased from 5eggs per annum in 1951 to 60 eggs per annum in 2012-13. Whereas National Institute of Nutrition recommends 180 eggs per annum. The back yard sector contributed 33% of the total egg produced (2000), its contribution is declined mainly due to the increased placement of improved layers from years to year. Whereas such pattern of replacement is not for indigenous fowl.

Private sector partnership in broiler production through contract farming system and the vertical integration has played a major role in the growth of broiler industry especially in southern and western states of India like Andhra Pradesh, Tamil Nadu, Maharashtra, Haryana, etc.

### **Production Performance:**

The productivity of commercial hybrid layers has increased significantly over the years due to advances in feeding technology like least cost feed formulation, feed additives, integration of feed production

and advance in health care like vaccines against ND, IBD, IB. The egg number increased from 270 to 340 eggs between 1970 to 2010. Average increase was observed to be 1.8 eggs per year. Both, egg quantitative and qualitative performance have been improved comparative to deshi birds like egg number (28.8%), egg mass (42.7%), egg weight (11.7%) and feed efficiency (32.4%). In case of broilers, there has been a major change in structure, size and number of broiler farms during last 3 decades. Due to modernization of production practices, the FCR has been improved from 2.5 to 1.6, the body weight was achieved at 8 weeks of age during 1980 is now realized in 35 to 40 days of age. This was achieved through the import of pure genetically improved lines, grand parent stocks; least cost feed formulation, introduction of vaccines against major diseases and availability of support services.

#### **POULTRY STATISTICS:**

**Table1. World and India's egg and chicken meat production**

	<b>India's Production</b>	<b>Ref.</b>
Annual Egg production (12-13)	69.73 billion egg	BAHS, 2010, 2014
Annual meat production (12-13)	3.00 million tones	BAHS, 2010, 2014

**Table 2. Average per capita consumption of egg and chicken meat  
(Source: FAOSTAT, 2003)**

	<b>Eggs (Nos)</b>	<b>Poultry Meat (kg)</b>
World	152	11.7
<b>India</b> (Source: Shukla and Nayak, 2015)	<b>61</b>	<b>2.68</b>
Developed countries	280-310	25.3
Developing countries	130	8.0
National Institute of Nutrition recommended consumption	180	11

**Table 3. Present status of Indian Poultry Industry:**

Sr. No.	Particular	Egg	Meat
01	India's Rank	3 <sup>rd</sup>	4 <sup>th</sup>
02	India's total production million tonnes /annum	69.73 billion eggs/annum	3.90
03	Annual growth rate	6-8%	12-15%
04	Per capita availability in India capita/annum	61eggs/capita/annum	2.87 kg/
05	Per capita availability in developed countries meat/capita/annum	280-310 eggs/capita/annum	15.6 kg
06	Recommendations of National Institute of Nutrition meat/ capita/annum	180 eggs/capita/annum	11 kg
07	Contribution to agriculture GDP	12-15%	
08	Total contribution to national GDP	1%	

#### **India's Meat and Egg Production (2016-17):**

##### **It has been reported a very strong growth in meat and eggs**

- Broiler 25 crore birds per month (Approximate . 300 crore)
- Egg production 70 billion per year
- Contribution to national income : 26,000 crore

The ranking of first four countries in the world for broiler production

1. China 2.USA 3. Brazil 4. India

The ranking of first three countries in the world for broiler production

1. China 2.USA 3.India

#### **Regional variations**

One important aspect of poultry development in India is the significant variations in production across regions. Only 4 states of Andhra Pradesh, Tamil Nadu, Maharashtra and Punjab account for over 60% of total egg production. Maharashtra ranks 4<sup>th</sup> in poultry production in India. The per capita eggs and chicken meat availability is highest in southern states, followed by Northern & Western States and lowest in Eastern & Central States.

**Top 10 state in egg and meat production:**

State	Egg production 2012-2013 (in lakh Nos )	Rank	Meat production 2012-13 (*000 tonnes)	Rank
Andhra Pradesh	2,22,973	1 <sup>st</sup>	446	1 <sup>st</sup>
Tamil Nadu	1,19,333	2 <sup>nd</sup>	350	2 <sup>nd</sup>
<b>Maharashtra</b>	<b>45,661</b>	<b>4<sup>th</sup></b>	<b>346</b>	<b>3<sup>rd</sup></b>
West Bengal	47,114	3 <sup>rd</sup>	301	5 <sup>th</sup>
Haryana	42,342	5 <sup>th</sup>	324	4 <sup>th</sup>
Punjab	37,911	6 <sup>th</sup>	79	7 <sup>th</sup>
Karnataka	36,773	7 <sup>th</sup>	38	9 <sup>th</sup>
Orissa	23,229	8 <sup>th</sup>	62	8 <sup>th</sup>
Kerala	22,375	9 <sup>th</sup>	-	-
Uttar Pradesh	17,073	10 <sup>th</sup>	175	6 <sup>th</sup>
Bihar	-	-	37	10 <sup>th</sup>

- Average consumption of eggs in major cities is 170
- Average consumption of eggs in smaller cities is 40
- Average consumption of developed rural areas is 20
- Average consumption of eggs undeveloped rural areas is only 5

**SEGMENTS OF INDIAN POULTRY**

Indian poultry farming is typically classified into three different segments.

1. Backyard poultry
2. Rural Poultry
3. Commercial Poultry

1. Backyard poultry  
A lot of advantages are there for back yard poultry. It gives employment to rural small scale and marginal farmers. Provides additional income to the rural household, helps in enhancing the soil fertility. The poultry products fetches high price compared to commercial poultry farming, provides no or very less investment and it will reduce protein malnutrition in susceptible groups like pregnant women, feeding mothers and children.

Backyard poultry in India is characterized by small flock size consisting of 5-10 predominantly non-descript birds maintained in extensive system under zero input conditions. But fetch the owners much needed animal protein and supplementary income. The importance of backyard poultry is well recognized by Government of India and special programmes are formulated for its promotion. Maintaining small flock of local non-descript fowls under free range condition by landless poor, small and marginal farmers are a common sight in rural areas. These birds are very popular due to their adaptability to local agro climatic conditions and management practices with prominent brooding behaviour, mothering ability and disease resistance. But these birds are small in size, poor in egg production (40-50 eggs/ annum), with small clutch size and prominent brooding behaviour. They are best suited to contribute to the economic benefits of the farmers in their respective home tracts. They are good foragers, efficient mothers, require less cost and special care to grow and thus have characteristics essential for raising poultry under village conditions.

## **2. Rural Poultry Production**

In this poultry keeping, the improved types of birds are used by the farmers having better production potential than the non-descript indigenous chicken. Improved chicken are partially reared under semi-intensive or intensive system for initial brooding period and later on shifted to extensive system of poultry production with supplementary feeding to achieve the production of 150 to 175 eggs per annum. This system requires minimum investment as compare to commercial production but helps in reducing the malnutrition and provides continuous source of income to the farmers.

Small poultry holder is practically capable of more significant contribution to alleviate malnutrition, poverty and unemployment. China's 76% of total egg comes from rural backyard production. India requires both mass production as well as production by masses. The need for development of varieties suitable for rural poultry production in India was visualized by poultry breeders during eighties.

### **Improved varieties of back yard poultry birds**

Understanding the importance of rural poultry farming in India, several research organizations have developed different back yard chicken varieties which have successfully been reared by farmers from many parts of the country. This improved variety includes CARI Nirbheek, CARI Shyama, UPCARI, Vanraja, Giriraja, Gramlakshmi Grampriya, (Pathak and Nath, 2013). Some of improved varieties in rural poultry farming are as below:

**CARI NIRBHEEK:** It is cross of Indian native breed Aseel with Cari Red. Birds are active, larger in built, pugnacious in nature with high stamina and majestic gait. They are able to save themselves from their predators due to its fighting characters and activeness and are adapted to all climatic zones of the country. The standard weight varies from 3 to 4 kg for cocks and 2 to 3 kg for hens. Age at sexual maturity (days) 196 days. Annual egg production (number) 92. An egg weight at 40 week is 50(g).

**CARI SHYAMA:** It is cross of Kadakanath breed of Indian native chicken with Cari Red. Birds have plumage of various colours dominated by black. The skin, beak, shank, toes and soles are dark gray colour. The peculiarity of this bird is that most of the internal organs show the characteristics black pigmentation. Varying degree of blackish colouration is also found in skeletal muscles, tendons, nerves, meninges, brain and bone marrow. The black colour of muscles and tissues is due to deposition of melanin pigment, which causes increase in protein, and decrease of fat and muscle fiber. The meat and eggs are reckoned to be a rich source of protein (25.47% in flesh) and iron. Body weight at 20 weeks is 920 (g). Age at sexual maturity (days) is 180. Annual egg production is 105. Egg weight at 40 week is 49(g), Fertility is 55(%) and Hatchability FES is 52 (%).

**UPCARI:** Indian native chicken with Frizzle plumage has been crossed with Cari Red for development of UPCARI birds. These multicolored birds have single comb and medium body size. Presence of Frizzle plumage helps in fast heat dissipation due to which birds are better adapted to tropical climate especially for arid zones. Age at sexual maturity is 170-180 days. Annual egg production is 165-180. Egg size 52-55 g. Egg colour is Brown. Excellent Internal quality of an egg. Livability is above 95% and temperament is active and good forager.

**Vanraja :** Suitable bird for backyard farming in rural and tribal areas, developed by the Project Directorate on Poultry (ICAR), Hyderabad. It is a multi-coloured dual purpose bird with attractive plumage. It has better immune status against common poultry diseases and is adaptable to the free range rearing. Vanaraja males attain moderate body weight at 8 weeks of age under regular feeding system. The hen lays 160-180 eggs in a laying cycle. Due to their relatively light weight and long shanks, these birds are capable to protect themselves from predators which are otherwise a major problem observed in birds reared in backyards.

**Giriraja :** Developed by Department of Poultry Science, University of Agricultural Sciences, Bangalore, presently Karnataka Veterinary Animal Fishery Sciences University, Hebbal, Bangalore. The bird can be reared for its eggs and meat. It attains maturity from the 22-23rd week after hatching. Hens attain a body weight of about 3 kg and the cocks about 4 kg.

**Krishibro:** Developed by the Project Directorate on Poultry (ICAR), Hydereabad. Multi-colored commercial broiler chicks. Attain body weight by 6 weeks of age with less than 2.2 feed conversion ratio. The survivability of this bird upto 6 weeks of age is around 97%. These birds have attractive colour plumage and are well adapted to tropical weather conditions. The commercial Krishibro has highly resistance against the common poultry diseases like Ranikhet and Infectious bursal disease. Hardy, Well adapted and Better survivability are the advantages of this breed.

### **Managerial Aspect: Technical tips of improved variety**

Improved breeds can be reared in small number (10 to 20) in free range condition if plenty of natural feed resources are available. But for the production of meat they can be reared in large number under intensive or semi intensive condition which requires provision for all inputs similar to commercial broilers. These birds need to be reared under proper nursing management up to 6 weeks and later they may be released in free range.

**Management of chicks:** To maintain the required body temperature, these chicks need brooding during first 3 weeks of age and this also helps in protecting them from predators. Low cost brooding material like wooden material, wooden pole can be used for purpose of brooding. Electric bulbs can be used as a heat source. Chick guards can be used to restrict the movement of chicks nearby the heat source. Under brooder about 60 square inch space is recommended.

Poultry house can be constructed with low cost material like bamboo, wooden planks, sheets, etc. which will be easily available and need minimum expenditure. Complete balanced feed which can meet out all nutritional requirements should be given. Feeder and waterer should be made in such a way that they will be easily accessible to all the birds. Fresh and clean water should be available at all times. The birds should be vaccinated against several diseases like mareks disease, ranikhet disease, fowl pox, etc. It will develop immunity against these diseases (Rath *et al.*, 2015).

**Management of adult birds:** After attaining six weeks of age, the birds can be kept in free range system. These birds are let free/loose in the day time for foraging and only at night time shelter is given. Night shelters should have adequate light, ventilation and protection from predators. Clean drinking water should be provided before letting them out from the night shelter. Under free range conditions generally the birds meet their protein requirement through scavenging but risk of energy deficiency is common. Therefore feeding the birds with some locally available cereals (like maize, bajra, jawar, broken rice) is always advantageous. Similarly, balanced feed as a supplementary/protective feeding @40gm per day is advised which helps in improvement of heath and also plays an important role in egg production. Periodic cleaning of night shelter is essential. Proper deworming should be done at 2 to 3 months interval (Rath *et al.*, 2015).

### 3. Commercial Poultry Production

In this type of poultry production, high yielding strains are used which require scientific management. This system requires high capital. The inputs are high as well as output from this type of farming is high. This system characterised by mass production of egg or meat in very small area as compare to earlier two segments. Intensive system of poultry production is followed in commercial poultry production. Under the intensive system the birds are reared Deep litter methods or in cages.

#### Conclusion:

Poultry farming has become a remunerative business and pre-eminence over all other livestock enterprises in developing countries. It carries a scope for quick and large profit. Most of the poultry development in last three decade is in commercial poultry production which is in the hands of private sector/integrators. In recent years back yard poultry farming production has been extremely emphasized in sustaining and enhancing rural livelihood. Birds are kept in a low input and can be easily managed by family members. There is growing demand about meeting of per capita requirement of animal protein in rural area. Poultry meat and eggs have been proved to be the best and cheapest solution for this. Back yard poultry is the best alternative for small scale farmers to their subsidiary income with negligible input. It is the need of hours to make more awareness among the farmers in the rural area regarding back yard poultry farming.

#### References:

- BAHS (2010). Basic Animal Husbandry Statistics, Ministry of Agriculture, Government of India.
- BAHS (2014). Basic Animal Husbandry Statistics, Ministry of Agriculture, Government of India.
- Pathak, P.K., B.J. Nath (2013). Rural poultry farming with improved breeds of back yard chickens. *J. Worlds Poult. Res.*, 3(1): 24-27.
- Prabhakaran, R. (2014). Indian poultry industry: Current status, practical challenges and Opportunities. XXXI Annual Conference of Indian Poultry Association and National Symposium on Poultry Production for Global Tred. December, 18-20.
- Rath, P.K., K.D. Mandal and P. Panda (2015). Back yard poultry farming in India: A call for skill upliftment. *Res. J. Recent Sci.*, 4: 1-5.
- Shukla, P.K. and S. Nayak (2015). Lead paper presented in XXXII Annual Conference of Indian Poultry Association and National Symposium on Clean and Green Poultry Production November, 19-21.

**SS-08 Recent advances in diagnosis of neoplasms in Animals : An Overview**

S.D. Moregaonkar and G. R. Gangane

Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani

**SUMMARY:**

Neoplasms are common in Indian domestic animals, however they are rarely reported. Due to lack of availability of ready to refer material and guidelines, many a times they prefer to throw these valuable materials in to the dust beans after successful surgical removal. Diagnosis of neoplasm would be perfect if the pathologist is old (experienced). Diagnosis of tumours is a tedious task, but is most important step for not only prognosis of tumour but also for taking a decision for surgical excision of the neoplastic growth. There are many methods which can be employed for diagnosis of neoplasms in animals. Histopathological diagnosis of neoplasm is a golden standard for tumour diagnosis. However, more than one method of diagnosis should be employed for more precise and correct diagnosis of neoplastic conditions in animals. This handout, would be beneficial to the field veterinarians and students as a ready to refer material and also would be useful while writing post-mortem reports of neoplastic conditions.

**INTRODUCTION:**

This short information gathered would be beneficial to the field veterinarians and students as a ready to refer material and also would be useful while writing post-mortem reports of neoplastic conditions.

Neoplasms are common in Indian domestic animals, however they are rarely reported. By using suitable surgical interventions the field veterinarians are routinely removing neoplastic growths. But due to lack of availability of ready to refer material and guidelines, many a times they prefer to throw these valuable materials in to the dust beans after successful surgical removal. This short information gathered would be beneficial to the field veterinarians and students to handle the cases of neoplasm in a more rational way.

A neoplasm (*G, neo = new + plasma = things formed*) is a growth of new cells that proliferate without control, serves no useful function and has no orderly arrangement.

**Oncology** (*G, Oncos – tumor + logos = Study of*): It is a branch of pathology, which deals with study of tumor or neoplasm.

**IMPORTANT POINTS:**

Neoplasia literally means a new growth. Whereas, Tumor (*G, swelling*) literally means a swelling but all swellings are not the tumors, such as haematomas, cysts, nodules or granulomas in chronic inflammation (in tuberculosis, glander etc.), cold abscesses, parasitic nodules etc. Also, all these swellings may subside after removal of the causative agent, while neoplasms grow continuously and indefinitely.

Neoplasm must be differentiated from the inflammatory, reparative processes and also from hyperplasia (particularly microscopically).

As in inflammation and repair cells proliferate but then there is a purpose for proliferation i.e. to protect and replace the lost tissue. Therefore, in these conditions as soon as the need is fulfilled proliferation ceases and the growth even regresses.

Hyperplasia results due to proliferation of cells from a definite demand (in terms of work). for ex. Hyperplasia of erythropoietic tissue occurs in cases of anaemia. Hyperplasia of thyroid occurs in iodine deficiency. Hyperplasia is purposive. It progresses only so long as the functional need or hormonal stimulus, which evoked it, persists.

#### **CLASSIFICATION OF NEOPLASM:**

Different authors have classified animal neoplasms in different ways as under,

- A) According to Chauhan (2003)** neoplasms are classified under the following heads.
- B) According to Vegad (1995)** the neoplasms are classified as,

**DIAGNOSIS OF NEOPLASMS:** Diagnosis of neoplasm would be perfect if the pathologist is old (experienced). Diagnosis of tumours is a tedious task, but is most important step for not only prognosis of tumour but also for taking a decision for surgical excision of the neoplastic growth. There are many methods which can be employed for diagnosis of neoplasms in animals.

**I. CLINICAL DIAGNOSIS:** It is made on the basis of clinical symptoms and is the primary aid in diagnosis

**II. DIAGNOSIS OF CANCER BY BIOPSY EXAMINATION:** Examination of tissue samples obtained from living animals.

Some of these common methods are as under

- A) FNAB or FNAC:**

- B) SCRAPINGS:
- C) SWAB SMEARS:
- D) BRUSHINGS:
- E) EXFOLIATIVE CYTOLOGY:
- F) PUNCH/NEEDLE CORE BIOPSY:
- G) INCISIONAL BIOPSY:
- H) EXCISIONAL BIOPSY:
- I) GRAB SAMPLING:
- J) Nipple aspiration fluid cytology:

### **III. GROSS AND HISTOPATHOLOGY:**

#### **A) Gross description of tumours:**

Many a times neoplastic growth can be a museum of different pathological conditions such as, degenerative conditions, necrosis, calcification, gangrene, cystic condition, different stages of inflammatory conditions etc. Therefore, in description of gross and microscopic changes of a neoplastic growth a basic knowledge of pathology is highly essential. It is also stated that for the diagnosis of neoplasm the pathologist should be old, here oldness means more experienced.

The following points should be taken in to consideration while writing gross description of an organ/ structure / system/ part thereof during post-mortem examination.

(1) Shape (2) Size (3) Surface (4) Colour (5) Consistency (6) On section (appearance of a cut surface) (7) Examination of fluids/ exudates/secretions etc. (8) Relationship to surrounding i.e. position (9) Measurement of fluids /exudates. (10) Description of tubular structures and (11) Weighing of the tumour mass (occasionally) etc.

#### **B) Histoathology:**

- It is the Oldest, yet confirmative tool in diagnosis.
- Helps in understanding the histogenesis,
- Classification of tumors - Nature of tumor
- Staging and grading of tumors
- It is considered as the gold standard in diagnosis of neoplasms

### **IV IMMUNOHISTOCHEMISTRY (IHC):**

- It is an elegant field of recent origin, having tremendous diagnostic significance
- It is useful to know the tissue of origin by using monoclonal and polyclonal antibodies
- Concept is demonstration of antigens with in tissue sections by means of specific antibodies.
- Once the Ag – Ab reaction occurs, it is demonstrated by a colored histochemical reaction visible by light microscope

**For ex: Estrogen Receptors**

- ER positive tumor - influenced by the estrogen hormone
- Anti estrogen therapy can be given

V. FLOW CYTOMETRY:

VI. SOUTHERN BLOT:

VII. IN SITU HYBRIDIZATION:

VIII. APOPTOTIC CELL COUNT:

IX. POLYMERASE CHAIN REACTION: Real-time:

X. CYTOGENETICS- FISH:

XI. MICROARRAY:

XII. PROTEOMICS:

**XIII. DIAGNOSTIC IMAGING:** Some of the common methods are as under,

- A) Radiography:
- B) Ultra Sonography:
- C) Laparoscopy:
- D) Endoscopy:

**XIV. ELECTRON MICROSCOPY:**

**TERMS USED FOR NEOPLASTIC CONDITIONS OF DIFFERENT TISSUES/ ORGANS**

As it has already been stated that different authors have classified neoplasm in different ways, the terms used for description of a particular neoplasm may vary slightly. It is also interesting that in a particular tissue depending upon the involvement of its particular component (mucosa, connective tissue, blood vessels etc.) multiple neoplastic conditions are possible. But in order to have precise information about the widely accepted terms for description of different neoplasm of different organ(s), we have prepared this precise and handy note.

## REFERENCES

- Chouhan, H. V. S. (1997) A text book of Veterinary Pathology, 1<sup>st</sup> Ed<sup>n</sup>, Efficient Printers, New Delhi, pp. 217-247.
- Gopal Krishnarao (2004) A textbook on Tumors of Domestic Animals, 1<sup>st</sup> Ed<sup>n</sup>, Army Printing Press, Luknow, pp. 109-194.
- Sastry, G. A. (1983) Veterinary Pathology, Sixth Ed<sup>n</sup>, CBS Publishers and Distributors, Delhi, pp. 202-266.
- Vegad, J. L. (1995) A Text Book of Veterinary Pathology, first Ed<sup>n</sup>, Vikas Publishing House Pvt. Ltd., Delhi, pp. 204-288.

**SS-09**

### **Recent advances in Immuno-biotechnology**

Dr. S.S. Gaikwad

Department of Microbiology

College of Veterinary and Animal Sciences, Parbhan

#### **Advances in vaccines and diagnostics**

The criteria for successful animal or veterinary vaccines can be very different from those for human vaccines depending on the animal groups under consideration. For example, criteria for companion animal vaccines are similar to those for human vaccines in that the health and welfare of the individual animal are primary concerns. The potential returns for animal vaccine producers are much less than those for human vaccines, with lower sales prices and smaller market sizes, resulting in a much lower investment in research and development in the animal vaccine area than in the human vaccine area, although the complexity and range of hosts and pathogens are greater.

The next generation vaccines tested are DNA vaccine, virus like particles and synthetic attenuation of pathogens. DNA vaccination has been shown to elicit immune responses against many viral, bacterial and parasitic agents. Advantages like an ease of production, safety and thermo stability makes DNA vaccine an interesting approach in animal vaccination.

Virus like particles are derived from recombinant surface proteins which natively fold in virus like structure. This addresses the issue of handling highly virulent pathogen. Same approach is being applied to bacteria i.e. ghost bacteria and bacteria like particles. Synthetic biology innovations have allowed construction of pathogen from its nucleic acid sequences. Due to degenerate nature of codon usage, pathogen prefers specific set of codons. A pathogen which is synthetically de-optimized by creating

mutations its DNA/RNA coding sequence. This virus though encodes same amino acid sequence have altered pathogenicity and work for priming immune system.

New generation diagnostics are innovations in Molecular Biology through Polymerase chain reactions and its variants. Next generation sequencing has caused paradigm shift the way are pathogen are diagnosed rapidly. Tools like lateral flow tests allows rapid and cheap diagnosis at field level. Geographical Information systems based forecasting and prediction of host spots of disease helps in management of disease at community level.

## **Advances in Reproductive Biotechnology**

### **Semen Sexing**

This technology is used for producing offspring of the desired sex. Selecting the sex of the progeny using sex sorted sperm has been an advantage for animal breeders. This technique works on the principle of flow-cytometric separation of fluorescent labeled X chromosome bearing spermatozoa from the sperms carrying fluorescent labeled Y chromosome. This technique has been used in various domestic species including buffaloes Although the number of sorted sperm tends to be low, acceptable pregnancy rates have been obtained by in vivo by deep intrauterine insemination. In addition, semen sexing can be used for enhancing progeny testing program, increase breeding male production, reduce the incidence of sex linked diseases, besides conservation of superior and rare animals.

### **Embryo Sexing**

Embryo sexing is a technique in reproductive biotechnology having practical applications. Various procedures for embryo sexing have been used, viz., biopsy or cells aspiration. For this technique, embryos are collected on day 7 and are washed in buffer saline. Only excellent or good embryos are biopsied or aspirated. Sex determination is performed by Y chromosome specific DNA probe technology coupled with polymerase chain reaction (PCR) amplification of specific Y chromosome region. Other methods involve detection of embryonic HY antigen in the embryos and use of loop mediated isothermal amplification and duplex PCR based assay showing more than 95% accuracy but involves high cost, time and expertise for carrying out these protocols.

This is a noninvasive technique used for recovering large numbers of competent oocytes in live animals. This procedure was first done in cattle which was later tried in other species as well. Embryo production from ovum pickup oocytes is affected by age, season, follicle stimulating hormone (FSH) stimulation and can average 13 embryos developed from oocytes collected per session. It also evident that

repeated OPU can be performed without side effects both in cattle and buffaloes with a minimal stress to the animal. In India, the first buffalo calf (Saubhagya) was produced through this technique first bovine calf (Holi) was produced at ICAR National Dairy Research Institute.

### **Embryo Cryopreservation**

For cryopreservation of embryos, conventional equilibrium methods involving freezing machines with controlled cooling rates and glycerol as cryoprotectant are usually followed. However, pregnancy rates obtained after transfer of the cryopreserved embryos are low. The method involves the use of highly concentrated aqueous solution of cryoprotective agents, viz., glycerol, ethylene glycol, and non permeating agents such as sucrose, glucose, and fructose during no freezing equipment and hence considered superior as compared to slow freezing. This technique is advantageous as it reduces the risk and expense in the transportation of expensive animals; reduce disease transmission and conservation of endangered species germplasm but the survival rate of frozen embryos diminishes thereby causes poor pregnancy rates following embryo transfer.

### **Somatic Cell Nuclear Transfer**

Somatic cell nuclear transfer also termed as “cloning” involves utilization of micromanipulation technique and cell fusion to transfer blastomeres of multicellular embryo or somatic cell into enucleated oocytes. In this technique, nucleus of blastomere is reprogrammed for development of new embryo. It is useful technique used for multiplication of elite animals with minimal genetic variation Animal cloning was used for propagation of valuable genotypes, induce genetic modifications, and for producing transgenics. The first animal obtained by somatic cloning was a sheep “Dolly”. Successful pregnancy and production of cloned calves, viz., “Garima I,” “Garima II,” “Mahima” female calf were born from cloned buffalo “Garima” and male calves, “Shresth” and “Swaran” have been produced in India using handmade cloning technique. Recently, a male cloned calf “Hisar Gourav” was produced at ICARCIRB, Hisar from adult somatic cells of tail obtained from progeny tested bull of superior genetic merit. Cloning holds the promise of bypassing conventional breeding procedures by allowing the creation of thousands of duplicates of genetically engineered animals. It can be used for the conservation as well as propagation of endangered species. It may be used as a tool for the production of stem cells for therapeutic cloning. This technique has opened novel opportunities for genetic engineering, animal genetic diversity conservation, tissue regeneration, and development of targeted ES cells for therapeutics.

## **Stem Cell Technology**

Based on their potency, stem cells are classified into different types, viz., totipotent stem cells, i.e. the cells derived from fertilized egg and can differentiate into embryonic and extraembryonic cell types. The other type namely pluripotent stem cells are the descendants of totipotent cells and can differentiate into cells of the three different germ layers. On the other hand, multipotent stem cells can produce only closely related cells types and unipotent stem cells are those which give rise to only one cell type, but possess the property of self renewal. Likewise, based on their source, stem cells have been classified into three types, viz., embryonic, adult and fetal stem cells. ES cells are derived from embryos at a developmental stage before the time of implantation in the uterus. This is usually during the blastocyst stage (32 cell stage), and these ES cells can give rise to cells from all three embryonic germ layers as they do possess the pluripotency markers. The ESs cells are advantageous as they do not form tumors when transferred into the body which potentiates their use in transplantation. On the other, adult stem cells are those undifferentiated cells found throughout the body which is needed for replenish and regenerate cells in any damaged tissue. Such types of stem cells are found in bone marrow, neural, adipose and olfactory adult stem cells. In addition, there are fetal derived from fetal origin, i.e., amniotic fluid, umbilical cord and blood. In addition, it is also imperative to address the ethical concerns for the use of these cells both of diagnostic and therapeutic in livestock species.

## **Transgenics**

Transgenics involve transfer of gene using various methods within the genome for producing transgenic animals. The transgenic animals carry recombinant DNA within their genome, introduced by intervention. The transferred gene consists of two parts: A functional part and a promoter region for carrying out function in the transfer host. Transgenesis has been carried out in various animals such as mouse, pig, sheep, goat, and cattle. Efforts have been made in buffalo using ES cell like cells isolated from in vitro fertilized and cloned blastocysts. Several methods have been used for gene transfer, viz., pronuclear microinjection, retrovirus based vectors, cytoplasmic microinjection, transferring DNA to embryos or ES cells via retroviral vectors, sperm mediated gene transfer and RNA interference. Transgenic animals can be used both in breeding and also bioreactors.

Tools recently invented like zinc finger endonucleases, TALEN endonucleases and CRISPR- CAS9 endonucleases opens avenues in faster breeding and evaluation of animals. The genome editing allows in vivo change in nucleotide sequence for certain trait. This technique will have widespread effect ranging from mosquito elimination through gene drives and increased milk production encompassing all biology sphere.

## **Nanotechnology**

This technology allows studying biological materials, i.e., cells, fluids in minute quantities. In addition to other field such as cellular biology, bio technology, therapeutic medicine and genetics, it can also be another useful technique in farm animal reproduction. The techniques will be used for biosensor development for disease diagnostics as well as tracking different parameters of animal health in field.

## **Conclusion**

Advances in immunology and Biotechnology will affect all spheres of human life. For Veterinarians these tools allow better, cheaper and faster diagnostics of pathogens, better health management and increased productivity of animals through breeding programmes. The innovations in reproductive Biotechnology is one of the hope for Indian farmers to increase productivity of animals' in these worrying time of climate change.

**Organizing Committee**

**Chief Patron**

**Shri. Anoop Kumar IAS**

Vice-Chancellor

M.A.F.S.U., Nagpur

**Patrons**

**Dr. A.S. Bannaliker**, DI & Dean and Director of Research, M.A.F.S.U., Nagpur

**Dr. A.P. Somkuwar** Director of Extension, M.A.F.S.U., Nagpur

**Dr. N.N. Zade**, Dean, Lower Education, M.A.F.S.U., Nagpur

**Dr. A.G. Karpe** Associate Dean, C.O.V.A.S., Udgir

**Chairperson and Organizing Secretary**

**Dr. (Mrs.) S.B. Majee** Associate Dean, C.O.V.A.S., Parbhani

**Joint Secretary**

**Dr. S.D. Deshpande**

**Treasurer**

**Dr. J.Y. Waghaye**

**Compendium and Editorial Committee**

Dr. M.S. Vaidya Chairman

Dr. G.R. Gangane

Dr. S.S. Gaikwad

Dr. P.R. Suryawanshi

Dr. P.B. Ghorpade

Dr. P.V. Nandedkar

Dr. J.G. Gudewar

Sh. R.V. Ukkalgaonkar

**Reception Committee**

Dr. S.D. Deshpande Chairman

Dr. N.M. Markandeya

Dr. S.U. Digraskar

Dr. M.F. Siddiqui

Dr. M.V. Dhumal

Dr. V.D. Aher

Dr. V.V. Deshmukh

Dr. A. K. Wankar

Dr. K.Y. Deshpande

**Registration Committee**

Dr. M.V. Dhumal Chairman

Dr. S.T. Borikar

Dr. M.G. Nikam

Dr. P.V. Nandedkar

Dr. Mrs. B.K. Bhadane

Dr. S.B. Daware

Dr. N.M. Karad

**Scientific Seminar Committee**

Dr. B.W. Narladkar Chairman

Dr. G.R. Gangane

Dr. J.G. Gudewar

Dr. B.L. Kumawat

Dr. S.S. Gaikwad

**Refreshment and Catering Committee**

Dr.A.R. Deshpande Chairman  
Dr.J.Y. Waghaye  
Dr.P.R. Suryawanshi  
Dr.V.S.Ingle  
Dr M.D. Kharwadkar  
Dr.T.F.Siddiqui

**Campus Beautification Committee**

Dr. M.F. Siddiqui Chairman  
Dr. B. V. Ballurkar  
Dr. P.V. Nandedkar  
Dr. P.J. Kapadnis  
Dr. S. A. Dhaware  
Dr. T.B. Bhuktar  
Dr S A. Katkuri  
Mr. Pramod Tayde

**Accommodation Committee**

Dr. S.R. Rajurkar Chairman  
Dr. S.B. Dawre  
Dr. B.V. Ballurkar  
Dr. B.K. Bhadane  
Dr. N.D. Jadhav  
Dr. V.S. Ingle

**Transportation Committee**

Dr.D.S. Deshmukh Chairman  
Dr.S.V. Londhe  
Dr.P.N. Thakur  
Dr.A.K. Wankar  
Dr B.V. Ballurkar  
Dr S A. Katkuri

**Stage and Decoration Committee**

Dr. V.D. Aher Chairman  
Dr. C.S. Mamde  
Dr. G.D. Ranvir  
Dr. A.P. Patil  
Dr. S.A. Dhawarc  
Dr. Syed Mujeeb  
Dr.P.M.Mane  
Dr.G.P. Dhage  
Dr. T.D. Rasal

**Award and Memento Committee**

Dr. G.D. Ranvir Chairman  
Dr. B.L. Kumawat  
Shri. R.J. Kamble

**Finance Committee**

Dr.J.Y. Waghaye Chairman  
Dr. B.V. Ballurkar  
Dr.S.D. Kadam  
Shri. S.G.Katkar  
Dr. Y.T.Deshmukh

**Fund Raising Committee**

Dr. S.D. Moregaonkar Chairman  
Dr. G.R. Gangane  
Dr. M.G. Nikam  
Dr. P.V. Nandedkar  
Dr. N.D. Jadhav  
Dr. J.G. Gudewar  
Dr. P.R. Suryawanshi  
Dr. Syed Mujeeb  
Dr. K.Y. Deshpande  
Dr. S.S. Gaikwad  
Dr. P.B. Ghorpade

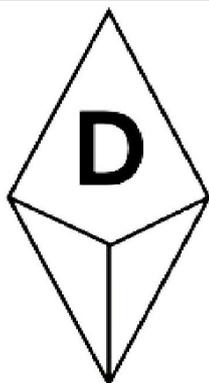
**Cultural Committee**

Dr. N.M. Markandeya Chairman  
Dr. A.R. Deshpande  
Dr. M.G. Nikam  
Dr. J.G. Gudewar  
Dr. M.D. Kharwadkar

**Card Distribution & Publicity Committee**

Dr.V.V. Deshmukh Chairman  
Dr. M.S. Vaidya  
Shri. K.U.Tekale  
Dr. A.P. Patil  
Dr. S.S.Daga  
Sh.R.V. Ukkalgaonkar

# **Advertisements**



## **ADVY CHEMICAL PVT. LTD.**

**CORPORATE ADDRESS:**

17th floor, Hoechst House, Nariman Point, Mumbai - 400 021, India.

Tel: +91-22-6656-0900

**PLANT ADDRESS:**

Plot No. A-334 / 336 / 338,

A-337 & A-339, Road Nos. 25 & 26, Wagle Industrial Estate,

Thane - 400 064. Maharashtra, India.

Phone: +91-22-2583-0326, Fax: + 91-22-2583 0311

E-mail: [sanjeev.lala@advychemical.com](mailto:sanjeev.lala@advychemical.com) /

[syd.daftary@advychemical.com](mailto:syd.daftary@advychemical.com)

Advy Chemical Private Limited (ACPL) was established in India in 1991. Indigenous pioneers in the development of polyclonal antibodies, native human proteins and diagnostic kits. We are catering to the requirements of diagnostic industry worldwide.

Consistently high quality, reproducible results and a flexible approach to address the needs of each customer has helped us to emerge as the provider of choice to IVD manufacturers' world over. Advy Chemical exports its products around the world, significantly to USA, Europe, Japan and China.

We have the necessary expertise to manufacture rapid diagnostic test kits. The range of rapid diagnostic test kits includes Malaria Antigen & Antibody Rapid Tests and Dengue Rapid Diagnostic Tests.

- **Strive to meet the needs and expectations of our customers – customization is a hallmark of Advy's services**
- **Compliance to regulatory requirements**
- **Certified for ISO 9001: 2008 and EN ISO 13485:2012**
- **We are listed in the EU TRACES database for exporting the antisera products to EU. Farm with over 800 goats**
- **Contract manufacturing services available on request**
- **Fully equipped facility for large scale manufacturing of Point-Of-Care Tests**
- **Largest manufacturer of C-Reactive Protein and Alpha-1 Microglobulin globally**

Virbac



For further information, please contact:  
**VIRBAC ANIMAL HEALTH INDIA PVT. LTD.**  
604, 6th floor, Western Edge 1, Magathane, Western Express Highway, Borivali East, Mumbai – 400066.  
Tel: (022) 4008 1333. Web: [in.virbac.com](http://in.virbac.com)



# LEADERS IN ANIMAL HEALTH...

*Herbal  
is Healthy*

## Food Safety With Sustainability



**Cultivate  
Quality  
Medicinal  
Plant**

**Advancing the  
knowledge of  
Herbs for  
Animal  
Health**

### Water Conservation for Sustainable Development



**AYURVET  
LIMITED**

Registered Office: 4th Floor, Sagar Plaza, Distt. Centre Laxmi Nagar, Delhi, INDIA-110 092.  
Phone: 011-22455993 • Email: info@ayurved.com • Website: www.ayurved.com

Corporate Office: Unit No. 101-103, 1st Floor, KM Trade Tower, Plot No. H-3, Sector-14, Kaushambi, Ghaziabad-201010 (U.P.)  
Phone: 0120-7100201 • Fax: 0120-7100202

Reg.No.MAHA248/200/F-5363 (Parbhani)

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपुर मान्यता प्राप्त  
कै.प्रमिलाताई देशमुख सेवाभावी संस्था, संचलित

**मुक्तेश्वर पशुधन व्यवस्थापन व  
दुग्धोत्पादन पदविका विद्यालय**

• हट्टा ता.वसमत जि.हिंगोली •

**मा.डॉ.राहुल वेदप्रकाश पाटील**  
आमदार, परभणी

अध्यक्ष  
**परिहार कमलाबाई बालकिशन**

सचिव  
**हुलगे सविता रमेश**

प्राचार्य  
**दहीफळे हनुमंत व्यंकटराव**

Contact : 0-2452 227568 9689200993 imdpschoolhatta@gmail.com

Reg.No.MAHA/67/99/Beed/Dt.21/01/1999

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपुर मान्यताप्राप्त  
भैरवनाथ निसर्ग मंडळ, संचलित

**पशुधन व्यवस्थापन व  
दुग्धोत्पादन पदविका विद्यालय**

• पिंगळी ता.जि.परभणी ४३१४०१ •

**मा.डॉ.वेदप्रकाश के.पाटील**  
मा.कुरलपुर, व.ना.म.कृ.वि.परभणी तथा

अध्यक्ष

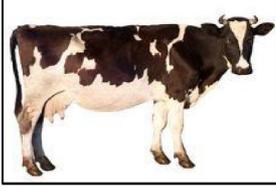
सचिव  
**मा.प्रतापसिंह वेदप्रकाश पाटील**

प्राचार्य  
**डॉ.बळवंत विनायक गुजर**

उपसचिव  
**मा.डॉ.राहुल वेदप्रकाश पाटील**  
आमदार, परभणी

Contact : 0-2452 227568 9881487813 imdpschoolpingli@gmail.com

ग्रामिण विकास प्रतिष्ठान, अंबड संचलित  
पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका अभ्यासक्रम



अंकुशनगर, ता. अंबड जि. जालना

अभ्यासक्रम मान्यता :- जा.क.अ.अ.एक्स आर-9 दि. २६/०७/२०००

दुरध्वनी क्र. ०२४८३-२७९६३९ ईमेल आयडी -csjrmcvc@gmail.com

श्री. बरसाले बी.जी.

प्र.प्राचार्य

मा. आ. राजेश अंकुशराव टोपे

(माजी उच्च व तंत्रा शिक्षण मंत्री म.रा.)

अध्यक्ष

विद्यालयाची वैशिष्ट्ये :-

१. सुसज्ज इमारत.
२. तज्ञ शिक्षक.
३. अत्याधुनिक प्रयोगशाळा.
४. नियमित प्रात्यक्षिक.
५. डेअरीफार्मला वेळोवेळी भेटी.
६. भव्य क्रिडागंगण.
७. निसर्गरम्य वातावरण.

महाराष्ट्र पशु व मस्य विज्ञान विद्यापीठ नागपुर मान्यता प्राप्त  
महात्मा फुले शैक्षणिक, सामाजिक, सांस्कृतिक व क्रिडा मंडळ जामखेड संचलित  
पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय जामखेड  
ता. जामखेड जि. अहमदनगर

ए-वन कृषी फार्म, कर्जत रोड जामखेड, ता. जामखेड जि. अहमदनगर ४१३२०१

Web Side :- [www.mahatmaphule.in](http://www.mahatmaphule.in)

Email:- [imdjamkhed@gmail.com](mailto:imdjamkhed@gmail.com)



प्राचार्य

प्रा. शेख अलताब ह.

९४२३६५३२४४

९४२३७५३२४४



अध्यक्ष

श्री. भानुदास बोराटे

९४२१५७९७३०

महाराष्ट्र पशु मत्स्य विज्ञान विद्यापीठ, नागपुर-मान्यता प्राप्त  
श्री.विठ्ठलगड शिक्षण प्रसारक मंडळ, सारणी(सां)संचलित  
**पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय,**  
देवगांव ता.केज जि.बीड

(02442) 274640

mail id-lmdpdevgaon@gmail.com



**विद्यालयाची वैशिष्ट्ये-**

१. प्रशस्त इमारत
२. कुक्कूट पालन
३. समृद्ध ग्रंथ संपदा
४. गाय व म्हैस पालन
५. उज्वल यशाची परंपरा
६. मुला व मुलींचे स्वतंत्र वस्तीगृह
७. तज्ञ व अनुभवी प्राध्यापक वृंद
८. अर्ध बंधीस्त शेळी पालन



श्री. विक्रम(बप्पा)मुंडे  
संस्था कार्याध्यक्ष



मा. श्री. विजयकांत विक्रमराव मुंडे  
अध्यक्ष



श्री. बळवंत भिमराव बागलाणे  
प्राचार्य



श्री. ठोंबरे व्ही. वाय



श्री. कानडे पी. ए



श्री. भोजने बी. एस



Gramin Shikshan Prasarak Mandal's



Founder

Vasantro Naik Gramin College Of Agriculture Neharunagar  
Tq.Kandhar Dist. Nanded - 431742

Shivrimji Pawar Gramin Institute Of Agri. Engineering & Technology  
NEHRUNAGAR  
Tq.Kandhar Dist. Nanded - 431742

Shivrimji Pawar Gramin Institute Of Food Technology Neharunagar  
Tq.Kandhar Dist. Nanded - 431742

Gramin Polytechnic  
Vishnupuri, Nanded

Gramin College Of Engineering  
Vishnupuri, Nanded

Gramin Science (voc.) College  
Vishnupuri, Nanded

Gramin I.T.I. Neharunagar  
Tq.Kandhar Dist. Nanded - 431742

Gramin Krishi Vidhyalaya Neharunagar  
Tq.Kandhar Dist. Nanded - 431742

Gramin Livestock Management And Dairy Production School Neharunagar  
Tq.Kandhar Dist. Nanded - 431742

Gramin I.T.I.  
Malakoli Tq. Loha

President

**Sanjay Pawar**

Mob. : 9423233333, 9823338333

Secretary

**Vijay Pawar**

Mob. : 9422171151

Reg.No.MAHA248/2001

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपुर मान्यता प्राप्त  
कै.प्रमिलाताई देशमुख सेवाभावी संस्था, संचलित

**अशोका पशुधन व्यवस्थापन व  
दुग्धोत्पादन पदविका विद्यालय**

• वाघी • ता.जि.नांदेड •

मा.डॉ.राहुल वेदप्रकाश पाटील  
आमदार, परभणी

अध्यक्ष  
परिहार कमलाबाई बालकिशन

सचिव  
हुलगे सविता रमेश

प्राचार्य  
डॉ.खंदारे विजयमाला गंगाधर

Contact : ☎ 0-2452 227568 📠 9890020084 ✉ lmdp.ashokadairy@gmail.com

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
विवेक वर्धिनी सेवाभावी संस्था, संचलित,  
रामकृष्ण पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय, मोरेवाडी.  
ता.अंबाजोगाई, जि. बीड-४३१५१७.  
संपर्क:- ९९६०७१३०७१

जाहिरात

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापिठ, नागपुर मान्यताप्राप्त  
श्री. मारुतराव घुले पा.शिक्षण संस्थेचे  
जिजामाता पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय  
ज्ञानेश्वरनगर (भेंडे) ता.नेवासा जि.अहमदनगर  
फोन नं. ०२४२७-२५५१६६ (कायम विनाअनुदानित)

अ.न.	अभ्यासक्रमाचे नाव	कालावधी	किमान शैक्षणिक पात्रता
१	पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका	२ वर्षे	इयत्ता १० वी उत्तीर्ण

विद्यालयाची वैशिष्ट्ये:-

- १) स्थापना सन १९९४
- २) अनुभवी,सेवाभावी व तज्ञ प्राध्यापकवर्ग
- ३) निसर्गरम्य व प्रसन्न परिसर
- ४) सर्व सोयींनी युक्त इमारत
- ५) प्रात्यक्षिकांसाठी सुसज्ज प्रयोगशाळा,चिलिंग प्लँट,कृत्रिम रेतन योजना,पशुवैद्यकिय दवाखाना, डेअरी फॉर्म, पोल्टी फार्म व शेळी फार्म.
- ७) उत्कृष्ट निकालाची उज्वल परंपरा.
- ८) उत्तीर्ण विद्यार्थ्यांसाठी नोकरीविषयक व व्यवसायाबद्दल संपूर्ण मार्गदर्शन.
- ९) कॉम्प्युटर प्रशिक्षणाची सोय उपलब्ध
- १०) मेस व वसतीगृहाची सोय
- ११) क्रिडासाहित्यासह भव्य क्रिडांगण
- १२) विद्यालयास विद्यापिठाकडून अ दर्जा मानांकन.



(श्री.मते एस.एल.)

प्राचार्य

जिजामाता पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय  
ज्ञानेश्वरनगर (भेंडे) ता.नेवासा जि.अहमदनगर

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
श्री धनेश्वरी मानव विकास मंडळ संचालित,  
कै. मुरहरराव पाटील पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय,  
गेवराई तांडा, ता. जि. औरंगाबाद. ४३१००२.

डॉ. वेदप्रकाश काशीराव पाटील  
अध्यक्ष

डॉ. उदयसिंह वेदप्रकाश पाटील  
कार्यकारी अध्यक्ष

संपर्क:- ०२४०-२४८०४५१, मो- ७३८७९२७७७७

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
सुभाषचंद्र घोस मिशन, संचालित,  
ओंकारेश्वर पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय,  
पिसादेवी, ता. जि. औरंगाबाद. ४३११०५.  
संपर्क:- ९९६०३१८४०१

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
सुभाषचंद्र बोस मिशन, संचलित,  
शिवशंकर पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय, पालफाटा,  
ता. फुलंब्री, जि. औरंगाबाद ४३११११.  
संपर्क:- ९७६६४३६७७७.

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
जयभवानी शिक्षण प्रसारक मंडळ, संचलित,  
सिध्देश्वर पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय,  
सातारा, ता. जि. औरंगाबाद ४३१००१.  
संपर्क:- ८६६८३३९०९८.

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
जगदंबा शिक्षण प्रसारक मंडळ, संचलित,  
घृष्णेश्वर पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय, वाळुज.  
ता. गंगापूर, जि. औरंगाबाद ४३११३३.  
संपर्क:- ८८०५०००७१०

महाराष्ट्र पशु व मत्स्य विज्ञान विद्यापीठ, नागपूर, मान्यता प्राप्त  
पशुवैद्यक व पशुविज्ञान महाविद्यालय, परभणी, अंतर्गत  
सर्वोदय शिक्षण संस्था, संचलित,  
माऊली पशुधन व्यवस्थापन व दुग्धोत्पादन पदविका विद्यालय, खरवंडी.  
ता. नेवासा, जि. अहमदनगर ४१४६०२.  
संपर्क:- ७४८००८०८०८

See like you have never seen before

 **TOWA**  
Towa Optics (I) Pvt. Ltd.  
223 Okhla Industrial Estate Phase-3  
New Delhi-110020  
towa@towaoptics.com



**N-SIM**

**N-STORM**



Ensures consistent, predictable performance for your flock.

**Benefits of Celmanax :**

- ✓ Improve digestibility, gut health and gut morphology.
- ✓ Promote growth without antibiotics.
- ✓ Condition marginal feeds.
- ✓ Enhance the immune system.
- ✓ Protect against *E.coli* and Coccidiosis.



For more details contact:



Corporate office: 129, 2nd Floor, Patparganj Industrial Area, Delhi-110092  
Customer Care No.: +91-9212309678  
Email: info@saifevetmed.com, Website: www.saifevetmed.com



of GI transit, lag period, regional release and absorption time, suitability in plasma level in few animal models. This information is to assess regional absorption pattern, variables such as pH, time, repeat dosing etc.

Role in formulation development: Different types of delivery formulations can be evaluated *in vivo* in three to four animals at a time to assess its repeatability and evaluation of formulation to choose which better one is. This will help to minimize time in development of molecule.

Potential alternative portals of drug entry to the systemic circulation include the buccal, sublingual, nasal, pulmonary and vaginal routes. These routes are also being studied for the local delivery of drugs directly to the site of action, thereby reducing the dose needed to produce a pharmacological effect and also possibly minimizing systemic side-effects / toxicities.

Many a time's formulations can be evaluated for its beneficial effects as well as detrimental effects (toxicological) on various blood components, alteration or change in bio-distribution behavior of known radiopharmaceuticals. This will be possible to demonstrate by *in vitro* and *in vivo* studies in animal model. Many ayurvedic or herbal formulations can be evaluated for the same purposes to demonstrate their efficacy to prove the claims.

Microdose studies are designed to evaluate pharmacokinetics or imaging of specific targets and are designed not to induce pharmacologic effects. Because of this, the risk to human subjects is very limited. A *microdose* is defined as less than 1/100th of the dose of a test substance calculated (based on animal data) to yield a pharmacologic effect of the test substance with a maximum dose of <100 micrograms (for imaging agents).

Gamma scintigraphy or tracer kinetic study can be used for the sequential imaging after radiolabelling of molecule for:

**Release of the drug from:**

- o Stomach (micro particulate, muco-adhesive formulations etc.),
- o Conjunctival sac (microtab etc.)
- o Per-rectal formulations for residence
- o Colonic targeting etc.
- o Distribution and the retention time of a novel bioadhesive intravaginal delivery system