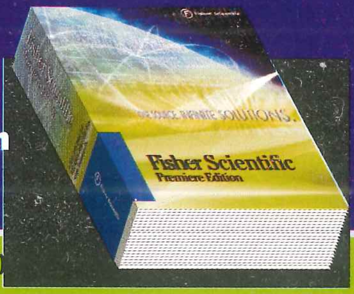
International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation"

&

XVII Annual Convention of Indian Society of Veterinary Immunology & Biotechnology

29 - 31 December, 2010

Department of Veterinary Microbiology & Biotechnology
College of Veterinary & Animal Science
Rajasthan University of Veterinary & Animal Sciences
Bikaner-334001(Raj.)



International Symposium on "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation"

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XVII Annual Convention of Indian Society of Veterinary Immunology & Biotechnology

29-31 December, 2010

-:: Editor ::-

Dr. G.S. Manohar
Dr. S.K.Kashyap
Dr. S.Maherchandani
Dr. Ashok Dangi

Department of Veterinary Microbiology & Biotechnology
College of Veterinary & Animal Science
Rajasthan University of Veterinary & Animal Sciences
Bikaner-334001(Raj.)

XVII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology

Patron
Prof. A.K.Gahlot
Vice Chancellor
RAJUVAS, Bikaner

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Dr. R.N.Kacchawa Head, Dept. of Livestick Products Technology	
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Catering Committee
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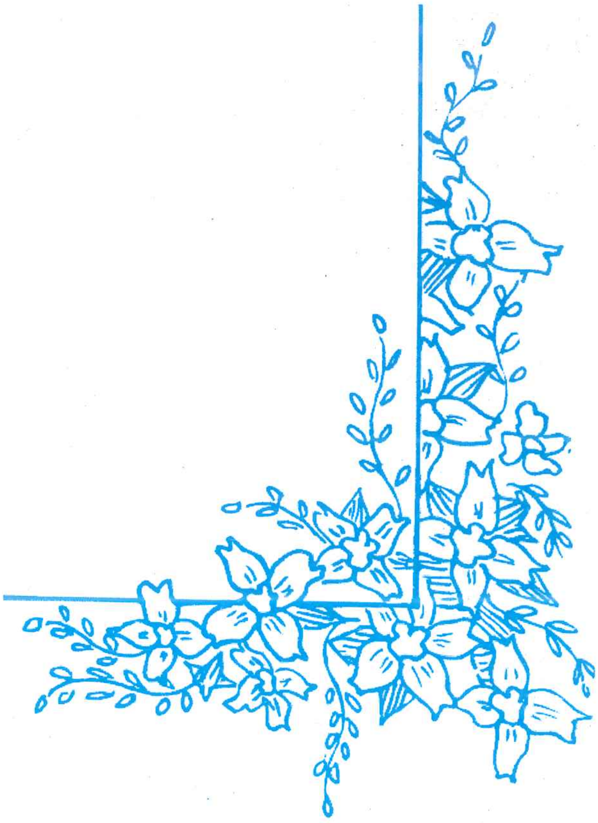
Dr. A.P.Singh
Dr. J.S.Mehta

Messages



Ashok Gahlot
Chief Minister, Rajasthan

सत्यमेव जयते

CHIEF MINISTER
RAJASTHAN

Message

It gives me great pleasure to note that Rajasthan University of Veterinary and Animal Science, Bikaner is going to host an International Symposium on the topic 'Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation' and is also organizing the 'XVII Annual Convention of India Society for Veterinary Immunology and Biotechnology' from December 29 - 31, 2010.

Biotechnology can play a major role in conserving biodiversity and also in developing livestock which, in turn, have the potential of ensuring food security and poverty alleviation. Biotechnology can offer means to improve our biodiversity. In view of the growing demand for food, we need to explore new avenues of sustained production. Biotechnology can contribute a lot in this direction.

I hope that the deliberations at the conference would further explore the role of biotechnology in enriching the popular breeds of livestock of the state.

I send my good wishes for the success of the conference as well as for the publication of the compendium on this occasion.

(Ashok Gehlot)

Dr. S. Ayyappan
Secretary & Director-General

सत्यमेव जयते

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद्
कृषि मंत्रालय, कृषि भवन, नई दिल्ली 110 014
GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE, KRISHI BHAWAN, NEW DELHI 110 114
Tel. : 23382629; 23386711 Fax : 91-11-23384773
E-mail : dg.icar@nic.in

Message

It is a pleasure to know that College of Veterinary and Animal Science, Rajasthan University of Veterinary & Animal Science, Bikaner is organizing International Symposium on "Role of Biotechnology in Conserving Biodiversity and Livestock Development for Food Security and Poverty Alleviation" and XVII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology (ISVIB) at Bikaner during 29-31 December, 2010.

India is endowed with rich floral and faunal biodiversity and remains central to agricultural development. Our bio-diversity has evolved in different environments and is a rich treasure of genetic resources. We must conserve and preserve these genetic resources while using them in rational manner for sustainable farm growth.

I wish the Symposium all success.

(S. Ayyappan)

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

भारतीय कृषि अनुसंधान परिषद्
कृषि भवन, डा. राजेन्द्र प्रसाद मार्ग, नई दिल्ली–110114
Indian Council of Agricultural Research
Krishi Bhawan, Dr. Rajendra Prasad Road, New Delhi-110114
Phone : (O) 23381119, 23388991-7 Extn. : 200
Fax : 0091-11-23097001, 23387293
E-mail : ddgas.icar@nic.in

Message

It is a matter of great pleasure to know that the newly established Rajasthan University of Veterinary and Animal Science, Bikaner is organizing International Symposium and XVII Annual Convention of Indian Society of Veterinary Immunology and Biotechnology on a very relevant theme, "Role of Biotechnology and conserving biodiversity and livestock development for food security and poverty alleviation" from December 29-31, 2010.

As has been accepted by United Nations, sustainable use of biological resources is based on conservation of biodiversity. The entire biomass on earth is a result of an evolution spanning more than 3.5 billions of years and encompassing more than 10 million species. The growing human population burden on the earth has created danger of loss of this biowealth much faster than ever before. Convention on Biodiversity adopted by United Nations and came in force from 1993 has brought a radical change in terms of recognition of all varieties of biospecies within a State as its sovereign right. Current knowledge about the consequences of biodiversity loss in actual ecosystems is limited, particulary when considering large ecosystems and changes in biodiversity. Biotechnology with its diverse tools for identification at definitive levels, large scale genetic manipulation and preservation can significantly contribute to conservation of biodiversity in general and animal genetic resources in particular.

I hope that the scientific deliberations in the international symposium will highlight the dangers of loss of biodiversity and suggest concrete measures to overcome these.

I wish the symposium a great success.

(K. M. L. PATHAK)

Dr. Arvind Kumar
Deputy Director General
(Education)

भारतीय कृषि अनुसंधान परिषद्
कृषि अनुसंधान भवन–II, पूसा, नई दिल्ली 110 012
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
KRISHI ANUSANDHAN BHAWAN-II, PUSA, NEW DELHI-110 012
Ph. : 011-25841760, Fax : 011-25843932
E-mail : ddgedn@icar.in; Website : www.icar.org.in

Message

I am glad to learn that Rajasthan University of Veterinary and Animal Sciences, Bikaner is hosting an Internation Symposium on " Role of biotechnology in conserving biodiversity and livestock development for food security and alleviation" and XVII Annual Convention of Indian Society and Veterinary Immunology and Biotechnology, from 29-31 December, 2010 at Bikaner. The topic selected is of great relevance considering the important of livestock in the overall economy of the Country.

India is one of the 12 mega diversities of the world having 7.8% of the global recorded Species. Being the treasure of vast animal and plant biodiversity, the Country has greater strength to realize the dream of ever green revolution. It is crucial to conserve biodiversity and in this endeavor, available biotechnological tools need to be fully exploited for their in situ and ex-situ conservations, particularly in the era of Climate change. The local communities play a greater role in preserving the biodiversity and sustainable utilization of biological resources through traditional knowledge, practices and innovations.

I am sure that the present symposium would provide a common platform to the researchers, scientists, and development planners to deliberate on the emerging issues and exchange ideas and come out with specific recommendations that could be useful in not only conserving the biological resources but also utilize them fruitfully for developing the breeds/varieties that are high yielding and resistant to environmental stress. I believe that the Compendium will prove a knowledge source for various stakeholders, particularly in the livestock sector.

I wish the symposium a grand success.

(Arvind Kumar)

Dr. A. L. Chaudhary
President

Veterinary Council of India
(Statutory body of Government of India)
A-Wing, 2nd Floor, August Kranti Bhawan
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Fax: 011-26182434
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website: www.vci.nicin
www.vci-india.in

Message

It gives me immense pleasure to note that the Rajasthan University of Veterinary and Animal Sciences, Bikaner is organizing International Symposium on 'Role of Biotechnology in Conserving Biodiversity and Livestock Development for Food Security and Poverty Alleviation" & XVII Annual Convention of Indian Society for Veterinary Immunology and Biotechnology (ISVIB) during 29 - 31 December, 2010.

I take this opportunity to complement the organizers of the Seminar for choosing this important and relevant topic. Biotechnology has proved to be an important tool in conserving biodiversity. It would be the right approach to harness this potential in ensuring food security for millions of countrymen through livestock development.

I hope, this Seminar will provide a platform for effective interactions among the veterinary educationists & scientist from all over the country, and the deliberations will be very useful in developing strategies and policies at appropriate levels.

I wish the Seminar a grand success.

(Dr. A.L Chaudhary)

Prof. K. S. Palaniswami
Former Director of Research TANUVAS
and Secretary ISVIB

Indian Society For Veterinary Immunology And Biotechnology
Madras Veterinary College
Chennai-600 007

Message

Greetings to all:

We are extremely pleased to hold XVII Annual Convention and concurrent International Symposium on role of Biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation at Rajasthan University of Veterinary and Animal Sciences, Bikaner. This University is a newly constituted body inherited with an extremely rich livestock wealth of the state. The campus rich in heritage of red sandstone buildings leave an indelible impression on visitors.

If the 20th Century was an expansive era seemingly without boundaries, the early years of the 21st century have showed us the limits of our small world. The demand for oil, battle over water, shrinking biodiversity, mortal threats and feeding a warming world have compelled us to make the life designed to order by ways of conservation of the biodiversity and power of biotechnology for saving the future.

We, the visionary scientist are committed to face these challenges and pursue for excellence. A commitment that does not rest on consistency but on bettering our performance year after year. With this object in mind I wish a very successful symposium. We also convey our best wishes for greater growth of this infant university.

(Prof.K.S.Palniswami)

Dr. A. K. Gahlot
Vice-Chancellor

Vice-Chancellor
Rajasthan University of Veterinary & Animal Sciences
Bikaner
Bijey Bhawan, Near Pt. Deen Dayal Upadhyay Circle,
Bikaner-334001 (Raj.)
Tel. : 0151-2543419(O), 2549348(Fax)
E-mail : vcrajuvas@gmail.com; ajeygahlot@hotmail.com

Message

It is a matter of pride that Rajasthan University of Veterinary and Animal Sciences, Bikaner is organizing, International Symposium & XVII Annual Convention of Indian Society for Veterinary Immunology & Biotechnology "Role of biotechnology in conserving biodiversity and livestock development for food security and poverty alleviation" from 29th to 31st December, 2010.

Rajasthan, especially the Bikaner region entails a unique ecosystem with a great variety of genobiome making it an interesting model of understanding biodiversity rich livestock wealth of the state that resulted in establishment of new university in Rajasthan, i.e. RAJUVAS, also needs to be considered in terms of conservation of genome in context to the unique environment of the region. The last 3 decades have witnessed knowledge revolution in the field of basic molecular biology as well as applied biotechnology that has risen the expectations and hopes of society especially pertaining to improvement of human and animal health and sustainable development of human civilization. Livestock wealth which already contributes significantly to the national/state economy may further accelerate its growth if setting up of the value added livestock product industries like food processing and others is facilitated and encouraged in the country which may ultimately prove beneficial to livestock farmers and help alleviate poverty in rural folks.

In this context, the theme of the international symposium on which scientists are going to present their findings /views is indeed the need of the hour and requires threadbare analysis.

I believe that scientific deliberations and discussions at this occasion will prove useful to scientific community, animal lovers and livestock farmers.

(A.K.Gahlot)

Dr. S. B. S. Yadav
Dean

College Of Veterinary & Animal Science, Bikaner
Rajasthan University Of Veterinary & Animal Sciences
Bikaner
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E-mail: yadavsbsmaiLcpmicvas9O1(a,nail.com

Message

Organizing an International Symposium on "Role of Biotechnology for conserving biodiversity and livestock development for food security and poverty alleviation" is truly a commendable step of Rajasthan University of Veterinary & Animal Sciences, Bikaner within seven months of its birth. Biotechnology is the focal issue to be discussed in the symposium by a galaxy of eminent scientists, researchers and planners coming from the country and abroad, for the conservation of biodiversity related to production, distribution and consumption of available natural biological resources which are being lost at an alarming rate.

We have a large variety of livestock and poultry besides cultivated and wild flora and fauna which serve as backbone of rural economy particularly in under developed nations. Food insecurity and poverty are the major problems being faced by the developing countries and biotechnology can open up new dimensions to resolve these problems and evolve new initiatives to conserve biodiversity and optimum utilization of biotechnolocal tools for achieving sustainable food security.

Biotechnology and biodiversity are two areas which would be useful for identifying and mining genes to combat stress due to extremes of temperatures, bacterial, viral, fungal and parasitic diseases, increased metabolic and production levels and other biotic and a-biotic factors. An amalgamation of such identified genes and gene combinations would help in resolving permanent solutions for food security and poverty alleviation.

I am confident that through interaction of specialized scientists, administrators and executives in this international symposium, recommendations would be evolved for the overall benefit of all kind of diversified varieties of germplasm useful to serve mankind and to maintain ecological balance.

I wish the symposium a grand success.

(S.B.S.Yadav)

RAJUVAS : AT A GLANCE

The Rajasthan University of Veterinary & Animal Sciences, Bikaner has become a separate entity on 13th of May, 2010 consequent to carving out of units pertaining to veterinary & animal sciences from Swami Keshwanand Rajasthan Agricultural University, Bikaner and Maharana Pratap University of Agriculture & Technology, Udaipur, in accordance to commencement of the Act of this University. Professor A.K. Gahlot has been appointed as the First Vice-Chancellor of the University. Within a short span of its existence, the University is striding ahead towards its progress by leaps and bounds under the able guidance, dynamic leadership, positive approach, innovative ideas and administrative flair of the Vice-Chancellor. The University got approval of the Government to open a new Constituent Veterinary College at Jodhpur. It has also added two more constituent institutes for providing two years' Diploma in Animal Husbandry at Jaipur and Vallabhnagar (Udaipur). One more affiliated Veterinary College at Dungarpur has been allowed admissions by the VCI.

The University is located in a palatial building and its precincts spreading over 200 acres of land. The University has sophisticated equipments and well equipped facilities for execution of major three organs of the University, i.e., Teaching, Research and Extension. Under this University, there are now three constituent colleges, one each at Bikaner, Navania, Vallabhnagar (Udaipur) and Jodhpur, where 60 students in each college are admitted annually besides a total of 25 payment seats. Rajasthan is the first state in the country to have Public Private Partnership mode in Veterinary Education. Under this innovative step of the state, there are seven private veterinary colleges affiliated to this University in the state. The State would be producing nearly 500 veterinarians every year in the service of animal health care which is the backbone of rural economy of Rajasthan.

The College of Veterinary and Animal Sciences, Bikaner was established in 1954 in heritage buildings. The college awards undergraduate degree of BVSc & AH recognized by VCI and offers MVSc course in 15 subjects and PhD by course work in 11 disciplines. There are two Livestock Farms working under the control of this college which are situated at the college campus and Kodamdesar (25 kilometers from Bikaner). There are four Livestock Research Stations at Beechhwal (Bikaner), Chandan (Jaisalmer), Nohar (Hanumangarh) and Navania, Vallabhnagar (Udaipur).

The University also has 49 institutes providing 2 years' Diploma in Animal Husban dry after 10+2 spread over whole Rajasthan producing about 2500 paravets annually. These institutes are in Bikaner, Alwar, Bharatpur, Churu, Dausa, Dungarpur, Hanumangarh, Jaipur, Jhalawar, Jhunjhunu, Jodhpur, Karauli, Kota, Bundi, Baran, Sriganganagar, Sikar, Tonk, and Udaipur. Recently, two new centers at Research Center on Livestock Health and Production, Jaipur and CVAS, Navania Vallabhnagar,(Udaipur) have also started offering Diploma in Animal Husbandry as constituent units, besides the one at the College of Veterinary & Animal Science, Bikaner.

There is one centre of Rajasthan Livestock Health and Production at Jaipur dealing with animal disease diagnosis, monitoring and surveillance.

I Breed improvement

Breed improvement is one of the major objectives of the University. To this end, the University has taken in hand various programmes which are as follows:

1. There are various projects under RKVY to improve the quality of cattle breeds, viz., Tharparkar at LRS, Chandan (Jaisalmer), Rathi at LRS, Bikaner, and Kankrej at Kodamdesar and Gir cattle at LRS, Vallabhnagar (Udaipur).
2. A programme on improvement of Surti Breed of Buffalo is running at LRS, Vallabhnagar (Udaipur).
3. There is another project on the breed improvement of Sirohi goats and mega seep project on Sonadi sheep.
4. A network project sponsored by ICAR on the improvement of Magra sheep is running in Norangdesar, Gadhwala and Kilchu villages of Bikaner district.
5. Improvement on Marwari Goats is going on in some of the villages viz. Deshnoke, Raisar, Dhaiya and Kalyansar of Bikaner District.
6. A recent research project on Sheep and Wool Improvement Scheme (SWIS) sponsored by Central Wool Development Board, Jaipur started functioning to improve the health and quality of Magra and Bikaneri Chokla sheep covering the areas of Bikaner and Lunkaransar tehsils under which a population of 2 lakhs sheep will be taken for its breed improvement, establishing two ram raising units at Kodamdesar farm producing 600 rams for distribution among breeders and a multiple facility centre for providing services for dipping and shearing.

II. Livestock Health

1 In outdoor clinical centre more than 35000 animals are diagnosed and treated annually and diseases related to obstetrics and gynecology are also treated.

2 The university possesses well equipped laboratories wherein lab. tests on blood, urine, stool etc are executed to diagnose the diseases in livestock. There are sophisticated sonography and radiography machines through which high level examinations are done.

3 Task on inter-dental wiring on camels is done world widely developed at this University.

4 Under the technical extension programmes, time and again the University organises camps in the villages for treatment vaccination and infertility in animals. Through these camps, nearly 20000 animals are treated annually.

5 A booklet on forewarning disease in the season is published and disseminated in various centers which foster awareness amongst livestock owners about the disease likely to be spread in the specific season and they take first aid preparations to save their livestock.

6 Apex centre periodically conducts various training programmes on disease diagnosis and control and on recent research on animal disease to the veterinarians and Para-veterinarians. As of now, nearly 800 veterinarians and 800 para-veterinarians had been benefitted from these training programmes. In addition to providing training, the centres also does work on investigation and control of epidemic diseases in the state in animals.

7 High level facilities on artificial insemination and embryo transfer are provided.

8 A unique research project on Embryo transfer under which world level laboratory is functional at Bikaner through which superior germplasm will be produced and conserved to enhance the productivity of the livestock.

9 An on-line animal health call centre (toll free number) is contemplated and will soon be started by which livestock owners will get the latest information regarding recent research and disease diagnosis and prevention of livestock from disease.

III. Animal Nutrition:

1. Feed blocks using non conventional and conventional feed and fodder have been designed to reduce losses on transportation and feeding of animals without affecting palatability and digestibility.
2. The research work is underway to identify local herbs and the techniques to reduce methane. Analysis of production feed and fodder and their utilization by the livestock in producing good quality animal's products is done through latest and modern techniques.
3. Production of feed without deteriorating productivity and livestock health with financial support through National Fund for Basic and Applied Research in Agriculture, ICAR.

IV Other salient features:-

1. The students passed out from this university have been selected in national and international institutes of repute and they are contributing significantly in the progress of the nation to expedite economic reforms and as a means to improve the quality of livestock sector.
2. Free of cost distribution of improved breed of Magra sheep and Marwari and Sirohi goats to the villagers.
3. Production and distribution of Tharparkar and Rathi improved bulls.
4. Various measures taken to sustain livestock health and there is mitigation in the mortality rate in animals.
5. The faculty from this University is contributing in publications of three journals from Bikaner of international levels.
6. Effective implementation and result oriented task is done on ICAR schemes/research projects running in the university.
7. Each and every department and hostels are equipped with internet connectivity so that students get research data for their study material and other equally related work.
8. In order to channelize the students' energy into constructive work, the university has recently created an office of Dean, Students' Welfare which promotes talents amongst students in extra-curricular activities like cultural, dance, music, literary etc. and infuse a spirit of community service. NSS activities also conducted in the its college. There is one unit of Remount and Veterinary Corps under which NCC students of the college enroll for a period of two years and get the certificates of various categories after passing the examinations.
9. In order to promote leadership talents amongst the students, the University conducts students' body elections annually at college as well as university levels.
10. A students' councelling centre is functional in the university which facilitates information related to fellowships, job opportunities etc. to the students.
11. A dog show is organized every year to create awareness among pet lovers.

RAJASTHAN UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES, BIKANER
COLLEGE OF VETERINARY AND ANIMAL SCIENCE, BIKANER
INTERNATIONAL SYMPOSIUM ON
"ROLE OF BIOTECHNOLOGY IN CONSERVING BIODIVERSITY AND LIVESTOCK DEVELOPMENT FOR FOOD SECURITY AND POVERTY ALLEVIATION" AND
XVII ANNUAL CONVENTION OF INDIAN SOCIETY FOR VETERINARY IMMUNOLOGY AND BIOTECHNOLOGY

29-31 DECEMBER, 2010

29.12.2010

TIME	EVENTS		
09:00 – 10:00 AM	REGISTRATION	DEPARTMENT OF VETERINARY MICROBIOLOGY & BIOTECHNOLOGY	
10:00 – 12:00 AM	INAUGURATION	AUDITORIUM, RAJUVAS, BIKANER	
12.00 - 12.15 PM	TEA BREAK		
	SCIENTIFIC SESSION 1 : BIODIVERSITY		
12:15 – 01:30 PM	CHAIR: PROF. (DR) M. P. YADAV DR. K. KUMANAN RAPPORTEUR: DR. A. K. KATARIA	LEAD PAPERS	DR. ALOK SHARMA PROFESSOR, DEPT OF VETY EXTENTION, PALAMPUR
			DR. NAVEEN SHARMA PRINCIPAL SCIENTIST, DIVISION OF SURGERY, IVRI, IZATNAGAR
	ORAL PRESENTATIONS 10	POSTER 33	
01:30 – 02:30 PM	LUNCH		
	SCIENTIFIC SESSION 2 : IMMUNOPATHOLOGY		
02:30 – 05:00 PM (03:30 – 03:45 TEA BREAK)	CHAIR: DR. R. K. SINGH DR. ALOK SHARMA RAPPORTEUR: DR. B. R. GULATI	LEAD PAPERS	DR. M. P. YADAV EX. DIRECTOR, IVRI, IZATNAGAR
			DR. J. M. KATARIA DIRECTOR, NATIONAL INSTITUTE OF ANIMAL HEALTH, BAGHPAT
			DR. D. K. MITRA PROFESSOR, DEPT OF TRANSPLANT IMMUNOLOGY & IMMUNOGENETICS, AIIMS, NEW DELHI
	ORAL PRESENTATIONS 17	POSTER 21	

30.12.2010

	SCIENTIFIC SESSION 3 : PROTEOMICS		
10:00 – 11:30 AM	CHAIR: DR. V. PURUSOTHOMAN DR. T. S. RAI RAPPORTEUR: DR. MINAXI PRASAD	LEAD PAPERS	DR. DAVE CAUDELL ASSISTANT PROFESSOR, PATHOLOGY & HEMATO-ONCOLOGY, VIRGINIA TECH, BLACKSBURG, VA
			DR. SIBA K. KAMAL PROFESSOR, VA-MD, REGIONAL COLLEGE OF VETERINARY MEDICINE, UNIVERSITY OF MARYLAND
	ORAL PRESENTATIONS 13	POSTER 11	
11:30 – 11:45 AM	TEA BREAK		
	SCIENTIFIC SESSION 4 : GENOMICS		
11:45 – 01:30 PM	CHAIR: DR. H. M. SAXENA DR. DILIP SHARMA RAPPORTEUR: DR. S. K. GHORUI	LEAD PAPERS	DR. BHANU P CHAWDHARY PROFESSOR & DIRECTOR, MOLECULAR CYTOGENETICS LABORATORY, TEXAS A & M UNIVERSITY, USA
			DR. R. KALAIMATHI PROFESSOR, DEPT OF MICROBIOLOGY & BIOTECHNOLOGY
	ORAL PRESENTATIONS 16	POSTER 14	
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	SCIENTIFIC SESSION 5: MICROBIAL BIOTECHNOLOGY		
02:30 – 05:00 PM (03:30 – 03:45 TEA BREAK)	CHAIR: DR. ARVIND KUMAR DR. R. KALAIMATHI RAPPORTEUR: DR. BRINDHA NARAYAN	LEAD PAPERS	DR. A. THANGAVEL PROFESSOR/ SECERATRY ISVIB, MVC
			DR. MEENAKSHI PRASAD PROFESSOR, ANIMAL BIOTECHNOLOGY, CVSC, CCSHAU, HISAR
	ORAL PRESENTATIONS 21	POSTER 20	

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	SCIENTIFIC SESSION 6 – APPLIED BIOTECHNOLOGY		
10.00 – 01.30 PM (12:00 – 12:15 TEA BREAK)	CHAIR: DR. B. S. CHANDEL DR. N. S. SHARMA RAPPORTEUR: DR. K. L. PHANIRAJ	LEAD PAPERS	DR. SATISH KUMAR PRINCIPAL SCIENTIST & GROUP LEADER, CCMB HABSIGUDA, UPPAL ROAD, HYDERABAD
			DR. SAGAR M GOYAL PROFESSOR OF VIROLOGY, DEPT OF VETERINARY POPULATION MEDICINE, UNIVERSITY OF MINNESOTA 1333, GORTNER AVENUE, SAINT PAUL
			DR. K. KUMANAN PROFESSOR & HEAD, DEPT OF ANIMAL BIOTECHNOLOGY, MVC
			DR. V. PURUSOTHOMAN DIRECTOR RESEARCH, TANUVAS, CHENNAI
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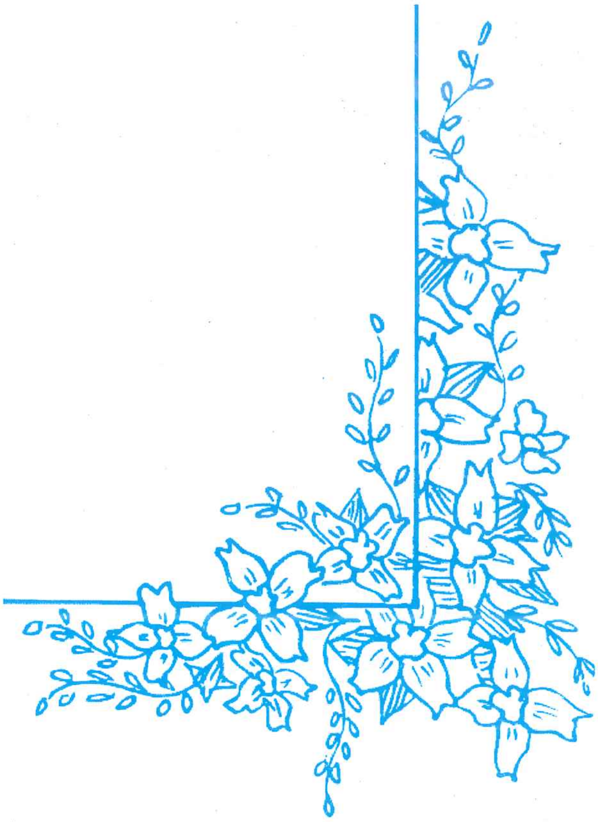
POSTER PRESENTATION

Lead Papers



DEVELOPMENT OF BIOMATERIALS FOR RECONSTRUCTIVE SURGERY IN VETERINARY PATIENTS

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The clinical need for strong, biocompatible materials that encourage integration while minimizing adverse reaction, such as tissue reaction and adhesion formation, is apparent. Yet, the design of optimal surgical repair materials to reinforce or replace soft tissue remains problematic. When normal tissues fail, surgeons are confronted with the challenge of manipulating available healthy anatomy in such a manner as to produce normal form and function in the affected region. Local circumstances will vary, depending on the etiologic factors involved. Congenital anomalies, acute trauma, premature tissue deterioration, and the consequences of ablative surgery all present different local circumstances with which the reconstructive surgeon must deal. At the cellular level, however, it makes no difference how the tissue fails. What does matter is a set of basic cell requirements and wound management principles common to all tissue reconstructive efforts. Most tissue engineering approaches to the restoration and repair of damaged tissues require a scaffold material upon which cells can attach, proliferate and differentiate into a functionally and structurally appropriate tissue for the body location into which it is placed. A variety of scaffold materials are available, each with different physical properties and each associated with a specific and unique host response when implanted in mammalian hosts. All scaffold materials are subjected to certain processing steps before use in therapeutic devices. These processing procedures can moderately or severely affect both the native material properties and the subsequent clinical utility of scaffolds.

Scaffold materials can be either synthetic or naturally occurring. Synthetic materials tend to elicit a foreign material type of response in the host: specifically, a mixed polymorphonuclear and mononuclear inflammatory cell infiltrate and associated fibrous connective tissue deposition. This type of response is not always compatible with optimal constructive tissue remodeling. Therefore, naturally occurring materials such as purified collagen and extracellular matrix, which theoretically should not cause as great a "foreign" material response needs to be investigated. The most commonly used naturally occurring scaffold material has been the structural protein collagen. Collagen is a naturally occurring, highly conserved protein that is ubiquitous among mammalian species and accounts for approximately 30% of all body proteins.. Bovine and porcine type I collagen provide a readily available source of scaffold material for numerous applications and have been shown to be very compatible with human systems. The collagen can be stabilized by various methods of cross-linking and must be sterilized prior to surgical use.

Collagen is generally treated as a "self" tissue by recipients into which it is placed and is subjected to the fundamental biological processes of tissue degradation and integration into adjacent host tissues when left in its native ultra structure. Certain treatment methods, however, significantly alter the mechanical and physical properties of collagen based materials and may negatively affect the natural physiological processes of cell attachment and proliferation and tissue remodeling. There is no denying that the autografts are the best choice followed by allografts and finally the xenografts. However, the difficulty of procuring and preserving autografts and allografts makes the alternative of the abundant xenograft sensible.

Collagenous tissues obtained from abattoir, cadaver or patient being degraded immediately. Therefore, in the exploitation of tissue as clinical material this deterioration must be arrested and deferred preferably beyond the recipient's natural life. Resorption control of collagen membrane is essential where it is used for tissue regeneration controlled by varying the cross links density of the biomaterials. Cross linking is an effective method to control resorption rate of collagen based biomaterials and to prevent a rapid elution of the material into wound fluids. This is an important for synchronizing degradation of biomaterial with wound healing as well as for increasing tensile properties and flexibility of the membrane to the level necessary for application as a synthetic derma for biosynthetic skin substitute. However cross linked biomaterials are more resistant to enzymes, with a higher elasticity modulus and a lower degree of swelling. The aim of cross linking is to prolong the material's original structural and mechanical integrity. Another aim of cross linking is to remove or at least neutralize the antigenic properties attributed to these materials.

Natural collagenous materials are being investigated for surgical repair because of inherent low antigenicity and their ability to integrate with surrounding tissue. Degradable collagenous materials have shown the potential but may lose strength *in-vivo* if they are not cross linked. Methods of cross linking, concentrate mainly to create new additional chemical bonds between the collagen molecules. These supplementary links (cross linking) reinforce the tissue to give a tough and strong but non-viable material that maintains the original shape of the tissue. The process of cross linking involves the chemical agents initiating ideally, irreversible and stable intra- and inter-molecular chemical bonds between collagen molecules. Preferably, the agent promotes bonds between the groups of the amino acids. The efficiency and extent of cross linking reactions depend upon the thickness of the layers of the collagenous tissue and defines the magnitude of the penetration. The other parameters like concentration of the cross linker, the time and the temperature of exposure affects the cross linking.

Ideally, the cross linking treatment should also maintain much of the original character of the tissue, such as the flexible mechanical properties of the tissue (biomaterial) without its shrinkage. Hence it is necessary to keep the tissue near to neutral pH, ensuring an aqueous media environment and minimizing denaturation of the collagen for optimizing cross linking. Therefore, a balance must be achieved for attaining enough reliable cross-links for the biomaterials to last lifetime of the recipients, yet permit the biomaterial to perform as it would be in its natural state. The methods that have been developed do not and probably cannot satisfy the dual requirements.

At a time when technological research should ideally contribute both to wealth creation and improvements to the quality of life, it is obvious that developments that lead to the implantation of advanced materials and devices within the body and order to treat disease and injury should have a very high priority. There can be no doubt that the most widely recognized applications of biomaterials involve those situations where a tissue or organ has suffered from some disease or condition that has resulted in pain, malfunction or structural degeneration, and which can only be alleviated by the replacement or augmentation of the affected part. The cause of the condition could be bacterial, viral or fungal, or could be related to an autoimmune, sclerotic, neoplastic or simply age-related process. They require that the pain is reduced or eliminated and that the offending tissue be circumvented by an alternative structure. For example the best solution to the arthritic hip may be to remove the affected bone and cartilage and replace them in their entirety with a total joint prosthesis, but with an atherosclerotic (i.e. blocked) artery, the objective may be more easily achieved with a by-pass rather than a replacement. The important thing is that the function is restored. Some implantable devices are used to alter the shape, appearance or structure of the body. Breast implants are used more frequently or augmentation rather than post-mastectomy reconstruction.

There are other situations in which control is required over regenerative processes in tissues and where a device is used either to enhance or repress tissue growth or proliferation. Spinal degeneration, for example, is usually treated by fusion or vertebrae and some cage systems are now available which incorporate materials (and sometimes ancillary drugs) that enhance the bone development that is required to produce the fusion.

A further type of application is the transient implantable system that is directed towards the temporary support of traumatized or deformed tissues. This includes sutures, clips, adhesives and staples for soft tissues, haemostatic and sealant materials and devices in the vascular system plates, screws, pins and fixators for bone fracture repair and devices in orthodontics for tooth movement. Sometimes these devices are directed towards the regeneration of new tissue rather than assisting a natural repair process. The regeneration of skin in areas of chronic ulcers or burns, and the regeneration of tendons and ligaments after sporting injuries are good examples.

It is not surprising that there are not just a few widely used biomaterials in clinical practice but rather a whole range of metals and alloys, ceramic and glasses, thermoplastics resins, fibres, textiles and elastomers, composites and natural materials from which selection is made depending on the precise circumstances.

TISSUE ENGINEERING

Tissue engineering is a multidisciplinary field which involves the application of the principles and methods of engineering and life sciences towards the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes that restore, maintain or improve tissue function. The goal of tissue enginering as a treatment concept is to replace or restore the anatomic structure and function of damaged, injured or missing tissue or organs following any injury or pathological process by combining biomaterials, cells or tissue, biologically active molecules and or stimulating mechanical forces of the tissue microenvironment and surpasses the limitations of conventional treatments based organ transplantation. One of the principle methods behind the tissue engineering involves growing the relevant cells *in-vitro* into the required three-dimensional (3-D) organ or tissue. There are four basic components in tissue engineering which includes biomaterial scaffold, functional cells, biomolecules (eg. Growth factors, extracellular matrix, other functional molecules) and dynaminic forces. The developments in clinical expertise and the engineering quality of medical devices during the last thirty years have led to considerable success and effectiveness to trauma repair and tissue replacement. In spite of this success however, the whole field of implant surgery is witnessing a gradual shift away from the robust maximally invasive approaches in the treatment of disorders and injuries towards the use of the less invasive techniques that attempt to encompass biological or physiological concepts of tissue repair. The concept of tissue engineering is best seen as an alternative to transplantation. In many areas of medicine, one of the most appealing solutions to irretrievable organ or tissue failure has been that of transplantation, using tissues and organs from donors. The donors could be the patients themselves, as with skin grafting, live immunologically matched humans, as with bone marrow transplants, deceased human donors as with heart transplants, or possibly animals. Whilst many of these procedures using transplanted tissues are very successful, as with, for example, corneal transplants and porcine heart valves, there are clear limitations and difficulties. These start with the obvious immunological problems associated with the use of foreign proteins, which means that recipients of viable organ transplants have to be immunologically suppressed, whilst xenogeneic tissues (from animals) have to be rendered non-immunogenic by chemical means.

A combination of *in-vitro* and *in-vivo* approaches has solved some simple tissue-engineering problems, and will continue to be equally important for autologous tissue removal, augmentation in the laboratory and eventual *in vivo* replacement. Organizing complex three-dimensional functional structures from a combination of cells and structural elements relies on numerous spatial and chemical relationships. The multitudinous approaches taken by tissue engineers can be roughly reduced to *in-vitro* and *in-vivo* designs. There is a requirement for an underlying framework or scaffold for the cells, the proper population of cells and a substitute for the extracellular matrix or extracellular environment. Interactions between cells and extracellular matrix are some of the keys to cell migration, proliferation, differentiation and apoptosis, which are all critical functions for a tissue-engineered construct.

In-vitro models have usually relied on the formation of a bioreactor system or cell patterning for monolayer co-culture studies. Bioreactors are dynamic tissue-culture devices that range from simple mechanical designs to more complex systems with more elements of a living physiological system such as $O\text{-}_2$ exchange, defined flow rates and electrical and mechanical stimulation. The engineering of less complex tissues such as cell sheets only requires a simple method to renew the growth medium to the engineered construct and to avoid stasis. Thus, there has been success with models that agitate a growth medium around a construct that has been seeded with cells, such as a spinner flask or rotating vessel.

In-vivo studies have focused on animals as a ‘physiologically-complete bioreactor’, with composite constructs implanted into vascularized spaces such as the omentum, mesentery, interscapular fat pad or latissimus dorsi. A substitute for the extracellular matrix in the form of a scaffold is implanted after cell loading onto the construct. The construct can be optimized by modifying the polymer itself through chemical engineering, microfabrication of topographic cues including the chemical microenvironment or by prevascularization. A combination of *in-vitro* and *in-vivo* approaches has solved some simple tissue-engineering problems, and will continue to be equally important for autologous tissue removal, augmentation in the laboratory and eventual *in-vivo* replacement.

TECHNOLOGY AND POLICY LED ROAD MAP FOR ANIMAL HEALTH FOR ENSURING FOOD AND NUTRITIONAL SECURITY IN INDIA

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Live Stock inclusive of poultry and fishery are the engine of growth for national economy, development, food and nutritional security, employment, empowerment of women and landless/marginal and small holder farmers. Besides the genetic potential of the animal, balanced nutrition and management, protection and promotion of Animal Health through judicious use of diagnostics, therapeutics, prophylactics, and adequate delivery of veterinary services, play pivotal role in enhancing animal production, both qualitatively and quantitatively. Consequent to globalization, export of livestock, livestock products, organs and feed ingredients, along with global human travel, have enhanced the risk for encountering new diseases, specially trans-border animal diseases (TADs) and zoonotic infections to food and companion animals. Likely adverse impact of global warming and climate change on animal health through enhanced abiotic and biotic (infectious diseases) stresses, further threaten livestock health, reproduction and production. To face these challenges, a workable road-map based on technology and policy, need to be developed and implemented on national basis on priority. The road- map for animal health should be an integral component of a comprehensive National policy on livestock.

In order to safeguard Indian livestock from the threat of infectious diseases, we need to develop state of the art diagnostics, vaccines, infrastructural facilities, human resources and laboratories to ensure adequate Bio-safety and Bio-security. Adequate check posts at interstate and international borders, quarantine and isolation facilities at sea- and air-ports are prerequisite to prevent the ingress of exotic diseases and TADs. Prevention and control of mastitis and production diseases in high yielding dairy animals has significance in livestock production. Breeding for disease resistance through judicious use and blending of our indigenous germ plasm (poultry, cattle, buffalo, sheep, goat, pig, camel, yak and mithun) through application of modern technologies of genomics, proteomics, phenomics, metabolomics, biotechnology and nanotechnology will go a long way in protecting animal health leading to enhanced livestock production. Incorporation of deficient micro-nutrients in the feed and fodder and supplementation with area specific mineral mixtures, need to be promoted though research, market linkages and policy instruments. Incorporation of prebiotics and probiotics in livestock feed promotes both health and productivity and hence needs to be provided research and incentive support.

Efforts should be directed to prioritize the existing, emerging and exotic diseases of livestock considering their economic, zoonotic and epizootiology characteristics, and threat perceptions for undertaking appropriate R&D and policy interventions. The diagnostic tests should qualify to OIE requirements which require harmonization, validation and accreditation of all the diagnostic tests in place, which is mandatory requirement for health certification for export and import of livestock and livestock products, semen and embryos. Unlike bacterial diseases, viral diseases are difficult to control due to their rapid spread and lack of effective and affordable antiviral drugs. Rapid, specific and precise diagnosis is of utmost importance for timely and effective control of viral diseases. The diagnostic test should be sensitive, specific and at the same time cost effective. Where ever possible, recombinant antigen and monoclonal antibodies should be used in ELISA and other diagnostic assays. Only OIE recommended tests,prescribed for various diseases, should be used for trade certification purposes. DIVA test should be developed for all those diseases where vaccination policy is in vogue, for differentiating the infected animals from the diseased ones. This simultaneously requires the use of marker vaccine(s). An array of diagnostic tests from conventional AGPT, neutralization, CFT, HA-HI and agglutination to the second and third generation series tests ,including IFT, IPT, IEM, ELISA, Dot-ELISA, Dip- Strip, EIAs, RIAs, PCR, PCR-ELISAs, LAMP, RT-PCR, Nested PCR, Real Time PCR, Multiplex PCR, nucleic acid hybridization, gene sequencing are now available and many more are under development. Signal (light, electronic) amplification and detection methods are the most exciting ones among these. Now there are tests having sensitivity of detecting as less as 10 virus particles. New innovation in molecular diagnostics include automated PCR, Real-time Computerized PCR analysis, second generation PCR kits, DNA amplification finger printing, DNA Chips and Bio Sensors, micro arrays, nucleic acid sequence based amplification (NASBA), ligase chain reaction (LCR), DNA probe test based on strand displacement amplification (SDA), self sustained sequence replication reaction (3SR), application of quantum dots and molecular imaging for the diagnosis of respiratory viruses and surface enhanced Raman spectroscopy (SERS) using laser and nanotechnology. From field application point of view, development of Pen-side diagnostic tests is the need of hour as there is limitation of laboratory based diagnosis in rural areas where most of the livestock is available.

Ideal vaccines should be potent, capable of imparting lasting immunity, having adequate protective immunogenic mass with out other unwanted extraneous antigens, easy to administer, safe for all category of animals including pregnant ones and capable of providing immunity in young animals and after in-ovo vaccination of poultry. The vaccine should have good keeping quality at refrigeration temperature as well as at ambient temperature. Recombinant bivalent and multivalent vaccines will reduce the cost of vaccination as well as stress to the animals. Immuno modulators including adjuvants and cytokines should be used to enhance the immune response of killed vaccines. Marker vaccines are required for using DIVA test. The dose of the vaccine should be small (about1 to 2 ml for large animals). The vaccine should be able to induce adequate humoral as well as cellular immune responses. The vaccines should be updated periodically to accommodate the antigenic variations in the pathogen.

Provision of vaccine R&D venture fund in PPP mode is required for undertaking researches in vaccinology and setting up of modern vaccine production units and providing subsidy to the BPL category livestock owners on the vaccine as a part of pro-poor policy. Availability and affordability of vaccines against major infectious diseases of livestock and poultry will provide strong arm to safeguard the livestock against enhanced biotic stresses due to climate change.

Ethno-veterinary medicines should be encouraged as these are not only cheaper but also Eco-friendly. Investment in livestock research, education and extension should be commensurate with its contribution in the national GDP. Livestock insurance need to be made available through out the country for all category of animals. A National Mission on livestock should be constituted by the government for all round development of livestock. For improving the delivery of veterinary services, the number of veterinary practitioners, stock man , compounders, veterinary hospitals and dispensaries need to be doubled immediately.

MOLECULAR DIAGNOSIS AND NEWER IMMUNOPROPHYLACTICS FOR IMPORTANT VIRAL DISEASES OF POULTRY

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The livestock sector has emerged as one of the key drivers of agricultural growth in developing countries in recent years. It is projected that by the year 2020 over 60% of meat and 50% of milk will be produced in the developing countries. Within the developing countries, Asia will be the key production hub and India and China will be the primary producers of meat and milk. There is a growing demand for livestock products in Asia and south East Asia. The cost of livestock production in Asia is much cheaper compared to the developed world, as bulk of the livestock are maintained by small holders, and hence the cost of the products are more competitive than that of the developed world, however opportunities are lost in international trade due to SPS standards.

The poultry industry is considered as the most dynamic industry in the Indian agribusiness sector, with a contribution of 1% to the national GDP. The Indian industry has the potential with commercial viability and certain intrinsic advantages for the export of poultry and poultry products to global market. But there is a constant risk factor of disease incidences, which comes on its way for further expansions and export in the WTO region due to public health implications. Emerging and re emerging diseases of poultry are constant threats impacting the economy of the poultry industry.

Genetic changes in pathogens (evolution/mutations) are one of the reasons for emergence and re-emergence of diseases. The changed disease scenario in Indian poultry flocks with complexities in their pathogenesis and resulting economic losses have put a challenge before the avian diseases specialists to address the disease problems in commercial poultry flocks with modified approaches based on newer concepts of poultry diseases management. There is a paradigm shift in the application of techniques for diagnosis and prevention of diseases. Molecular biology and biotechnology have been the passwords for global biological research. The presentation reviews the recent molecular diagnostic techniques and modern immune prophylactic measures for some of the economically important viral diseases of poultry.

HEMATOLOGICAL MALIGNANCIES: LESSONS FROM TRANSGENIC MOUSE MODELS

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The biomedical research community is heavily dependent on murine models to better understand the molecular mechanisms underlying many human diseases. Animal models have provided tractable systems with which to study a wide range of human diseases, including hematologic malignancies. Mice and humans share many important biological attributes including anatomy, physiology, and genetics. Given these trans-species similarities, the mouse has become a standard animal model used to study the pathophysiology of disease and explore novel therapeutic approaches. Until recently, potential cancer-causing genes and their associated pathways have been difficult to study. The use of carefully designed genetically engineered mice (GEMs) has provided much insight into the molecular biology of disease including hematopoietic malignancies.

The hematopoietic system is the source of erythrocytes, leukocytes, and megakaryocytes that function in gaseous exchange, immune defense and modulation, and coagulation respectively. Hematological malignancies, including acute and chronic leukemia, are clonal proliferations of undifferentiated progenitor cells in either bone marrow or lymphoid organs. These malignancies represent a heterogenous group of neoplasms with unique molecular genetic features, and variable clinical and prognostic outcomes. Historically, leukemia and lymphoma have been classified using the French-American-British system, which defined these malignancies based on morphology. This included the following four categories: acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), or chronic myelogenous leukemia (CML). The more recent World Health Organization (WHO) classification scheme stratifies hematopoietic neoplasms according to lineage, but also extends the classification to incorporate immunophenotype, genetic features, and clinical syndromes. Clinical presentations associated with acute leukemia are attributed to leukemic infiltration into either bone marrow or parenchymal organs. Clinical symptoms, as a result of bone marrow infiltration, lead to bone marrow failure, and are reflected by lethargy and listlessness due to anemia; bleeding disorders due to thrombocytopenia; and secondary infections because of leukopenias. The clinical presentation seen with organ infiltration varies with the organ involved and the degree of leukemic infiltration. Acute leukemia is uniformly fatal if left untreated.

Acute leukemia results as a consequence of multiple acquired mutations that accumulate in hematopoietic precursor cells. These mutations can be grouped into three general categories: point mutations, gross chromosomal rearrangements, and epigenetic changes. The analysis of theses altered genetic events has provided much insight into the biology of hematopoietic malignancies, leading to improved diagnosis and classification, as well as identification of novel therapeutic targets.

Chromosomal translocations (CT) occur as a result of DNA double strand break and mis-repair leading to rearrangement of DNA between nonhomologous chromosomes. Investigation into their molecular pathogenesis has revealed several key concepts about these mutations. **First**, specific CT are typically cell specific (CT tropism) leading to specific leukemic phenotypes. For example, the t(15:17) is associated only with promyelocytic leukemia that gives rise to a PML-RARá fusion seen in patients with M3 AML. **Second**, at least two outcomes are associated with CT: either they produce a novel chimeric protein, e.g., *BCR-ABL*; or they lead to unscheduled proto-oncogene expression as seen with *MYC-IGH*. **Third**, CT frequently involve genes that encode for either transcription factors or proteins associated with signal transduction pathways. **Fourth**, genes affected by CT can also be associated with point mutations. For example, the t(8;21), which produces an *AML1-ETO* fusion has been associated with point mutations. **Finally**, the pathogenesis of AML is a multistep process. The current paradigm in leukemic biology proposes collaboration between two categories of mutations. Class I mutations lead to enhanced cell proliferation as well as impaired apoptosis. The mutations alter signal transduction or tyrosine kinase pathways and include genes such as *RAS*, *cKIT*, *FLT3*, or *BCR/ABL*. Class II mutations result in mutant DNA binding transcription factors, these include *PML/RARá*, *AML/ETO*, *NPN1*, and *MLL* fusions. These novel fusions lead to impaired hematological precursor cell differentiation.

CALM-AF10 transgenic mice develop AML with lymphoid features and have *Hoxa* gene cluster upregulation including *Hoxa9*. In this particular model, mice develop leukemia after a prolonged latency period (9-18 months) with incomplete penetrance. These results suggest additional genetic events are needed to complement *CALM-AF10* mediated leukemic transformation. Retroviral insertional mutagenesis identified complementary genetic events that might collaborate with *CALM-AF10* during neoplastic transformation. Ligation-mediated PCR and sequence analysis of DNA derived from leukemic cells identified potential collaborating genes at the retroviral insertion sites including *Zeb2, Nf1, Evi1, kRas,* and *Mnl*. Identification of these genes as potential collaborating genes with *CALM-AF10* supports the emerging paradigm in leukemia biology that predicts that most, if not all leukemic cells must undergo at least two collaborative events to result in a fully transformed neoplastic cell. One of these events often leads to impaired differentiation and enhanced stem cell renewal, whereas the second event results in increased proliferation and/or decreased apoptosis.

GEM that express *NUP98-HOXD13* develop severe Myelodysplatic syndrome at 4-7 months of age and progress to acute leukemia within 14 months. These mice develop a broad array of hematological disorders including Myelodysplastic syndrome (MDS), megakaryocytic and erythroid leukemia, undifferentiated and myeloid leukemia, as well as pre T, lymphoblastic leukemia. Transgenic GEM have osteosclerotic bone with increased numbers of megakaryoblasts in the bone marrow. These features are similar to thrombopoietin transgenic mice that develop myelofibrosis involving their bone marrow and spleens. Additional studies show that when embryonic stem cells express *NUP98-HOXD13* there is impaired hematopoietic cell differentiation and that K562 cells which express *NUP98-HOXD13* have disruption of tissue plasminogen activator induced differentiation suggesting an inability to proliferate normally. Lineage negative hematopoietic precursors derived from *NUP98-HOXD13* mice show impaired differentiation as well as marked apoptosis, a feature observed in patients with MDS.

Using GEMs to study hematological malignancies is important for several reasons. **First**, numerous hematological malignancies and abnormalities can be reproduced in mice. **Second**, by conducting research in GEMs, new insights will be gained about the biology of bone marrow and malignant disease. **Third**, studies in these mouse models will allow researchers to evaluate the interaction of various bone marrow cell types both *in vitro* as well as *in vivo*. Using these mouse models, we will be able to study the molecular mechanisms that underlie malignant diseases. **Fourth**, novel molecular mechanisms often identified through the use of GEM, can reveal therapeutic targets that result in the development of new or improved drugs. These new drugs can then be screened in the mouse model before entering animal and human clinical trials. **Finally**, understanding the mechanisms of disease and developing new drugs, using GEM models, may ultimately lead to improved patient care and increased patient survival.

NEWCASTLE DISEASE VIRUS AS A VACCINE VECTOR

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Vectored vaccines based on recombinant Newcastle disease virus (NDV) have great potential for inducing protective immunity against human and animal diseases. As a vaccine vector, NDV induces substantial local and systemic immune responses against the foreign antigen. NDV is antigenically distinct from common human viruses, accommodates foreign genes with good degree of stability, and has a very low incidence of recombination. Because of its natural tropism for the respiratory tract, NDV is particularly suitable for the development of vectored vaccines against respiratory pathogens of humans and animals.

DIVERSITY IN BLUETONGUE VIRUS: GLOBAL SPREAD AND CLIMATE CHANGES

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Bluetongue (BT) is non-contagious arthropod transmitted viral disease of domestic and wild ruminants. Outbreaks of clinical BT have been reported from almost all regions of the country. Out of 26 serotypes, circulating antibodies for 21 serotypes have been reported in India. Exotic breeds of sheep are comparatively more susceptible to clinical BT compared to native breeds. In India BT is almost exclusively a disease of sheep, although antibodies to BTV have been detected in cattle, buffalo, camel, goat and some wild ruminants. Outbreaks of BT have been associated with the periods of peak *Culicoides* activity in different part of the country. Considering the effect of global warming, it has been speculated that by 2050, there will be 4 C rise in the global temperature. If this becomes a reality, there will be forereaching consequences leading to ecological changes of global scale. Since BT is a vector borne disease, climate change will have great impact on its epidemiology. Taking in account the global picture of BT, the Indian subcontinent includes several different climate zones, supporting a large and genetically diverse population of domestic and wild ruminants. As bluetongue virus (BTV) is known to infect a wide variety of animal species, including most ruminants, with outcomes ranging from inapparent, to frank or even fatal clinical disease, depending on the species and breed of host animal, and the serotype/strain of BTV. Owing to the immense economic importance of BT in Indian ruminant livestock, the Indian Council of Agricultural Research (ICAR) has launched a National Network Project on BT with eleven centers located in different regions of the country including Hisar.

The mandate of the Network Project is to gain an understanding of BT epidemiology, vector biology, develop suitable vaccines based on local strains/serotypes and suggest strategies for control. Serological data concerning BTV have been reported for several native breeds of sheep, goats, cattle, buffalo and camels within India. *Culicoides* vectors are significantly affected by the climate and weather, and annual variations frequently influence the incidence and overall severity of the diseases transmitted by these insects. Most of the annual BT outbreaks recorded on the subcontinent, have occurred in areas affected by the north-east monsoon (during October to December - the monsoon period), followed by areas affected by the south-west monsoon (during June to September). The number of adult vectors in the south of India reaches a peak during the monsoon season (June to December). Outbreaks of BT in Karnataka, Tamil Nadu and Andhra Pradesh have also been associated with these periods of peak Culicoides activity. Several species of *Culicoides* in the Chittor and Prakasam districts of Andhra Pradeshhave been reported with *C. actoni, C. anophelis, C. inoxius, C. majorinus, C. peregrinus and C. oxystoma* being the predominant species. In contrast, the predominant species in Tamil Nadu are *C. imicola and C. peregrinus*, while in the Marathwada region and Kolkota, *C. schultzei* has been the predominant species. The abundance of *C. imicola* in Tamil Nadu has been found related to seroconversions in domestic animals.

The effect of change in ecosystem as a result of global warming has clearly been visualized in European countries. Since 1998, there has been huge increase in the incidence of BT in Europe, starting in the Mediterranean region, with outbreaks gradually extending further north into central and northern Europe arriving in the UK during August 2007. The repeated introduction and emergence of new BTV strains in Europe has provided clear evidence of an epidemiological 'stepchange' in the region with high mortality in livestock. The northern European outbreak, caused by BTV-8 which started in the Netherlands and Belgium during the summer of 2006, is the largest and most economically damaging BT epizootic on record. The occurrence of such epizootics demonstrates a major increase in the threat posed to European livestock by BTV. The occurrence of unique serotypes and combination of BTV strains that currently exist in India and Europe, together with the change in the ecosystem including involvement of novel vector species of *Culicoides,* could lead to the emergence of reassortant virus strains and could eventually even lead to the emergence of distinct Indian and global topotypes. Therefore, monitoring the movement of the potential vectors, infected ruminant animals and their products would be challenge for the governments and quarantine authorities. Serotype specific molecular typing assays would be required for effective monitoring and surveillance of different serotypes of the virus.

KNOCKOUT OF *WDR13* GENE LEADS TO INCREASED ISLET MASS, HYPERINSULINEMIA AND MILD OBESITY IN MICE

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The WD-repeat proteins are found in eukaryotes and play an important role in the regulation of a wide variety of cellular functions such as signal transduction, transcription, and cell proliferation. These proteins provide a platform for protein-protein interaction. The WD repeat, to which the family owes its name, is a conserved motif of nearly 40 amino acids that often end with the di-peptide Trp-Asp (WD). These WD repeat protein contain 4-16 WD repeats. Wdr13 is a member of WD repeat family contains 5 WD repeats. Like its human counterpart, mouse Wdr13 gene is localized on the X chromosome and encodes a WD – repeat protein of 485 amino acids. Many diseases and pathological conditions have been reported for WD repeat proteins mutation. Diabetes and obesity are amongst the serious human health problems. Insulin producing beta islet cell mass of pancreas is dynamically regulated according to the metabolic health of the individual and is one of major determinants of progression towards diabetes and obesity. Several cell cycle regulators have been identified that have role in pancreatic beta cell proliferation.

We have created and characterized the mutant mouse strain lacking *Wdr13*, a gene encoding a member of WD repeat protein family. In the absence of this protein the mice had higher insulin levels, increased islet mass and better glucose clearance as a result of the enhanced beta cell proliferation. Further, this mutation led to the age dependent mild obesity phenotype. In mechanistic details this protein has interaction with pleckstrin homology interacting protein 1 (PHIP1), HDAC 1, HDAC 3 and HDAC 7. Given the Insilco information that wdr13 protein does not have DNA binding domain, this protein is interacting with DNA through PHIP1 and recruiting histone deacetylases in repressor complex. It appears that by repressing some cell cycle gene (cyclin D2 & E1) this protein control beta cell proliferation. We propose that wdr13 is a novel negative regulator of pancreatic beta cell proliferation. Given the higher insulin levels and the lack of any evidence for insulin resistance in *Wdr13* knockout mice, we suggest that this protein may be a potential candidate drug target for ameliorating the impaired glucose metabolism in diabetes.

THE ROLE OF BIOTECHNOLOGY IN PATHOGEN DETECTION AND CONTROL

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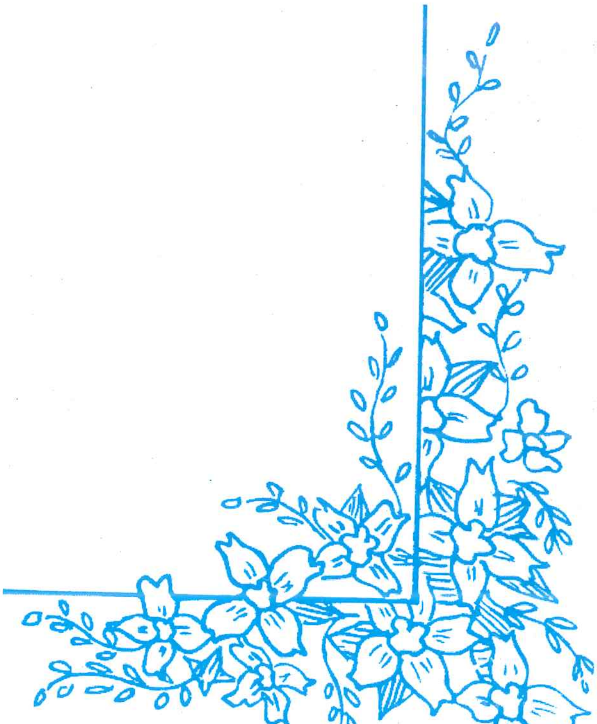
An important part of disease control is accurate disease diagnosis. Detection, isolation, and identification of pathogen(s) is key in controlling bacterial and viral diseases. Another important component is 'characterization' of the pathogen in terms of antibiotic resistance, antiviral resistance, subtyping, and strain variation, etc. Isolation and identification by means of bacterial culture and virus isolation is necessary for various purposes but is time consuming and laborious. Newer techniques of biotechnology are instrumental in rapid and sensitive detection of animal pathogens. These include PCR, RT-PCR, Ligase Chain Reaction, Transcription Mediated Amplification – Hybrid protection assay, Strand Displacement Amplification and Qb replicase, Branched Chain DNA, and Invader Assay.

The application of these techniques to study strain variation and phylogeny are essential because vaccination against one strain may not be fully protective against another, similar strain. This is particularly true in highly mutable pathgens such as the influenza virus. With the help of both new and old technology, progress can be made in detecting the exact pathotype/serotype of the pathogen present on an animal farm so that efforts can be made to customize strategies for its control.

Oral Presentations



I-T-1

AUGMENTING HILL LIVESTOCK PRODUCTION FOR IMPROVED FOOD SECURITY: AN APPRAISAL OF BIODIVERSITY CONSERVATION INITIATIVES IN CHAMBA DISTRICT OF HIMACHAL PRADESH

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Himachal Pradesh is a small hill state located in the western Himalayas. The state has huge variations as far as agro-climatic conditions are concerned. Chamba district represents all four agro-climatic zones of Himachal Pradesh. Different combinations of indigenous livestock species are reared in the district. Buffaloes are prominent animals in low lying districts, while local hill cattle are reared throughout the district. In high altitude areas sheep & goats are predominant animals reared. In spite of the thrust of the Government sponsored-development projects of cross-breeding (genetic up-gradation) for over 30 years, the local inhabitants still have a preference for local cattle, though their productivity is low. The present study was conducting for operationalization of the GEF/ World Bank-funded Project "Harmonizing biodiversity conservation and agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystems". Baseline survey indicates that majority of the households have annual income between Rs. 10,000 to 50,000 per annum. Majority (81.9%) households had cows yielding 1-2 litres of milk production per day, while 8.5% households reared cows yielding less than 0.5 litres milk per day. No proper breeding records are maintained at the farmer's level to conserve local breeds of livestock. The most important reason for the use of male bull/ram/buck from a particular source own/ depends on its availability and not upon the source. Thus, there is urgent need to increase the awareness level of farmer towards rearing local livestock which may be less productive but are better adapted to local environment conditions and social needs. At the same time, these are less input-intensive (in terms of feed, fodder, veterinary services etc.). Under the project, appropriate animal health and production technologies are being transferred at farmer's doorsteps in user-friendly manner by theme-oriented 3-D teaching models, use of vernacular language, live animal demonstrations to enhance the conservation and productivity of indigenous livestock breeds thereby enhancing the livelihood security in target cluster villages of this district of western Himalayan region.

I-T-2

FAUNAL DIVERSITY OF THE THAR DESERT AND ITS CONSERVATION STRATEGIES

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Due to increasing recognition of the economic potential of biodiversity, it has assumed prominence at the global level over the past two and half decades. Depleting biodiversity all over the world has become such an issue that the year 2010 was declared international year of biodiversity by UN. Desert ecosystems are very fragile and often devoid of floral and faunal elements. The Thar desert, situated in NW part of India, is exceptionally rich in biological diversity. It is one of the smallest deserts of the world but the most populated desert of the world. The anthropogenic activities are exerting extensive pressure on the natural resources of the ecosystem. The Thar is a great repository of wild animals and as many as 66 species of mammals, 364 species of birds, 44 species of reptiles and eight species of amphibians make the ecosystem naturalists' paradise. Hoesoever, introduction of Indira Gandhi Canal, Global climatic changes and ever-increasing human and livestock population are transforming the xeric ecosystem. Our studies on avian and mammalian species, which we have been carrying from last one and half decades, indicate that many aquatic birds and mesic rodent species are extending their range into the Thar. As many as 23 species of birds are believed to be extirpated from the Thar over a century. The Thar is an interesting zone zoogeographically and admixture of Saharan, Oriental and Palaearctic realms is found here and the region is house to many endemic wild species. Along with canal irrigation, vegetation degradation, invasion of exotic plant and animal species, emergence of new weeds and pests, alarming increase in human and livestock population, and decreasing pasture lands are great threat to the xeric biodiversity. Biotechnology can play important role in conserving the faunal elements of the region. The natural resources should be used judiciously and modern techniques of captive breeding, cloning, improving genetic variations in inbreeding populations, formation of gene banks etc. can help a great deal in conserving the endangered fauna of the area.

I-T-3

TECHNOLOGICAL INTERVENTIONS TO BOOST INDIGENOUS LIVESTOCK PRODUCTION FOR CREATING SUSTAINABLE LIVELIHOODS AND FOR POVERTY ALLEVIATION IN A FRAGILE ECO-ZONE OF HIMACHAL PRADESH

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Livestock rearing is one of the major avocations of rural hill farmers of Himachal Pradesh. However, livestock rearing in the state suffers from nutritional deficiencies as a result of unavailability of local feed and fodder around the year. Indigenous livestock breeds are best adapted to hilly topography and climate and yet they are poor in productivity. One of the reasons of low production is the poor plane of nutrition followed by livestock farmers. A baseline survey under the current NAIP project entitled "Harmonizing Biodiversity Conservation and Agricultural Intensification through Integration of Plant, Animal and Fish Genetic Resources for Livelihood Security in Fragile Ecosystems" in Chamba district indicated that majority of farmers were unaware about improved feeding technological interventions viz. feeding mineral mixture and enrichment of local crop residues through urea molasses treatment. Majority of farmers (87.61%) fed whole crop residues without any nutritive supplementation. Further, only 4.76% of the farmers fed mineral mixture and that too only for short periods during the terminal period of gestation. Based on the benchmark survey report, suitable technological interventions were planned for the local hill cattle and these are now being operated by the scientists from HPKV Palampur under the project. Urea molasses feeding technology is currently being transferred to the targeted farmers in the adopted villages. Urea and other supplements are mixed with molasses to make local crop residues (mainly maize and wheat straw) more nutritive and palatable to livestock. Feeding of UMB blocks and Urea molasses enriched straw is reported to enhance the productivity and reproductive health status of livestock. The gains are being quantified.

I-T-4

LIVESTOCK COMMODITY & HOUSE HOLD NUTRITIONAL SECURITY: FUTURE RELEVANCE

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Food, nutrition and environmental security have gained serious concern in the new millennium. India has witnessed phenomenal progress in the sphere of agricultural production however; in-spite of satisfactory progress in food production, widespread nutritional insecurity prevails at household level. The vulnerable fraction of society cannot still meet nutritional requirements and suffer from diseases related to deficiency of protein, energy, vitamins and minerals especially iodine. The livestock sector plays a significant role in the welfare of rural population of India. Small and marginal farmers account for three quarters of these households. Income from livestock production accounts for 15-40 per cent of the total farm household's income in different states. It is appreciably visible that with the increase in per caput income, the people are shifting their eating habits from cereal to animal foods. Therefore, livestock products are going to make a likely contribution for creating nutritional security. India is facing per capita deficit in milk, egg and meat to meet the ICMR dietary recommendations. An assessment was made to compare per capita availability, ICMR guidelines and to arrive at per capita deficit and projected demand of 334g/d, 48.27 eggs/annum and 7.4 kg/annum milk, egg and meat respectively for population in the year 2020. It is being fairly concluded that for future nutritional security a sound animal husbandry system needs to reinforce in rural masses of India.

I-T-5

OPPORTUNITIES FOR ENTREPRENEURS IN LIVESTOCK SECTOR

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Livelihood security is defined as "food that is available at all times, to which all persons have means of access, that is nutritionally adequate in terms of quantity, quality and variety, and is acceptable within the given culture making food accessible to all" as per declaration of the World Food Summit. Indian livestock sector has witnessed significant expansion in milk, meat and eggs output, during last thirty years. This happened due to factors like enhanced production and productivity, preference change, income effect, market factors, open economy, urbanization, education, and population change, as well. The sector has achieved significant contribution in national GDP and remained few of the high growth sectors. It contributed in agriculture GDP to the tune of 31.7% with over 5.26% to national GDP. The 11th Five Year Plan envisaged overall growth of 6-7% per annum for animal husbandry sector. The dairy sector contributed largest share followed by meat and eggs. The total livestock output has increased more than five times during last five decades. According to Central Statistics Organization estimates the gross value of out put from livestock sector has increased from Rs. 20,856 crore in 1950 to over 110200 crore during 2009-10. During same period the contribution of agriculture and allied sectors to the national GDP has declined from 55% in 1950 to nearly 18%. The growth in livestock sector during previous two decades has grown at an annual rate of 5.6%, which is higher than that of agriculture sector (around 3%). The milk production grew @ 4.5% per annum during the period 1980-2008. The growth in meat production has been faster than that of milk and transformed from small ruminants (45%) to large ruminants (35%), and poultry (20%). At base year 1991, it is estimated that the demand for meat for all developing countries would increase from 17 kg/capita/year to 25 kg in 2010. Thus total meat production in developing countries has been projected to reach 143 mmt in 2010. India has tremendous potential to occupy the market. The egg production has achieved an annual growth rate of 5.8%. Thus livestock sector is the largest contributor to livelihoods with over 70% of the population. Out of total under and malnourished people in the world, 95% are in developing countries mainly in Asia and Africa. The FAO report underlines that the livestock sector is one of the sunrise sector of the agricultural economy. It contributes 40 percent of the global value of agricultural production and supports the livelihoods and food security of almost one billion people. Globally, livestock contributes 15 percent of total food energy and 25 percent of dietary protein. The Indian animal food processing industry is widely recognized as a sunrise industry having huge potential for uplifting livestock economy, and the resultant generation of employment and export earnings. The industry as whole estimated to be worth around US$ 67 billion and employing about 13 million people directly and about 35 million people indirectly. The food processing sector in India is geared to meet the international standards. The Agricultural & Processed food products Export Development Authority (APEDA) has formed for promoting exports from India. Major opportunities lie in infrastructure investment through Public Private partnership (PPP), Mega Food parks, Integrated cold chain, and food safety management systems and others. Thus the role of livestock is well established with ever growing potential.

I-T-6

SEROPREVALENCE OF TUBERCULOSIS IN ASIAN ELEPHANTS (*Elephas maximus*) OF NORTH-EAST INDIA

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Elephants have been reported to be susceptible to tuberculosis and infections with both *Mycobacterium bovis* and *Mycobacterium tuberculosis*. But most of the cases of tuberculosis in elephants are reported to be caused by *Mycobacterium tuberculosis*. Humans and elephants both carry and suffer from active *Mycobacterium tuberculosis* infection. Furthermore, the disease exhibits the same presentation in both species. Eighty–eight serum samples were examined by indirect haemagglutination test for detection of antibodies against *Mycobacterium tuberculosis* in domesticated privately owned Asian elephants of north east India which had been captured from wild and then domesticated. The investigation was carried out in the months of July, August and September during the year 2005. The seropositivity of TB in elephant was 29.54 per cent in the present study and the titer of most of the positive samples ranged between 1:20 and 1:80. Age wise not much difference in the seropositivity was observed.

I-T-7

CONSERVATION STRATEGIES FOR FISH BIODIVERSITY

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Due to factors such as human modifications to the environment, overexploitation, habitat loss, exotic species and others, aquatic biodiversity is greatly threatened. Ecosystems and species important in sustaining human life and the health of the environment are disappearing at an alarming rate. In order to preserve these threatened areas and species for future generations, immediate action in the form of aquatic biodiversity conservation strategies are necessary. In general, aquatic conservation strategies should support sustainable development by protecting biological resources in ways that will preserve habitats and ecosystems. In order for biodiversity conservation to be effective, coordinated and constructive efforts are required ,that too in a very short span throughout the globe. Since maintenance of fish biodiversity along with other biotic resource has been viewed as prerequisite for the well being of even human beings, the conservation policy should promote the management practices that maintain integrity of aquatic ecosystem, prevent endangerment and enhance recovery of the threatened species. The main goal in a conservation programme is to conserve the genetic diversity. This can be achieved through many mechanisms including in situ and ex situ methods. In situ conservation of fish as landraces and wild relatives is useful where genetic diversity exists and where wild forms are present. The major advantages of insitu conservation to co-evolve with other forms, providing the breeders with a dynamic source of resistance that is lost in ex situ conservation and (ii) natural parks and biosphere reserves may provide less expensive protection for the wild relatives than ex situ measures. Stock enhancement through ranching is feasible only if there is incomplete colonization of available carrying capacity. The successful induced spawning and larval rearing of several endangered species have opened up the avenues of replenishment. It implies aquaculture in rehabilitation programmes of endangered fish populations by increasing the effective population size of the threatened species. A marine reserve is a defined space within the sea in which fishing is banned or other restrictions are placed in an effort to protect plants, animals, and habitats, ultimately conserving biodiversity. Marine reserves can also be used for educational purposes, recreation, and tourism as well as potentially increasing fisheries yields by enhancing the declining fish populations. The creation of ADMAs, are a systematic management approach for watersheds, where the primary goal is to protect the aquatic biodiversity in a given area. ADMAs range from individual species protection acts to full-scale biodiversity oriented programs. Bioregional management is a total ecosystem strategy, which regulates factors affecting aquatic biodiversity by balancing conservation, economic, and social needs within an area. This consists of both small-scale biosphere reserves and larger reserves where activities such as fishing, hunting, harvesting, and development activities are restricted. In recent years, the protection of lakes and small portions of watersheds organized by local watershed groups has helped the problem of political jurisdiction. Many specialized programs have been instituted to protect biodiversity. The goal of this program is to restore the health of riverine systems. Increasing public awareness can be accomplished through educational programs, incentive programs, and volunteer monitoring programs. Aquatic areas that have been damaged or suffered habitat loss or degradation can be restored including restoration of species depletion. The Indian Fisheries Act of 1897 (modified in 1956) is a landmark in the conservation of fishes. Besides provision to and monitor gears, mesh size and observance of fishing or closed seasons, the Act also prohibits the use of explosives or poisons to indiscrimately kill fish in any water. In this measure, the threatened are conserved outside their natural habitats. The main pillars of ex situ conservation programme are (I) live gene bank and (II) gene bank with gamete and (III) gene bank with DNA. In a live gene bank which is a genetic resource centre, the endangered species are reared in captivity, bred therein and genetically managed avoiding inbreeding depression, domestication and unintended selection. The NBFGR is maintaining the wild stocks of threatened species. In Gamete/ Embryo gene bank, adequate samples representative of the natural genetic variation of endangered species are kept in suspended animation under extra low temperature (-196^{0}C) in liquid nitrogen (LN$_2$) availability of genetic materials of threatened categories and for intensive breeding programmes of economically important species. Long-term cryopreservation of milt of the endangered and economically important fishes has been achieved by NBFGR. Since the technique is successful only for sperm, Efforts should now be focussed on the androgenesis through which the whole genome can be constituted from the cryopreserved milt alone.

I-T-8

EFFECTS OF EXPERIMENTALLY INDUCED ZINC DEFICIENCY ON *STAPHYLOCOCCUS AUREUS* INFECTION IN RATS

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The zinc is one of the major trace mineral essentially required for the normal growth of various body tissues especially of epithelium of various organs of digestive tract in animals. In clinical observations, the zinc deficiency has also been found to result in an increased susceptibility to a variety of infectious disorders as well as decreased cell mediated immunity causing significant impairment of cellular and humoral immune response. The various factors causing clinical or sub- clinical deficiency of this mineral in various animal species hinders performance by affecting a number of body functions including retardation of growth, inappetance, alopecia, impaired development and functions of different organs. The present study had been carried out to delineate the effects occur due to experimental zinc deficiency on *Staphylococcus aureus* infection in rats. The present investigation was conducted on 48 male weanling albino rats divided into two groups offered zinc deficient and zinc adequate purified diets, respectively. Blood samples of each rat were collected on 0, 7, 14, 21 and 28th day of experiment for haematological, biochemical, bacteriological and immunological examinations. On 21st day of experiment, when clinical signs of zinc deficiency appeared, animals of different sub-groups were infected with *S. aureus* infection intraperitoneally. After appearance of clinical signs due to infection, the bacterial viable cell count were examined in different vital organs. The bacterial viable cell count of *S. aureus* in liver and spleen of zinc deficient animals was significantly higher than that of animals offered zinc adequate purified diet.

I-T-9

IMPACT OF AI AND ET IN ANIMAL BIO-DIVERSITY

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Biotechnology in animal breeding includes AI and ET in practical breeding. There are certainly merits for these techniques. AI was initially developed to reduce the incidence of reproductive disease in animals, but now allows the use of superior male animals on a larger scale than possible with natural service. If breeding or AI centers are available, smallholders, who often prefer keeping female animals, may no longer have to keep entire males. ET makes it easier to introduce exotic animals into countries that have strict quarantine requirements. There are, however, a number of prerequisites for successful use of AI. Farmers need to recognize whether an animal is in heat; semen and insemination technicians must be within easy reach; liquid nitrogen has to be available etc. This infrastructure is lacking in large parts of India and experience with AI has often been disappointing. Even where it seems to work well, there are some dangers. In most cases, the semen is from animals of potentially high production in meat (rapid growth) or milk. Such high-yielding animals are less resistant to disease, more prone to heat stress, require more water than indigenous breeds and need good-quality feed to achieve their production potential. For a dairy cow that produces 6000 to 8000 liters of milk per lactation, straw with a digestibility of 50–55% is not good forage, whereas indigenous breeds that need only to survive as a savings account can manage on this kind of nutrition. AI can also lead to loss in biodiversity. AI bulls can become semen millionaires, which may be good for the breeders and the AI businesses but has a disastrous impact on genetic variability in indigenous breeds. The push for maximum production means that many bulls are closely related. This leads to an international uniformity among HF dairy cattle. ET will further accelerate this dangerous trend towards uniformity among dairy cattle. These trends are occurring in the face of the need to conserve animal genetic diversity. The poorer livestock keepers in India have to cope with a great variability in ecological conditions and need animals adapted to the local environments. Uniform, high-producing animals cannot serve this purpose. Indigenous animal genetic resources are needed for that purpose. Although predominantly used among specialized high-yielding breeds, there are exceptions. Gir cattle semen and embryos are sold in Brazil.Gir are known well adopted harsh environment, can cope with low-quality forage, are fairly tick resistant and tolerate a range of diseases. However, the number of donor animals does not reflect the variability of types found in the larger Gir cattle population. And this use of biotechnology raises another difficult issue: the Gir were originally selected by cattle breeders but the Indian Government successfully discouraged the farmer from keeping Gir. So white commercial farmers conserved Gir cattle and brought them back to fame. If the Gir continue to be commercially successful, who should benefit?

I-T-10

GUT MICROBIAL DIVERSITY IN DROMEDARIES: FUTURE IMPLICATIONS

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Camels (*Camelus dromedarius*) are ship of the desert ecosystem utilizing the flora and fauna of the deserts as their feed. They are mostly confined to the North-western arid zone of India and are used for transport, milk etc. The feed consumed by the animals is converted by the rumen microorganisms into meat and milk. The microbial populations are vital to life on the earth and are of enormous practical significance in medicine; engineering and agriculture. Current research indicated that more than 99% of the microbes in the environment can not be readily cultivated. According to Woese's classification all microbes represented in the rumen ecosystem can be classified into three domains, Bacteria (bacteria), Archaea (methanogens) and Eucarya (protozoa and fungi). The digestive system of ruminant animals is composed of a complex pregastric microbial fermentation, coupled with a specialised metabolism that allows ruminants to digest plant material and utilise the fermentation end-products for growth and production. The rumen contains large numbers of bacteria, protozoa, archaea, fungi and viruses. The inability to culture all (or even most) of the microbes in the rumen is a major barrier to defining their true functional roles in the rumen. To understand the complex microbial communities function and how microbes interact within their niches represents a major challenge for rumen microbiologists today. The present paper will enlist certain aspects of studying the gut microbial diversity in dromedaries and their future implications.

II-T-1

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST CLASSICAL SWINE FEVER VIRUS

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Classical swine fever (CSF) is one of the most feared and devastating viral disease of pigs. The disease is prevalent in most of the pig producing states of India and is considered to be the major obstacle for growth of pig husbandry in Assam and other North Eastern states. The CSF virus, which belongs to the members of the genus Pestivirus of the family Flaviviridae may cross react with other members of the pestivirus and use of polyclonal serum in serological assay occasionally, shows false positivitiy if pigs are infected with pestivirus like bovine viral diarrhoea virus. To overcome this problem monoclonal antibodies specific to CSF virus antigen have been produced and characterized. PK-cell line adapted local isolate of CSF virus was partially purified and 100 µl of concentrated antigen having protein concentration of 78µg was mixed with an equal volume of freund's complete adjuvant. The mixture was injected intraperitoneally per mouse on 0 day followed by immunization at 14 days interval upto 70 days and 200 µl of the antigen without adjuvant three days prior to fusion. The spleenocytes of the immunized mice and myeloma cells (Sp2/0Ag) were fused at the ratio 10:1 using 50% polyethylene glycol (MW 1500) and added in Dulbecco's medium supplemented with 20% foetal bovine serum and HAT medium and distributed equally into 96 wells tissue culture plates. The plates were incubated for 7-10 days at 37^0C in Co2 incubator having 5% Co2 level and screening of the hybridoma supernatant was done by indirect ELISA. Hybridomas secreting antibodies against CSF virus antigen were cloned by limiting dilution technique. A panel of five monoclonal antibodies of IgG2a isotype and specific to CSF virus was obtained. Reactivity of the monoclonal antibodies with CSF virus in PK-15 cell line was tested by indirect peroxidase test (IPT) and the results were compared with reference panel of monoclonal antibodies against E2 protein of CSF virus. All the monoclonal antibodies reacted with the homologous CSF virus and cell culture adapted lapinized CSF vaccine virus. The reaction pattern is similar to the E2 monoclonal antibodies. Fourteen CSF virus field isolates from different places of Assam were also tested by IPT using the monoclonal antibodies. The monoclonal antibodies, which were produced for the first time in India against CSF virus will be useful for detecting and antigenic characterization of CSF virus isolates from different places of India.

II-T-2

FLOW CYTOMETRIC ASSESSMENT OF LEVELS OF B AND T CELLS AND THEIR IL2 RECEPTOR EXPRESSION IN BUFFALOES VACCINATED AGAINST HAEMORRHAGIC SEPTICAEMIA

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Haemorrhagic Septicaemia (HS) causes more than 50,000 deaths and losses of Rs.10 million annually in India (Singh *et al,* 1996). Inspite of vaccination, outbreaks of HS continue to occur in the endemic areas. The present study was therefore undertaken to assess the levels and activation status (IL2 receptor expression) of lymphocytes, in the peripheral blood of pre- and post-vaccinated buffaloes to gain an insight into the immunity induced by vaccination. The mean B lymphocytes levels at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 40.25 ± 15.17, 28.38 ± 9.89, 29.77 ± 12.45, 27.04 ± 14.27 and 30.31 ± 15.19, respectively. There was no significant variation in B lymphocyte levels between pre-vaccinated and post vaccinated buffaloes at different time intervals (0 day, and 30, 60, 90 and 120 days). The mean percentages of CD3-positive T-lymphocytes were 29.31 ± 11.04, 27.95 ± 12.49, 30.98 ± 10.96, 32.77 ± 12.50 and 13.56 ± 2.20, respectively. The differences between the mean percentages of CD3-positive T-lymphocytes were non-significant at various intervals post-vaccination. However, percentage of CD3-positive cells showed a non-significant decline from 0 to 30 days followed by a non-significant rise from 30 to 90 days, which subsequently showed a non-significant fall at 120 days post-vaccination. The mean percentages of CD8-positive T lymphocytes at day 0 and 30, 60, 90 and 120 days post-vaccination were 28.40 ± 5.32, 25.97 ± 9.25, 17.55 ± 7.87, 25.97 ± 9.24 and 12.43 ± 3.45, respectively. There was no significant variation in CD8+ lymphocyte levels between pre-vaccinated and post vaccinated buffaloes at different time intervals (0 day, and 30, 60, 90 and 120 days). The mean levels of activated (IL2R+) lymphocytes at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 13.81 ± 2.79, 24.31 ± 5.43, 22.18 ± 8.30, 22.07 ± 6.55 and 27.08 ± 11.35, respectively. There was no significant variation in levels of IL-2R$^+$ lymphocytes between pre-vaccinated and post-vaccinated buffaloes at 30 day, 60 days, 90 days and 120 days post-vaccination. The results indicate that the vaccination was unable to enhance the levels of lymphocytes and their activation to a significant level. Furthermore, the levels declined very early giving little hope for protective immunity upto 6 months as believed widely.

II-T-2

MONITORING OF VACCINATION AGAINST HEMORRHAGIC SEPTICEMIA (H.S.) IN THE STATE OF HARYANA: A THREE YEARS STUDY(2006-09)

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H.S. vaccination was monitored by determining immunity status of cattle and buffaloes. The serum antibody levels estimated using an indigenously developed monoclonal antibody based ELISA kit for HS antibodies and animals were classified as "Protected, Partially Protected and Protected" on the basis of serum antibody levels. A total of 14320 serum samples were tested during the period of study. Of these, 3695 samples in 2006-07, 5429 in 2007-08 and 5196 in the year 2008-09 were tested. Over all immunity status of cattle and buffaloes were recorded as 58.67%, 19.9% and 21.40% as Protected, Partially protected and Not protected in the year 2006-07. In the year 2007-08, 57.25%, 33.16% and 9.59% were recorded as Protected, Partially protected and Not protected and in the year 2008-09, 57.14%, 28.46% and 14.4% were recorded as Protected, Partially protected and Not protected. Therefore, almost similar levels of protections were recorded in all the three years. The study also showed higher levels of protection in buffaloes than in cattle indicating preferential vaccination of cattle. Districts wise picture of the immunity levels was also constructed. Reasons for occurring of sporadic HS outbreaks despite vaccinationand importance of monitoring of vaccination for control of the disease discussed.

II-T-4

TITERS OF ANTIBODIES TO FMD VIRUS IN VACCINATED CATTLE AND BUFFALOES BY DIVA ELISA

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Serum samples from a total of 67 cattle and buffaloes from various villages around Ludhiana were subjected to DIVA ELISA. Sera from 10 animals were found positive for Foot and Mouth Disease Virus (FMDV) infection. Serum samples of 10 vaccinated animals from the University Dairy Farm were subjected to DIVA ELISA. Two out of the 10 animals were found to be positive for FMDV infection. Serial samples of sera at 100 days, 130 days and 145 days post-vaccination from 6 Foot and Mouth Disease vaccinated buffaloes were assessed for titers of antibody against FMDV serotypes O, A and Asia-1 by Liquid Phase Blocking ELISA. The mean titers were as follows: Antibodies against O at 100 days, 130 days and 145 days = 2.4; Antibodies against A at 100 days = 1.858, 130 days = 1.866 and 145 days = 1.85; Antibodies against Asia-1 at 100 days = 2.225, 130days= 2.166 and 145 days = 2.225, respectively. The differences among the mean titers of antibodies against FMDV O, A and Asia-1 at days 100, 130 and 145 were non – significant. However, two animals showed a titer of 1.65 against FMDV A at 100, 130 and 145 days which was below the protective level of ≥ 1.8. The findings suggest the induction of an inadequate immunity by vaccination.

II-T-5

EVALUATION OF RECOMBINANT-DNA and RECOMBINANT-BCG VACCINEs AGAINST BOVINE TUBERCULOSIS IN CALVES BY FLOWCYTOMETRY

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In most of developing country, humans and animals share the same microenvironment, especially during droughts and the dry season, thereby potentially promoting the transmission of *M. bovis* from animals to humans. Cell-mediated immunity is essential for the control of mycobacterial infections. DNA vaccines induce strong CD4 and CD8 mediated immune responses in mice, but the two compartments may differ in relative importance in the protection conferred by the vaccine against TB according to the antigen, the genetic background, and the phase of infection (active infection or latency). To assist in the evaluation of efficacy of rDNA and rBCG vaccine, three groups of 8 calves each, aged approximately 4 months from herds with known negative tuberculosis status were injected intramuscularly with 1000ug of recombinant-DNA expressing Rv3881c protein, subcutaneously 10^6 CFU of recombinant-BCG expressing Rv3881c protein and PBS via the same route. In the Flowcytometry assay, the difference in the population of CD4 cells produced in rDNA vaccinated calves and recombinant-BCG injected calves was significant on 30th day ($P < 0.05$), increased to maximum and maintained at high level on 120th day ($P<0.01$). Whereas, the difference in population of CD8 cells produced in rBCG vaccinated calves was also significant on day 60, 90, and 120 ($P>0.05$). the difference in the CD4 population in rDNA and Control was significant only on 150th day ($P < 0.05$), and moderately significant on 120th day ($P<0.01$) and non-significant on 0, 30, 60 and 90th day. Whereas, the difference in population of CD8 cells produced in Control and rDNA vaccinated calves was non-significant on all days ($P>0.05$). Therefore, Under the prevailing conditions the rBCG vaccine were able to show higher significant CD4 and CD8 cell responses in calves against the B*ovine* tuberculosis than rDNA vacine.

II-T-6

SEROEPIDEMIOLOGICAL STUDY OF BRUCELLOSIS IN BOVINES IN FEW DISTRICTS OF SOUTH KARNATAKA

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Brucellosis is a typical zoonotic infection which threatens public and animal health and contributes to economic problems in many countries of the world including India which is caused by bacteria of the genus *Brucella,* which are facultative intracelular micro-organisms. economically important reproductive disease of livestock including cattle, buffalo, sheep, goats and pigs. There is growing concern that the disease may further flare up due to intensive dairy development programmes, especially among the farming community and professionally associated personnel. Maintenance of such unproductive milch animals, especially buffaloes known for their low and delayed pregnancies is a potential loss to dairy farmer. The disease induces infertility, delayed heat, interrupted lactation, loss of calves, wool, meat and milk production and is of zoonotic importance in developing countries, including India. There is growing concern that the disease may further flare up due to intensive dairy development programmes, especially among the farming community and professionally associated personnel. In the present study, 1902 serum samples were collected from large herds with a history of abortion and infertility from HASSAN, CHIKMAGALUR, SHIVAMOGGA districts of Karnataka and screened by conventional Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (SAT) tests. Out of which, 54 of 754 cattle samples (1.3%) and 41 out of 678 buffalo (1.65%) were positive. In another serosurvey, out of 470 bovine sera tested for brucellosis, the seroprevalence was 1.24% in cattle and 0.44% in buffaloes. These findings of brucellosis survey indicated that several factors influenced the varied prevalence of brucellosis.

II-T-7

EFFECT OF DIETARY AFLATOXIN B_1 ON IMMUNE RESPONSE IN DUCKLINGS

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The present study was undertaken to assess the effect of dietary aflatoxin B_1 on cell mediated and humoral immune response in ducks. Two test groups and one control group (each comprising of day old fifteen ducklings) were taken in the present study. The ducklings from test groups received aflatoxin B_1 at the rate of 0.2 and 0.5 ppm respectively in the feed for 45 days. Immunological investigation revealed significant suppression of humoral immunity as measured by haemagglutinin (HA) response in ducklings against Sheep RBC. The ducklings of both the test groups showed a significant (Pd"0.01) decrease in HA titer as compared to the ducklings of control group. Measurement of Cell mediated immunity (CMI) by *in vivo* contact sensitization test with 2-4, dinitrofluorobenzene showed significantly ($P \leq 0.01$) lower increase in mean skin thickness in the ducklings of test groups as compared to the ducklings of control group indicating depression of CMI in the ducklings fed with aflatoxin B_1. The results in the present study indicated that the concentration of aflatoxin B_1 as low as 0.2 ppm in duckling causes significant suppression of both cell mediated and humoral immunity, , leading to increased susceptibility to environmental and infectious agents.

II-T-8

IMMUNOBIOCHEMICAL CHARACTERIZATION OF A PURIFIED ALLERGEN FROM BLACKGRAM

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Legumes are important source of dietary proteins worldwide. They are also among the major elicitors of IgE-mediated food allergy in the US, UK, Mediterranean and many Asian countries. Purified major allergens are prerequisite for component based diagnosis and therapy of food allergy, facilitating the detection of individual sensitivity and understanding of clinical expression. The present study was aimed to isolate a major allergenic protein from blackgram and characterize by standard immunobiochemical methods. Respiratory allergy patients with history of food allergy were skin prick tested with a battery of common food allergens at the Out Patients Department, V. P. Chest Institute, Delhi. Sera were collected from patients with marked positive skin reactions and elevated specific IgE to blackgram extract. An allergenic protein from blackgram was purified using a combination of anion exchange (Q Sepharose) and hydrophobic interaction chromatography (Phenyl Sepharose). The purified protein was characterized by indirect ELISA, ELISA inhibition, immunoblot and immunoblot inhibition skin prick tests (SPTs), stripped basophil histamine release, lymphoproliferation and cytokine analysis assay. Further, biochemical and physiological properties of the protein were characterized by digestibility assay, periodic Acid Schiffs (PAS) staining, and N-terminal amino acid sequencing. The purified protein separated at 28 kDa on 12% SDS-PAGE and showed IgE- reactivity to 81 % of blackgram hypersensitive patients' sera on immunoblot indicating it to be major allergen. The potency of the purified protein was assessed by IgE-ELISA inhibition. A dose dependent inhibition was observed with EC_{50} of 142 ng. The protein concentration of 10 ng could inhibit up to 61% of IgE binding to self in ELISA inhibition. Clinical relevance of purified 28 kDa allergenic protein was evaluated by in vivo and in vitro assays. Of the 39 food allergy patients (history) skin prick tested (SPT), 9 showed marked positive skin reactions to blackgram extract. The 28 kDa protein recognized 7/9 (77.8%) of blackgram hypersensitive patients by skin prick tests. All nine blackgram hypersensitive patients also showed histamine release with 10 ng each of blackgram extract and 8/9 patients to purified protein as compared to the controls. The purified protein induced lymphocyte proliferation (mean O.Ds (0.421 ± 0.09) and IL-4 secretion (285± 78 pg/ml) substantially in 7/9 of patients. This indicated induction of TH2 type response by the purified protein. Digestibility of the purified protein was assessed by simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The 28 kDa purified protein remained stable for 15 min. on incubation with pepsin. A weak 16 kDa band appeared after 15 min. of pepsin digestion that remained stable till 60 min. Purified protein degraded within 5 min. after incubation with SIF. The purified protein was detected to be a glycoprotein. IgE-binding property was slightly reduced upon meta-periodate treatment. The N-terminus-12 residues sequence of 28 kDa purified protein obtained was GRREDDYDNLQL A stretch of residues 'DDYDNLQL' showed homology with Rho-specific inhibitor of transcription termination (E=0.42, Identity=87%) and NBS-LRR type disease resistant protein from peanut (*Arachis hypogaea*) (E=2, Identity=77%). The purified 28 kDa protein seems to be a potent major allergen that may have implication in diagnosis and therapy of allergic disorders due to blackgram.

II-T-9

DETECTION OF FOOT AND MOUTH DISEASE VIRUS SEROTYPE SPECIFIC ANTIBODIES IN SHEEP AT ORGANIZED FARMS AND RURAL COHORTS BY USING LIQUID PHASE BLOCKING ELISA

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We described the prevalence of FMD serotype specific antibodies in sheep population at organized farms as well as rural cohorts from four districts of Haryana using liquid phase blocking ELISA (LPBE). A total of 634 serum samples were analysed. At two organized farms, where vaccination against FMDV was carried out routinely, the LPBE antibody titres ($> 1.8 \log_{10}$) were observed against O, A and Asia-1 serotypes, in 45.40, 39.00 and 22.70 percent of sheep, respectively, while in other organized farm where no vaccination is carried out these titres were recorded in 1.2, 1.2 and 2-5 percent sheep population. In rural cohorts (whose vaccination status was not known) these percentage were 13.55, 18.00 and 3.8 percent of sheep population respectively. The possible role of sheep in light of these observations in epidemiology of FMD in the field shall be discussed.

II-T-10

SERO-EPIDEMIOLOGICAL STUDY OF *PESTE DES PETITS RUMINANTS* IN CATTLE AND BUFFALOES IN SOUTHERN INDIA

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This study describes sero-surveillance of *Peste des petits ruminants* (PPR) in cattle and buffaloes carried out during the period 2009-2010 using the serum samples randomly collected from different parts of Southern India. This report presents the results of PPR virus (PPRV) specific antibodies in situations where the sub-clinical infection was suspected in cattle and buffaloes. A total of 1742 serum samples from cattle and buffaloes, and 425 from sheep and 50 from goat, which were associated with bovine population were collected from different states of Southern India and were screened for PPRV antibody by using indigenously developed PPR monoclonal antibody-based competitive ELISA kit. Analysis of 1742 serum samples indicates that an overall 4.99 % prevalence of PPRV antibody in cattle and buffaloes. The presence of PPRV antibodies in cattle and buffaloes demonstrate that bovines are exposed to PPR infection in naturally either directly or indirectly. Further, it implies the importance of bovines as sub-clinical hosts for the virus besides widespread presence of the disease in sheep and goats in India.

II-T-11

VARIABILITY IN HYPERVARIABLE LOOPS INDICATES A POSSIBLE ROLE OF PMOMPA IN DIFFERENTIAL PASTEURELLA PATHOGENESIS

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Our recent work on understanding the **role of genetic elements in pasteurellosis pathogenesis** has thrown in few surprises. It was found that pasteurellae recovered from diseased animals vis-à-vis apparently healthy animals harbour different sets of ompA alleles belonging to three major lineages. The association of almost identical alleles with different capsular types suggests that different alleles have undergone assortive recombination and horizontal gene transfer. Secondary structure predictions of Pm ompA classes using Psipred and their swiss-model comparison with *E.coli* ompA three-dimensional structural models revealed that these consists of eight membrane spanning â-strands structures and four surface exposed loops in the N-terminal transmembrane domains. Most amino acid substitutions are located in the hypervariable domains within the surface exposed loops with probable role in adhesion to host cells. Consistent recovery of pasteurellae with definitive ompA allelic variants from clinical infections is indicative of the differential role of ompA in pasteurella pathogenesis which is currently being explored.

II-T-12

DEVELOPMENT OF IMMUNO DIAGNOSTICS USING CHICKEN ANAEMIA VIRUS (CAV) VP1 RECOMBINANT PROTEIN

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A single serum dilution enzyme linked immunosorbent assay (ELISA) and flow through assay (FTA) were developed using VP1 recombinant protein of chicken anaemia virus (CAV) as coating antigen. The VP1 ORF3 recombinant clone was constructed in pPROEXHTb plasmid and expressed in DH5á *E.coli*, by the department of animal biotechnology, Madras Veterinary College, TANUVAS, Chennai. The recombinant plasmid was analyzed with regard to size, PCR amplification, restriction enzyme digestion, immunoblotting and sequencing of PCR product. The dynamic working range of recombinant antigen and conjugate was found to be 7.5 ng / μl and 1 in 3000 respectively. The correlation coefficient at 1: 1000 dilution was 0.90, and which was more than that of all other dilutions. Hence, 1:1000 serum dilution was preferred to predict titre. The slope (A) and intercept (B) at 1:1000 dilution were found to be 0.06 and 0.106 respectively. After developing the formula, a total of 302 serum samples were screened for CAV antibodies. The developed flow through assay detected 3 μg / μl concentration of recombinant CAV antigen. The single serum dilution ELISA was highly useful when screening large number of serum samples while monitoring breeder flock infection status or immunity. The developed ELISA reduce cost of reagents and time and interpretation of results was made easy. The flow through assay was also easy to perform and save reagents and can be done at farm level without much technical expertise for interpretation in detection of antigen.

II-T-13

DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF THE ORGANOPHOSPHATE PESTICIDE, CHLORPYRIFOS

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Chlorpyrifos is a widely used broadspectrum organophosphate pesticide. Due to its worldwide use in agricultural and domestic settings, and the harmful effects it causes on the body of individuals exposed to it even at low levels, it becomes necessary to detect its minutest levels of contamination, for which immunoassays are the best option. For the development of an immunoassay, one of the prerequisite is the availability of specific reagents: antigen/antibodies. Chlorpyrifos being low molecular weight, needs to be conjugated to a carrier molecule of high molecular weight, to render it immunogenic. To conjugate it to the carrier, a functional group needs to be introduced in the molecule of chlorpyrifos, since it lacks a reactive functional group. In the present study, a hapten of chlorpyrifos was synthesized by introducing a carboxylic group to be used for linking with the carrier protein through the amino group. Introduction of this carboxylic group was confirmed by thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) spectral analysis. The hapten thus prepared was conjugated with Bovine Serum Albumin (BSA) by the active ester method and purified by dialysis. The resulting conjugate was used to produce anti-chlorpyrifos antibodies and the presence of anti-chlorpyrifos antibodies was tested by Double immunodiffusion (DID) test. ELISA was developed for the detection/titration of anti-chlorpyrifos antibodies and as high as 640,000 titre antibodies were detected. These anti-chlorpyrifos antibodies had no detectable anti-BSA antibodies and antibodies against other pesticides tested in the present study. A competitive inhibition ELISA was developed for the quantitative detection of chlorpyrifos employing anti-chlorpyrifos antibodies developed. Sensitivity of the ELISA developed above was found to be 10 fg. Potential applications of the ELISA developed here will be discussed.

II-T-14

SURVEY OF BRUCELLOSIS, IBR, PPR BY ELISA TEST

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An epidemiological study was conducted to determined the prevalence of Brucellosis,pestes des petitis ruminants, infectious bovine rhinotracheitis in cattle and buffaloes in and around Bikaner. A total of 100 cattle and buffaloes each were screened. An overall seroprevalence of brucellosis was 21% in cattle and 19% in buffaloes by A-B ELISA test. An overall seroprevalence of IBR was 43% in cattle and 40% in buffaloes by A-B ELISA test. An overall seroprevalence of PPR was 19% in cattle and 15% in buffaloes by c- ELISA test.

II-T-15

SURVEY OF SOME INFECTIOUS DISEASES OF GOATS IN BIKANER

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An epidemiological study was conducted to determine the seroprevalence of Brucellosis, Johne's disease and Contagious caprine Pleuropneumonia (C.C.P.P) in goats in Bikaner. A total of 300 goats were screened. An overall seroprevalence of Brucellosis was found to be 10 and 6 per cent by using RBPT and STAT, respectively. An overall seroprevalence of Paratuberculosis was 29 per cent by indirect ELISA test. An overall seroprevalence of contagious caprine pleuropneumonia (C.C.P.P) was 60 per cent in goat by Latex agglutination test.

II-T-16

DETECTION OF PESTI DES PETITS RUMINANTS VIRUS USING MONOCLONAL ANTIBODY BASED SANDWICH ELISA AND N GENE BASED REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION.

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The present study envisaged appraisal of detection of PPRV antigen and genome in suspected typical as well as atypical clinical and post mortem samples of sheep and goats. A total of 353 such samples from 226 sheep and 127 goats were tested. Over all incidence rate of 23.80 per cent was observed. Among 268 ante mortem samples, 63 (23.50 %) were found positive. Almost similar rate of incidence was also recorded in post mortem samples wherein 21 (24.70 %) samples yielded positive results. Species wise 49 (21.68 %) and 35 (27.55 %) samples were found to be positive in sheep and goats, respectively. Among ante mortem samples, nasal swabs yielded highest positivity (37.40 %) followed by rectal swabs/ faecal material (19.60 %) and eye swabs (13.33 %). None of the blood, serum, plasma and urine samples was found to be positive for PPRV antigen. Among post mortem tissues, lymph nodes yielded highest positivity (71.42 %), followed by spleen (50.00 %), lung (40.00 %), intestine, liver, heart (each 28.57 %) and trachea (20.00 %). However, antigen could not be detected in kidney, bile/gall bladder, thymus, brain, abomasum and tongue. Present study also intended to detect PPRV nucleic acid directly from clinical as well as tissue samples using N gene based RT-PCR. Out of 25 samples tested, 12 samples viz., 4 nasal swabs, 2 spleens, 2 hearts, 2 lymph nodes, one liver and one faecal sample were found to be positive.

II-T-17

SEROPREVALENCE OF PESTI DES PETITS RUMINANTS IN SHEEP IN GUJARAT

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The present study was undertaken to screen the sera of sheep for the detection of PPRV antibodies employing c-ELISA. A total of 591 sera belonging to 13 different locations of 3 regions of Gujarat were screened and of these 335 (56.68 %) samples yielded positive result. The maximum incidence of seroprevalence recorded was 83.72 per cent from Panjarapole, Idar followed by 72.00 per cent from Panjarapole, Kant. Region wise seroprevalence recorded was 64.10 per cent in Kachchh followed by 55.95 per cent in North Gujarat and 43.08 per cent in Saurashtra regions. District wise highest seroprevalence was recorded from Sabarkantha (83.72 %), followed by Kachchh (64.10 %), Mehsana (60.00 %), Patan (58.89 %), Banaskantha (49.10 %) and Rajkot (43.08 %). The rate of seroprevalence was highest in Chokhla breed (64.44 %) followed by Marwari (60.16 %), Patanwadi (55.40 %), Magara (44.44 %) and Crossbred (Patanwadi x Rambouillet) (44.00 %). Age wise maximum seroprevalence recorded was 76.30 per cent in the age groups of > 3 years of age followed by (63.41 %) in age group of 2 to 3 years (42.05 %) in 1 to 2 years and (35.71 %) in *d*" 1 year of age groups.

III-T-1

GENETIC CHARACTERIZATION AND EVOLUTIONARY LINEAGES ANALYSIS OF TLR2 AND TLR 4 OF *BOS GRUNNIENS* (YAK) FROM NORTH EASTERN REGION, INDIA

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Immune system functions to detect and destroy the invading pathogens via innate and acquired immune systems, which are initiated through TOLL like receptors. These are the major components of the pattern recognition receptors (PRR) systems that detects invading pathogens through conserved molecular structures found on these invading microorganisms. These receptors recognize specific ligands of bacteria, virus and fungi and mount the antimicrobial responses. As TLRs are crucial for effective immune system response, insight into organization and expression of the TLRs is important for understanding molecular basis of disease resistance/susceptibility. In bovine, 10 TLRs have been identified. No information is available on these innate immune receptors in Yak, the animal reared in high altitude areas of our country. As TLR plays a pivotal role in initiating immune responses, understanding the molecular characterization and evolutionary lineage of these TLR genes will enlighten the molecular basis of disease resistance/susceptibility in these animals. We analyzed the TLR 2 and TLR 4 from *Bos grunniens* (Yak) of North East Region of India. TLR2 and TLR4 recognize variety of microbial components. These two TLRs recognizes lipoproteins/lipopeptides from various pathogens, peptidoglycan and lipoteoic acid from gram positive bacteria, lipoarabinomannan from mycobacteria, glycerol phosphatidylinositol anchors from *Trypanosoma cruzi*, a phenol soluble moduline from staphylococcus epidermis, zymosan from fungi and lipopolysaccharide. The peripheral blood mononuclear cells were isolated from blood collected from yak from Nyukmadung Farm, NRC-Yak, Dihrang, Arunachal Pradesh using Ficoll gradient centrifugation, followed by genomic DNA isolation. The PCR for full length amplification was performed with self designed primers with three and two overlapping fragments covering the full length of TLR2 and 4, respectively. The individual amplicon was purified from gel, cloned into pJET 1.2/blunt cloning vector and confirmed by colony PCR and sequenced. The TLR2 ORF was recognized to be 2355bp from 28 to 2382 position consisting of 785aa. TLR2 showed more than 99% sequence homology with *Bos indicus*, *Bos taurus* breeds, buffalo and 97-85% with other species at the nucleotide level. Amino acid similarity also revealed similar picture with 97-99% identity with bovines and 77-93% with other species. SMART analysis of Yak protein domain architecture revealed Toll-Interleukin I receptor (TIR), Leucine rich repeats (LRR) and signal peptide region. The TLR 4 ORF was recognized to be 2385bp from 22 to 2406 position consisting of 841 amino acid. It showed more than 94.4% sequence homology with *Bos indicus* breeds and 91.5-94.5% with other species at nucleotide level. Amino acid analyses revealed 99.4%-91.1% identity with bovines and 90.3% with other species. The variations in Yak mainly lie in the LRR region. Bootstrap phylogenetic analysis by Neighbor-joining method at the amino acid level clustered yak more closely to *Bos indicus* and other bovines. Though variations were limited, present study shows existence of genetic variability in TLR2 and TLR4 gene of Yak, in particular the LRR region, which plays an important role in the pathogen recognition and the evolutionary lineage analyses shows its closeness with other bovine species.

III-T-2

IMMUNOCYTOCHEMICAL AND FLOWCYTOMETRIC CHARACTERIZATION OF BUFFALO ES LIKE CELLS FROM MORULA

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Buffaloes not only play a major role in India's milk producing supremacy but also show promising results as a disease model and genitic research platforms.therefore embryonic stem cells from this species .In this study buffalo embryos derived by *invitro* fertilized ,invitro matured buffalo oocytes were cultured in modified TCM-199 medium co cultured with buffalo oviduct epithelial cells .Embryos reaching morula were dissected using a 26G needle under zoom stereo microscope.zonapellucida is left out and the blastomeres were collected and washed in knockout DMEM supplemented with Lukemia Inhibitory Factor and cultured on Buffalo Fetus Feeder Laye(BEFF) inactivated by Mitomycin-C treatment..Medium was changed every second day.Colonies and embryoid bodies formed was immunostained for OCT-4 and SSEA-4. RT-PCR results showed these colonies were positive for OCT4 expression .Immunoflurosence were observed,flowcytometric analysis of various passages shown that the cells are positive for OCT4 and SSEA4.but cultured cells rapidly looses the expression in each passge.The present study revealed that globoseries marker SSEA-4 is comparatively superior marker for embryonic stem cell characterization.

III-T-3

STUDIES ON CELL PENETRATING ABILITY OF A RATH PEPTIDE ANALOG R31 IN HELA CELL LINE

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The aim of this work is to design and develop a cell penetrating peptide and studies on its cell penetrating ability. In this study, a novel cell penetrating peptide named R31 was synthesized by solid phase peptide synthesis using Fmoc chemistry. The R31 is a 27 amino acids long peptide derived from Rath peptide (a CPP developed in our lab with cargo carrying ability) with few modifications using Phe in hydrobhobic domain of peptide and additional basic amino acids, i.e. Lys and His extended at the N-terminal in order to increase the cationic charge and reported enhancement of delivery. A spacer Ser-Pro-Gln was introduced to separate the transmembrane domain and nuclear localization sequence (NLS domain). Finally, this peptide was conjugated with FITC at N-terminal end to facilitate its cellular uptake studies. Cellular uptake experiments were performed in HeLa cells and analyzed by fluorescent microscopy, confocal microscopy and flowcytometry (FACS). FACS analysis gave quantitative estimate of cellular uptake of FITC labeled peptide with 78.14%, 86.96% and 88.59% delivery at peptide concentrations of 20 μg, 30 μg and 40 μg, respectively. This peptide has potential of an ideal delivery vehicle as it has the ability to protect the DNA in the presence of serum, nucleases and has least cytotoxic effect even at higher peptide concentrations.

III-T-4

INVOLVEMENT OF MITOCHONDRIA IN APOPTIN INDUCED ONCOLYSIS OF HELA CELLS

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Cancer still remains a challenge for survival of living beings inspite of mammoth successes achieved in medical science in past few decades. Considerable efforts are currently being invested in the development of new anticancer therapies, which are based on the induction of apoptosis and are not hampered by the lack of functional p53 and/or over-expression of antiapoptotic genes. Oncolytic virotherapy is such a promising form of gene therapy for cancer which employs nature's own agents to find and destroy malignant cells. This therapy has a large therapeutic index but only limited side effects. Chicken Infectious Anaemia Virus (CAV) is one such virus with an inherent oncolytic property. The CAV protein Apoptin (also known as VP3) has inherent ability to induce a p53-independent and bcl-2-stimulated apoptosis specifically in cancer cells while leaving normal healthy cells unharmed. In order to utilize anticancer property of apoptin efficiently, it is necessary to elucidate the role of various signaling pathways and proteins in apoptin induced apoptosis. This study was undertaken to determine the oncolytic activity of VP3 (apoptin) gene and elucidate the molecular mechanism involved in apoptin induced apoptosis in cultured HeLa cells. The VP3 gene of CAV was amplified by PCR and cloned in eukaryotic expression vector pcDNA3.1(+) at *BamH*I and *Xba*I restriction sites. The recombinant clone containing VP3 gene (pcDNA.cav.vp3) was confirmed by restriction enzyme digestion and PCR. *In vitro* expression analysis of VP3 was done by RT-PCR using total RNA extracted from cells transfected with recombinant pcDNA.cav.vp3. To elucidate the molecular mechanism involved in apoptin induced death of HeLa cells, the cells grown to 80-90% confluency were transfected with recombinant pcDNA.cav.vp3 and harvested at 24 and 48 h post transfection. These cells were analysed for change in mitcochonrial membrane potential, cytochrome C release and activation of caspase9 (the initiator caspase of intrinsic pathways). The results of flow cytometry and western blot indicated change in mitochondrial membrane potential and efflux of cytochrome c, confirming involvement of mitochondria in apoptosis of HeLa cells by apoptin. The activation of caspase9 and caspase3, as detected by western blotting and flow cytometry revealed that the apoptosis of HeLa cells induced by apoptin is caspase dependent and further confirmed involvement of the intrinsic / mitochondrial pathway.

III-T-5

EXPRESSION AND CHARACTERIZATION OF RECOMBINANT HEAT SHOCK PROTEIN 70 (HSP 70) FROM INDIAN WATER BUFFALO *BUBALUS BUBALUS*

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Heat shock protein 70 (Hsp70) plays a vital role by bestowing cytoprotection against diverse kinds of stress. The ubiquitous Hsp 70 is the most abundant and sensitive protein to stress among all the members of Hsp family. *hsp 70* is an intronless gene located on chromosome 23 of bovines (BTA 23). Hsp 70 has been reported to protect cells from stress by promoting the folding of nascent polypeptides and by correcting the misfolding of denatured proteins. The present study was aimed to produce recombinant Hsp70 from buffalo (*Bubalus bubalus*) in a suitable prokaryotic expression vector so that it can be used as antigen for development of ELISA for detecting stress in animals. To do this, chromosomal DNA was isolated from blood of Murrah buffaloes following standard protocol. Primers were designed for complete Hsp70 *orf* after thorough analysis of the available gene sequences of buffalo species on NCBI database. While designing primers, *Nco*I and *Xho*I restriction sites were included in forward and reverse primers respectively for directional cloning of the PCR product. Hsp70 gene was amplified by PCR which resulted in a specific single band of ~1946 bp (*orf* size 1926). After restriction double digestion of the PCR product and selected prokaryotic expression vector for the study, pPRoExHTa (Invitrogen, USA) by *Nco*I and *Xho*I, ligation was put by standard methods in 20µl reaction. 10µl of the ligated mixture was transformed in to the freshly prepared DH5á competent cells by heat shock method and plating was done on Luria Bertani agar plates containing ampicillin (100µg/ml) as selectable marker. Plates were incubated at 37^{0}C for overnight. Next day randomly six colonies were picked and grown in Luria Bertani broth containing ampicillin (100µg/ml). After plasmid isolation from the selected clones, restriction double digestion was put with *Nco*I and *Xho*I enzymes for confirmation. Additionally, PCR was also put by using isolated plasmids as template. One of the clones releasing specific size insert (~1946 bp) and giving specific size amplicon (~1946 bp) by PCR was selected for induction of expression. Induction was done by adding 0.6 mM IPTG to the Luria Bertani broth containing selected clone in log phase (0.6 OD). After induction, samples were collected at hourly intervals up to 8 h to study the expression kinetics. Un-induced sample was also collected before adding IPTG for comparison. The recombinant his-tagged Hsp70 protein produced was of ~ 72 kDa and expression was optimal at ~6h post induction as evident by SDS-PAGE analysis of collected samples. Recombinant expressed protein was purified by Ni-NTA affinity chromatography under denaturing conditions using standard protocol (Qiagen, Germany). Affinity purification resulted in the single specific band of expressed protein (~72 kDa) as evidenced by SDS-PAGE. For removing salts (urea), expressed protein was dialyzed several times against PBS using dialysis tubing of 12 kDa cut off value. For further confirmation, DOT blotting and western were performed by using Ni-HRP conjugate and 4-chloro 1-naphthol as substrate. DOT blotting resulted in a dark circular brownish spot on nitrocellulose membrane while in western blotting dark brown band at specific location indicating ~72kDa size was obtained. In the present study, recombinant Hsp70 was successfully produced and purified to homogeneity. By using recombinant DNA techniques, large quantity of the highly purified protein can be obtained in a cost effective manner as seen in the present study. However, to determine the quantity of protein expressed, more vectors need to be tested. Further experiments are underway to use this recombinant Hsp 70 as antigen for development of ELISA for detection of stress in animals.

III-T-6

COMPARATIVE STUDY ON SEMINAL PLASMA PROTEIN PROFILE IN THREE DIFFERENT BREEDS OF EQUINES

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A comparative study for seminal plasma protein profile was carried out in Marwari, Zanskari and jack stallions. For this study, a total of twelve semen ejaculates, two each from two Marwari, two Zanskari and two jack (*Martina franca*) stallions were obtained using artificial vagina method. Seminal plasma was separated from all the ejaculates immediately after semen collection by centrifugation at 2000 rpm for 20 minutes. Total protein concentration was estimated by Lowry method and molecular weight was determined by SDS-PAGE analysis from the seminal plasma samples of Marwari, Zanskari and jack (*Martina franca*) stallions. The overall mean values of total protein recorded were 0.71±0.02 gm/dl, 2.24±0.22 gm/dl and 2.21±0.05 gm/dl in Marwari, Zanskari and jack stallions, respectively. The differences in protein concentration were found to be significantly higher ($P<0.01$) in Zanskari and jack stallions as compared to Marwari stallion. There was no significant difference of total protein values between Zanskari and jack stallions. In Marwari and Zanskari stallions, twelve protein bands ranging between 11.45-130.23 kDa were observed while in jack stallions there were fifteen protein bands ranging between 11.45-162.83 kDa. Out of fifteen protein bands observed, three were unique in jack stallions having molecular weight of 36.52 kDa, 39.25 kDa and 1602.83 kDa. It can be concluded that seminal plasma protein profile of jack stallion is different from the Marwari and Zanskari stallions.

III-T-7

NS1 INDUCED ONCOLYSIS OF HELA CELLS - INDEPENDENT OF DEATH RECEPTOR MEDIATED PATHWAY

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Cell-cycle progression is a highly organized and tightly regulated process that controls cell growth, cell proliferation, and ontogenesis and is well connected to the regulation of DNA damage repair. The development and maintenance of multicellular biological systems depends on a sophisticated interplay between the cells forming the organism. During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues. Cell death is part of normal development and maturation cycle, and is the component of many response patterns of living tissues to xenobiotic agents and to endogenous modulations, such as inflammation and disturbed blood supply. Cell death is an important variable in cancer development, cancer prevention and cancer therapy. Apoptosis is typically mediated by intracellular cysteine proteases, called caspases that function as initiators and executioners of the apoptotic process. The activation of caspases is accomplished by two major signalling routes: the extrinsic death receptor and the intrinsic mitochondrial pathway. In the extrinsic pathway, ligation of death receptors by their cognate ligands triggers the recruitment of the adaptor FADD and initiator caspase-8 into a death-inducing signaling complex. This leads to autolytic cleavage of procaspse-8 to form active caspase-8. Active caspase-8 cleaves and activates downstream effector caspases (caspase-3, 6 and 7). The active effector caspases, in turn, cleave a number of cytoplasmic and nuclear substrates, including poly ADP ribose polymerase (PARP), inhibitor of caspase activated Dnase (ICAD), and so on; leading to the DNA fragmentation. This study was undertaken to determine the oncolytic activity of NS1 gene (Canine parvo virus) and elucidate the molecular mechanism involved in NS1 induced apoptosis in cultured HeLa cells. The gene was cloned into a eukaryotic expression vector, pcDNA3.1(+). The recombinant clone having NS1 gene (pcDNA.cpv.ns1) was used to check if NS1 causes apoptosis in mammalian cells. For this, 70-90% confluent HeLa cells were tranfected with pcDNA.cpv.ns1 and transfected cells were analysed for apoptosis by detection of phosphatidylserine translocation using Annexin V binding by flow cytometry, internucleosomal fragmentation by Ladder assay and TUNEL assay. Results proved to be positive. To elucidate the molecular mechanism involved in NS1 induced death of HeLa cells, the cells grown to 70-90% confluency were transfected with recombinant pcDNA.cpv.ns1 and harvested at 24h, 48h and 72h post transfection. These cells were analysed for activation of caspase 8 (the initiator caspase of extrinsic pathways), TRAIL and other death receptors. The results of flow cytometry, real time PCR and western blot of the transfected samples did not show any significant change from the mock control which indicated that these are not involved in the induction of apoptosis of HeLa cells by NS1 thus excluding the possibility of death receptor mediated (extrinsic) pathway being involved in NS1 induced oncolysis of HeLa cells. Involvement of other pathways is being explored.

III-T-8

NEWCASTLE DISEASE VIRUS (NDV) CAUSES APOPTOSIS IN HELA CELLS

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Newcastle disease (ND) is one of the most acute and contagious viral infection of chickens. It causes great economic loss in developing countries. In the present study, we assessed the apoptotic potential of NDV in human cervical cancer cell line (HeLa). For this, 60-70% confluent monolayer of HeLa cells were infected with 0.1 multiplicity of infection (moi) of virus and cells were harvested at 24h, 48h & 72h post infection and analysed in various assays for apoptosis. Trypan blue dye exclusion test did not show any significant loss in cell viability at 24 h p.i. but at 48 h and 72 h p.i, the difference in viable cell population between mock and NDV infected cell was significant. This indicated that the NDV causes cytotoxicity in HeLa cells. To establish that the cell death at 48 h p.i. was due to induction of apoptosis rather than necrosis, various cytochemical assays (DNA fragmentation, PI staining, Annexin v binding assay and TUNEL assay) were performed. A classical DNA ladder was observed in NDV infected cells at 72 h p.i. whereas, as expected the no cleavage of DNA was not observed in mock and NDV infected cells at 24h and 48h p.i. The presence of intense DNA ladder at 72 h p.i. and its absence at 24h, and 48 h p.i. suggested that induction of apoptosis in Hela cells is a late event. The DNA fragmentation was further confirmed by TUNEL assay using flow cytometer. Further, to assess induction of apoptosis, harvested cells were stained with propidium iodide. Mock transfected cells revealed a typical diploid DNA peak and the minor peak in the hypo-diploid region at all time intervals. On the other hand, NDV infected cells manifested time dependent increase in the number of hypo-diploid cells. The emergence of only few hypodiploid cells (apoptotic cells) at 24h (18.71) and increase in apoptotic cells at 48 h (31.29) and 72 h (66.16) revealed that induction of apoptosis by NDV was time dependent. To further, confirm induction of apoptosis, Phosphotidyl serine (PS) translocation was studied by AnnexinV binding assays. PS which is present on inner surface of plasma membrane is translocated to outer leaflet of plasma membrane in apoptotic cells. In present study, the percentage of Annexin V positive cells was notably higher in NDV- infection (62.28%) as compared to the mock (55.28) at 24h post infection which further increased to (70.08%) as against (63.58% in mock infected cells at 48 h indicating induction of apoptosis.

III-T-9

ENDOCRINE RESPONSES AS BIOMARKERS OF STRESS IN DESERT SPECIES

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Desert species of the arid tract are frequently encountered with the problems of recurring droughts and high ambient temperature. Physiological modulations are there to tackle the crisis developed due to stress. One such change occurs in the endocrine responses as this system is one of the controlling systems of the body. Early detection of these changes can help in the alleviation of stress. Therefore determination of the endocrine responses is extremely important to solve the exigent problem of stress. It can be considered as an epoch-making step for animal welfare. Examination of the stress and productivity is important to develop management strategies. The hypothalamic-pituitary-adrenal axis is important in making the physiological approach to regulate the unusual condition. Increased release of corticotrophin releasing hormone (CRH), adreno-corticotrophin hormone (ACTH) and thyroid stimulating hormone (TSH) during stress are related with increased concentrations of thyroid hormones and adrenal steroids. As there is paucity of such work in desert species of arid tract like camel, sheep and goat, an investigation was carried out to find out the role of endocrine responses as biomarkers of stress. Sera were harvested during hot ambience and drought conditions to analyse cortisol, aldosterone, prolactin, thyroxine, and gastrin. The hormones were determined by radioimmuno assay (^{125}I). To assess the physiological role of the hormones certain supportive parameters like sodium, potassium, chloride, calcium, phosphorus, glucose, protein and cholesterol were also determined. The results showed that endocrine responses can be used as biomarkers of stress in the animals.

III-T-10

CHARACTERIZATION OF CELL PENETRATING RATH-NAVIGATOR PEPTIDE FOR ITS DNA BINDING ABILITY

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Cell –penetrating peptides (CPPs) are short peptides of less than 30 amino acids that are able to penetrate cell membranes and translocate different cargoes into cells. CPPs are novel vehicles for the translocation of cargo into cells and this property makes them potential drug delivery agents of interest. The DNA binding ability of peptide at different Peptide:Nucleotide ratios was checked by Gel Retardation Assay and Nuclease Protection Assay. In this study the cargo property of Rath-Navigator peptide was evaluated for delivery of commonly used expression vectors. The plasmid and peptide complexes were prepared at varying concentrations of peptides (10μg, 20μg, 30μg, 40μg, 50μg, 60μg, 70μg) and constant concentration of plasmid DNA(pVITRO GFP/LacZ) 500ng. The peptide showed strong interaction in retarding plasmid on gel at P:N ratio starting from 60:1,followed by 80:1,100:1,120:1 and 140:1. The Rath-Navigator Peptide formed a cage around the DNA and protected it from DNase degradation at P/N ratio 120:1,140:1,160:1,180:1 and 200:1. The peptide interacts strongly with plasmid DNA, forming a cage around them, which stabilizes and protects them from degradation in cell culture media. The Rath-Navigator peptide has the potential of an ideal delivery vehicle as it has the ability to protect the DNA in the presence of nucleases.

III-T-11

DETECTION OF ANTIBODY AGAINST STRUCTURAL AND NON STRUCTURAL PROTEIN OF FMD VIRUS IN DIFFERENT GROUP CATTLE

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The present study was undertaken with the objectives to detect the presence of antibody against structural proteins (SP) of different serotypes of FMDV in different groups of cattle. Besides, monitoring of the presence of antibody against non-structural proteins (NS) of FMDV, comparision of the occurrence of antibody against NS proteins as well as against different serotypes of FMDV were also carried out considering the significance of presence of antibody against NS proteins. A total of 1082 samples from cattle were collected from different districts of Assam for the present study. The samples were subjected to both Liquid phase blocking ELISA (LPBE) and recombinant 3AB3 nonstructural protein based indirect ELISA (DIVA test). The tests were carried out in different groups of cattle- infected, vaccinated, recovered animals and random. The samples were tested for presence of antibody against SPs of different serotypes of FMD virus as well as antibody against NSP of FMDV. Further, sandwich ELISA was taken up for confirmation by seroptyping of clinical samples collected at the phase of FMD outbreaks. Comparison on the basis of all the tests results and the available history was taken up and accordingly interpreted. For the comparative study in cattle out of the total 462 serum sample 170 showed LPBE titre of e"1.8 $\log_{10}$ against different serotypes of FMDV in which 103 were DIVA positive rest 67 serum samples were found to be negative for NSP ELISA. The outbreaks were attributed to the serotype O of FMDV in cattle. Indirect ELISA for DIVA revealed a high percentage of positive reactors for NSP antibody and it is indicative of circulation of FMD virus among the cattle population and presence of large number of carrier animals. LPBE and ELISA for NSP can be applied for retrospective diagnosis of FMD outbreak and a direct correlation could be observed between the identification of FMDV serotype associated during the outbreaks and monitoring of antibody titre against different serotypes as well as the detection of antibodies against NSP of FMDV in infected or non-infected animals, irrespective of vaccination.

III-T-12

IN OVO ADMINISTRATION OF TLR LIGANDS INDUCE PROTECTION AGAINST NEWCASTLE DISEASE VIRUS IN BROILER CHICKENS

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Innate components of the immune system detect pathogen associated molecular patterns (PAMPs) by means of pathogen recognition receptors (PRRs), including Toll-like receptors (TLRs) that can activate cells and lead to development and coordination of subsequent immune responses. Newly hatched chickens are immediately confronted with both pathogenic and commensal microorganisms that were not encountered *in ovo* via both the omnivorous diet and the local environment. The first few days post-hatch are characterized by immune system immaturity as cellular components of the adaptive response are in a naive state and therefore slow to respond to infectious agents, leading to increased early mortality in environments of high pathogenic challenge. The in *ovo* vaccination indicated a definite advantage of early onset of immunity in neonatal chickens, coupled with the convenience by which large number of embryos can be vaccinated and the advantage of giving a uniform delivery of vaccine. Besides vaccination, the in *ovo* administration of CpG modulates the immune response of young broiler chickens and increases the innate immune function of chicken heterophils. The *in ovo* administration of TLR ligands elicit a response, such as cytokine production, that can be detected post hatch draws attention to their potential use as therapeutic agents for the poultry industry. Therefore, the present work is carried out with a view to determine the stimulatory effects of TLR agonists viz; flagellin (FGN), lipopolysaccharide (LPS), genomic DNA and CpG oligodeoxynucleotide (CpG ODN) administered *in ovo* on the immune system and the protection against sub challenge dose of virulent Newcastle disease virus in broiler chicken. The TLR agonists like flagellin, genomic DNA and LPS were isolated from Salmonella Typhimurium using standard procedures and CpG-ODN^{2007} were used for *in ovo* stimulation on eighteen day old embryonated chicken eggs. The eggs were allowed to hatch and all the different groups of chickens in experimental trial were challenged intra-nasally with 0.1ml containing $10^{2.5}$ mean egg infective dose (EID_{50}) as per OIE on day 18 of age after the maternal antibody wanes. Innate immune response was studied by performing functional assay like NBT assay and H_2O_2 assay on the second day post challenged. Sera were collected at 0 day, 7th day and 14th day of post-challenge for studying the HI titre and antibody responses (Ig A and Ig Y). Chicks were observed for clinical signs and symptoms using score card. Day wise mortality/ survivability was also recorded to find the protection percentage in different groups post challenge. The hatching percentage in the FGN injected group recorded highest (95%) followed by control group (90%) then LPS injected group (80%). The DNA and CpG injected groups recorded 75% hatching indicated that the TLR agonists doses used did not cause toxicity and embryonal death. The innate immune response like the production of superoxide anion and hydrogen peroxide by stimulated heterophils and monocytes in the NDV alone group, H_2O_2 production was significantly high but lower than those found in agonists treated groups. The superoxide anion production after exposure to NDV was found to be almost double with FGN treated group in both heterophils and monocytes followed by DNA treated group. LPS, CpG and NDV alone groups showed similar production which are not significantly different from each other but significant from non-treated control group. The HI titre at 7 dpi was found higher in the CpG treated group which was better than other TLR ligands treated groups. On 14 dpi, the CpG and DNA treated groups showed maximum titre followed by FGN, LPS and NDV alone groups. The Newcastle disease virus specific IgY and IgA antibodies were detected with indirect ELISA. On 7 dpi, the FGN, CpG and DNA treated groups showed significant increased in IgY levels but in LPS and NDV alone groups, the levels were low. On 14 dpi, the DNA treated group showed highest IgY antibody level but not statistically different from FGN treated group. The Ig Y level in CpG treated group decreased while LPS treated group recorded significant increased. No significant difference was observed between the NDV alone and non-stimulated control challenged group for any of the day point. The NDV specific IgA antibody in the sera on 7 dpi, the DNA, CpG and FGN treated groups showed significant increased in IgA levels but in LPS and NDV alone groups, the increased was non-significant. On 14 dpi, the DNA treated group showed highest IgA antibody level followed by FGN, CpG and LPS treated groups which was found to be significantly different from each other. Interestingly, no significant difference was observed between the NDV alone and non-stimulated control challenged group for any of the day point. These results correlates with the protection rate shown by challenge study where FGN, DNA and CpG treated groups accorded 100% protection while LPS treated group showed 81%. The NDV alone group which has not received any TLR agonists during *in ovo* recorded only 40%. The varying degree of protection shown by various TLR agonists in challenge study correlates with the findings of adaptive immune response indicating TLR agonists have the ability of modulating immune response.

III-T-13

EXTRACTION OF BOVINE PYRUVATE DEHYDROGENASE COMPLEX AND COMPARISON OF ITS ENZYMATIC ACTIVITY WITH THE RECOMBINANT HUMAN PYRUVATE DEHYDEROGENASE COMPLEX

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The mitochondrial pyruvate dehydrogenase complex (PDC) is a multienzyme complex composed of multiple copies of 3 distinct enzymes: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and dihydrolipoamide dehydrogenase (E3). This complex is responsible for controlling a key committed step in carbohydrate utilisation by catalysing the oxidative decarboxylation of pyruvate to yield acetyl CoA, CO_2 and NADH. PDC, particularly from prokaryotic sources, has been widely studied as a model system for investigating the molecular basis of cooperativity between physically and functionally linked enzymes in a metabolic pathway and the catalytic and regulatory advantages conferred by their organisation into precisely-engineered 'molecular-machines'. Defects in human PDC have been implicated in a wide variety of genetic, metabolic and autoimmune disorders. Over 200 PDC-linked mutations have been reported to date in the human population leading to clinical symptoms of various magnitudes and manifestations, mostly in the X-linked gene for the á- subunit of the E1 component. In these mutations, there is a decreased activity of PDC enzyme leading to lactic acidosis due to lack of proper utilization of glucose and hence developmental delays and defects. Prevalence of these mutations might go unnoticed in bovine population; however their study is important in studying the patho-physiology of such genetic diseases. In this study PDC was extracted from a muscle sample collected from a male cow and its enzymatic activity was compared to human recombinant PDC (sample collection from of native type of PDC from human is difficult due to ethical reasons). It was found that the activity of the bovine PDC was comparable to recombinant human PDC, the later was found to have activity comparable to reported values of native PDC collected from human heart.

IV-T-1

CAPSULAR POLYSACCHARIDE GENOTYPING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICALLY MASTITIC CATTLE AND GOATS

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Mastitis is an economically important disease occurring worldwide in all dairy animal species and is mostly caused by Staphylococcus aureus. In the present investigation 30 *S. aureus* were isolated from milk obtained from cattle (20) and goats(10), the two very important milch animal species in Rajasthan, having clinical mastitis. The phenotypic characterization revealed that all isolates of *S. aureus* produced round, smooth and glistening colonies with golden yellow pigmentation and produced coagulase and haemolysins. All the isolates were confirmed to be *S. aureus* by ribotyping using species-specific primers. The capsular typing revealed that 60% of *S. aureus* isolates from cattle possessed *cap5K* gene, 20% possessed *cap8K* gene and rest 20% did not have either of these two genes. From goats, 30% of the isolates showed presence of *cap5K* gene, 20% isolates exhibited *cap8K* gene and 50% of the isolates did not show presence of any of the two genes. The RFLP and PCR-SSCP analysis of *cap5K* and *cap8K* amplicons suggested that these genes were highly conserved.

IV-T-2

ANALYSIS OF SOX-2 EXPRESSION IN BUBALINE PREIMPLANTATION EMBRYOS BY QRT-PCR

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Sox-2 acts as a molecular rheostat in early embryonic development and its expression level needs to be tightly controlled to maintain pluripotency of embryo-derived stem cells. An attempt was made to analyze the expression pattern of sox-2 gene in *in vitro* produced embryos. Experimental groups included mature oocytes (M-II stage), 2-cell, 4-cell, 8-cell, 16-cell and morula where n=20 per group. Total RNA was extracted and cDNA synthesized using Oligo dT primers followed by PCR amplification. Amplicons of 413bp were detected in all the developmental stages studied. At the protein level, expression of sox-2 gene was demonstrable in embryos from 2-cell to morula stage by immunostaining. Relative quantification of sox-2 transcripts was attempted by using primers (FP- 5'CCGAGTGGAAACTTTAGTCC3'; RP - 5'AGTGTGTACTTATCCTTCTTC3'; Probe - 5'AAGCCGTTCATCGACGAGGCCA3') designed from the cloned PCR product. It was found that there was an increase in expression of sox-2 from mature oocyte to 2-cell stage followed by decrease until 8-cell which corresponds with the zygotic maternal transcriptional profile. This decline is followed by a drastic increase in the level of transcripts in morula. Thus it can be concluded that the pluripotent nature of the embryonic stem cells tend to improve as it approaches blastocyst stage by regulation of a network of genes involved in embryogenesis, one of which is sox -2.

IV-T-3

CLONING AND EXPRESSION OF FUSION (F) PROTEIN GENE OF NEWCASTLE DISEASE VIRUS (NDV)

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Newcastle Disease (ND) is a devastating, highly contagious disease of poultry that causes severe outbreaks with great economic losses in many countries. ND has a wide host range, infecting 27 of the 50 orders of birds. The disease is caused by Newcastle disease virus (NDV), which is designated as avian paramyxovirus type-1. The nonsegmented, negative-sense, single stranded RNA genome of NDV contains six genes in the order 3′-NP-P-M-F-HN-L-5′, which encode the six major structural proteins: nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN) and the large (L) RNA-dependent RNA polymerase. The present study was undertaken to amplify, clone and express Fusion protein gene of Newcastle disease virus. The freeze dried virus of pigeon origin was propagated in embryonated chicken eggs. RNA was isolated from the infected allantoic fluid and cDNA was synthesized by reverse transcriptase polymerase chain reaction (RT-PCR). Primers specific to fusion gene incorporated with restriction enzyme sites viz., *Nde*I and *Xho*I were used to amplify the fusion gene using cDNA as a template with Polymerase Chain Reaction (PCR). The amplicon of 1680 bp was obtained and gel purified. The purified PCR product was first cloned into TOPO cloning vector and the clone was confirmed by colony PCR and release of insert from the vector was done after restriction enzyme digestion. Prokaryotic expression vector pET22b was digested with *Nde*I and *Xho*I and linearized. TOPO released insert was ligated with digested expression vector using T_4 DNA ligase and transformed into *E.coli* using Ampicillin resistance selection. Colony PCR was carried for the selection of the recombinant clone. Recombinant clones were induced with 0.5 mM final concentration of Isopropyl-1-thio-â-D-galactosidase (IPTG) for the expression of the recombinant Fusion protein. The expressed protein of 55 kDa was obtained during the 4th hour post induction. The obtained recombinant protein reacted with rabbit anti serum, against fusion protein peptide, in Western blot confirming it to be NDV specific. The expressed fusion protein can be purified and its potential as a candidate antigen in NDV vaccine cocktails can be studied. Moreover, it is well documented that the fusion protein cleavage site sequence is one of the molecular markers for assessing the pathotypes of NDV isolates. It was concluded that the fusion protein of a pigeon isolate of NDV has been successfully cloned and expressed in a prokaryotic system and further studies would be initiated to assess its potential as an antigen in diagnostic assays or in recombinant cocktail vaccines.

IV-T-4

GENOTYPING BY TEMPLATE-DIRECTED DYE-TERMINATOR INCORPORATION FOR COOKED/PUTREFIED MEAT AUTHENTICATION

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Development of better assays for precise and rapid detection of origin of meat are always in demand. Identification of the origin of meat from processed meat product has always been a concern for a variety of economic, religious, and health reasons. A novel reproducible method for identification and differentiation of cattle and water buffalo meat by fluorescence-based SNaPshot assay (Applied Biosystems, USA) is proposed which utilized genotyping (template directed dye terminator incorporation) of cyt b gene region of mt DNA extracted from meat samples. Meat samples were subjected to different levels of autolysis experimentally, to simulate various meat processing technologies. Meat samples were cooked by various methods and were also subjected to putrefaction upto 72 hours. Further various levels of adulteration were tested to study the sensitivity of the assay. In conclusion this assay indicate absolute specificity and a high sensitivity, even to low DNA concentrations of 0.01 pg (1%) of adulteration in cattle-buffalo meat mixture and can be applied with equal efficiency to fresh, cooked and putrefied meat. The extensive development of nucleic acid based technologies over the past decade reflects their importance in food analysis. Various PCR based approaches were attempted in the past for meat authentication, but only a limited number of studies targeted buffalo as one of the species under study. The present study is also of significance in Indian context to detect adulteration of buffalo meat with meat from cattle slaughtered illegally in spite of its ban in many states. Fewer studies have been published so far reporting the application of DNA-based techniques for differentiation of water buffalo meat from cattle meat (Girish et al. 2005; Rastogi et al. 2004; Jain, et al. 2007), but with limited utility in case of mixed or processed meat. The application of fluorescence-based assay formats greatly simplifies protocols for DNA detection and overcome the limitation and ambiguities of the PCR based detection. The disadvantage of heteroduplex and SSCP analyses is that confirmatory testing (usually by sequencing) is required for all positive results to definitively identify the sequence variant. In comparison, the SNaPshot assay is a highly specific assay, producing sequence data such that confirmatory testing is not required (Murphy et al. 2003). In this context, the present study describe a method for identification and differentiation of cattle and water buffalo meat by making use of a SNP in *Cyt b* gene region, of mitochondrial genome and identifying it by SNaPshot (Applied Biosystems, USA). Fresh muscle meat samples, thirty in number for each species, were collected from local slaughter house and stored at -20 °C until use. Mitochondrial DNA, along with genomic DNA was extracted by using the method described by Ausubel et al. (1987) with few modifications. The quantity and quality (A_{260}/A_{280} ratio, i.e absorbance at wavelengths of 260 and 280) of DNA was assesses by using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA). Small fragment of *cyt b* gene of mitochondrial DNA extracted from fresh, cooked and putrefied samples was amplified. For this purpose SNaPshot primer (genotyping primer) was designed by modifying the reverse primer ($(T)_{12}$ GGC ATT GGC TGA ATG GCC GGA A- 3'), described by Rea et al. (2001) and forward primer was taken as such. Primers were designed within the cytochrome b gene region of the mitochondrial DNA. The water buffalo cytochrome b gene sequence, deposited in GenBank by Chikuni (1993), accession number D 32193, was compared with the bovine cytochrome b gene sequence reported by Anderson et al (1982), to choose the most suitable region for designing the primers (Fig. 1). Twenty two mer genotyping primer was elongated by adding 12 thymidine nucleotide at 5' end, making it reasonably long to be resolved by capillary electrophoresis. PCR was performed in Master Cycler gradient thermocycler (Eppendorf, Germany) and the total PCR solution volume of 25 µl was used containing: 1x PCR master mix (MBI Fermentas, Canada) 10 pmole of each primers and 50 ng of DNA template (cattle and/or buffalo). Reaction was performed under the following cycling conditions; after an initial heat denaturation at 95 °C for 5 min, 35 cycles were programmed as follows: 95 °C for 30 sec, 65 °C for 30 sec, 72 °C for 30 sec and final extension at 72 °C for 5 min. Amplified product was confirmed by using Genesnap and Genetool programmes (Syngene, UK) and running the products parallel to 100 bp MW marker. After amplification, PCR products were purified by Perfectprep® PCR clean up plate (Eppendorf, Germany) according to manufacturer's protocol, to remove primers and un-incorporated dNTPs. SNaPshot assay was performed in Master Cycler gradient thermocycler (Eppendorf, Germany) as per the manufacturer's protocol. SNaPshot reaction consisted, 5 µl of SNaPshot ready reaction mix (ABI), 0.01 – 0.4 pmol of purified PCR products, 2 mM genotyping primer in the final reaction, and nuclease free water to a final reaction volume of 10 µl. Extension used 25 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds and extension at 60°C for 30 seconds. After the SNaPshot reaction, a post extension treatment to remove the 5'- phosphoryl group of the ddNTPs helps to prevent unincorporated terminators from comigrating with the extended primers and producing high background signal. For this a 10 ìl final volume was treated with 1 ìl of CIP (MBI Fermentas, Canada) for 60 min at 37°C, followed by 15 min at 85°C for enzyme inactivation. Further 1 µl of the SNaPshot products was mixed with HiDi™ formamide and LIZ 120 (Applied Biosystems) size standard and denatured at 95 °C for 5 min, further placed on ice for at least 2 min. The fluorescently labeled fragments were resolved by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) for 24 minutes at 15 kV and 60 °C temperature using GS POP-4 (1 ml) E5 Control module. The resulting data in the form of color, position of peak and area under curve was analyzed by GeneMapper™ 3.7 Software (Applied Biosystems). The processing technology applied during the manufacture of meat products are those steps, which mainly affect the integrity of the extractable DNA causing its degradation into small size fragments (Dias et al. 1994; Martinez and Man 1998). Thirty meat samples from each species were minced and mixed at various levels. Further divided into five replicates to be subjected to various experimental procedures of cooking and putrefaction. Meat samples (1g) were cooked at 100 °C and 120 °C in dry (hot air oven) and moist heat (water bath and autoclave) for 45 minutes to simulate various methods of cooking. Many times meat samples are brought to the laboratory for speciation after one or two days of slaughter under unpreserved conditions, therefore different levels of autolysis was produced by allowing the meat samples to putrefy for variable period (48 hrs to 72hrs) of time at room temperature in unpreserved conditions to stimulate the autolysis in meat. Also mitochondrial gene was chosen as a target because of the high copy number of small, circular mitochondrial DNA in cells which ensures a sufficiently high quantity of PCR product, even when small amounts of fresh or processed tissues are included (Girish et al. 2005). PCR amplified 152 bp fragment for both cattle and buffalo samples (fresh, cooked and putrefied). Amplification was not observed when DNA samples of sheep, goat, pig, horse and chicken were used. SNaPshot required an unlabeled genotyping primer that anneals to the PCR product template immediately 5' to the target SNP on the template. The reaction contains only dideoxynucleotide (ddNTP) terminators (no dNTPs) such that during thermal cycling, the DNA polymerase can extend the primers by only one base (ddNTP), which is complimentary to the template strand at the site of interest such that the products can be separated, sized, and detected by capillary electrophoresis. Each ddNTP is labeled with a different fluorescent signal/fluorochromes (A-dR$_6$G- Green, G-dR110- Blue, T dRox- Red, C-dTAMRA- Yellow, on screen indicated as Black) allowing for the detection of all four nucleotides in the same reaction. In the present study, genotyping primer gave red color peak for cattle DNA (as Rox-ddT incorporated) and the same gave yellow (Black) color peak for Buffalo DNA (as dTAMRA-ddC incorporated). Same concentration of cattle and buffalo DNA templates produced different peak heights depending on the terminator and dye label. In general, thymidine labeled with dRox (6-carboxy-x-rhodamine) produces a higher signal than cytosine labeled with dTAMRA (*N, N, N', N'*- tetramethyl-6-carboxyrhodamine). Some fragments display stronger fluorescent signal than others in the electropherogram (even after color compensation with the appropriate matrix) due to variation in SNaPshot chemistry. However, this fact does not unduly affect the readability of the electropherogram (Filippini et al. 2007). Quantification was possible as the primer set hybridized at the similar position in the cattle and buffalo DNA with same annealing affinity and amplified the DNA fragment of identical size. To measure the sensitivity of the presented SNaPshot reaction, 100ng of DNA sample was 10 fold serially diluted and tested to observe positive signals up to 0.1pg. The sensitivity was also evaluated for mixed meat samples (cattle : buffalo) of 99:1, 90:10, 75:25, 50:50, 25:75, 10:90, 1:99 (Fig. 2). SNaPshot was found to be capable of detecting 1% adulteration in cattle-buffalo meat mixture as tested. The assay was not tested for better sensitivity and possibility of the same cannot be ruled out. False positive and false negative results were not encountered in this assay, demonstrating the reliability of the procedure and repetitive assays proved the reproducibility of the method. Detection of adulteration from degraded DNA obtained from cooked and putrefied samples is one of the very important merits of this technique as a tool for meat species detection. Further trials using five genotyping primers multiplexing, for seven species (cattle, buffalo, sheep, goat, horse, chicken and pig) together, are planned. The SNaPshot assay described here provides a highly sensitive and specific assay, for identification and differentiation of cattle and buffalo meat. Due to its low detection limit and fast, simple execution it can be used as a common tool for authentication purposes involving cattle and water buffalo meat, and can be applied with equal efficiency to fresh, cooked and putrefied meat.

IV-T-5

MARKER ASSISTED INTROGRESSION USING GENOMIC SELECTION BASED ON MICROSATELLITE MARKERS

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Introgression is a crossbreeding system that introduces a beneficial allele from a donor line (or individual) into a recipient line. Traditionally, introgression involves crossing of the donor and recipient lines to form a F_1 progeny, several generations of backcrossing of the crossbred progeny to the latest generation of the recipient line (BC_1 etc.); and intercrossing to establish a new synthetic line fixing the introgressed allele. The number of generations of backcrossing is determined by the desired proportions of genes coming from the donor line for other parts of genome. Using such conventional backcrossing breeding schemes, the expected proportions of the recipient genome are increased by 50% in each successive generation. However, these figures are averages, and the actual proportion of the genome in individual progeny after one-backcross ranges between 0.5 and 1.0 (50% and 100%). This variation can be exploited to accelerate the recovery of recipient genome by selecting the individuals having higher proportions of recipient genome. The selection of the progenies having higher proportion of the recipient line or lower proportion of the donor line will increase the rate of recovery of the recipient parental genomic proportion. The parental genomic proportion may be estimated as genetic similarity between the parent and progenies, which in turn could be assessed as the proportion of band sharing between them. A suitable resource population was developed as per the need. The F_1 population was developed by crossing the donor line having trait to be introgressed and the recipient line. Subsequently, the F_1 progenies having the introgressed traits were crossed with recipient line to produce BC_1 population. A panel of 50 microsatellite markers from the chromosome carrying the introgressed trait (carrier) as well as from the non-carrier chromosomes was made. The genomic DNA from all the BC_1 progenies (carrying introgressed trait) and grand sire were extracted by using a commercial DNA extraction kit and the extracted genomic DNA was diluted to the final concentration of 25-50 ng/ ml. The microsatellite assay was carried out and the genomic proportion of the grand sire in the BC_1 progenies was estimated in terms of the genetic similarity between them. Among the BC_1 progenies, the individuals having lower genetic similarity with the grand sire were selected. The selected individuals were again backcrossed with the recipient line to produce the BC_2 progenies. Again the BC_2 progenies having introgressed trait were genotyped with the same panel of microsatellite markers and the genomic proportion of the grand sire in the BC_2 progenies was estimated in terms of the genetic similarity between them. The genomic selection in BC_2 progenies was made against the genomic proportion of donor grand sire. Several cycles of genomic selection were made. Then the progenies having introgressed trait were intercrossed to produce a stable population. A resource population was developed for introgression of naked neck gene into White Leghorn by crossing naked neck heterozygous male with WLH females. The naked neck F_1 progenies were backcrossed with WLH line. The naked neck BC_1 progenies were genotyped with a panel of 10 informative microsatellite markers. The genetic similarity between the grand sire and BC_1 progenies ranged from 0.58 to 0.85. In BC_1 generation, genomic selection was made against the genome of naked neck donor grand sire i.e. BC_1 individuals showing least genetic similarity with naked neck grand sire were selected. The average genetic similarity in selected BC_1 males and females were 0.54 and 0.59, against the population average of 0.73 in males and 0.70 in females. The BC_2 population was developed and naked neck BC_2 progenies were genotyped. The average genetic similarity between donor sire and F_1, BC_1 and BC_2 progenies were 0.80, 0.72 and 0.67. The productive performance of BC_2 population was equivalent to the WLH line.

IV-T-6

GENETIC CHARACTERIZATION AND DIVERSITY STUDY OF INDIGENOUS CATTLE BREEDS OF RAJASTHAN

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Indiginous breeds of cattle i.e. Rathi and Tharparkar were assessed for genetic diversity using eight microsatellite loci markers (ETH225, CSRM60, HEL9, ILSTS002, ILSTS-006, ETH10, ILSTS002 and BM1818) selected from the available list suggested by FAO (ISAG) for the estimation of genetic diversity in cattle. The mean number of observed and effective alleles was 6 and 3.77 for Rathi and 5 and 3.77 for Tharparkar. The average observed heterozygosity values (0.453) showed substantial number of heterozygotes in Rathi whereas tharparkar showed lesser number of heterozygotes(0.3716) . The average expected heterozygosity (0.7224) in Rathi and (0.6365) in Tharparkar indicated high diversity for this set of eight markers in the selected population. BM1818 and ETH225 were found to be most informative in Rathi and Tharparkar respectively with high PIC values. High PIC values observed for most of the markers are indicative of the usefulness of these microsatellite markers for biodiversity evaluation in both the breeds. The allele diversity (7.37), mean effective number of alleles, (4.69) and gene diversity (0.7754) values imply a substantial amount of genetic variability in both the populations. Overall H (DC) was found to be maximum (0.3716) for ETH225 (0.6304) hence it can be used for paternity testing in these breeds. The average direct count heterozygosity across all the loci was 0.4203, indicating substantial number of heterozygotes for these markers in both the populations. Maximum Hardy-Weinberg expected heterozygosity (Gene Diversity) was obtained for ETH225 (0.8447) and minimum for ILSTS006 (.6758) indicating highest and lowest range of heterozygosity for these markers. The average Hardy-Weinberg heterozygosity was found to be 0.7754; hence both the populations showed good gene diversity. Marker ILSTS002 did not show amplification in Tharparkar breed while it was successfully amplified in Rathi. Hence, this marker can be used to identify Rathi breed. Similarly, marker ILSTS006 had no heterozygotes in the analyzed animals of Tharparkar breed. Hence, these markers can be testified for Rathi and Tharparkar breed distinctness and characterization with extended sample size and comparison with the other breeds of the region.

IV-T-7

CHARACTERIZATION OF PROLACTIN GENE IN MARATHWADI BUFFALOES

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Fifty Marathwadi buffaloe blood samples were collected aseptically from three districts viz. Parbhani, Jalna and Beed of Marathwada region of Maharashtra for characterization Prolactin gene (exon III) of Marathwadi Buffaloes. DNA was extracted from the collected blood samples. Quality and Quantity of DNA was checked with agarose gel electrophoresis and biophotometer respectively. Good quality DNA samples were taken for further analysis The PCR amplification of Prolactin gene (exon III) fragment was carried out using bovine Prolactin gene specific primers. Polymerase chain reaction was carried out by using standard protocol. The PCR amplification was confirmed by running PCR product on agarose gel electrophoresis. It was observed that Bovine specific Primers successfully amplified prolactin gene (exon III) at 156 bp in Marathwadi Buffaloes in the present study. This indicates the sequence homology of that region of gene between cattle and buffaloes.

IV-T-8

CHARACTERIZATION OF A MICRO-TUBULAR GENE OF *TRYPANOSOMA EVANSI* FROM CAMEL

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Trypanosomes are obligate parasites having digenetic life-cycle alternating between vertebrate and invertebrate host. They not only infect human, but also cause disease among sheep, goat, horse, cattle, buffalo and camel being no exception. In camel it is known to cause "surra" disease, wherein the animal becomes thin, weak and prostrate and eventually dies. The first signs of the disease are a drop in milk yield and the tendency of pregnant females to abort. There is loss of appetite and the animals become very emaciated. India possesses a large dromedary population and the State of Rajasthan alone has almost 70% of this, as the rural population depends on this animal for its livelihood. Due to lack of information on the diverse strains of the parasite, therapeutic strategies have their own limitations and further, because of antigenic variations vaccines are also lacking against this organism. Microtubules are cytoskeleton proteins that are present in all eukaryotic cells and are especially abundant in the Trypanosomatidae family. These are composed of tubulin heterodimers and of microtubule associated proteins. Beta tubulin is an important isoform distributed beneath the surface membrane, the flagellum, paraflagellar rod and mitotic spindle apparatus of dividing nuclei in trypanosomes. The present study was therefore undertaken for characterization of beta tubulin gene of *Trypanosoma evansi* from camel as the sequence analysis could then be helpful for developing specific flagellar vaccines against the organism to check its motility.

IV-T-9

IDENTIFICATION OF PORK USING SPECIES-SPECIFIC PCR ASSAY OF MITOCHONDRIAL 12S r RNA GENE

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The aim of this study was to determine the applicability of pig species-specific primer for identification of pork (pig meat) and testing for cross reactivity with cattle (Ox), buffalo, sheep and goat meat. The species-specific primer of amplicon size 322 bp was designed from mitochondrial 12S r RNA gene sequences of pig. DNA extracted from meat samples were carried out by standard Kit and the quality, purity and concentration of extracted DNA were checked. The PCR conditions for designed primer pair was optimized with respect to annealing temperature. The pig specific primer was subsequently tested for cross amplification with cattle (Ox), buffalo, sheep and goat meat. After amplification and gel documentation, an intense band in case of goat, a less intense band of similar size in goat, sheep and buffalo were observed at annealing temperature of 55.6 °C. To eliminate the cross reactivity of goat specific primer, still higher annealing temperatures was attempted for amplification. At the annealing temperatures of 70.0 °C, no cross reactivity of the goat specific primer was exhibited and the desired band of 322 bp was amplified only in pig. Thus, it was concluded that that designed primer pair was species specific for pig meat samples at an annealing temperature of 70.0 °C.

IV-T-10

DEVELOPMENT AND EVALUATION OF PCR PRIMERS FOR THE DETECTION OF MONODON BACULOVIRUS IN CULTURED MARINE SHRIMP, *PENAEUS MONODON*

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Monodon baculovirus (MBV) was the first viral agent found responsible for causing disease in cultured marine shrimp (*Penaeus monodon*) and also the second viral disease of penaeid shrimps. A heavy infection of hatchery-reared larvae and postlarvae of *P. monodon* with MBV results in stunted growth and mortality causing substantial economic losses to the shrimp farmers. Samples of marine crustaceans consisting of shrimps, crabs, prawns and squilla were analyzed for the presence of MBV by using highly sensitive and specific molecular technique such as polymerase chain reaction (PCR). Several sets of published primers as well as primers designed in this study targtting the polyhedrin gene were used. Among these, 8 shrimp sample (3.4%) were positive for MBV with non-nested primer (1.4F/R) while nested primer (1.4NF/NR) gave positive reaction with 31 samples (11.10%). The primer set 261F/R (Surachetpong *et al.*, 2005) yielding a amplicon size of 261bp helped to detect MBV in 26 samples (10.6%). None of the samples were positive for MBV with primer pair P1/P2 or the nested primer pair PA1/PA2. Primers designed in this study (PmNP 189F/R & NPV 210F/R) detected MBV in 27 (10.6%) samples by first step PCR. Thus, the primers designed in this study were found to be highly sensitive and specific for the rapid diagnosis of MBV. Histopathology is one of the important method to detect MBV. The samples, which gave positive reaction for PCR, were also subjected for histopathological examinations. The microscopic observation of tissue section of hepatopancreas showed no noticeable changes indicating the absence of viral infection and consequent lack of any pathology. The efficiency of the primers in detecting the low level of virus in samples not showing any pathology indicates that it can be used for detection of healthy carriers where the virus is dormant.

IV-T-11

DETECTION OF VIRULENCE ASSOCIATED GENES IN *PASTEURELLA MULTOCIDA* ISOLATED FROM BOVINE AND POULTRY

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Twenty two isolates of *Pasteurella multocida* were recovered from field cases of haemorrhagic septicaemia (6 from buffalo, 4 from cattle) and fowl cholera (12 from poultry) outbreaks in bovines and poultry respectively during the period from April 2009 to March 2010 from different parts of Gujarat. All the isolates were identified by cultural, biochemical and further confirmation was done using PM PCR. All the *P. multocida* isolates were subjected for detection of various virulence associated genes like *pfhaB*1/2 for filamentous hemagglutinin, *oma87* for outer membrane proteins: Oma87 protein, Iron acquisition Factors like *TbpA* and *Hgb A* genes which codes for transferrin binding protein A and Haemoglobin binding Protein A respectively, *NanH* and *NanB* for Enzyme Neuraminidase and *SodA* and *SodC* for superoxid dismutase using set of primers targeting respective gene. Our finding revealed that all the 22 isolates are positive for presence of the *pfhaB*1/2 gene, *Oma87*gene, *NanB* gene, *SodA* gene and *SodC* gene. While all the bovine isolates harbor *TbpA* gene and *NanH* gene and all the poultry isolates were negative for *TbpA* gene and *NanH* gene. Three isolates from buffalo and were negative for *Hgb A* gene while rest of all bovine and poultry isolates were positive for *Hgb A* gene. Present finding indicate that most of the virulence-associated genes are found present in *P. multocida* isolates and this genes are important for determination of virulence associated factors of the isolates.

IV-T-12

DETECTION OF VIRULENCE ASSOCIATED GENES IN *PASTEURELLA MULTOCIDA* ISOLATED FROM BOVINE AND POULTRY

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Twenty two isolates of *Pasteurella multocida* were recovered from field cases of haemorrhagic septicaemia (6 from buffalo, 4 from cattle) and fowl cholera (12 from poultry) outbreaks in bovines and poultry respectively during the period from April 2009 to March 2010 from different parts of Gujarat. All the isolates were identified by cultural, biochemical and further confirmation was done using PM PCR. All the *P. multocida* isolates were subjected for detection of various virulence associated genes like *pfhaB*1/2 for filamentous hemagglutinin, *oma87* for outer membrane proteins: Oma87 protein, Iron acquisition Factors like *TbpA* and *Hgb A* genes which codes for transferrin binding protein A and Haemoglobin binding Protein A respectively, *NanH* and *NanB* for Enzyme Neuraminidase and *SodA* and *SodC* for superoxid dismutase using set of primers targeting respective gene. Our finding revealed that all the 22 isolates are positive for presence of the *pfhaB*1/2 gene, *Oma87*gene, *NanB* gene, *SodA* gene and *SodC* gene. While all the bovine isolates harbor *TbpA* gene and *NanH* gene and all the poultry isolates were negative for *TbpA* gene and *NanH* gene. Three isolates from buffalo and were negative for *Hgb A* gene while rest of all bovine and poultry isolates were positive for *Hgb A* gene. Present finding indicate that most of the virulence-associated genes are found present in *P. multocida* isolates and this genes are important for determination of virulence associated factors of the isolates.

IV-T-13

ANALYSIS OF AMPICILLIN RESISTANCE AND DETECTION OF BLA_{PSE} GENE IN POULTRY ISOLATES OF *SALMONELLA* TYPHIMURIUM

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The present study was undertaken to study the ampicillin resistance in the poultry isolates of *Salmonella* Typhimurium. In this study 51 isolates of *Salmonella* Typhimurium which were isolated from poultry eggs were studied for their responses against ampicillin , Most of the isolates were found resistant for ampicillin, so the minimum inhibitory concentration for ampicillin was calculated. As these isolates were not possessing small size plasmid which has been reported to be responsible for multiple drug resistance. The chromosomal location of drug resistant gene was also studied. Minimum inhibitory concentration for ampicillin was calculated and the highest MIC was found to be 64 μg/ml in isolate No. A833. â-lactamase has been reported to be responsible for ampicillin resistance. The gene code for this enzyme is known as bla_{PSE} in phage type *Salmonella* Typhimurium DT104. In our study out of 51 isolates the gene was found to be present in 49 isolates. In two isolates this gene was found to be absent. These isolates were found to be sensitive for ampicillin. Our study indicates that the *Salmonella* Typhimurium is becoming resistant for ampicillin which is very commonly used for the treatment of gastroenteritis in India. This résistance is more chromosomal in nature. Few isolates have shown resistance for more than 6 antibiotics. These isolates can cause serious problem in animal and human beings.

IV-T-14

IDENTIFICATION OF LACTOFERRIN GENE FROM THE MARWARI GOAT MILK

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Milk composition traits, influenced by genes and environmental factors, play important roles in value assessments of efficiency and productivity in goats. Lactoferrin (LF), involved in the efficient expression of protein in milk, is also an anabolic factor in skeletal tissue and a potent osteoblast survival factor. Therefore, it is an important candidate gene for milk composition traits in marker-assisted selection. The objective of this study is to detect *LF* gene in Marwari goats and explore their possible association with productivity. *LF* gene was isolated from the milk sample collected from the Marwari goats under field condition. The collected milk samples were transferred to laboratory (in an ice-cooled box, where they were kept under -20°C in a deep freezer until DNA isolation). Genomic DNA was extracted from frozen milk sample by Standard phenol-chloroform method and kept at -20°C. Primers were he LF gene of the DNA isolated from the milk was amplified using specific primers designed from nucleotide sequence of GenBank. The amplicon run on the (1.2%) agarose gel electrophoresis and study under the UV-illuminator. The amplicon size of LF gene was in between the 200bp and 300bp of standard 1Kb plus DNA molecular weight marker.

IV-T-15

AMPLIFICATION OF BETA-LACTOGLOBULIN (â-lg) GENE BY POLYMERASE CHAIN REACTION FROM SHEEP BLOOD

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Milk protein genetic polymorphisms have evoked considerable research interest in recent years because of possible association between milk protein genotypes and economically important traits in dairy cattle. Milk protein genes such as k-casein and b- lactoglobulin are associated with milk production performance and have a major influence on the composition of milk and on the processing properties of milk. The objective of this study is to detect â-lg gene in Marwari sheep and explore their possible association with properties of milk. Blood samples of 5 ml each were collected from Marwari sheep using 5 ml vacuitainer tubes containing EDTA from jugular vein and stored at 4^0C until processed. Genomic DNA was extracted from blood samples using standard phenol-chloroform protocol. The â-lg gene specific primers design according to the Gen Bank accession No. The DNA isolated from the sheep blood samples were amplified using specific primer. The amplicon run on the (1.2%) agarose gel electrophoresis and study under the UV-illuminator. The amplicon size of â-lg gene was in between the 200bp and 300bp of standard 1Kb plus DNA molecular weight marker.

IV-T-16

ESTIMATION OF PARENTAL GENOME PROPORTION IN PROGENIES USING MICROSATELLITE MARKERS

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It can be said that the offspring receives 50 % of its genome from each parent i.e. 50 % from dam and 50 % from sire. Since the DNA markers are the part of genome, hence the offspring inherits these markers also as a part of genome from the parents. The proportion of a particular parent in offspring can be judged from the proportion of the markers in the offspring, specific to that particular parent. Either the DNA markers are assayed by southern hybridization or PCR, the presence or absence of the bands on autoradiographs or on gels show the presence or absence of the marker. Since the common bands between the individuals represent the similarity in that particular region, the proportion of common bands between individuals can be used as the measure of genetic similarity between them. A panel of microsatellite primers was identified from the website depending on the need. The markers identified were highly polymorphic. The sequences of these primers were retrieved from the database and were synthesized commercially. Further the primers were diluted to the final concentration of 10 pmole/ µl. The genomic DNA of the resource population i.e. parents and progenies were extracted from whole blood using any commercial kit. The extracted genomic DNA was to be quantified and diluted to the final concentration of 30-50 ng / µl. The PCR reactions were set up in 25 µl reaction volume containing 2.5 µl of 10 X Assay buffer (100 mM Tris- HCl, pH 9.0, 500 mM KCl and 0.1% gelatin), 0.75 to 3.0 mM $MgCl_2$, 200 mM of dNTPs (dATP, dGTP, dCTP and dTTP), 10 pmole each of primers, 1 U Taq DNA polymerase (Promega), 50 ng of genomic DNA and autoclaved milliQ water to make up the volume. The amplification was carried out using the amplification programme: initial denaturation at 94° C for 4 minutes, followed by 30 cycles consisting of 45 sec at 94° C, 45 s at 45-65 °C and 45 sec at 72 °C; and followed by a final extension step of 10 min at 72 °C. After the completion of PCR reaction, the the tubes were spun briefly, 5 µl of gel loading dye was added to stop the reaction and the amplified product was resolved in 3.5 % Metaphor agarose gel for 45-60 min at 7-10 V/cm. The gel was stained with ethidium bromide and the resolved gel was visualized under U-V light. The gel image was to be read carefully and number of bands and their sizes was estimated. While the number of bands can be counted manually, their sizes were estimated against the known sizes of molecular markers using suitable software. Then total number of alleles was identified and each lane (individual) was genotyped for the presence or absence of the alleles. The presence of an allele was scored as "1", while the absence was scored as zero. Construction of the "1" and "0" matrix from allelic profile has been described in detail in the given example. The genomic proportion from the parent to the progenies was be estimated in terms of the genetic similarity between the parent and the progenies. The genetic similarity between parent and the progenies was calculated as average band sharing (BS) proportion between the parent and each of the progenies. The genetic similarity between parent and one progeny was calculated using the followng formula: $BS_{Parent.Progeny} = (2N_{Parent.Progeny}) / (N_{Parent} + N_{Progeny})$ where $N_{Parent.Progeny}$ was the number of shared bands between parent and progeny, N_{Parent} and $N_{Progeny}$ were the total number of bands scored in parent and the progeny, respectively. This technology was to be applied in Marker Assisted Introgression. The band sharing was estimated between parent-parent and all the progenies and then averaged. In the same way, at all the loci, band sharing was estimated and averaged

V-T-1

MULTIPLEX PCR FOR THE DIFFERENTIATION OF *BRUCELLA ABORTUS* VACCINE STRAIN 19 FROM *BRUCELLA ABORTUS*, *BRUCELLA MELITENSIS* AND *BRUCELLA SUIS*

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Bovine brucellosis is frequently caused by *B. abortus*, less frequently by *B. melitensis* and rarely by *B. suis*, so confirmation of specie specific infection is necessary. Multiplex PCR was carried out for the differentiation and confirmation of *Brucella* spp. viz., *B abortus* (standard strain S99 and field isolates), *B melitensis* and *B suis* from the vaccine strain (S19). The latter is used for the control of bovine brucellosis and its differentiation from the strains which cause infection among vaccinated herds in the field is essential. A cocktail of five different primers was used in the assay. All the strains (*Brucella abortus* field isolates, *B abortus* S99, *B melitensis* 16M and *Brucella suis* 1330) except the vaccine strain 19 amplified a 178 bp fragment indicating an intact *eri* sequence in all except S19 in which this gene has a deletion in the *eri* locus. This gene is associated with the ability of *Brucella* spp. to catabolize the sugar erythritol. Further *B abortus* field isolates and strain S99 could be identified by the amplification of 498 bp fragment. This fragment was also detected in *B abortus* S19. *B melitensis* was identified by the amplification band of 731 bp while *B suis* exhibited an amplicon of 285bp. The assay offers the potential advantage of rapid differentiation among the field isolates and vaccine strain in a single assay.

V-T-2

A RAPID TAQMAN REAL TIME PCR FOR DETECTION OF CAMELPOX VIRUS NUCLEIC ACID FROM CLINICAL SAMPLES

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A TaqMan real time (RT-PCR) assay for the detection and semi-quantitation of camelpox virus (CMLV) nucleic acid was developed using the virus DNA and ankyrin repeat protein (C18L) gene-specific primers with Cy5-labeled fluorescent probe and compared with established C18L gene based conventional and SYBR green real time PCR. The assay was found specific as amplification was observed only with CMLV nucleic acid. Based on a serial dilution of standard plasmid construct containing the gene of interest, the sensitivity of the TaqMan RT-PCR was found to be up to $4x10^2$ copy numbers. Whereas, the detection limit for the established assays namely SYBR green and conventional PCRs was found to be $4x10^1$ and $4x10^4$ copy numbers respectively. The TaqMan assay was linear within a range of $4x10^{10}$ to $4x10^2$ copies with an intra-assay coefficient of variation (CV) in the range of 0.26 to 1.78 % and an inter-assay CV were ranged between 0.63 % and 4.7 %. The standardized TaqMan RT-PCR has an excellent assay efficiency of 100.4% with a slope (3.313) and Rsq value (0.994) and it was easily employed for the detection of CMLV nucleic acid directly in the field clinical samples. This TaqMan RT-PCR was rapid, specific and 100 times more sensitive than conventional PCR and equally senstitive and more specific to SYBR green RT-PCR in the detection of CMLV nucleic acid from known CMLV isolates (n=11) and suspected clinical samples (n=23) in the form of skin scabs, skin crappings and nasal swabs. Therefore, developed TaqMan RT-PCR in this study, is an alternative test to the already existing various diagnostic assays and could be useful for clinical diagnosis of camelpox.

V-T-3

ISOLATION AND GENETIC CHARACTERIZATION OF JAPANESE ENCEPHALITIS VIRUS FROM EQUINES IN INDIA

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Japanese encephalitis (JE) is an important vector borne viral disease of human and horses caused by JE virus (JEV) of genus *Flavivirus* of family *Flaviviridae* in Asia. Although human JE outbreaks in human population occur every year, clinical JE cases from equines in India have not been reported. The present study was done to detect Japanese encephalitis virus (JEV) infection in equines showing neurological signs and to isolate & characterize the JEV from equines. Two foals in an equine farm at Hisar (Haryana) developed clinical signs of ataxia, with hind legs extended, low appetite and became recumbent. Post-mortem lesions included widespread congestion of meninges, cerebral hemispheres and cerebellum. Histologically, widespread congestion of blood vessels, swelling of endothelial cells in cerebral hemispheres and multi-focal areas of neuronal swelling, degeneration and necrosis with glial cell proliferation was seen. These foals were detected positive for Japanese encephalitis virus (JEV) by RT-PCR. A pool of mosquitoes collected from the surrounding field was also detected positive for JEV RNA by RT-PCR. Sequential serum samples were collected from the foals for virus isolation. JEV could be isolated from the serum sample collected on day 2 of illness from one of the foals. The virus isolation was confirmed by VNT using hyper-immune serum to JEV raised in rabbits and also by RT-PCR with amplification of products of expected size corresponding to partial E-gene (291 bp) and 32 NTR gene (146 bp). This is the first report of isolation of JEV from equines in India. The structural gene sequence of the isolated virus (JE/eq/India/H225/2009) from nucleotides 134-2633 on BLAST search showed >97% homology with JEV isolates circulating in human population in India. The phylogenetic analysis of E-gene (1500 nt) indicated that the isolate belonged to genotype III. Within GIII, the equine JEV clustered together with the clade 'Vellore group' of JEV isolates from India. Phylogenetic analysis of JEV/eq/India/H225/2009 based on 240 bp nucleotide sequence of the C/prM region also indicated that the equine isolate grouped into genotype III. The study confirmed that JEV is prevalent in equines showing clinical signs of encephalitis in India and that genotype III of JEV is associated with clinical cases in equines.

V-T-4

DETERMINATION OF VIRULENCE FACTORS OF *STREPTOCOCCUS AGALACTIAE* ISOLATED FROM BOVINE MASTITIS BY PCR

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Bovine mastitis is a major cause of economic loss in dairy industry. Approximately 70 per cent of the total cost associated with a reduction in milk production is due to subclinical mastitis, which is the main form of mastitis in modern dairy herds. *Streptococcus* species are one of the most important group of causatives of mastitis. The major Streptococci species among causative agents of bovine mastitis are *Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis.* Several cell-associated and extracellular factors of *Streptococcus* species have been identified and they can exert a direct effect on stromal cells while others can thwart one or more host defense mechanisms to allow for survival and persistence of the pathogen in the invaded tissue. The present study was carried out with an objective to detect the virulence genes of predominant *Streptococcal* species namely *S. agalactiae* isolated from bovine mastitis cases by PCR. A total of 82 *Streptococci* were isolated. Earlier, out of 82 *Streptococci* isolates, confirmed by *tuf* gene based PCR, seven were found to be *S. agalactiae* based on 16S rRNA and *sip* gene based PCR. These *S. agalactiae* isolates were further subjected to cAMP factor (a pore forming cytotoxin) using designed *cfb* and *hyl* (*hyaluronidase*) primers. The *cfb* and *hyl* primer set amplified an expected PCR amplicon of 320 and 950 bp respectively. This rapid, user friendly PCR amplification of the *hyl* and *cfb* virulence genes of *S. agalactiae* indicated its potential for rapid detection of virulent *S. agalactiae* isolated from bovine mastitis cases infection.

V-T-5

GENETIC CHARACTERIZATION AND VIRULENCE TYPING OF *PASTEURELLA MULTOCIDA* STRAINS INFECTING ANGORA RABBITS IN HIMACHAL PRADESH

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Pasteurella multocida is responsible for acute respiratory infection among rabbits known as snuffles. In Himachal Pradesh angora rabbits are raised for wool production and various infectious diseases affect the economical farming. To study the incidences of snuffle among the rabbit farms in the Himachal Pradesh total 429 samples were collected from different places in the state and 29 isolates were obtained. These isolates were later confirmed by PM-PCR. Then ERIC-PCR was performed to study the genetic variation among these 29 isolates. Three genetically variant strains of *P. multocida* were detected by ERIC-PCR. Capsular typing was also performed with multiplex PCR and 27.6% were found to be of type A and 72.4% type B. No other capsular type was detected. Virulence gene typing of all 29 *P. multocida* isolates of rabbit origin based on *pfh*A, *hgb*B, *tbp*A and *tox*A genes was done to determine genes responsible for virulence of these isolates. Virulence typing of tested *P multocida* strains revealed that in 60.60% isolates, *pfhA* gene was present, followed by *hgb*B gene in 12.12%) and *tbp*A gene in 6.06% isolates. *tox*A gene was detected in any isolate. These results indicate that snuffles in rabbits is caused by genetically variant strains of *P. multocida* but the strains having *phf*A gene, which is responsible for bacterial adhesion are involved in clinical infections. Phylogenetic analysis of few *P. multocida* isolates based on the nucleotide sequences of *ompA* gene revealed clustering of rabbit isolates along with bovine isolates.

V-T-6

SEQUENCE ANALYSIS OF HOST RANGE GENES OF ZOONOTIC BUFFALOPOX VIRUS ISOLATED FROM OUTBREAK (2010) IN JALGAON DISTRICT OF MAHARASHTRA

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Buffalopox is an emerging contagious viral zoonosis of domestic buffaloes (*Bubalus bubalis*), often associated with high morbidity (80%) and rarely affects cows and human. The causative agent of the disease, buffalopox virus (BPXV) is a close variant of vaccinia virus (VACV). BPXV is recognised as zoonotic disease and causing threat to humans as outbreaks is frequently occurring in animal attendants. The WHO Joint Expert Committee on Zoonosis (FAO/WHO 1967) has also declared buffalopox to be one of the important zoonotic diseases. Here, we report the outbreak of buffalopox in buffaloes and milkers in Jalgaon district of Maharashtra and molecular characterization of host range genes viz. E3L, K3L and C7L of the isolated BPXVs as these genes plays critical role in host tropism. The outbreak occurred in February, 2010 in a herd and the disease was characterized by localized pock-like lesions on the udder, teats and inside of thighs of buffaloes. The ulcerative lesions on hand, wrist and fingers were observed in human cases. Scab materials were collected from buffaloes and affected human for isolation of virus. BPXVs were isolated from scab materials in Vero cells. The viral DNA was extracted from cell culture supernatant using commercial kit and BPXV was confirmed by PCR using OPV-specific primers which resulted in amplification of a 552-bp fragment of the A-type inclusion gene. The purified DNA was subjected to PCR amplification of four host range genes (E3L, K3L and C7L) using specific primer pairs. The PCR products were purified and cloned into the pTZ57R/T vector (MBI Fermentas, Burlington, Canada). The confirmed clones were sequenced commercially. An open reading frame (ORF) nucleotide sequence homology search was carried out using the NCBI BLAST server. The nucleotide (nt) and deduced amino acid (aa) sequences were aligned using the CLUSTAL W program and phylogenetic trees were constructed using neighbour-joining method of MEGA version 4 software. In the present study, we have selected host range genes as the products of those genes implicated in modulation of innate immune system of the host and responsible for host tropism, as has been shown for VACV. Comparative sequence analysis was carried out to find out the variation in these genes of current BPXV isolates from buffalo and human as well as to determine the evolutionary relationship among OPXVs. We have successfully amplified the targeted host range genes of BPXVs. The sequence analysis revealed the ORFs of 573bp, 267bp and 453bp for E3L, K3L and C7L genes, respectively. Current isolates shared maximum homology (98.95 to 99.65 % at nt and 95.06 to 100 % at aa level) with VACV for all the genes. Phylogenetic analysis also revealed clustering of BPXV isolates with VACV. The close homology between BPXV and VACV suggests the similar role of these proteins in viral pathogenesis as in VACV. This is the first report of genetic analysis of host range genes of buffalopox virus which will help in elucidating the host antiviral response and to design strategies for development of vaccine and antiviral agents.

V-T-7

ATYPICAL *RHODOCCOCUS EQUI*: BIOCHEMICAL, EPIDEMIOLOGICAL AND MOLECULAR STUDIES

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Rhodococcus equi, the Gram-positive pleiomorphic rod which is a free-living saprophyte with widespread distribution, causes chronic granulomatous pneumonia, lung abscesses and occasional enterocolitis in foals. It is recognized as an opportunistic pathogen capable of infecting a wide range of hosts including, equine, porcine, camel and human as well. Its importance as zoonotic pathogen has been increasing, particularly in immunocompromised hosts. Due to the emergence of many corynebacteria as animal and human pathogens, rigorous biochemical and molecular tools have increasingly been applied for identification and epidemiological studies of *R.equi.* The present study was designed to characterize 67 *R. equi* isolates from the faeces of clinically healthy horses (29), donkeys (9), pigs (8) and one camel. Isolation was performed on selective media, nalidixic acid-novobiocin-actidione-potassium tellurite (NANAT) and Sheep Blood Agar (SBA). The isolates (67) were obtained from horse (54), pigs (4), donkeys (5) and camel (4). All the isolates were identified and characterized by cultural and biochemical properties and PCR analysis. Among these 67 isolates, the typical *Rhodococci* (35) were catalase positive, oxidase negative, urease positive, nitrate positive, arginine positive and negative in sugar fermentation. Significantly, the typical *R. equi* were isolated only from the horse. However, 32(47.76%) *R. equi* isolates detected in equine, porcine, donkey and one camel faecal sample gave atypical reaction in oxidase, nitrate reduction and/or urease test. Out of these, 22 isolates showed 1 atypical reaction, whereas 8 isolates showed 2 atypical reactions. Among the single atypical reaction isolates, 13 were from horses, followed by camel (4), pigs (3) and donkey (2) isolates. Among the atypical isolates, 11 urease atypical isolates were found in all 4 animal species, whereas the 10 nitrate atypical were isolated only from horse. There was a single oxidase positive atypical isolate from horse. In order to further confirm the identity of atypical *R. equi*, these were subjected to species-specific PCR to detect a unique 700-bp fragment of *R. equi* chromosomal DNA. Further, isolates were targeted for three virulence associated genes: *cho*E, *vap*A and *vap*B. Among the 27 atypical isolates for which the test was done, 14(51.85%) cultures were positive for species-specific PCR. The species wise observation for PCR positivity was: 13/16 (81.3%) horse isolates and 1/3 (33.4%) camel isolates. Out of 14 isolates, 11 isolates for *cho*E (950 bp amplicons) and 10 isolates for *vap*B (648 bp amplicons) were positive. However, none of the isolates were positive for *vap*A virulence plasmid, the hallmark of acute pathogenicity. The camel isolate which was positive for species-specific gene was also positive for *cho*E gene and *vap*B gene. Out of 13 species-specific gene positive atypical horse isolates, 9(69.2%) were positive for *vap*B gene. Among them, 11(84.6%) were positive for *cho*E gene. The present study indicates prevalence of atypical biotypes of *R. equi* in equine population and camel. Positive species-specific PCR detection of atypical-*R. equi* isolates confirms the presence of such atypical biotypes in environment, however the negative species-specific PCR result for 13 isolates also shows that biochemical identification is presumptive and not specific for *R. equi* identification. The findings of VapA^-B^+ strains indicate atypical *R. equi* carrying intermediate virulence gene plasmid in equines, a finding of epidemiological significance. The molecular tools in conjunction with analysis of biochemical parameters should be applied for identification and characterization of *R. equi*. It would be interesting to investigate the identity of isolates showing phenotypic similarity with *R. equi*.

V-T-8

PHYLOGENETIC ANALYSIS OF STREPTOCOCCI ISOLATED FROM SUBCLINICAL CASES BOVINE MASTITIS BY PCR-RFLP

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The epidemiology of Streptococcal mastitis in dairy cattle is poorly understood. All attempts to differentiate genotype by the conventional microbiological, serological and genomic DNA fingerprinting have met with limited success. In the present study, Restriction Fragment Length Polymorphism (RFLP) analysis of 16S rRNA gene amplicons of *S.agalactiae, S.dysgalactiae* and *S.uberis*, *sip* gene based amplicon of *S.agalactiae*, were digested using restriction enzymes (REs) selected based on RE mapping *viz.*, *Ava*II, *Alu*I, *Eco*RI and *Eco*RV, with an objective to study their RFLP profile as a tool for molecular epidemiology. Initially, 16S rRNA gene PCR amplicons of three isolates of *S.agalactiae,* one reference *S.agalactiae* isolate (AD1), one reference isolate of *S.dysgalactiae* (AD3) and two reference isolates of *S.uberis* (AD2 and AD6) were subjected to RFLP digestion using *Ava*II. *S.agalactiae* 16S rRNA gene based amplicons after digestion with *Ava*II revealed single restriction site and produced two fragments each of 57 bp and 272 bp. This restriction pattern did not show any variation among the *S.agalactiae* isoloates. Likewise, *S.uberis* 16S rRNA gene with *Ava*II also revealed a restriction site and even this resulted in two fragments of 315 bp and 539 bp. Digestion of 16S rRNA PCR products of *S.agalactiae* using *Alu*I enzyme revealed two restriction fragments of 97 bp and 188 bp. Whereas, RFLP of *S.dysgalactiae* 16S rRNA amplicon with *Alu*I yielded two fragments of 180bp, 369bp and that of *S.uberis* 16S rRNA PCR amplicon showed fragments of 162 bp, 187 bp and 430 bp. Furthermore, the PCR amplicons of *S.uberis* 16S rRNA were subjected to digestion with *Eco*RI, which revealed two bands of 409bp and 445bp in both the isolates and did not reveal any variations in the banding pattern. Subjecting PCR amplicons of *S.agalactiae sip* gene to digestion with *Eco*RV revealed two bands of 76bp and 190bp in all the four isolates without showing any variations in the banding pattern. In conclusion, there was a distinct inter species banding pattern revealing no intra species variations. Study indicated that PCR-RFLP patterns may be used as a module for the identification and confirmation of the isolates and may serve as a substitute to the time consuming and costly sequence comparison and analysis.

V-T-9

DETECTION AND CHARACTERIZATION OF *GLPO* GENE IN MYCOPLASMAL ISOLATES BY PCR FROM GOATS OF GUJARAT

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The present study was undertaken to detect the *Glpo* gene in mycoplasmal isolates from goats of Gujarat. It is a virulence associated gene which acts as a powerful mediator for cell injury. Nineteen isolates of mycoplasma were screened by PCR using *Glpo* gene specific primers of the *Mycoplasma mycoides* subsp. *capri.* All the isolates yielded expected 1189 bp amplification product of *Glpo* gene of *Mycoplasma mycoides* subsp. *capri.*Restriction enzyme analysis (REA) of the amplicon with *Hpa*I resulted in fragments of 652 bp and 537 bp, with *Eco*471 resulted in fragments of 826 bp and 363 bp and with *Bpi*I resulted in fragments of 941 bp and 248 bp. The results were found in accordance with the expected restriction map, thus indicating absence of genomic variation among the field isolates. The results of this study show the importance of PCR-REA for detection and characterization of these mycoplasmas.

V-T-10

PROTECTION STUDY OF *Pasteurella multocida* RECOMBINANT AND NATIVE OUTER MEMBRANE PROTEINS

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Haemorrhagic septicaemia is a disease of cattle and buffaloes caused by *Pasteurella multocida* serotype B: 2. Research is going on for developing an improved vaccine based on the most potent antigen to encounter the demerits of presently available vaccines. The outer membrane proteins (OMPs) of the *P.multocida* is of utmost importance for the development of vaccine against Pasteurellosis. One of the major OMPs, porin (OmpH) has high immunogenicity and its protective ability has been demonstrated. This may also have importance for the development of subunit vaccine using recombinant protein. The present study was undertaken to express and purify recombinant OmpH and to study protection of native and recombinant protein in mice model. OMP-rich extracts were prepared from the sonicated antigen of *Pasteurella multocida* P52 vaccine strain. Recombinant clone (pQE32 vector containing insert *ompH* gene) was used for the expression and purification studies. Nucleotide sequence obtained was submitted in GenBank under accession number HM117642.The *ompH* gene amplified by PCR of recombinant plasmid using gene specific oligonucleotides primers yielded the expected product of 1 kb. Recombinant plasmid was transformed into *E.* coli M15 cells. For the induction of expression, the transformed *E. coli* M 15 cells were induced by 1 mM IPTG. A broth culture having OD_{600} of 0.6 was induced. The recombinant OmpH was purified under denaturing conditions using Ni-affinity chromatography. Purified recombinant protein eluted had a molecular mass of about 37.5 kDa, which consisted of 34.42 kDa truncated OmpH and 3 kDa-His protein. The purified recombinant protein reacted with the Penta-His antibodies in western blot. Protection studies were carried out in mice with the purified OMPs fraction and the recombinant OmpH. OMPs showed about 50% protection efficacy in mice. Although the protection level of OMPs was not adequate but it was same as shown by commercial vaccine. In the group that received recombinant protein as immunogen protection level was only 16.66% which indicate lower protection efficacy of recombinant protein probably because of denaturation.

V-T-11

DETECTION OF PUTATIVE VIRULENCE-RELATED GENES *VACB* AND *MVIN* AMONG *PASTEURELLA MULTOCIDA* B: 2 STRAINS USING MULTIPLEX PCR

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Vacuolating cytotoxic activity in the mouse macrophage cell line RAW 264 has been reported in *Pasteurella multocida* B: 2 strains associated with Haemorrhagic septicaemia in buffaloes and cattle (Shah *et al.* 1996). A putative virulence-related gene *mviN* was reported in the sequenced genome of *P. multocida* serotype A (avian strain Pm70). MviN is an integral membrane protein that belongs to MATE (multi-antimicrobial extrusion) family. The present study was undertaken to detect the presence of the putative virulence- related genes viz. *vacB* and *mviN* among *P. multocida* B: 2 strains (n=8). Sequence information for the *vacB* gene was obtained from the sequenced genome of *P. multocida* serotype A (avian strain Pm70). Oligonucleotide primers were designed for these genes and synthesized by Invitrogen, UK. All the eight *P. multocida* B: 2 strains, one *P. multocida* type A 3 (bovine) and one *P. multocida* type D (pig) strain had both of these genes of the expected size (1.5 Kb for *mviN* and 2.4 Kb for *vacB*) as confirmed by PCR. *E. coli* K12 used as negative control did not show any band. Both of these putative virulence-related genes were amplified in a single reaction mixture using multiplex PCR. The expected size (1.5 Kb for *mviN* and 2.4 Kb for *vacB*) of both the genes was obtained. This is the first report of the detection of these virulence-related genes among *P. multocida* B: 2 strains. Besides providing additional targets for diagnostic applications, the identification and subsequent analysis of virulence-related genes in *P. multocida* B: 2 strains may help in improving our understanding of the molecular mechanism of adaptation, survival and virulence of *P. multocida* B: 2.

V-T-12

CONJUGAL TRANSFER OF DRUG RESISTANCE AND SEPTICAEMIA RELATED CHARACTERISTICS FROM SEPTICAEMIC *ESCHERICHIA COLI* ISOLATES

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Eleven *Escherichia coli* isolates were selected as donor strain .These strains were showing multi drug resistance and positive for three septicaemia related characteristics, *viz.*, Congo red dye binding, and Sereny test and serum resistance. Recipient *E.coli* strain (K-12, F^-, Nalidaxic acid ^{res}) was obtained from MTCC and gene bank, Chandigarh and found negative for septicaemia characteristics. Gene transfer was done by broth mating conjugation and then tested for ability of donor to transfer drug resistance .Five out of 11 antibiotic resistant donor could able to transfer their genetic material to recipient cell via conjugation so three recipient cell became CR positive and two serum resistant. But no strain could be made Sereny test positive by conjugation probably genes governing such characteristics were residing on the bacterial chromosome and that were difficult to transfer.

V-T-13

MYCOPLASMA ISOLATION AND CHARACTERIZATION OF FROM POULTRY WITH CHRONIC RESPIRATORY DISEASE

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Indian poultry population is 3% of the world's poultry production. The country ranks 5th in egg production (2004) and 9th largest in world poultry meat production (2004).The above data indicates that poultry farming in India has made a phenomenal growth over the past of two decades and it is an important agri-based contributor to the Indian economy. It is said that over the past two decades the poultry industry in India has contributed approximately US $ 229million, to the Gross National Product and the present market research report i.e., "Vision for Poultry Industry-Current Scenario and Future Prospects" predicts relatively strong growth for the egg and poultry meat industry in both the urban and rural areas, in next two decades. Modernization of poultry entrepreneurship with the main aim of cost benefit ratio has invariably increased stress on birds which pre-disposes them to infectious as well as non infectious diseases. Among the infectious diseases Avian mycoplasmosis especially CRD, the most costliest disease confronting poultry industry in India and all over the world, causing huge economic losses due to drop in egg production, retarded growth; condemnation of carcass, decreased weight gain and decreased feed conversion ratio. The disease needs to be diagnosed early and confirmatively for prevention and control. Even today, though molecular techniques are available confirmation of disease by isolation of etiological agents considered as 'Gold Standard' for the diagnosis of disease. With the above concepts an effort has been made in the present study to isolate and characterize the mycoplasma from poultry suffering from CRD. A total of 101 CRD affected birds were screened for the isolation of *Mycoplasma spps* which comprised of 101 lungs, 57 tracheal and 82 air sac samples collected from various places of Karnataka state and Namakkal Tamil Nadu. A total of three isolates of mycoplasma were obtained in the present study. Indigenously dehydrated mycoplasma broth and agar with 20 per cent horse serum and 10 per cent fresh bakers yeast extract was found to be satisfactory for the isolation of mycoplasma in the laboratory. Morphologically a single type of mycoplasma species could be described; biochemical studies also indicated the similarity among the three isolates of mycoplasma. All the three isolates fermented glucose and didn't hydrolyze urea and arginine. Further the isolates were differentiated from *Acholeplasma spps* by using sodium polyanethol sulphonate (NPS) test, the test depicted clear zone of inhibition, which indicated that the isolates were of mycoplasma and not acheoloplasma. Characterization of the mycoplasma isolates was done by using growth inhibition test with known antiserum. The test shown inhibition zone of 2- 3 mm which indicated that all the three isolates belongs to *M.gallisepicum.* The agar gel precipitation test, counter immunoelectrophoresis test, immunoelectrophoresis and single radial immunodiffusion tests were carried out for characterization of the isolates by using the known antiserum. All the three isolates gave positive reactions for all the immunological tests which indicate isolates as *Mycoplasma gallisepticum.* The present study suggests the existence of mycoplasma infections in the poultry population. Based on the morphological, biochemical, immunological studies the isolates were identified as *Mycoplasma gallisepticum.*

V-T-14

STANDARDIZATION OF PCR FOR DETECTION OF PREDOMINANT STREPTOCOCCAL ISOLATES CAUSING SUBCLINICAL BOVINE MASTITIS

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Bovine mastitis caused by *Streptococcus* species is mainly subclinical and therefore can be diagnosed only in the laboratory. *Streptococci* are a highly infectious bovine mastitis pathogen that can rapidly spread throughout the herd from an infected animal. Consequently, early diagnosis of the presence of the infection in a herd is important for effective control. Many workers targeted 16S rRNA for the specific identification of *Streptococcal* species. 16S rRNA has been a reliable site for the development of rapid and accurate detection methodologies. Keeping these in view, 16S rRNA gene was targeted in designing the primers for the identification of *S.agalactiae*. The primers designed in the present study amplified all the four *S.agalactiae* isolates yielding 329 bp amplicons which was confirmed by sequence BLAST analysis of amplified products. Thus the present study runs in track with the earlier findings by many workers in identifying *S.agalactiae* based on the 16S rRNA sequences. The identification of *S.dysgalactiae* was accomplished by designing the primers based on 16S rRNA sequence. The designed primers amplified the specific region of *S.dysgalactiae* 16S rRNA gene yielding 549bp amplicon as expected. Similar observations were recorded by many of the earlier workers. Earlier *S. uberis* epidemiological studies utilized species specific 16S and 23S rRNA gene PCRs for primary identification following aesculin and hippurate hydrolysis. Prior to the adoption of 16S rRNA gene sequence analysis for species identification and confirmation for *S. uberis*, it is highly likely that it was difficult to differentiate *Streptococci* closely related to *S. uberis* from it and were included in previous *S. uberis* epidemiological studies. In the present study, 16S rRNA was again targeted to design the *S.uberis* specific primers. Further, these primers amplified a 854 bp amplicon and sequence BLAST analysis of the same confirmed its specificity. To conclude, the results of the present study indicated that the PCR methods can be successfully used for the identification of the major Streptococcal isolates both at genus level and at species level, especially the predominant streptococcal species such as *S.agalactiae, S.dysgalactiae* and *S.uberis.* Use of these rapid, sensitive and specific methods will help at the field level in the effective treatment, antibiotic selection and control of mastitis.

V-T-15

ISOLATION AND CHARACTERIZATION OF *ESCHERICHIA COLI* FROM DIFFERENT PATHOLOGICAL CONDITIONS OF POULTRY

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The present study was undertaken with a view to isolate *Escherichia coli* from different pathological conditions (colisepticaemia, yolk sac infection and egg peritonitis) of poultry for the study of their certain characteristics. A total of 597 samples from different sources of 253 necropsied birds with above stated conditions from 69 broiler as well as layer poultry farms in and around Anand, Gujarat State were collected and recovered 506 (84.76 per cent) *E.coli* isolates by using MacConkey agar and eosin methylene blue agar. The select 150 *E.coli* isolates were studied for biotyping, serotyping, *in vitro* antimicrobial sensitivity, congo red binding ability and haemolytic activity. Twenty different bio-types of *E.coli* were obtained on the basis of fermentation reactions of six different sugars *viz.,* salicin, starch, sucrose, raffinose, dulcitol and rhamnose. Similarly on the basis of serotyping 125 typable isolates were distributed into 36 different O groups (O2, O8, O151, O78, O109, O1, O171, O101, O26, O45, O107, O111, O119, O9, O20, O23, O38, O40, O50, O58, O83, O91, O131, O135, O157, O55, O61, O68, O81, O100, O106, O120, O144, O145. O156, and O157 in decreasing order of frequency) along with 15 rough and 10 untypable strains. Amongst the pathological conditions noted, 56 per cent of *E.coli* isolates were from colisepticaemia, 38 per cent from yolk sac infection and six per cent from egg peritonitis. Conditionwise predominant serotypes found were O2, O78, O109, O1 and O151 (colisepticaemia); O8, O101, O171 and O151 (yolk sac infection); O2 and O151 (egg peritonitis). All the tested *E.coli* isolates were found resistant to lincomycin followed by tetracycline, furazolidone, ampicillin, co-trimoxazole, amoxycillin, doxycycline, neomycin, colistin, flumequine, gentamicin, ciprofloxacin, pefloxacin, enrofloxacin and chloramphenicol in decreasing order of resistance. All the tested *E.coli* isolates were found positive for congo red binding ability whereas 54 per cent isolates revealed presence of haemolytic activity. No. relationship could be observed amongst the different characters of *E.coli* studies.

V-T-16

MOLECULAR CHARACTERIZATION OF *DICHELOBACTER NODOSUS* ISOLATED FROM OVINE FOOTROT IN CENTRAL KASHMIR, INDIA

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The study records the 12.54 per cent prevalence of ovine footrot in central Kashmir comprising of three districts. The prevalence was highest (15.84%) in district Srinagar and minimum (10.89%) in district Budgam, while it was 13.28 per cent in district Ganderbal. Overall economic impact of footrot was estimated to the tune of Rs 15.82 million annually to the sheep production in central Kashmir. Out of 370 samples collected from footrot lesions of naturally infected sheep, 200 (54.05%) detected *D. nodosus* positive by polymerase chain reaction (PCR). Out of these, 132 (66.00%) samples carried serogroup B of *D. nodosus*, five (2.50%) serogroup E, one (0.50%) serogroup I, 53 (26.50%) had mixed infection of serogroups B and E, four (2.00%) serogroups B and I, two (1.00%) serogroups B and G, while three samples (1.50%) carried the mixed infection of serogroups B, E and I . Serogroup G was detected for the first time in India. A total of 265 *D. nodosus* strains were isolated out of which 194 (73.20%) were typed as serogroup B, 61 (23.01%) serogroup E, 8 (3.01%) serogroup I and remaining 2 (0.75%) were typed as serogroup G. Out of 265 *D. nodosus* isolates, 164 (61.88%) possessed *intA* gene, thus were considered as virulent strains. Serogroupwise *intA* gene was found in 121(62.37%) isolates of serogroup B, 36 (59.01%) of E, two of G and five of I. Out of 20 randomly selected isolates subjected to gelatin gel test, 16 isolates with *intA* gene produced thermostable protease while four isolates without *intA* gene revealed the production of thermolabile protease. The study suggests the incorporation of serogroups B and E in the formulation of an effective bivalent vaccine to combat footrot in central Kashmir.

V-T-17

MOLECULAR TYPING OF FOWL ADENOVIRUSES ISOLATED FROM CHICKENS IN HARYANA

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In order to study the genetic variations in Inclusion body hepatitis (IBH) causing virus, the DNA was extracted from the liver tissues of suspected chikens and after PCR amplification of 1.219 kb partial region of hexon gene, 60% suspected samples were found positive for fowl adenoviruses (FAdV).A Phylogenetic tree was constructed using partial length hexon gene nucleotide sequences and it was found that our isolates formed a separate clad. However, their closeness was towards FAV11 and FAV12 serotypes of GenBank. The Pairwise nucleotide sequence homology of partial length hexon gene revealed that they were not identical and differs from each other as their sequence homologies ranged between 64.5 to 99.6 %. Further this study of sequence analysis of field isolates of FAdV offers the potential for novel and additional information in analysis of the molecular epidemiology of FAdV in Haryana.

V-T-18

PYOCYANIN PRODUCING PSEUDOMONADS OTHER THAN *PSEUDOMONAS AERUGINOSA*

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Present study was conducted to observe pyocyanin production among *Psudomonas* isolates. Pyocyanin production has been considered as an important inherent characteristic feature only of *P.aeruginosa.* In the present study 61 isolates of genus *Pseudomonas* including 43 isolates of *P.aeruginosa* were first characterized by PCR using standard genus and species specific primers respectively based on 16S rRNA gene fragment. These isolates were studied for pyocyanin production on nutrient agar plates. Total 13 isolates were found to be nonpyocyanogenic, out of which 11 isolates were of *P.aeruginosa* and two isolates were of other pseudomonads and 16 isolates were found to be pyocyanogenic pseudomonads other than *P.aeruginosa.* Non pyocyanogenic *P.aeruginosa* were found to be 25.6 per cent. In addition pyocyanogenic non *P.aeruginosa* were found to be 88.9 per cent which is otherwise considered as hallmark of *P.aeruginosa* only.

V-T-19

STANDARDISATION OF REAL TIME PCR(RT-PCR) FOR DIAGNOSIS OF IBR INFECTIONS IN BREEDING BULLS

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The members of the herpesviridae cause several diseases in both domesticated as well as wild animals. Bovine herpes virus -1 belongs to the subfamily Alphaherpesvirinae and is an important pathogen of cattle. It is the causative agent of respiratory and genital tract infections and abortions. Upon primary infection, BHV-1 replicates in the periphery, i.e. the mucous membranes of either the respiratory or the genital tract, it gains access to local sensory neurons for establishment of latency in the corresponding ganglia. Cattle that become infected can excrete virus long after recovery from the primary clinical disease. A cow with IPV may recover from the clinical disease but continue to transmit the virus for more than a year. A bull that is infected can transmit virus in the semen through natural mating or artificial insemination. Cattle with IBR may recover with no complications, but some may develop secondary pneumonia or remain carriers for life. When animal become stressed during periods of transport, mating, calving, weaning, parasite infection or long-term yarding under intensive conditions they re-excrete virus at the primary site(s) of viral entry (mainly nasal or genital mucosa) and become a source of infection for non-immune cattle. Present study was undertaken with an objective to standardize real time polymerase chain reaction for detection of infectious bovine rhinotrachaties virus from known negative neat semen samples of breeding bulls spiked with BHV-1 virus. Known negative neat semen samples spiked with BHV-1virus was taken and DNA was extracted using chelating resin along with Proteinase-K and DL-Dithiothretiol and subjected to routine PCR, using primers amplifying the gC region of BHV-1,which yielded a specific PCR product of 173 bp. The extracted DNA when subjected to Real time PCR using a pair of sequence-specific primers and a 5'-nuclease sequence-specific oligoprobe (Taqman) labelled with two different flurophores, the reporter/donor, 5-carboxyfluorescein (FAM) at the 5' end, and the acceptor/quencher 6-carboxytetramethylrhodamine (TAMRA) at the 3' end yielded a cycle threshold (CT) value between16 to 19 which indicates that a significant level of DNA concentration is present in the test sample in turn which reflects on the load of the virus that is present in the neat semen samples. The procedure for followed for virus isolation and real time PCR methodology is as per the OIE Manual 2008,2.4.13. Shedding pattern of IBR virus by an carrier breeding bull in its semen ejaculates forms the matter of concern in the spread of virus from an infected herd to healthy herd. Currently there are only a few OIE recommended tests for undertaking international trade in animal germplasm, though Virus isolation is an OIE recommended test, it is time consuming and is labour and cost intensive and requires specific laboratories hence only few laboratories can undertake this protocol. Present study revealed that Real time PCR is highly reliable and can very well be performed at ease and less time consuming, the results are highly acceptable with respect to repeatability, sensitivity and specificity.Real time PCR can considered as an substitute for other tests for detection of IBR virus.

V-T-20

CLONAL RELATIONSHIP AMONGST THE STRAINS OF *E.COLI* OF LIVESTOCK AND POULTRY

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The present study involved isolation and identification of strains of E.coli isolates from diarrhoeic and non diarrhoeic faecal samples of cattle, camel, sheep and poultry of Bikaner range. The bacterial strains were subjected to serotyping and biotyping, pathotyping on sheep blood agar producing varying degree of hemolysis and toxigenicity in mice, chick as well as in rabbit ileal loop assay. *E.coli* isolates with common or different subgroup in all the four host species were subjected to resistotyping against different antibiotics and ribotyping i.e. determination of 16S- 23S rRNA intergenic space polymorphism. Dendogram were prepared on the basis of analysis of *E.coli* strains considering two, three or all four above parameters i.e. biotyping resistotyping, pathotyping and ribotyping. The resistotyping and ribotyping allowed maximum discrimination of bacterial strains. The bacterial strains could be grouped into clusters on the basis of their relative similarities. Each group consisted of major clonal groups, clusters and subclusters. The isolates could be differentiated into 10 clusters showing similarity coefficient up to 0.8 when further discriminated into subcluster the similarity coefficient value fall between 0.8 to 0.9 i.e. similarity is more than 90% It is evident that overall similarity between different clusters is highest when all four parameters were considered for cluster analysis.

V-T-21

IDENTIFICATION OF THE PUTATIVE MOLECULAR TARGET OF INFECTIOUS BURSAL DISEASE VIRUS ON CHICKEN LYMPHOCYTES.

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Infectious Bursal Disease Virus (IBDV), a member of the dsRNA *Birnaviridae* family, is an important immunosuppressive avian pathogen. The target molecule on B cells for binding of IBDV is not known. The present study was undertaken for the identification of molecular target of IBDV on chicken lymphocytes. A virus binding assay was established, which enables to directly visualize the putative target for the IBDV on chicken lymphocytes by microscopy using immunofluorescence and quantitation by Flow cytometric analysis. Identification of the target molecule was pursued by several different approaches. The effects of modulating the expression of activation markers of lymphocytes by Lipopolysaccharide (LPS), Transforming Growth Factor Beta (TGF-â), Interferon Gamma (IFN -ã) and Concanavalin A (Con A) on the binding of IBDV to cells were evaluated. Bursal lymphocytes from chicken aged 6 weeks were treated with LPS prior to incubation with IBDV. The activation of lymphocytes with LPS was found to tremendously increase the IBDV binding to the cells. The mean percentage of IBDV bound lymphocytes in the bursal cell suspension was 22.96 ± 4.12 without LPS treatment and 34.24 ± 6.01 after the LPS treatment. The difference was found to be highly significant ($p<0.005$). Flow cytometric studies clearly indicated that TGF-â very significantly down regulated the expression of MHC II as well as IBDV binding on lymphocytes. The mean percentage of MHC II stained lymphocytes was 24.46 ± 5.54 without TGF-â treatment and 20.07 ± 5.40 after TGF-â treatment. The difference was found to be highly significant ($p < 0.005$; $t = 3.94$). The mean percentage of IBDV bound lymphocytes was 21.42 ± 4.80 without TGF-â treatment and 16.12 ± 3.99 after TGF-â treatment. The difference was highly significant ($p < 0.005$; $t = 4.88$). The effect of IFN -ã treatment on MHC II expression and IBDV binding on the chicken lymphocytes was studied by immunofluorescence and flow cytometry. The mean percentage of MHC II^{+vc} lymphocytes was 5.08 ± 2.35 without IFN treatment and 6.50 ± 2.88 after IFN treatment. The difference was found to be significant ($p < 0.05$; $t = 3.42$). The mean percentage of IBDV bound lymphocytes was 7.46 ± 1.54 without IFN treatment and 10.60 ± 3.50 after IFN treatment. The level of IBDV binding was found to increase non significantly. Hence, the treatment of lymphocytes with IFN ã was found to increase MHC II expression as well as IBDV binding on B cells. Activation of T lymphocytes with the mitogen Con A resulted in the expression of MHC II molecule on the surface of CD3+ T cells. Simultaneous expression of CD3 and IBDV binding on activated T lymphocytes was observed for the first time. The expression of MHC II molecule on activated T cells and IBDV binding on activated T cells was found to be comparable. Studies on blocking of the putative target were conducted by prior incubation of lymphocytes with IBDV followed by MHC II staining of the cells as well as by prior incubation of lymphocytes with unlabelled anti-MHC II antibody followed by exposure of cells to IBDV and staining for IBDV. The mean percentage of MHC II^{+vc} lymphocytes was 20.99 ± 4.25 without IBDV treatment and 18.14 ± 2.87 after treatment .The difference was found to be highly significant ($p < 0.001$; $t = 5.30$). The mean percentage of IBDV bound lymphocytes was 19.36 ± 6.25 without anti MHC II Antibody treatment and 14.77 ± 7.28 after anti MHC II Antibody .There was a non significant decrease in the level of IBDV binding after the antibody treatment of cells. Interestingly, the blocking of the putative target with IBDV very significantly decreased the staining for MHC II on B cells and prior incubation of B cells with anti MHC II antibody resulted in decreased IBDV staining on B cells. The novel findings in the various approaches for identification of molecular target of IBDV suggest that the MHC II molecule may be the putative target for IBDV binding on chicken B cells. The findings of the present studies offer an exciting possibility of a target centric approach for development of a new vaccine for the control of Infectious Bursal Disease.

VI-T-1

EFFICACY OF MICRO-ENCAPSULATED LIVE *BRUCELLA ABORTUS* RB51 VACCINE DELIVERY SYSTEM IN BALB/C MICE.

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Bovine brucellosis is endemic in India. It is a bacterial disease of economic importance to the dairy industry since it causes infertility, abortion, retention of placenta in cows and orchitis in bulls. The pathogen is excreted in the milk and semen. Brucellosis is of zoonotic significance since human acquire infection through exposure from infected herd and consumption of contaminated raw milk. Bovine brucellosis is currently controlled in India by immunization of female calves with live attenuated *Brucella abortus* S19 smooth strain. However, the use of S19 strain does not allow differentiation of immune response elicited by the vaccine and wild type infection. Because the rough vaccine strain RB51 is devoid of the O moiety in lipo-polysaccharide (LPS) chain, the strain has the advantage over S19 in that it does not react in standard serological tests that depends on use smooth LPS as antigen. The concept of using live, subunit or subunit glyco-conjugate vaccines are new approaches that are being currently pursued to essentially address the issues of magnitude and duration of immune response. In this study the efficacy of live *Brucella abortus* RB51 encapsulated in alginate beads permanently cross-linked by poly-L-Lysine as a vaccine delivery tool has been explored. A comparative study based on subcutaneous immunization of BALB/c mice with $1x10^5$ colony forming units (CFU) each of micro-encapsulated live RB51 and un-encapsulated RB51 strain followed by intra-peritoneal challenge with 2×10^5 CFU of B. abortus 544 on day 30 post-immunization and recovery of the challenge strain from spleen 15 days post-challenge (PC) revealed that the microencapsulated preparation of RB51 elicited better protection than the un-encapsulated version [Protection Mean $\log_{10}$ CFU 3.71 as compared to 3.04]. Also the recovery of the vaccine strain from spleen at 15 days PC from encapsulated group was higher than the un-encapsulated group [Mean $\log_{10}$ CFU 2.68 as compared to 1.95] indicating the persistence in greater magnitude, probably ensuring better protection and more sustained immune response. The antibody isotype specific immunoglobulin response induced in BALB/C mice immunized with live micro-encapsulated RB51 as measured by indirect ELISA (i-ELISA) using acetone killed RB51 cells as antigen indicated significantly different response as compared to the un-encapsulated group when the mean optical density values [log 10 dilution titers] were subjected to analysis of variance. The mean IgG1 titer elicited by encapsulated vaccine differed significantly from un-encapsulated group and unvaccinated controls between days ($P<0.002$), between group ($P<0.0001$) and days versus group ($P<0.0003$). The mean IgG2a and IgG2b also differed significantly between days, group and days versus group ($P<0.0001$); similarly the mean IgG3 titer were significantly different from control group between days ($P<0.0001$) and between group ($P<0.0001$) and days versus group ($P<0.0001$). Most strikingly, the mean $\log_{10}$ i-ELISA titers of IgG2b and IgG3 in encapsulated group differed significantly from un-encapsulated group and unvaccinated controls between day 30 post-immunization and 15 days PC. This study has shown that microencapsulation of live Brucella RB51 strain led to modification of the uptake, trafficking, and processing of antigen in BALB/c mice. It demonstrated that adequate persistence and sustained presentation of the vaccine to the immune system of the host enhances the magnitude of immunity and protection.

VI-T-2

PREPARATION AND ASSESSMENT OF VACCINES AGAINST SHEEP PASTEURELLOSIS

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Iron Restricted Outer Membrane Protein (IROMP) and Iron Restricted Biofilm (IR-HSB) vaccines against Sheep Pasteurellosis were prepared using *Pasteurella multocida* serotype 'A'. The IR-HSB culture was inactivated with 0.5 percent formalin. The IR-Biofilm vaccine was adjuvanted with Montanide ISA206, while Aluminum hydroxide was used as adjuvant for the IR Outer Membrane Protein vaccine. Both the vaccines were found to be sterile. Safety and potency were established by experimental trials conducted in mice. The vaccinated mice were found to be protected against challenge with 100 LD_{50} of virulent culture. Experimental vaccination trials were also conducted in sheep with both IROMP vaccine (1 ml s/c) and IR-HSB vaccine (2 ml s/c). Sera collected 21 days post vaccination were subjected to Passive Mouse Protection Test for immune response assessment. The passively immunized mice were found to be protected against virulent challenge.

VI-T-3

IMMUNOGENIC POTENCY OF AN *E. coli* TRIVALENT FIMBRIAL VACCINE IN CHICKEN.

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In the recent years *E.coli* infections are posing troublesome to poultry industry because of emergence of drug resistance together with frequent breakdown of immune status, prevalence of large number of *E.coli* serotypes and failure to develop a potent vaccine. Fimbriae, proteinaceous, filamentous structures present on surface of *E.coli* are important factor in bacterial survival and persistence in the host. The fimbrial protein has found to be immunogenic and it could be explored as a suitable vaccine candidate. In this study immunogenicity of an *E. coli* trivalent fimbrial vaccine against avian colibacillosis was carried out in 4 weeks old chicken. An oil-emulsified vaccine with aqueous phase to oil phase ratio of 1:5 was prepared using purified fimbrial proteins of common and predominant *E. coli* serotypes O1, O2 and O97 of Bidar locality. Vaccine contained protein concentration of 150µg from each serotype O1 and O97 and 160 µg from serotype O2. Chicken were vaccinated twice subcutaneously with 0.5 ml of vaccine at 4th and 6th weeks of age. At 8th week, the vaccinated chicken and unvaccinated controls were challenged via posterior thoracic air-sac route with *E. coli* serotype O1, O2 and O97. Vaccinated chicken protected from active respiratory distress as they suffered no morbidity or mortality, had gross lesions so mild that, the scored values were comparable statistically to zero lesion score of the negative controls and did not yield *E. coli* when their heart blood, pericardial sacs, liver and airsacs were cultured. Unvaccinated challenged chicken had some respiratory distress, suffered 11 to 23% mortality and had average airsac, pericardial sacs and liver lesions scores significantly (Pd”0.05) differ from both their vaccinated counterparts and negative controls. From the overall assessment it is evident that, fimbrial proteins from endemic strains with oil adjuvant can incite a high level of immunity to broiler chicken against *E. coli* infection. The main advantages of this subunit vaccine are there is no reversion to virulence, no toxicity and good protection against infection. Fimbrial proteins from endemic strains of *E. coli* could be more effective and protective vaccine candidate against colibacillosis in chicken.

✓ 7/5 Amd

VI-T-4

EXPRESSION OF BOVINE HERPESVIRUS 1 (BHV-1) GLYCOPROTIEN C (gC) BY A RECOMBINANT BACULOVIRUS IN INSECT CELLS

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BHV-1 glycoprotein C is immunodominant and is believe to play a role in initial viral attachment. Here a recombinant baculovirus was constructed by incorporating BHV-1 glycoprotein C (gC) coding gene to characterize the expression of the glycoprotein in infected *Spodoptera fugiperda* (Sf9) cells. The BHV-1 complete gC gene was PCR amplified with specially designed primers to clone into pENTR/SD/D Directional TOPO vector (entry clone). The purified entry clone plasmid was subjected to LR recombination with the linear baculovirus DNA which was then transfected into Sf-9 cells. The recombinant baculovirus carrying the gC coding gene (P1) was selected against gancyclovir in the Sf-9 cells, serially passaged for 2 more generation and the P3 (3rd passage) viral stock was used to infect fresh Sf-9 cells for gene expression study. The reactivity of polyclonal antibody with the Sf-9 cell expressed gC was detected by immunoperoxidase test (IPT).The recombinant gC protein was purified by Ni-NTA column chromatography and immunoprecipitation and when subjected to SDS-PAGE and Western blot analysis protein bands of MW 54 kDa and 28 kDa were detected consistently with both monoclonal and polyclonal antibodies. Expression of gC gene in the infected Sf-9 cells was further confirmed by dot-ELISA indicating its potential use as a coating antigen in an indirect ELISA.

VI-T-5

CLONING OF CELLULOLYTIC GENES FROM *PHANEROCHAETE CHRYSOSPORIUM* INTO YEAST FOR CELLULOSE DEGRADATION

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Cellulose is the world's most abundant biopolymer in nature. Cellulose is usually found in association with both lignin and hemicelluloses as a component of lignocelluloses, which is usually considered indigestible for animals. Biological degradation of lignocelluloses involves the action of cellulases produced by a wide range of bacterial and fungal species. Fungal cellulases like exocellobiohydrolase (CBH 1, 4) and endoglucanase (Beta-1,4-glucanase) frequently act on the â-1, 4 chain of lignocelluloses for effective cleavage biopolymer into simple glucose moieties. The white rot fungus *Phanerochaete chrysosporium* secretes both exocellobiohydrolase and endoglucanase enzymes to degrade all of these components of lignocelluloses. Aim of the present study is to develop recombinant yeast expression system with cellulolytic enzymes (exocellobiohydrolase and endoglucanase) for the degradation of crop lignocelluloses which improve celluose utilization in the rumen. *Phanerochaete chrysosporium* was cultured and RNA was isolated. Both exocellobiohydrolase (CBH 1,4) and endoglucanase genes were amplified using specific primers by Reverse transcriptase polymerase chain reaction . The amplified product was cloned into pENTR-TOPO vector and the recombination was carried out for the transfer of both exocellobiohydrolase (CBH 1,4) and endoglucanase genes from entry clone into pYESDEST-52 vector, which have the ability to survive both in bacterial and yeast strain. Then the pYESDEST-52 vector was transfected into yeast diploid strain INVSc. The positive yeast clones were identified based on the ura3 genotype (Ura$^-$) selection and were induced by galactose to produce the recombinant exocellobiohydrolase and endoglucanase enzymes. Our future studies will be focused on the purification of recombinant proteins and to study the efficiency of these enzymes for degradation potential of lignocelluloses from different plant sources.

VI-T-6

EFFECT OF PLANT SECONDARY METABOLITES FROM *ACACIA CATECHU* TREE LEAVES ON *IN VITRO* DIGESTIBILITY

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The present study was made to see the effect of *Acacia catechu* tree leaves containing plant secondary metabolites estimated in terms of total phenols and condensed tannins on *in vitro* digestibility. *Acacia catechu* trees commonly occurring in the arid region and known to contain plant secondary metabolites i.e. phenols and tannins. The leaves samples were dried, ground and stored for chemical analysis. For in vitro digestibility of complete feed containing wheat straw (2.65% CP) 60 parts and concentrate (19.60% CP) 40 parts was prepared and supplemented with 1, 2.5, 5, 7.5 and 10 per cent levels of leaves supplementation. The dietary combinations were subjected to *in vitro* evaluation by following the method of Tilley and Terry (1963). *In vitro* digestion was carried out in 100 ml conical flask fitted with Bunsen valve after incubation at 39^{0}C for 48 hours. The rumen liquor was collected from adult rams kept on standard maintenance diet. Following incubation for stipulated period, the substrate were filtered through GI crucible to determine the *in vitro* digestibility of dry matter, organic matter, neutral detergent fibre, acid detergent fibre, hemicellulose and cellulose. The nutrient composition of *Acacia catechu* was recorded to be 51.74% DM, 1.28% EE, 14.00% CP, 26.78% CF, 48.94% NFE, 9.00% TA, 55.23% NDF, 21.75% ADF, 33.42% HC, 25.25% cellulose and 14.45% Lignin, whereas, the total phenol and condensed tannins were observed to be 2.51% and 0.51% respectively. On increasing supplementation of *Acacia tortilis* leaves from 1 to 10% present at graded level as such, an increase in *in vitro* digestibility of dry matter, organic matter, NDF, ADF, hemicellulose and cellulose were recorded. The *in vitro* digestibility of dry matter was 64.15, 64.97, 65.68, 65.75 and 64.45% in T_1, T_2, T_3, T_4 and T_5. Whereas, the *in vitro* organic matter digestibility were recorded to be 61.05, 61.78, 62.23, 62.91 and 62.02% present in T_1, T_2, T_3, T_4 and T_5 treatme.nt group respectively. Likewise, at 1 and 10 % level of supplementation, *in vitro* digestibility of NDF, ADF, hemicellulose and cellulose increased were 44.82 and 46.22, 37.02 and 41.63, 48.95 and 52.88 and 38.61 and 41.67 per cent, respectively. On the basis of average value obtained for digestibility of dry matter, organic matter and various fibre fractions, it was observed that the leaves of *Acacia tortilis* rich in total phenols and condensed tannin can be incorporated safely up to 10% level in complete feed without any adverse effect on digestibility. Tilley, J.M.A. and Terry, R.A.1963. *Journal of Biitish Grassland Scociety*, 18: 104-111.

VI-T-7

IMMUNISATION OF SHEEP WITH AN ANTI-IDIOTYPIC DNA CODING FOR THE INTERNAL IMAGE OF A REGION ON HN PROTEIN OF PESTE DES PETITS RUMUNANTS VIRUS ELICITS ANTIBODIES CAPABLE OF NEUTRALISATION OF VIRUS INFECTIVITY

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Peste des Petits Rumunants (PPR) is an economically important disease of small ruminants which is endemic in South Asia, the Middle East and equatorial Africa. The causative virus PPRV is a member of the *Morbilli virus* genus in the *Paramyxoviridae* family. A live attenuated vaccine has been in use; however due to lack of robustness due to heat liability, use of this vaccine requires adherence to rigid cold chain management. As part of our efforts in developing new generation subunit vaccine for possible application in situations demanding differentiation of antibodies elicited in animals due to infection from that due to vaccination, we have generated a candidate DNA vaccine coding for the variable region of heavy chain of an anti-idiotypic antibody which recognizes the Hemagglutinin-Neuraminidase protein of PPRV. In the present work, we have shown that intramuscular administration of plasmid DNA carrying the coding region of the internal image of the HN antigen to sheep elicits generation of HN specific antibodies in the complete absence of any virus or viral antigen. We further demonstrate that these antibodies are capable of neutralising PPR virus infectivity *in vitro*.

VI-T-8

PCR BASED DETECTION OF *MYCOPLASMA BOVIS* FROM BOVINE CLINICAL SPECIMENS

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Mycoplasma bovis is an important but sometimes overlooked pathogen in cattle. It causes major economic losses by causing respiratory disease, arthritis and a variety of other disease manifestations including mastitis and reproductive problems. The identification and isolation of *Mycoplasma bovis* with conventional methods are notoriously difficult and time consuming where as DNA based methods for *Mycoplasma bovis* identification, based on amplification of specific, highly conserved genes, reduces the assay time to hours as opposed to days or weeks, with high sensitivity and specificity. Therefore, in the present study, the identification of *Mycoplasma bovis* from the clinical specimens of bovine was performed by polymerase chain reaction. A total of 384 animals (216 cattle and 168 buffaloes) from various organized farms, villages and veterinary hospitals were screened for *Mycoplasma bovis* from various samples such as nasal and vaginal swabs, milk and semen. Out of 293 nasal swabs from bovine, an amplicon of 415bp was found in 76 (25.93%) samples with the 52 (28.88%) out of 180 nasal swabs from cattle and 24 (21.23%) out of 113 nasal swabs from buffalo. A total of 60 milk samples from cattle and buffalo were screened for *Mycoplasma bovis* by PCR. *M. bovis* is also a major pathogen for bovine mastitis therefore, 60 milk samples from cattle and buffalo were screened for it out of theses, 14 (23.33%) were found positive. The percent positivity for mycoplasma infection in milk samples of cattle and buffalo were 33.33% and 40.00%, respectively. A total of 22 semen samples from apparently healthy bulls of cattle and buffalo were screened for *Mycoplasma bovis.* Out of these 22 semen samples 08 (36.36%) were found positive. The percent positivity for mycoplasma infection in semen samples of cattle and buffalo were 27.27% and 21.05%, respectively. When 9 vaginal swabs of suspected cases of genital disorders were examined, 3 (33.33%) samples were found positive for mycoplasma infection. Therefore, the study reveals that *M. bovis* is present in various secretions and excretions of bovine whether showing the disease condition or in subclinical infection.

VI-T-9

BIOTECHNOLOGICAL APPROACH IN NUTRITIONAL STUDIES

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Importance of ruminants in livestock community is due to their ability to convert locally available feedstuff to animal products with help of rumen microorganisms. Manipulation of microbiological activities in the rumen and bioengineering of rumen microorganism to enhance animal productivity with available feed resources is promising area of research. Genetic and biological diversity, complex microbial community function, interaction among microbes and rumen ecology are some of the constraints in rumen microbiological studies. Conventional culture based methods of enumerating rumen microbes are being rapidly replaced by nucleic acid- based techniques and metagenomics. Recent advances in molecular biology and genomics now offer holistic approach to understand the complex microbial diversity, function of microbes and how microbes interact with each other with in their niches. This technique has potential to overcome the limitation of conventional techniques including isolation and taxonomic identification of strain. Thus, future of rumen microbiology research is dependent how these technologies can be used to improve ruminant's production through a better understanding of rumen ecology and microbial function.

VI-T-10

SUPEROVULATION AND EMBRYO RECOVERIES IN RATHI COWS USING DIFFERENT HORMONAL TREATMENTS

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Superovulation and subsequent embryo recoveries were attempted in Rathi cows using different hormonal treatments. Cows were either given a porcine FSH in 12 hourly divided doses (Total 8 doses) on day 8-10 of a natural estrus or a single IM dose (2000 IU) of eCG on the same days. Alternatively when a reference estrus was not known a CIDR-B was inserted intravaginally and superovulation started on day 8-10 of the implant insertion. Cows were given a dose of prostaglandin at the end of FSH treatment and inseminated at estrus. After 7 days of insemination, the uteri of cows were flushed for non-surgical embryo using DPBS supplemented with BSA and antibiotics. The ovulatory responses were high with eCG compared to porcine FSH, but the embryo recoveries and transferable grade embryo recovery was better with porcine FSH. There was no difference in the ovulatory response whether the superovulatory treatment was started after a natural estrus or after a CIDR-B implant. It was concluded that both eCG and porcine FSH can be used along with CIDR-B implants for superovulation of Rathi cows but porcine FSH yields better quality embryos.

VI-T-11

SUCCESSFUL TREATMENT OF CANINE CUTANEOUS LEISHMANIASIS BY HEAT THERAPY

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Two pet dogs have ulcerative mucocutaneous lesions on snout since last two months were presented in the canine outdoor .Out of them one mix GSD bitch have five lesions ,another Labrador female have three ulcerative and two nodular lesions and lesions were of 5-10 mm in size. Both pets were Suspected for cutaneous leishmaniasis . Contact smears from the lesions were made and stained with Leishmans stain ,examined under oil immersion microscopy.Several Leishmania tropica bodies (L.T.bodies) were found in the smears and confirmed the cutaneous leishmaniasis. The dogs were treated with Thermomed 1.8 device by putting electrode over the lesions for 30 seconds once only.Pets were examined every fifteenth day for progress of healing of ulcers and observed complete healing of ulcers after 45 days of heat therapy.

VI-T-12

DETECTION OF *OSMOTIN* GENE IN SEEDS OF *GOSSYPIUM* SPECIES

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The pathogenesis-related (PR) proteins are involved in the systemic acquired resistance and in general to antifungal responses. Osmotin has antifungal activity against a variety of microorganism, including *Phytophtera* infections, *Candida albicans*, *Neurospora crassa* and *Trichoderma ressel*. Recent reports also indicate that not only *Tobacco* but also osmotin like protein from several other plants species have similar inhibitory effects on fungal pathogens and may function as plant defense protein. The objective of this study was to detect the *osmotin gene* in seeds of *Gossypium* species. Healthy Seeds of *Gossypium arboreum* CINA 343, *G. herbaceum* G.Cot.23 and *G.hirsutum* LRK 516 were obtained from Central Institute for Cotton Research (CICR), Nagpur. Genomic DNA was extracted and purified from using modified method of Paterson (1993). The quality of DNA was evaluated from the 260/280 nm UV absorption ratios and by agarose gels electrophoresis and quantified by Smart Spac^{TM} Plus, Bio-Rad spectrophotometer. The primers were designed as per the published sequences available in the public domain Gene Bank (Accession No FJ647193). PCR was performed in 20ìl reaction mixture containing template DNA, 10X PCR buffer, 25 mM $MgCl_2$, 10mM dNTPs, 0.5 U *Taq* DNA polymerase and 5ìM each of forward and reverse primers. Osmotin was amplified using 24-mer (5'-AACCATGAGCTAGGGGCCATTTCC-3') for forward and 23-mer (5'-AAAGGTCCCGTACCCTTGAGTGC-3') long for reverse. Annealing temperatures ranging from 56-65°C were used for osmotin primer sets. Amplification reactions for *osmotin* gene using PCR was performed in Programmable Thermal Cycler (BioMetra) under the following programme: initial denaturation at 94°C for 5 min followed by denaturation at 94°C for 45 sec, primer annealing at optimum annealing temperature 56-65°C for 45 sec, primer extension at 72°C for 1 min (in total, 35 cycles of the above programme was performed), and final extension at 72°C for another 5 min. The PCR amplified products were resolved on 1.5% agarose gels stained with ethidium bromide using 0.5X TBE as the running buffer on horizontal electrophoresis. The 100bp and 1 Kb DNA step ladder used to determine the presence of the *osmoin* gene and visualized under UV light and photographed using Gel Documentation System (Alpha Innotech). The total genomic DNA was isolated, purified and subjected to qualitative and quantitative checks. The modified Peterson DNA extraction method (1993) gave maximum DNA yield in cotton species used in present investigation. In total genomic DNA, the intact DNA bands were observed. The result showed that the genomic DNA was isolated without contamination of protein in seeds of cotton cultivars. The A260/280 ratio found to be <0.50 in all the seeds of cotton cultivars justified the presence of good quality of DNA. Using the above mentioned primer the target gene *osmotin* was amplified in programmable thermal cycler and then amplification programme in the thermal cycler was standardized with different programme, especially annealing temperature, which play crucial role in amplification with lengthy primer. The annealing temperature was reduced from high temperature 56°C to 65°C for efficient amplification of targeted gene (*osmotin*). The best amplification was found at 57°C, the amplified product was resolved on 1.5% agarose gel electrophoresis. After the run the gel was stained with ethidium bromide and observed under UV-transilluminator and result were documented. A fragment of 0.7 kb *osmotin* was amplified with this primer. Fungal diseases destroy much of the cotton crop and subsequently result in large economic losses every year. Although fungicides have helped to control plant fungal infections, this chemical control is economically costly as well as environmentally undesirable. Therefore, new strategies should be used based on plant defense mechanisms to control fungal diseases to improve agricultural production and prevent the over usage of chemical fungicide treatments that could be harmful to humans and the environment. Use of *osmotin* gene in such plants may solve this problem. Due to the wide-ranging fungal effect of the *osmotin* gene, it may be possible to use the *osmotin* gene as a novel defense that is effective against numerous pathogenic fungi.

VI-T-13

ANTIMICROBIAL POTENTIAL OF *ZIZIPHUS NUMMULARIA* (BURM. F.) WIGHT ET. ARN. AGAINST SOME BACTERIAL AND FUNGAL PATHOGENS.

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Ziziphus nummularia (Burm. f.) Wight et Arn. belongs to family Rhamnaceae is a multipurpose wild economic species provide an excellent fodder for livestock. It possesses antibacterial, antifungal and antihelminthic properties and used to cure throat infections, eye diseases, urinary infections, leucorrhoea, gum bleeding, tuberculosis and blood diseases as folk medicine as well as in traditional veterinary medicine. In the present study leaves, fruit, seed and tissue culture extracts prepared in ethanol and water of *Z. nummularia* were screened against *Bacillus cerus* NCIM 2156, *Staphylococcus aureus* NCIM 2654, *S. epidermidis* NCIM 2493, *Mycobacterium smegmatis* NCIM 5138, *Escherichia coli* NCIM 2685, *Pseudomonas aeruginosa* NCIM 5032, *Proteus vulgaris* NCIM 2027, *Salmonella typhimurium* NCIM 2501 and two fungal pathogens *Candida albicans* NCIM 3466 and *Trichoderma viride* NCIM 1221 using agar well diffusion method. The results indicated that all the test extracts exhibit inhibitory effect against one or more test microorganisms however the extracts of tissue culture showed better antimicrobial activity against all the bacterial and fungal pathogens except *T. viride*. The study promises not only an interesting future for designing a potentially active antimicrobial agent from this arid zone plant but also conservation of this plant by the use of tissue culture.

VI-T-14

THERAPEUTIC EVALUATION OF IVERMECTIN AGAINST ENDOPARASITES OF DONKEY

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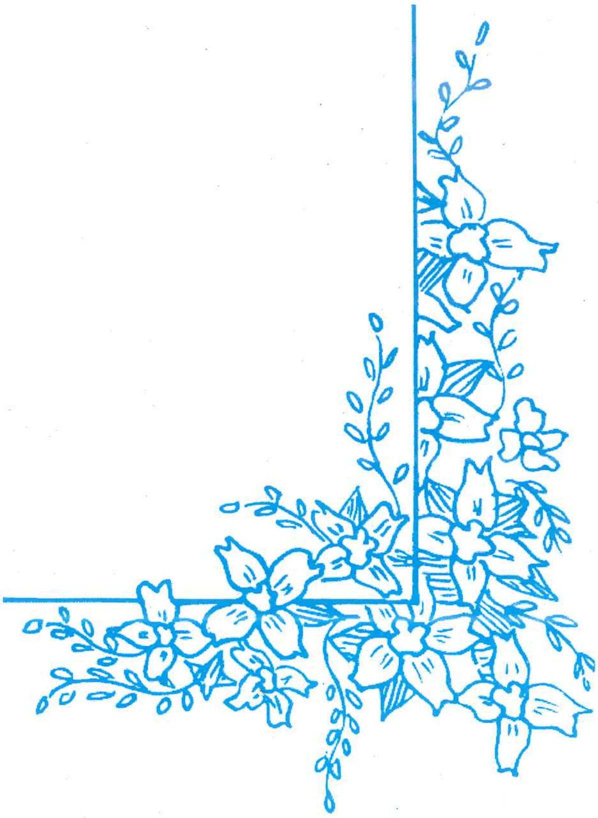
Therapeutic efficacy of ivermectin was evaluated in indigenous donkeys (14) purchased from farmer's field and reared on stall fed. Donkeys were naturally infected with helminth parasites *Strongyles, Strongyloides*, *Parascaris* and *Anoplocephala*. Ivermectin was given @ 0.2 mg/kg per orally. Mean EPG during control period on Day '0' (before the administration of ivermectin) was 1461±176, with range of 600 to 3150, which depicted very high level of infection. EPG was reduced to 39±16 on day 21 after treatment. Presence of helminth eggs on day 21st were mainly due to *Anoplocephala* which is not susceptible to ivermectin.Study illustrated efficacy of ivermectin against *Parascaris, Strongyloides, Strongyles* and *Anoplocephala* 100%,100%, 99.42% and no efficacy respectively. So it is suggested that ivermectin should be used in combination with any effective dewormer for tapeworms for effective deworming programme.

Poster Presentations



I-P-1

SEROCONVERSION STUDIES OF HAEMORRHAGIC SEPTICEMIA AND FMD IN ASIAN ELEPHANTS (*Elephas maximus*) OF NORTH-EAST INDIA

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Seroconversion of seven apparently healthy domesticated Asian elephants between 1-60 years of age and of both sexes from northeastern India were studied post vaccinal for haemorrhagic septicemia (HS) and FMD. The investigation was carried out in the months of July, August and September during the year 2005. These animals were vaccinated with alum precipitated killed HS vaccine (Bovilis) and Alum precipitated inactivated FMD vaccine (clovax) and tested for post vaccinal seroconversion on 30 and 90 days post vaccination. The doses of vaccines were the one which are usually used to vaccinate elephants in the region and are similar to that of cattle. The animals responded to the vaccines to some extent but the response were not similar within the animals and overall response was below the protective limits. It was concluded that standardization of vaccine and vaccination schedule are required to invoke protective immune responses in elephants.

I-P-2

CONSERVATION STATUS OF GYPS VULTURE IN INDIA

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Gyps vulture populations across the Indian subcontinent collapsed in the 1990s and continue to decline. Repeated population surveys showed that the rate of decline was so rapid that elevated mortality of adult birds must be a key demographic mechanism. Post mortem examination showed that the majority of dead vultures had visceral gout, due to kidney damage. The realisation that diclofenac, a non-steroidal anti-inflammatory drug potentially nephrotoxic to birds, had become a widely used veterinary medicine led to the identification of Diclofenac poisoning as the cause of the decline. Seven species of vultures are present including king vulture (Sarcogyps calvus), long-billed vulture (G. indicus), slenderbilled vulture (G. tenuirostoris), whitebacked vulture (G. bengalensis), Egyptian vulture (Neophron percnopterus), cinereous vulture (Aegypius monachus), Himalayan griffon (G. himalayensis) and Eurasian griffon (G. fulvus) in this rajasthan, first five are resident and the rest are migratory species. Surveys of diclofenac contamination of domestic ungulate carcasses, combined with vulture population modelling, show that the level of contamination is sufficient for it to be the sole cause of the decline. Using a range of plausible assumptions about normal mortality rates and intervals between meals, it was shown that less than 1% of livestock carcasses (0.13–0.75%, depending upon the vulture species, population) would have to carry lethal concentrations of diclofenac to have caused the observed rates of OWBV and LBV population decline in India and Pakistan between 2000 and 2003. The model was also used to calculate the proportion of dead adult and subadult vultures that would have visceral gout, the characteristic sign of diclofenac poisoning, if the observed declines were caused only by diclofenac. It was found that the proportion of dead vultures observed to have gout in Pakistan and India was consistent with diclofenac being the most important cause of the decline. Testing on vultures of meloxicam, an alternative NSAID for livestock treatment showed that it did not harm them at concentrations likely to be encountered by wild birds and would be a safe replacement for diclofenac. The manufacture of Diclofenac for veterinary use has been banned, but its sale has not. Consequently, it may be some years before diclofenac is removed from the vultures' food supply. In the meantime, alternative feeding managements of livestock carcasses for vulture species have been planed to provide sources of Diclofenac free food for future vulture as ex-situ conservation programmes.

I-P-3

PROFILE OF ANIMAL HEALTH SERVICES IN ARID ZONE OF RAJASTHAN

Rajput Devi SINGH AND TRIPATHI HEMA

The present investigation discusses the profile of veterinary service and veterinary service receivers in arid zone of Rajasthan, India. The present study was conducted in two purposively selected districts viz. Bikaner and Jaisalmer from arid zone of Rajasthan. The data were collected and 120 pastoralists that receive the veterinary service through twelve veterinary hospitals under organized system and 15 traditional healers, providing veterinary service under unorganized system. The study highlight that Majority of pastoralists (63.33%) belonged to middle age group and had medium level of family education status (58.33%) and belonged to large farmer's category (53.33%) having above 10 acres of land. Animal husbandry was considered as major family occupation of pastoralists. They are keeping various combination of animal herd for their livelihood sustainability. Predominant diseases among cattle in study area reported by the pastoralists were repeat breeding, retained placenta, botulism, night blindness and pica. Pox, pneumonia, diarrhoea and pica were found as major problems among sheep and goats and mange reported as major problem among camels. Veterinary officers (VOs) of state department of animal husbandry and traditional healers were found as prevalent and regular animal health service providers to the pastoralists.

I-P-4

Histological Studies On Stroma Of Spleen Of sheep (*Ovis aries*)

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The present study is conducted on the ten spleens. The stroma or parenchyma of spleen was composed of white and red pulp. The white pulp of spleen was lymphoreticular tissue consisting of lymphocytes, plasma cells and macrophages enmeshed in reticular network. The white pulp was composed of two components called as splenic nodules or malphagian corpuscles and periarterial lymphatic sheath. The periarterial lymphatic sheaths were less abundant in spleen of sheep. The splenic nodules were composed of aggregation of lymphatic tissue along the course of small pulp artery. Eccentrically situated arteries were observed in the splenic nodules, the noduler artery or central artery. The germinal center of nodule was light stained, where the lymphocytes were loosely arranged. In addition to lymphocytes, the plasma cells and macrophases were also observed in splenic nodules. The mean diameter of splenic nodules was 221.06 ??2.77 ?m, and overall mean thickness of centeral artery was 13.90 ??0.16 ?m. The red pulp filled the spaces between the white pulp and trabbeculae. It consisted of pulp arterioles, sheathed capillaries, terminal capillaries, splenic sinusoids and splenic cords. The splenic sinusoids were less abundant and poorly developed. Numerous splenic cords were observed between the sinusoids. The framework of splenic cords was formed by loosely arranged reticular fibers and it contained numerous erythrocytes, reticular cells, lymphocytes, macrophages and plasma cells. The iron and hemosiderin pigments were observed in splenic parenchyma.

I-P-5

STRANGLES IN A HORSE: A CASE REPORT

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Sample of bloody nasal discharge from a horse aged 3 years old with symptoms of nasal discharge and fever was presented to the Department of Veterinary Microbiology GADVASU Ludhiana. The sample was inoculated on the Blood Agar and incubated at 37°C for 24 hours. Large mucoid colonies were seen which were haemolytic with wide zone. Morphological examination by Gram's staining revealed small Gram positive cocci arranged in chains and negative staining revealed the capsulated bacteria. The culture was subjected to biochemical testing and was found to be catalase negative and oxidase negative. The culture was subjected to the sugar fermentation tests and was negative for trehalose, sorbitol, lactose but was positive for maltose. So the culture was confirmed to be Streptococcus equi subsp. equi which causes strangle in young horses. Antimicrobial susceptibility showed the bacteria to be sensitive to penicillin G, gentamicin, enrofloxacin, neomycin, ciprofloxacin, cephotaxime and resistant to erythromycin.

I-P-6

BIODIVERSITY OF MEGACHIRPTERANS AND MICROCHIROPTERAN BATS IN BIKANER CITY

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Bats belong to largest order of class mammalian, Chiroptera which is further classified two sub-orders viz. Megachirpterans and Microchiropteran. In India 109 bats species of bats, including 13 Mega and 96 Microchirpteran are present. Our study site the Bikaner (28^{0} 01' 22N and 73^{0} 19' 13E) lines in the north western part of Rajasthan. The district has a dry climate with large variations of temperature, and scanty rainfall. The winter lasts from November to March and is followed by summer from April to June. The vegetation of Bikaner district falls under the broad natural division of tropical thorn forest, but due to low rainfall, extreme temperature, high evaporation and tremendous biotic disturbances have converted it into a typical arid tract. In such a tract, no forest worth the name can grow. In low-lying areas of district, however, where the moisture accumulates to some extent during rainy season, a few scattered trees are found. The Plant vegetation of Bikaner is typical deserted as *Dalbergia sissoo, Delonix regia, Albizia lebbeck, Azadirachta indica, Kigelia africana* , *Cassia fistula, Melia azedarach, Mimusops elengi, Acacia leucophloea* Willd., *Acacia nilotica* Willd., *Acacia raddiana, Acacia senegal* Willd., *Ailanthus excelsa, Balanites aegyptiaca, Capparis deciduas, Cassia siamea, Clerodendrum phlomoides, Cordia crenata, Cordia dichotoma, Cordia gharaf, Ficus benghalensis, Ficus mollis, Ficus religiosa, Maytenus emarginatus* (Willd.) Ding Hou., *Mimosa hamata* Willd., *Moringa oleifera, Morus alba, Nyctanthes arbor – tristis, Parkinsonia aculeata, Pithecellobium dulce, Prosopis cineraria, Prosopis juliflora, Salvadora oleoides, Salvadora persica* , *Syzygium cumini, Tamarindus indica, Tamarix aphylla* , *Tamarix dioica* and *Tecomella undulata* . In this study four Microchirpterans bat *species viz. Rhinopoma hardwickii, Rhinopoma microphyllum kinnerri, Rhinolophus Lepidus* and *Pipistrellus tenuis* and one species of Megachirpterans in rainy season, Indian Flying Fox (*Pteropus giganteus*) are reported in fist time from Bikaner city. Roosting site are follow as: 600 *Rhinopoma hardwickii*, and 250 *Rhinopoma microphyllum kinneari* in Junagarh Fort (28^{0} 01' 22N and 73^{0} 19' 13 E), 56 *Rhinopoma hardwickii* in Devi kund Sagar, Sagar village (28^{0} 01' 11 N and 73^{0} 23' 34 E), 390 *Rhinopoma hardwickii,* and 210 *Rhinopoma microphyllum kinnerri* in Hanman temple, Pawan Puri (27^{0} 59' 33 N and 73^{0} 20' 19E), Mix population of *Rhinopoma hardwickii, Rhinopoma microphyllum kinnerri, Rhinolophus Lepidus* and *Pipistrellus tenuis* in Session Court Building (28^{0} 00' 18 N and 73^{0} 19' 21 E) and 130 Indian Flying Fox (*Pteropus giganteus*) Session Court area (28^{0} 00' 20N and 73^{0} 21' 15E). All four Microchirpterans and one Megachirpterans bat species population depend upon Temperature, Humidity and wind capacity. Breeding biology was also reported of *Rhinopoma hardwickii.*

I-P-7

INVESTIGATION OF CHLAMYDIAL ASSOCIATED ABORTIONS AND PNEUMONIA AMONG MIGRATORY SHEEP AND GOATS IN HIMACHAL PRADESH

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Chlamydial infections have been reported frequenlty among sheep and goat populations abroad and incidences have been reported from India also. Mostly *Chlamydophila abotus* is associated with abortions but recent reports also indicated the involement of *C. psittaci*, *C. pecorum* and *Chlamydia suis*. We conducted a molecular epidemiological investigation to detect the prevalence of different chlamydial species grouped under order *Chlamydiales* in abortions and pneumonitis cases among migratory ovine and caprine flocks. Total 134 samples from clinical cases of 75 abortions and 59 pneumonitis from 70 sheep and 64 goats were screened using *Chlamydiales* specific primer sets based on 16S and 23S RNA and *Chlamydiacae* family specific primers based on *ompA* gene. DNA was extracted from vaginal swabs (67), nasal swabs (59), aborted feti (3) and placental tissues (5). We observed the involvement of chlamydial species in 13% and 25% abortions in sheep and goat respectively and 9% pneumonia cases in goats as deceted by the group specific and family specific primers. All the PCR positive samples were further confirmed by attempting isolation of involved chlamydial species in embryonated chicken eggs. Our study indicated substantially higher involvement of chlamydial speceies in ovine and caprine reproductive and respiratory tract incetions that require further detailed studies to identify the prevalent chlamydial strain as well as chlamydia specific preventive and control interventions to curtail the economical losses to farmers.

I-P-8

SERUM PROTEINS, VISCOSITY AND SPECIFIC GRAVITY PROFILE DURING WATER DEPRIVATION IN GOAT

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Scarcity of water disturbs the balance between intake and loss of water in animals belonging to arid and semi arid tract. Under these conditions the animals are left to depend on their physiological ability to with stand body water deficit. To study the physiological mechanisms taking place in the body a study was conducted on 6 healthy adult female Marwari goats. Results showed that with the advancement of dehydration a significant ($P \leq 0.05$) increase was observed in the mean values of total serum protein, albumin & globulin. On day 9 of dehydration the values of TSP, albumin and globulin were 10.66±0.21, 4.83±0.21 and 5.66±0.43, respectively. The mean values of viscosity, specific gravity increased significantly with the advancement of dehydration and the values were 6.06±0.14 and 1.078±0.002 respectively on day 9 of dehydration.

I-P-9

PREVALENCE OF BOVINE TROPICAL THEILERIOSIS IN AND AROUND BIKANER

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A total of 150 animals were screened for Bovine Tropical Theileriasis (BTT), out of which 125 animals were showing clinical Sign viz. pyrexia, pale mucous membrane, enlarged lymph nodes. The rest of 25 apparently healthy animals were screened for carrier status. Out of these 125 animals, 122 were calves and three were adult cattle. Among calves, 86 crossbred and 20 Rathi calves were of 1 week to 3 months of age and 14 crossbred and 2 Rathi calves were of 3-6 months of age. Prevalence of BTT was found to be 59.2% (74/125) by blood smear and 80.0% (101/125) by PCR. Carrier status of Theileria infection in apparently healthy cattle by blood smear examination was 4% (1/25) and by PCR 56% (14/25). Both cross bred and indigenous Rathi calves were found to be susceptible but maximum prevalence 70.4% (86/122) of BTT was recorded in calves of 1 week to 3 months of age.

I-P-10

CLINICAL TRIAL OF LEVAMISOLE HYDROCHLORIDE AS AN IMMUNO-STIMULANT IN CASES OF FOOT AND MOUTH DISEASE IN CATTLE

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A clinical trial was undertaken to test the efficacy of levamisole hydrochloride as an immuno-stimulant in cases of foot and mouth disease in cattle. Twelve crossbred cattle affected with foot and mouth disease were included in this study. Half of them were treated as control group. The treatment of control group included antibiotic, anti-pyretic - anti-inflammatory and vitamin C preparation parenterally alongwith potassium permagnate mouth wash and antiseptic dressings of foot lesions. The rest of the animals were administered levamisole hydrochloride @ 7.5 mg/kg b.wt. S.C. as a single dose adjuvant therapy alongwith the treatment as prescribed for control group. The course of the disease was recorded shorter in cases treated alongwith levamisole hydrochloride as compared to control group. Thus the recovery was faster in the animal patients treated with levamisole hydrochloride as an adjuvant therapy. The faster recovery accounted to reduced duration of treatment and lesser economic losses in terms of milk production. Levamisole hydrochloride has no direct antiviral action but it enhances the normal cellular immune responses particularly related to macrophages, T-cells and B-cells which are responsible for production of antiviral antibodies. Thus levamisole hydrochloride may effectively be used as an adjuvant therapy in treatment and control of foot and mouth disease in cattle.

I-P-11

BRUCELLOSIS OUTBREAK IN BUFFALO *(BUBALUS BUBALIS)*

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Brucellosis is one of the very important zoonosis inflicting the animal population through out the world causing great economical losses in terms of abortion, birth of weak calves, reduced production and delayed or reduced conception rates and beyond this, on most of the occasions the disease remains undiagnosed in the herds. An outbreak of brucellosis with abortion storm was recorded in buffaloes in Nagaur district of Rajasthan state (India) at a farm with 250 adult heads during months of May-June 2010. Most of the animals were healthy with the history of reduced milk production and a general tendency of repeat breeding. There was premature delivery of weak calves. Many of the animals had hygroma at knee or hock joint in one or more legs. The abortions were recorded at six months onwards but most of the buffaloes aborted at seventh month of gestation. The disease was confirmed serologically using Rose Bengal plate test, Standard Tube Agglutination test and Avidin-Biotin ELISA test. The sero-prevalence recorded by RBPT was 48.00%, by STAT 43.10% and that with ELISA it was 32.00%. It was recorded that unhygeine, overcrowding and high environmental temperature were the main associated risk factors.

I-P-12

SEASONAL INFLUENCE ON PLASMA TRIIODOTHYRONINE (T_3) AND THYROXINE (T_4) CONCENTRATIONS IN MARWARI SHEEP

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The investigation was carried out to study the influence of season on thyroid hormone in Marwari sheep. Six adult sheep (rams) were subjected to study the influence of season on plasma thyroidal Triiodothyronine (T_3) and Thyroxine (T_4) hormones. Plasma Triiodothyronine (T_3) and Thyroxine (T_4) concentrations were estimated by Enzyme-immunoassay (EIA) technique in ng/ml., using pathozyme triiodothyronine (T_3) and Thyroxine (T_4) kits, respectively. A highly significant (P £ 0.01) effect of winter (extreme cold) season on plasma Triiodothyronine (T_3) and Thyroxine (T_4) with increasing concentrations, than that of the summer (extreme hot) season was observed. The exposure of animal to high environmental temperature depressed the functional activity of thyroid gland and thereby caused a relative lower concentration of thyroid hormones. The thyroid hormones are metabolic hormones. The oxidative metabolism and heat production increases under the influence of T_3 and T_4 during the winter months, so their concentration in blood rises in winter. The decline of these hormones during summer season enables the animals to compensate for the greater environmental heat load.

I-P-13

STUDIES ON INDUCED HYPOTHYROIDISM IN MARWARI SHEEP

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The investigation was carried out to study the effects of induced hypothyroidism on plasma Triiodothyronine (T_3), Thyroxine (T_4) and Thyroid stimulating hormone (TSH) in Marwari sheep. Six adult sheep (rams) were subjected to induced hypothyroidism by thiourea feeding (@ 50 mg/kg body weight). The blood samples were collected and analyzed on 0 (control), 3rd, 5th and 7th day of thiourea feeding. T_3, T4 and TSH were determined by Enzyme-immuno assay (EIA) technique using respective pathozyme kits. A highly significant (P £ 0.01) effect of induced hypothyroidism on plasma T_3 and $T_4$with gradually declining trend was observed ,while plasma TSH showed a highly significant (P £ 0.01) increase at the Phase-IV (7th day) of induced hypothyroidism. It is concluded from the present study that induced hypothyroidism in sheep appreciably alters the blood thyroid hormonal profile.

I-P-14

INVESTIGATION ON CUTANEOUS LEISHMANIASIS IN MEN AND DOGS IN BIKANER

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From the public health point of view cutaneous leishmaniasis is one of the most important zoonotict diseases of this area occurring mainly in dogs and men. It is caused by leishmania tropica and transmitted through the bite of sandfly i.e. Phlebotomous species. Presently cutaneous leishmaniasis infection is recorded for the first time in the pet dogs. In the present study survey of 542 street and 98 pet dogs were made around the Bikaner city, where 36 cases of cutaneous leishmaniasis in human being were reported. Out of 542 street dogs only 11 cases and out of 98 pet dogs only 6 cases are reported as cutaneous leishmaniasis. It seems that this disease is restricted only around the area of human patients of oriental sore. Disease was independent of 74 persons are positive for oriental sore. Human population below 20 years age group showed high incidence. This disease was independent to age and sex. In maximum cases the leions were observed on the extremities. Both intra and extracellular forms of L.tropica were present as revealed by the contact smear prepared from cutaneous lesions. The host cells were mononuclear leucocytes which were in certain cases found literally packed with parasite.In the cases of human beings, 36 out of 74 persons are positive for oriental sore. Human population below 20 years age group showed high incidence. This disease was independent tosex. The duration of lesions was 1-2 months in maximum cases. Lesions were mainly confined over exposed parts of the body. It is observed that cutaneous leishmaniasis infection is a localized skin infection as not a single case of Leishmania tropica body was observed in general circulation. In comparison to NNN media, Depasic media was found to be superior for the cultivation of L.tropica where the parasite multiplied well at 48 hours in B.O.D. incubation at 22^{0}C. Five to eight injection of 2% aqueous solution of barbarian sulphate at weekly interval (1 ml. intradermally in each lesion) are found to be effective for proper healing of cutaneous lesions in men and dogs.

I-P-15

FUELLING OF TCA CYCLE IN HEPATIC CELLS OF DROUGHT AFFECTED MARWARI GOATS

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Marwari breed of goat plays an important role in the economy of arid tracts in India. Recurring droughts modulate the physiological mechanisms to sustain the life. However, these prolonged changes can produce stress to these animals. Despite of immense quality characteristics of *Marwari* goat very little scientific attention has been given to explore these mechanisms. The TCA cycle is the central metabolic hub of the cell. It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid. Glutamate dehydrogenase enzyme is located in the mitochondria and is an important branch-point enzyme between carbon and nitrogen metabolism. It catalyses the reversible NAD (P)+-linked oxidative deamination of L-glutamate into alpha ketoglutarate and ammonia, making alpha-ketoglutarate available to tricarboxylic acid cycle (TCA cycle).Looking towards the significance of glutamate dehydrogenase enzyme in energy metabolism and paucity of work on this aspect in *Marwari* goat, the present study was launched to assess the effect of drought on fuelling of TCA cycle. To carry out the study, serum samples were collected from *Marwari* goat of drought affected and non-drought affected areas. An increased serum GD activity in drought affected animals as compared to non-drought affected areas was related with a decrease in serum glucose concentration. This showed the association of serum GD with glucose homeostasis. It was concluded that serum GD activity increased to provide more fuel for TCA cycle by making alpha ketoglutarate available in drought affected animals. Further serum levels of this enzyme can be used to assess fuelling of TCA cycle.

I-P-16

MICROSCOPICAL STUDY OF THE ADRENAL MEDULLA OF THE GOAT (*Capra hircus*)

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The microscopic studies on adrenal medulla of 36 adult male goat showed that the follicular cells of medulla grouped in to outer and inner zones. The reticular fibres along with collagen fibres formed a network between medullary cells. The outer zone was made up of large columnar cells in which nuclei were situated in apical portion while the cells of inner zone were polyhedral in shape with centrally placed nuclei. A large central vein was found in almost all specimens. Muscle fibres were not present in the wall of central vein.

I-P-17

HISTOLOGY OF GALL BLADDER IN BUFFALAO CALF (*Bubalus buballis*)

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The gall bladder is lined with tall simple columnar epithelium. The average cell height and width varies from 29 to 40 micron and 4 to 9 micron respectively in all the three regions of gall bladder and width varies from 28 to 37 micron and 4 to 7 micron respectively. The cytoplasm of epithelial cells is divided into two zones i.e. (a) a light apical zone and (b) a dark basal zone. Distinct goblet cells could not be observed in the surface epithelium but few goblet cells were found. The free surface of the lining cells bears a thin, homogenous striated brush border. The tunica propria of gall bladder evinced heavy lymphocytic infiltration The lymphoid tissue of the gall bladder was present in three different forms. At some places, the surface epithelium was eroded or the cells appeared almost compressed. A further point of interest is the slightly dome shaped appearance of epithelium seen at few places. The tunica propria contains serous and mucous glands.. The tunica muscularis of gall bladder is composed of at least two layers of smooth muscle cells. The tunica serosa forms the external thick connective tissue coat of the gall bladder. The tunica is rich in blood capillaries nerves and fat cells.

I-P-18

STUDY OF THE CONNECTIVE TISSUE OF TESTES IN DOG (*CANIS FAMILARIS*)

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The testes were enveloped in the capsule of tunica albuginea composed of collagen and reticular fibres with blood vessels. The testes had several lobules containing convoluted tubules the seminiferous tubules. The seminiferous tubules contained the spermatogenic cells and sertoli cells. The interstitial tissue occupied the area between the adjacent seminiferous tubules. It was composed of collagen fibres and cellular component consisted of interstitial cells of Leydig. The mediastinum testis occupied a central position along the longitudinal axis of the testes and contained the network of tubules, the rete testis.

I-P-19

MACROSCOPIC STUDY OF HUMERUS OF INDIAN GAZELLE (*GAZELLA GAZELLA BENNETTII*)

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In the present study, four humerii of adult *Chinkara* were used. The shaft was slightly twisted, cylindrical and presented four surfaces. Caudal surface presented a nutrient foramen in its distal third towards lateral surface. The musculo-spiral groove was shallow. The deltoid tuberosity was found as small rough convexity and less prominent. The head was roughly rounded and the neck was well-defined. The lateral tuberosity was large and curved over the inter-tubercular groove. The minor tubercle was small and did not divide noticeably. The inter-tubercular groove was present between both the tubercles. The coronoid fossa and olecranon fossa communicated by a small supra-trochlear foramen in some specimens.

I-P-20

FREE RADICAL SCAVENGERS IN ERYTHROCYTES OF INDIAN DROMEDARIES

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Free radicals damage immune system and are contributing factors for many diseases. They wipe out cytokine pathways. Oxygen-derived free radicals are important in both natural and acquired immunity. Oxidative stress may be detrimental in acquired immunity by activation of nuclear factor kappa B, which governs gene expression involving various cytokines, chemokines, and cell adhesion molecules. Animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells along with alteration in the immune status of animals by increasing susceptibility to infectious agents. Looking towards the paucity of work on free radical scavengers in the erythrocytes of Indian dromedaries, an investigation was carried out to determine various erythrocytic free radical scavengers during moderate and hot ambience. Blood samples were collected and erythrocytes were separated. Free radical scavengers included vitamin A, vitamin C, vitamin E, superoxide dismutase, glutathione reductase, catalase and peroxidase. The mean values of erythrocytic vitamin A, vitamin C and vitamin E decreased during hot ambience whereas of superoxide dismutase, glutathione reductase, catalase and peroxidase increased significantly during hot ambience as compared to moderate ambience. The result indicated the development of oxidative stress during hot ambience.

I-P-21

ASSESSMENT OF OXIDATIVE STRESS IN INDIAN DROMEDARIES DURING DROUGHT AND HOT AMBIENCE

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Oxidative stress affects the cellular mechanisms of an animal. It can be assessed only by laboratory methods. Dromedary camels contribute significantly in short distance transport in Rajasthan state in India. They face extreme variations in ambient temperatures and recurring droughts. For healthy management of these animals, it is important to assess antioxidant status to explore the existence of oxidative stress. Therefore an investigation was carried out in Indian dromedaries during drought condition, extreme hot and moderate ambience. Serum levels of antioxidants like vitamin A, vitamin C, vitamin E and glutathione and enzymes like catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, peroxidase, oxidase and xanthine oxidase were determined. The mean values obtained during moderate ambience were considered as control values. The mean values obtained during drought and hot ambience were compared with the respective control values. Vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly during drought and hot ambience. Serum catalase, superoxide dismutase, monoamine oxidase, glutathione reductase, xanthine oxidase, oxidase and peroxidase activities increased significantly during drought and hot ambience. The changes were significantly higher in drought affected animals as compared to those observed during hot ambience. It was concluded that hot ambience and drought condition induced oxidative stress in Indian dromedaries.

I-P-22

SOME STUDIES ON BUN AND SERUM CREATININE IN INDIGENOUS AND CROSSBRED CATTLE IN AND AROUND BIKANER

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Determination of the nonprotein group of nitrogenous substances, especially urea and creatinine, is important as significantly increased values are usually the result of accumulation of these substances in the blood because of defective kidney elimination. For this study a total of 317 cattle of either sex and different age groups (5 days to 18 years) were included which were either brought to the Medicine clinic or belonged to College dairy farm (organised farm), private farms and individual holdings of the farmers in and around Bikaner. From the organised farm 64 cattle (indigenous breed- Rathi) similarly from the unorganised/ private sector indigenous as well as crossbred/ Holstein 253 cattle of both sexes and different age groups having various physiological stages were randomly taken for study. The cattle below five years of age were considered as young. The organised and unorganised cattle were classified on the basis of sex (male and female); age (young and adult); production (lactating and non-lactating) and breed (indigenous and cross-bred). The over all mean BUN mean values were 12.37 ± 0.32 mg/dl. The statistical analyses revealed non significant effect of any of the factors of variation viz., organisational set-up, sex, age, breed and production. The mean values of BUN ranged between 9.66 ± 0.94 12.86 ± 0.75 mg/dl. The serum creatinine probably one of the very important indices to judge the renal function efficiency in different groups under organised as well as unorganised farms cattle under organised and unorganised management were recorded to be 2.24 ± 0.11 mg/dl and 2.87 ± 0.08 mg/dl, respectively, whereas the mean value for entire population was calculated to be 2.74 ± 0.07 mg/dl. The statistical analyses of data though could not reveal significant effect of sex, age and breed but revealed sequentially highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$) effect of organisational management and production, respectively. Further, the comparison of means showed that serum creatinine values were significantly lower in animals at organised farms and in non lactating animals in comparison to private/ institutional animals and lactating animals, respectively. However, sex, age, production and breed could not exhibit any statistical effect on serum creatinine values but in organised cattle the serum creatinine values were considerably lower. This could possibly be due to provision of stall-feeding in these animals, which must have resulted in lower muscular activity.

I-P-23

MICROBIOLOGICAL EVALUATION OF ABORTIONS IN BOVINES

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Infertility of infectious etiology can pose a serious problem in bovines. There is wide spread belief among dairy farmers and Veterinarians that diseases such as vaginitis, metritis, abortion or still birth may be caused by specific micro-organisms. These infections are of interest for economic and public health reasons and such infections may create serious problems for the commercial producer, since several of the diseases are chronic and affect calves health. However, the knowledge of the relationship between presence of bacteria in reproductive tract and abortion in bovines is still confusing. During recent years, reproductive failures among female have been reported more frequently by dairy farm owners. The present study was conducted to find out the association of aerobic bacterial flora in bovines abortion. Cattle with history of abortion presented to the local Veterinary hospitals including district Hospitals in and around HASSAN were selected for the study. Out of 1200 cattle with reproductive disorder over a period of two years, 463 cattle which aborted at different stages of gestation and presented to hospitals for therapy were included for this study. The bacterial species were identified following the standard methods described by Cruickshank *et.al.*,(1975) and Mackie and Macartney (1989). A total of 13 isolates were obtained from 113 cases of abortion. Pure isolates consisting of *E.coli* and *Streptococcus canis* were recovered from fortyfive samples, while fourteen samples yielded mixed cultures containing *Streptococcus canis, Staphylococcus epidermidis, Staphylococcus aureus* and *E.coli,* and senentyone sample failed to yield any growth. The frequency of different bacterial isolates in the descending order were *E.coli* (3.33%), *Streptococcus bovis* (6.67%), *Staphylococcus epidermidis* (4.00%) and *Staphylococcus aureus* (1.69%).

I-P-24

EVALUATION OF PHARMACOLOGICAL ACTIVITY OF COW'S URINE

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The detailed studies on pharmacological activities and safety evaluation of cow urine employing controlled studies in experimental animals are scanty. Cows were regarded as wealth and were the backbone of the economy of ancient Indians. There are numerous uses of cow urine for various human ailments like, cancer, osteoarthritis, allergies, kidney failures, skin diseases, healing of wounds, *etc.* Cow urine is said to be a very effective insect repellent when mixed with certain herbs. Cattle husbandry was well developed during the Rigvedic period (1500–1000 BC) and the cow (*Kamadhenu*) was adored and considered the 'best wealth' of mankind. There are no studies conducted earlier on cow urine for antipyretic activity, hence it's difficult to interpret. The pharmacological studies and safety evaluation of HF cross bred cow urine and routine comparative study of urine from different breeds of cattle were carried out in the present investigation. Anti pyretic and analgesic activity of cow urine was assessed in male and female rats. There was significant decrease in experimentally induced pyrexia at different intervals in male and female rats and there was no significant difference found between control and urine treated groups. Analgesic activity and Reaction time in seconds in treatment groups of Male rats were almost similar at all intervals when compared to control group. Similarly, Analgesic activity of cow urine was carried out in female rats, cow urine did not show analgesic property, reaction times in treatment groups did not differ significantly when compared to control group. Therefore it may be concluded that the cow urine treated groups did not reveal any statistically significant pharmacological activities.

I-P-25

EPIDEMIOLOGICAL AND HAEMATOBIOCHEMICAL STUDIES ON STRONGYLES IN SHEEP (OVIS ARIES) IN RAJASTHAN

A.K. Rawat, Fakruddin, S. Rani and R.K. Joshi

The present investigation was carried out in 300 sheeps and slaughtered at local abattoirs in and around Bikaner on the basis of faecal examination. On over all prevalence of 89.66 percent (269/300) of mixed infection of Helminths and Eimeria species was found. The mixed helminthes infection mainly comprised of strongyles (Haemonchus). All 268 of 269 parasites infected sheep were having strongylus infection. The clinical signs in parasites affected sheep included poor growth pale visible mucous membrane particularly of conjunctiva and rough wool coat. The faeces consistency varied from soft semisolid to pellet form. Parasites infected sheep showed significant decrease in hemoglobin, pack cell volume, total erythrocytes and lymphocytes count and significant decrease in basophiles and nutrophiles in comparison to parasites free sheeps in biochemical estimation also the total protein, albumin and Albumin and globulin ratio were significantly low in parasites infected sheeps.

I-P-26

PATHOLOGICAL STUDIES ON ACUTE AND SUBACUTE DERMATITIS IN CATTLE SKIN IN RAJASTHAN.

S. Rani, U.K. Vyas, R.K. Joshi and A. K. Rawat

For the present investigation a total of 3380 cattle were examined of which 663 (19.61%) skin samples were collected, for further studies. These were grouped according to their gross appearance and were processed mechanically (acetone and benzene technique) and stain with Haematoxylin & Eosin staining. Acute and sub acute dermatitis were found in 2.55 % cases. Grossly they appeared as papule, pustule, alopecia and crust. Microscopically acute dermatitis showed ballooning degeneration intraepidermal vesicle, sub acute dermatitis showing epidermal hyperplasia spongiosis and ballooning degeneration and infiltration.

I-P-27

RADIOLOGICAL STUDY OF PREGNANCY DIAGNOSIS BY RADIOGRAPHY IN BITCHES

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Present study was conducted in 26 bitches. The animals were divided in 2 groups. The animals of group A comprised the clinical cases referred for radiography. Group B comprised the bitches presented for sterilization under ABC programme. In animals of group A, 83.3% animals were found pregnant whereas animals of group B, 70% animals were found pregnant. In animals of group A, 40% bitches had one and 3 foetuses, each, in the uterine horn, whereas in animals of group B, 50% bitches had 3 foetuses and 14.3% bitches had 2, 4 and 5 foetuses in uterus, respectively. The skeletal density of foetal mass was not seen up to 30 days gestation; however, a mild skeletal density was seen at 45 days, moderate at 53 days and appreciable at 60 days of gestation. All pregnancies at 53 days gestation showed a greater size of foetus with abdomen distension and moderate degree of skeletal density of foetal mass. The mammary engorgement was also visible. A well developed foetal skeleton showing complete vertebral column and appendages with greater distension of abdomen together with increased quantity of foetal fluid were seen at 60 days gestation.

I-P-28

A COMPARATIVE STUDY ON DIAGNOSIS OF SURGICAL DISORDERS USING IITV AND CONVENTIONAL RADIOGRAPHY IN CANINES

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The radiological diagnosis of diverse surgical affections was done in canine species. An analysis of cases recorded revealed that out of 17 cases the ratio of male and female was 65% and 35%, respectively. The diagnosis of soft tissue (7 cases) and hard tissue (10 cases) was done by the fluoroscopy (IITV) and plain radiography. The hard tissue affections pertained to musculo-skeletal system, i.e. fractures and dislocations. IITV provided an instant diagnosis in majority of musculo-skeletal affections and that helped deciding the treatment in routine clinical practice without loosing time. However, owing to the smaller area coverage of affected part and poor density and contrast, the IITV cannot be considered as a substitute to the radiography. Fluoroscopy does not allow complete thorax or abdomen or both in a single scan. Similarly it does not allow visualization of complete bone in a single scan. However, the demerits of fluoroscopy eventually are merits of radiography where entire bone or thorax or abdomen or a substantial part of thoraco-abdominal region can be viewed in a single radiograph. Radiography involves cost of x-ray film and processing solution and the procedure is time consuming. Contrarily, the fluoroscopy (IITV) offers advantage of being less time consuming but has an additional demerit of radiation exposure to the patient and radiologist. It is therefore concluded that fluoroscopy (IITV) should be used in those cases where considerations for taking a radiograph are minimum.

I-P-29

NODULAR DERMATITIS IN SHEEP- A CASE REPORT

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The sheep is an animal of great importance of livestock industries in our country. It also represents one of domesticated indigenous fauna of arid and semi- arid region. It converts food and roughage into good cash products like mutton, wool and hides and fertilize the land. Skin diseases are very common in sheep flock due to their gregarious nature, which predisposes them to readily infect one another, if one animal suffer from infectious disease. Skin diseases cause great economic losses to the leather and tannery industries due to much destruction of skin and hide. In the present investigation, a sheep aged about 2 years submitted with the complaint of skin disorders. Clinical examination revealed multiple, firm, brown papules or nodules with ill-defined borders and button sign, seen most usually on the extremities, head and neck region. Biopsy was collected for further histopathological examination. The tissue was collected in 10 per cent formal saline and processed mechanically for paraffin embadding by acetone and benzene technique. The section revealed a circumscribed nodule with necrosis was seen accompanied with abundance of epitheloid histiocytes. More mature lesion tended to show numerous giant cells and spindle shaped fibroblastic cells.

I-P-30

BIOCHEMICAL EVALUATION OF DIFFERENT PLANT PARTS OF *ZIZIPHUS NUMMULARIA*

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Many species of higher plants biosynthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as chemical feed stock or as raw material for various scientific, technological and commercial applications. *Z. nummularia* is an important source of fresh edible fruits in arid regions of India due to the high dry weight protein content and also important source of protein for animals. The main objective for this study was to evaluate biochemical composition of plant parts of *Z. nummularia*. Fresh plant parts (leaves shoot and fruits) of *Z. nummularia* were used for quantitative determination of biochemical compounds. A known aliquot of sample was taken in test tubes in triplicate and volume was made to 1 ml with distilled water. 1 ml water served as blank. 5 ml of ninhydrin was added to each test tube including blank .Then all test tubes were placed in boiling water bath for 10-15 minutes; with marbles on the top to prevent water loss by evaporation. The test tubes were removed and cooled immediately under the running water. The spectrophotometer was adjusted at the wavelength of 570 nm and optical density was taken against blank. Standard curve was prepared using glycine as standard. To a known aliquot of protein extract 5.0 ml of reagent C was added. After 10 minutes, 0.5 ml of Folin's phenol (E) was added. The contents were mixed well by shaking tubes gently and kept for 30 minutes. The O.D. of colour developed was recorded at 520 nm using spectrophotometer. Standard curve was prepared using BSA as standard. Sugar (, 1954) 0.1ml sample was taken in test tubes in triplicate. 0.9 ml distilled water was added in each test tube to make it 1.0 ml.1.0ml of distilled water served as blank. To each test tube including blank 4 ml of anthrone reagent was added. Then all test tubes were placed in boiling water bath for 10 minutes, with marbles on the top to prevent water loss by evaporation. The test tubes were removed and cooled immediately under the running water. The spectrophotometer was adjusted at the wavelength of 625nm and optical density was taken using blank. Standard curve was prepared using glucose as standard. 0.1 ml of ethanolic extract was taken in test tubes in triplicate. 0.4 ml distilled water was added in each test tube. 0.5ml water served as blank. To each test tube including blank 0.25 ml of Folin reagent was added .Then 1.25 ml Na_2CO_3 solution was added and all test tubes were placed for about 40 minutes. The spectrophotometer was adjusted at the wavelength of 725 nm and optical density was taken using blank. Standard curve was prepared using gallic acid as standard. The data showed that fruit pulp had maximum sugar content (130.18 mg/g f.wt.) while lowest sugar content was found in the shoot tissue (37.21 mg/g f.wt.). Pulp was also found to be the rich in total phenols (116.64 mg/g f.wt.). Pulp had good proportion of free amino acids (73.33 mg/g f.wt.), while protein content was found to be 9.26 mg/g f.wt. However shoot showed maximum value of protein (20.26 mg/g f.wt.), followed by leaf (14.97 mg/g f.wt.). Seeds showed appreciable amount of sugars (62.91 mg/g f.wt.) and (76.40 mg/g f.wt.) of total phenol. Leaf was also found to be good source of sugars (80.46 mg/g f.wt.) and free amino acids (103.55 mg/g f.wt.). Our results revealed *Z. nummularia* fruit pulp to be a good source of sugars. Sugars are the main components of many fruits which influence sensorial and nutritional properties of the fruit. They contribute to the nutritive properties of a fruit by providing energy. Biochemical evaluation of different plant parts revealed that fruit pulp is rich source of sugars and total phenols followed by free amino acid and protein. As pulp is good source of carbohydrates and antioxidant so should be used in food formulations. The seeds also contain appreciable amount of sugars and phenols so can be utilized. Leaves were found to contain sugars, free amino acids and protein and so could be used for animal feed formulations.

I-P-31

PREVALENCE OF GASTROINTESTINAL PARASITES IN HORSES OF ANIMAL FAIRS IN RAJSTHAN

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A total of 719 faecal samples were collected from the horses of five animal fairs during 2009-10. The faecal samples were examined by direct smear, flotation and sedimendation methods for detection of any of parasitic infection based on the presence of parasitic egg/oocyst. The overall prevalence of gastrointestinal parasites in horses from all the five fairs was 33.24%. *Habronema* (14.04) showed maximum prevalence followed by strongyle (10.01), *Parascaris equorum* (7.09), *Strongyloides westeri* (4.86), *Draschia* (0.27), *Eimeria leuckarti* (0.27), *Oxyuris equi* (0.13), *Anoplocephala* (0.13) and *Schistosoma* (0.13%). Gastrointestinal parasites showed maximum prevalence at Tilwara (46.66) fair. *Habronema* infection was most prevalent in Tilwara (26.66), Hanumangarh (25.00) and Merta City (17.75) fairs. Strongyle infection was predominated in Nagaur (12.41) and Tilwara (10.66) fairs. *Draschia* was recorded only in Hanumangarh (0.64) and Tilwara (0.66) fairs. *Schistosoma* and *Anoplocephala* were recorded only in Tilwara (0.66) fair. *Oxyuris equi* was recorded only in Pushkar (0.65) fair. *Eimeria leuckarti* was recorded only in Nagaur (1.30) fair. *Habronema* predominated followed by strongyle, *Parascaris equorum* and *Strogyloides westeri* infections in all the three age groups i.e. below 3 years, 3-5 years and above 5 years. *Eimeria leuckarti* was recorded in horse below 3 years and 3-5 years of age.

I-P-32

ANTIBIOGRAM OF *E.COLI* ISOLATES RECOVERED FROM PIGEONS

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A total of 60 fecal samples were collected aseptically from apparently healthy pigeons. The samples were processed for the isolation of *E.coli* by inoculating into nutrient broth for 6 hrs followed by plating onto MacConkey's and Eosin methylene blue agar. The isolates recovered were identified by morphological, cultural and biochemical characterization using standard procedures. A total of 44 isolates of *E.coli* were recovered. All the isolates were studied for their antibiotic sensitivity pattern employing disc diffusion technique using commercially available antibiotic discs. Highest sensitivity (92.00%) was shown towards amoxicillin followed by gentamycin (88.00%) and oxytetracycline (64.00%). The isolates were found to be resistant towards ciprofloxacin, norfloxacin, cephalexin and ceftriazone.

From the present investigation it is concluded that though *E.coli* is considered a normal constituent of pigeon feces, but their antibiotic sensitivity is required to be studied to know their resistance pattern towards different antibiotics if at all they come out to be pathogenic.

I-P-33

TREATMENT OF PASTEURELLA INFECTIONS IN MARWARI SHEEP OF CSWRI, BIKANER

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The pasteurella infections broadly called pasturelossis by various stains, types and Serological, cultural, immunogenic, hannnemennia strains in all domestic animals including sheep. (Shand, Rose, Madsey) who studied the organism. The study was conducted on minor outbreak of pasturelosis and minor outbreak mixed with viral infection in Marwri sheep maintained at CSWRI, ARC, farm unit of Network project of CSWRI, ARC, bikaner. Maintained over last 40 years. The Marwari sheep belongs to west Rajasthan and migrates during famine to adjacent places within Rajasthan and adjacent state like Gujarat, Madhya Prades. Haryana, Punjab, Himachal Pradesh and Uttranchal. The carrier of animal infects the other sheep breeds of India and other domestic animals of livestock species by way of air, water, soil born infection from urine and faeces. An efforts have been made to control the outbreak at farm by the treatment with Ciprofolxacin after antibiotic sensitivity antibiogram. The samples of infect animals were collected from suppurative pneumonia cases at the farm and diagnosed in collaboration of the Department of Bacteriology and Pathology of College of Veterinary and Animals Sciences of Rajasthan University of Veterinary and Animals Sciences. The morbidity and mortality was also checked and the situation of the situation of the outbreak fo save the sheep mutton to prevent its infection to human population giving a food security as a part of supplementing animals Protein hunger to humanity.

II-P-1

ELISPOT ASSAY

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The Enzyme-Linked Immunosorbent Spot (ELISpot) assay is a common method for monitoring immune responses in humans and animals. The ELISpot assay is a very sensitive immunoassay, allowing the detection of a secreted cytokine at the single cell level. With detection levels that can be as low as one cell in 100 000, ELISpot assay enumerates B cells secreting antigen-specific antibodies and especially identify and enumerate cytokine-producing cells. This also allows visualization of the secretory product of individual activated or responding cells. Each spot that develops in the assay represents a single reactive cell. Thus, the ELISpot assay provides both qualitative (type of immune protein) and quantitative (number of responding cells) information. ELISpot analyses are also less impaired by binding proteins and protease activity since the analyte is bound to the capture antibody immediately after secretion. Due to its high sensitivity, the ELISpot has proven particularly useful when studying small populations of active cells such as those regularly found in specific immune responses. ELISpot assay employs the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) technique. Either a monoclonal or polyclonal antibody specific for the chosen analyte is pre-coated onto a PVDF (polyvinylidene difluoride) -backed microplate. The plate is blocked, usually with a serum protein that is non-reactive with any of the antibodies in the assay. Appropriately stimulated cells pipetted into the wells and the microplate is placed into a humidified 37°C, CO2 incubator for a specified period of time. During this incubation period, the immobilized antibody, in the immediate vicinity of the secreting cells, binds secreted analyte. After washing away any cells and unbound substances, a biotinylated monoclonal or polyclonal antibody specific for the chosen analyte is added to the wells. Following a wash to remove any unbound biotinylated antibody, alkaline-phosphatase conjugated to streptavidin is added. Unbound enzyme is subsequently removed by washing and a substrate solution 5-bromo-4-chloro-3-indolyl phosphate (BCIP) plus Nitroblue tetrazolium (NBT) is added. A blue-black coloured precipitate forms and appears as spots at the sites of cytokine localization, with each individual spot representing an individual analyte-secreting cell. The coloured end product (spots) can be counted with an automated ELISpot reader system to capture the microwell images and to analyze spot number and size or manually using a stereomicroscope. The ELISpot identify specific T lymphocytes with characterization of their polarisation. The ELISpot assay enables analysis of activated or responding cells at the single cell level. By virtue of exquisite sensitivity of the ELISpot assay, analysis of rare cell populations (e.g., antigen-specific responses) which were not possible by bulk assay methods is now possible. Limits of detection are below 1/100,000 rendering the assay uniquely useful for monitoring antigen-specific responses. It is particular advantageous in detecting functional T lymphocyte and even may be used without the knowledge of MHC-peptide complex. It is highly sensitive & automation is possible. It is applicable to a wide range of areas of immunology research, including cancer, transplantation, infectious disease, and vaccine development.

II-P-2

SEROEPIDEMIOLOGY AND CHARACTERIZATION OF PESTE DES PETITS RUMINANTS VIRUS (PPRV) IN ORGANIZED LIVESTOCK FARMS OF GUJARAT STATE

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Peste des petits ruminants (PPR), an acute viral disease of goats and sheep, is known to be present in a broad belt of sub-Saharan Africa, Arabia, the Middle East and Southern Asia. In India, PPR was first reported in 1987 from Tamil Nadu. Now, the disease is thought to be endemic in India including many reports of PPR in Gujarat, reported by various workers. During present study in which serological survey of PPRV antibodies was carried out by c-ELISA in different species of Livestock (sheep, goat, cattle, buffaloes and camels) in North Gujarat, Kachchh, Central Gujarat Saurashtra and South Gujarat regions. Out of 756 serum samples, PPRV antibodies could be detected in 218 samples (28.83 %). In sheep & goat, regionwise seroprevalence recorded was 56.07 & 76.04 per cent in Kachchh with highest in Chokhla breed (64.51 %) & Mehsani breed (28.22 %). Agewise maximum seroprevalence recorded was 53.42 & 39.13 per cent in the age groups of > 3 years of age of sheep & goat respectively.. In large ruminants out of 260 sera screened from cattle, buffaloes and camels, 34 were found positive yielding over all seroprevalence of 13.07 per cent. A total of 460 sheep and goats were tested for the detection of PPRV antigen by s-ELISA. Overall incidence rate of 24.78 per cent was observed with 21.50 % & 56.67% positivity in ante mortem & post mortem samples. Among post mortem tissues lymphnodes yielded highest positivity (100 %). In N, F gene based RT-PCR & nested PCR, out of 40 samples, 14 samples (41.17 %), 10 samples (29.41 %), 10 samples were found to be positive for PPRV respectively.The genetic characterization of PPRV by RT-PCR/RFLP revealed similar RFLP patterns with reference vaccine strain (Sungari/1996) suggesting no genetic variation among field virus from Gujarat state and Vaccine virus.

II-P-3

COMPARITION OF IMMUNE RESPONSE TO recombinant-BCG AND recombinant-DNA TB VACCINE BY GAMMA INTERFERON ASSAY

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Mycobacterium tuberculosis is the most common cause of human tuberculosis, but an unknown proportion of cases are due to *Mycobacterium bovis*, the agent of TB in cattle and *M.africanum*, has been prevalent since ancient times. Of the total Asian cattle and buffalo populations, 94% of the cattle and more than 99% of the buffalo populations in Asia are either only partly controlled for bovine TB or not controlled at all. Therefore, the global incidence of TB is greatly underestimated. In 2005, 3.3 million cases were reported to the Global tuberculosis Programme of WHO (2005), whereas a more likely number is 8.8 million. A vaccine against TB was developed by Albert Calmette and Camille Gu´erin in 1921. To date, Three billion people have been vaccinated with BCG worldwide but efficacy is very variable, ranging from 80% to 0%. BCG vaccination in cattle induced some reduction in the severity of disease against experimental challenge, although it appeared to be ineffective in field trials as a vaccine against natural infection. The recombinant vaccine used in the present study was developed in the department of Microbiology and cell biology, Indian Institute of Science, Bangalore consisting of a gene fragment designated as Rv3881c from *M. tuberculosis* coding for a 49 KDa protein.The recombinant-BCG and recombinant-DNA vaccine against bovine tuberculosis was evaluated in calves by conducting experiment in three groups of 8 calves each, aged approximately 4 months. They were injected intramuscularly with 1000ìg of recombinant-DNA expressing Rv3881c protein, subcutaneously 10^6 CFU of recombinant-BCG expressing Rv3881c protein and a control group received PBS via the same route. The efficacy of the vaccine was tested in a group of calves received from herds with known negative tuberculosis status. Booster vaccinations have been given after 30 days of initial injection. The cell mediated (Th-1) immune status was monitored using the IFN-ã assay and was able to distinguish between infected and healthy calves from an early stage. The immune status scores of individual animals were generally much higher in the recombinant DNA vaccine than control. The differences between recombinant-DNA to other groups were significant and it is concluded that under the prevailing conditions the recombinant-DNA vaccine was able to show higher IFN-ã response in calves against the B*ovine tuberculosis.*

II-P-4

CYTOKINE IL-4 RESPONSE IN CALVES TO Rv3881c PROTEIN EXPRESSING RECOMBINANT-DNA AND RECOMBINANT-BCG VACCINE AGAINST BOVINE TUBERCULOSIS

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The global incidence of TB is greatly underestimated. In 2005, 3.3 million cases were reported to the Global tuberculosis Programme of WHO (2005), whereas a more likely number is 8.8 million. The infectious agents like *Mycobacterium tuberculosis and Mycobacterium bovis* prevalent since ancient times cause a Tuberculosis in animals as well as in Humans, one of the most widespread infectious diseases and is the leading cause of death among adults in the world. Although humoral antibodies are not considered of important in protecting the host from virulent tubercle bacilli. The balanced IFN-ã to IL-4 ratio correlated to protective immunity due to vaccination. T-helper type 2 cells (TH2) produce interleukin-4 (IL-4), necessary to regulate antibody production while T-helper type 1 cells (TH1) produce IFN-ã and IL-2 and are associated with cellular resistance. BCG vaccination in cattle induced some reduction in the severity of disease against experimental challenge, although it appeared to be ineffective in field trials as a vaccine against natural infection. Cell-mediated immunity is essential for the control of mycobacterial infections. However, trials have not been done to evaluate the Th-2 responses. In the present study, the recombinant vaccine used in the present study was developed in the department of Microbiology and cell biology, Indian Institute of Science, Bangalore consisting of a gene fragment designated as Rv3881c from *M. tuberculosis* coding for a 49 KDa protein. The evaluation of recombinant-BCG and recombinant-DNA vaccine against bovine tuberculosis in calves done in three groups of 8 calves of 4 months old each. which were injected intramuscularly with 1000ìg of recombinant-DNA expressing Rv3881c protein, subcutaneously 10^6 CFU of recombinant-BCG expressing Rv3881c protein and a control group received PBS via the same route. The efficacy of the vaccine was tested in a group of calves received from herds with known negative tuberculosis status. Primary and Booster vaccinations were administered 21 days apart to 8 calves each, while another 8 were left unvaccinated as control animals. Humoral (Th-2) immune response was evaluated by Cytokine IL-4 capture ELISA. The immune status scores of individual animals were generally lower in all groups than what was expected. The statistical differences between all groups were non-significant and it can be concluded that under the prevailing conditions the recombinant-BCG and recombinant-DNA vaccine was unable to show TH-2 responses in calves.

II-P-5

EVALUATION OF TH-1CELL IMMUNE RESPONSE IN CALVES TO RECOMBINANT-BCG VACCINES by INTERLEUKIN-4 CAPTURE ELISA

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Mycobacterium tuberculosis and Mycobacterium bovis, a infectious agent prevalent since ancient times cause a bacterial disease Tuberculosis, one of the most widespread infectious diseases and is the leading cause of death among adults in the world. BCG vaccination in cattle although it appeared to be ineffective in field trials as a vaccine against natural infection but induced some reduction in the severity of disease against experimental challenge. Cell-mediated immunity is essential for the control of mycobacterial infections. BCG vaccines induce strong CD4 and CD8 mediated immune responses in mice, but the two compartments may differ in protection conferred by the vaccine against TB. The recombinant-BCG vaccine evaluated in calves by conducting experiment in three groups of 8 calves each, aged approximately 4-6 months were selected from herds with known negative tuberculosis status. They were injected subcutaneously with 10^6 CFU of recombinant-BCG vaccine bacilli expressing Rv3881c protein. A control group received PBS via the same route and Control group received 10^6 CFU of recombinant-BCG bacilli used as vector for rDNA vaccine. Primary and Booster vaccinations were administered 21 days apart to 8 calves each, while another 8 were left unvaccinated as Control animals. Humoral (Th-2) immune response was evaluated by Cytokine IL-4 capture ELISA. The immune status scores of individual animals were generally lower in all groups than what was expected. The statistical differences between groups were non-significant and it can be concluded that under the prevailing conditions the recombinant-DNA vaccine was unable to show protective immune TH-2 responses in calves.

II-P-6

EVALUATION CMI RESPONSE TO recombinant BCG and CONVENTIONAL BCG VACCINE BY BOVIGAM ASSAY

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Tuberculosis, is still a major public health disease of economic importance in many countries of the world, although, emerged about 35,000 years ago. No effective vaccine is not currently available for bovines, the development of cattle vaccine would be one of the best options for long-term control of Tuberculosis. In the present experiment, the efficacy of the vaccine was tested in a group of Calves received from herds with known negative tuberculosis status. The recombinant-BCG vaccine evaluated in calves by conducting experiment in three groups of 8 calves each, aged approximately 3-6 months. They were injected Subcutaneously with 10^6 CFU of conventional BCG and recombinant-BCG expressing Rv3881c protein and a control or unvaccinated group received PBS via the same route. Booster vaccinations have been given after 30 days of initial injection. The cell mediated (Th-1) immune status was monitored using the IFN-ã assay and was able to distinguish between infected and non-infected animals from an early stage. The immune status scores of individual animals were generally much higher in the recombinant BCG vaccine than conventional BCG and control Model which is statistically significant on 30th day ($P<0.01$) and continued to increase up to maximum level till 90th day. The maximum level of IFN-ã was maintained till 120th days after vaccination. It can be concluded that under the prevailing conditions the recombinant-BCG vaccine was able to show higher IFN-ã response in calves against the *Bovine tuberculosis.*

II-P-7

STUDY OF VACCINAL IMMUNITY AND SEROEPIZOOTIOLOGICAL SURVEY OF BRUCELLOSIS IN BOVINES IN NORTH GUJARAT.

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Brucellosis has economic importance as it cause abortions with loss of successive calf crops, varying periods of interrupted milk production, infertility, and sustained economic loss. It is a infectious disease, clinically characterized by abortion in late pregnancy and a subsequently high rate of infertility is caused by *Brucella abortus*. There is growing concern that the disease may further flare up due to intensive dairy development programmes, especially among the farming community and professionally associated personnel. Maintenance of such unproductive milch animals, especially buffaloes known for their low and delayed pregnancies is a potential loss to dairy farmer. Over and above, it is an important zoonotic disease causing undulant fever in human and it is transmitted by drinking infected milk. By using standard RBPT, STAT and i-ELISA test, a seroepizootiological survey of brucellosis was carried out. The influence on immune response with levamisole and zinc on vaccinal immunity to *B. abortus* S-19 vaccine was evaluated by standard agglutination test and cell mediated antibody response by contact sensitivity to DNCB. 1365 serum samples collected from villages and from two organized farms comprising 520 cattle and 845 buffaloes from field randomly. The overall seroprevalence rate of *Brucella abotus* antibody by i-ELISA was 12.53 in field and 5.57 in farm animals (14.96%-Mehsana, 13.00%-Patan, 12.65%-Sabarkantha and 9.22%-Banaskantha district). Significant increase ($P < 0.05$) in skin fold thickness at 24h was observed in levamisole treated calves but was non significant in zinc supplemented group. Haematological change in vaccinated and immune modulated cattle and buffaloes calves were also significant ($P<0.05$) in levamisole treated calves but non significant in zinc supplemented group.

II-P-8

SEROEPIDEMIOLOGY OF PPR IN SHEEP AND GOATS OF NORTH GUJARAT

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Seroprevalence of PPR was studied by competitive-ELISA in sheep and goats of North Gujarat. An overall seroprevalence of 46.26 per cent was observed in the Sheep and Goat population of the North Gujarat showing highest seroprevalence (58.66%) in goat population of Patan district and the lowest (12.75%) in Goat population of Sabarkantha districts. Sheep showed higher rate of seroprevalence (49.29%) compared to Goats (45.08 %). Seroprevalence was comparatively lower in organized sheep farm. Considering nasal swab as standard material, investigation of PPRV incidence was carried out from a total of 219 animals by s-ELISA. An overall incidence of 33.33% including 19.04% in Sheep and 39.10% in Goats was observed. Anal swabs and Whole blood samples were tested parallel to nasal swab. Nasal swab was found to be choice of antemortem clinical material for PPRV antigen detection by s-ELISA. Similarly, 19 goats and 15 sheep were tested for distribution of PPRV antigen in tissue samples. Incidence rate of PPRV were observed as 29.41%, 26.47%, 32.35% and 8.82% in lymph node, lung, spleen and intestine, respectively.

II-P-9

SEROPREVALENCE OF ANTIBODIES TO INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN BREEDING BULLS

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Infectious bovine rhinotracheitis / Infectitious pustular vulvo-veginitis (IBR/IPV) is caused by bovine herpesvirus - 1 (BHV–1) a disease of domestic and wild cattle. Disease is noticed worldwide including India, cardinally disease is indicated by fever, dyspnoea, nasal and ocular discharges and loss of body condition. BHV- 1 can in addition affect number of systems other than the upper respiratory tract resulting in abortions, encephalitis, conjunctivitis and genital infections (Anon, 1986). BHV-1infected bulls may potentially shed virus intermittently in their semen, for long periods after the primary infection. Therefore, cattle infected with BHV-1 are regarded as lifelong carriers and potential shedders of the virus. Several serological methods have been used to detect antibodies to BHV–1 for diagnosis and/or for epidemiological surveys. These include virus neutralization tests, (Gibbs and Rweyemamu, 1977) immunofluorescence (Wellmans and Leunen, 1973) passive haemagglutination test (PHT) (Kirby *et. al.,* 1974) and enzymelinked immunosorbent assay (ELISA) (Kramps *et. al.,* 1993). The present study was undertaken with an objective to study the seroprevalence of BHV-1 virus in breeding bulls. A total of 549 sera samples were collected from five different frozen semen stations of Karnataka state and screened for serological evidence of BHV-1, commercially available indirect ELISA kit (Checkit Trachitest serum screening® Idexx laboratories) was employed in the study. The test protocol was adopted as per the manufacturer's instructions. In the present study out of 549 samples tested 165 (30.05 %) serum samples shown presence of antibodies for BHV-1 virus and 384 (69.94 %) samples did not show seropositivity against BHV-1.The average percentage positives among five farms was 20% (33 Nos), buffaloe bulls have shown low seropositivity 21.21 % (35 Nos) when compared to cattle bulls 64 % (106 Nos) where as female animals have shown a lowest seropositivity of 14.54 % in the current study. Among five farms the highest seroprevalence was observed with 59.09% (65 positives out of 110 serum samples) and the lowest seroprevalence was recorded with 7.97% (11 positives out of 138 serum samples). This study correlates with the findings of Kiran *et al.* (2005) who noticed on the basis of sero-epidemiological study that 44% of cattle serum samples in India were positive for IBR antibodies by AB-ELISA. Deka *et al.* (2005) revealed 45.01% prevalence of BHV-1 antibodies in cattle bulls of Punjab by A-B ELISA whereas in this study it was found to be 30 %.While Khan (2004) reported 21.30% seropositivity of IBR by using i-ELISA in cattle and buffaloes of Gujarat, more or less similar results were obtained in cattle and buffaloes using ELISA by various workers.The higher seropositivity of 42%, 60.16%, 49.47%, 51.9%, 89%, 66.54%, 50.79% and 50% were recorded using ELISA, , by Lyaku *et al.* (1991), Suresh (1992), Pandita and Srivatava (1993 and 1995), Shome *et al.* (1997), Motha and Hansen (1998), Sontakke *et al.* (2002) and Sharma *et al.* (2006), respectively. While Rajesh *et al.* (2003), Jai *et al.* (2005) and Jain *et al.* (2006) observed a lower seropositivity of 14.88%, 17.68% and 10.39%, respectively, employing ELISA.The results confirm that the positive animals were exposed to the BHV-1 infection at some point of life. High density of animals rearing in restricted premises together and sharing common feed and water, as well as movement of animals and farm labours from a healthy herd to infected herd might be the major determinant of the BHV-1 seropositivity in these breeding bulls, as the virus is easily transmitted through aerosol route. There is a need for detailed study on the shedding of virus in semen ejaculates of these positive bulls. Presence of sero reactors along with non reactors and pattern of shedding of virus,whether to use the semen of a seropositive bulls needs to be answered for providing cleaner and safer frozen semen for breeding purpose.

II-P-10

SERO-PREVALENCE STUDIES ON BRUCELLOSIS IN ANIMALS AND ANIMAL HANDLERS IN AND AROUND BIKANER CITY

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Brucellosis is a serious zoonosis with serious economic implication. The disease is encountered world wide and in all climates. In India, the disease is reported to be endemic in live stock. Virtually every type of domestic animal and many wild ones are affected and mainly affect reproductive organs in animals. The men get infection through the consumption of raw milk or milk products from contaminated milk. Besides, this man also gets infected through secretions/excretions of infected animals and handling of aborted materials, animal tissue and meat etc. So, the present study is an attempt to know the status of disease in livestock and animal handlers. A total of 90 sera samples (collected randomly) comprising of 35 cattle and 35 goats and 20 samples from human handlers of livestock were screened for Brucella agglutinin using Rose Bengal plate agglutinin test (IVRI). The result indicated that 11.42% (4/35) cattle, 5.71% goat (2/35) reacted positively. None of the sera samples of animal handler reacted positively. Hence, it is important not to overlook brucellosis as it may result in abortion in animals and may act as reservoir for other animals and man involved in their handling.

II-P-11

PARATUBERCULOSIS-JOHNE'S DISEASE IN GOATS- A PATHOLOGICAL STUDY IN WESTERN RAJASTHAN.

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The present study was carried out on 1186 samples of intestine collected from goats of different age groups, breeds and either sex. Out of these 337 samples showing gross lesions were subjected to histopathology. These were processed (acetone benzene technique) and then stained with H and E stain. Para tuberculosis was found in 3.85% cases.Grossly, small intestine showed transverse corrugations folds of mucosa with velvety surface. Microscopically, section of ileum showed heavy cellular infiltration in mucosa and sub-mucosa with atrophy of intestinal glands.

II-P-12

PATHOLOGICAL AND HAEMATOBIOCHEMICAL STUDIES OF E.COLI IN LUNG LESION IN POULTRY

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For the present investigation a total of 2056 lung and air sac samples of died /sacrificed poultry of either sex and different age group were collected out of which 389 lung and 91 air sac samples showed lesions .they were collected for histo-pathological examination and wherever possible for microbiological examinations and haematobiochemical estimation. Bacterial isolation studies were conducted in 202 samples of lungs (156) and air sacs (46) which revealed presence of E.coli (37.1%).E.coli was found to be predominantly isolated organism from the cases of respiratory affections either as pure or mixed cultures._ Antimicrobial drug sensitivity test performed in vitro on 20 E.coli isolated exhibited that all (100%) of these isolates were sensitive to gentamicin and resistant to tetracycline. Haematobiochemical estimation of respiratory ailment affected bird due to E.coli showed significantly lower TEC, PCV, haemoglobin, mcv and lymphocytes whereas significantly >($P<0.01$)TLC, heterophils, monocytes, eosinophils were noticed as compared to normal birds.Grossly, E.coli affected lung showed congestion, edema and cheesy material over lung and nodules. Microscopically, intense infiltration of heterophils and bacterial clumps were noticed in few cases.

II-P-13

PERICARDITIS: A PATHOLOGICAL STUDY IN POULTRY.

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The present investigation was conducted on heart samples from ten adult birds submitted for postmortem examination. These samples were processed (acetone and benzene technique) and then stained with haematoxylene and eosin stain. Two cases showed pericardium involvement. Grossly, pericardium showed opaque, lusterless and variably thickened appearance. One showed granular appearance or variable sized nodular lumps. Microscopically, pericarditis mostly involved the visceral surface. The contents in the air sacs were not significant but were cloudy clotting on exposure to air.

II-P-14

A COMPAR ISION OF DIFFERENT SEOLOGICAL TESTS FOR DIAGNOSIS OF BRUCELLOSIS IN BUFFALOES

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Serological methods for diagnosis of brucellosis in animals are traditionally being used but no serological test and antigen combination fully resolved the diagnosis of brucellosis. Thus, the objective of our investigation was to study the comparative performance of different serological tests in buffaloes. Samples were selected from buffalo herds having history of abortion in late gestation in buffaloes. As buffaloes are valuable milch animals and disease like brucellosis can cause severe economic loss to farmer, the present study was conducted on buffaloes to find an appropriate test for proper and timely diagnosis of diseased animals. A serological study was conducted using 403 buffalo serum samples which were subjected to various serological tests i. e. RBPT, STAT, AGPT, 2MET, HIT and ELISA. Out of 403 samples, overall present seroprevalence was detected as 37.84%, 34.09%, 29.59%, 26.15%, 25.23%and 13.99% by ELISA, RBPT, STAT, HIT, 2MET and AGID respectively. So ELISA was detected as most accurate test for screening of buffalo herds for brucellosis.

II-P-15

DIFFERENTIATION OF VACCINATED FROM THE INFECTED ANIMALS (DIVA) IN FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease virus (FMDV) of genus *Aphthovirus* in the *Picornaviridae* family is a highly infectious and contagious vesicular disease affecting cloven-hoofed animals. FMD virus (FMDV) includes seven serotypes, O, A, Asia1, C, and SAT1, -2, and -3. FMD is endemic in many parts of Asia, Africa, and South America. Due to endemicity of the disease, FMD control programme (FMD-CP) has been launched in selected states including Haryana to create FMD free zones. The major constrain to attain the FMD free status is the carrier animals. A carrier of FMDV is defined as an animal from which live-virus can be recovered from scrapings of the oropharynx after 28 days following infection (Sutmoller and Gaggero, 1965). To differentiate the infected from vaccinated animals (DIVA) i.e. detection of carrier animals, NSP (Non-Structural Protein) serology using recombinant non-structural protein-3A expressed in Baculovirus was employed. In the present study, a total of 2000 serum samples were used in 3A-NSP ELISA for carrier/persistent infection of the FMDV. On analyzing it was observed that the anti-3A NSP antibodies in FMD-CP and districts under ASCAD (Assistance to States for Control of Animal Diseases) were found in 13.5 and 12.7 per cent of sera samples tested, respectively. Also anti-3A NSP antibodies positive cattle were significantly higher than buffaloes ($p<0.001$) in both the FMD-CP and ASCAD districts. Further isotyping of 3A-NSP specific antibodies to know which isotype of immunoglobulin IgG predominated during the FMDV infection revealed that both isotypes i.e. IgG1 and IgG2 were prevalent in both the FMD-CP and ASCAD districts.

II-P-16

SEROPREVALENCE OF BRUCELLOSIS AND PESTE DES PETTIS RUMINANTS IN CAMEL IN BIKANER

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The present epidemiological investigation was undertaken to determine the prevalence of brucellosis and PPR in camel in and around Bikaner. In the investigation 94 camels were screened for demonstration of antibodies against brucella and PPR infections. All the serum samples were seronegative for brucellosis tested by RBPT and STAT. For demonstrating PPR antibodies, competitive ELISA was used which revealed an overall 4.25 per cent sero-prevalence of the disease. Female camels revealed more sero-prevalence of PPR (10%) than the males (3.5%). During the same years lot of PPR outbreaks were recorded in the sheep and goat populations in the areas under study.

II-P-17

DETECTION OF VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS FROM A FIELD OUTBREAK IN CENTRAL INDIA

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Infectious bursal disease (IBD) is an acute, highly contagious disease of young birds, caused by IBD virus (IBDV) which infects B-lymphocytes of bursa of Fabricus, resulting in immune depression and an increase in the incidence of vaccine failures and secondary infections. In present study Bursal tissue was collected from commercial broiler flocks exhibiting clinical signs suggestive of infectious bursal disease (IBD). The presence of IBD virus (IBDV) was confirmed by partial amplification of the VP2 gene by reverse transcription and polymerase chain reaction. Isolates were identified as very virulent strains of IBDV (vvIBDV) by nucleotide sequence analysis. The comparison of the VP2 nucleotide sequences among the isolates revealed the presence of single-nucleotide polymorphisms in VP2 gene of IBDV in the same flock. The comparative analysis indicated that these viruses were genetically close to the vvIBDVs previously detected in India. Our analyses provided information about the existence of vvIBDV in central India.

II-P-18

ASSESSMENT OF FAILURE OF PASSIVE TRANSFER IN CALVES BY RADIAL IMMUNODIFFUSION TEST

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In bovines, the transplacental passage of immunoglobulin (Ig) is totally prevented and hence the Ig gained through the colostrum plays an important role in protecting the calf during its neonatal life. Failure of passive transfer (FPT) of this immunity makes the new born calves vulnerable to various disease conditions which may even result in mortality. The serum IgG level of the new born calf can be used as an indicator to check the status of passive transfer of Ig. A study was conducted to find out whether there was failure of passive transfer in neonatal calves reared at Instructional Farm of College of Veterinary and Animal Sciences, Pookot. Blood was collected from eight colostrum fed neonatal calves, 24 to 48 hours after birth, and the level of serum immunoglobulin G (IgG) was assessed by using the radial immunodiffusion test standardized using commercially available purified bovine IgG. An IgG level of less than 1000 mg/dl was considered suggestive of FTP. A range of 1000 to 1499 mg/dl was considered as adequate transfer and 1500 mg/dl and more was considered as excellent. Of the eight samples tested, two (25 per cent) of the calves had failure of passive transfer (serum IgG less than 1000mg/dl).There are three major reasons for FPT: (i) The cow may produce insufficient or poor quality colostrum (production failure); (ii) sufficient colostrum is produced but there is inadequate intake by the new born (ingestion failure); (iii) failure of absorption from gut despite of adequate of colostrum intake (absorption failure). Hence a good management system should aim to diagnose FPT sufficiently early and take corrective measures to minimize losses due to neonatal calf mortality.

II-P-19

SEROLOGICAL AND ENTEMOLOGICAL SURVEY OF BLUETONGUE VIRUS INFECTION IN SHEEP IN GUJARAT

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The present study envisaged the sero-epidemiology of BTV in sheep using BTV c-ELISA. A total 980 sera were screened and of these, 361(36.84%) were found positive for BTV antibodies. Regionwise,location wise and breed wise seroprevalance was also studied. Culicoides were collected by employing miniature insect light trap at various places. *C.oxytoma* was found to be potential vector of BTV in Gujarat.Hence, seroprevalance and entomological studies revealed that the BTV is prevalent in Gujarat.

II-P-20

PATHOLOGICAL AND HAEMATO- BIOCHEMICAL OBSERVATIONS OF ULCERATIVE DERMATITIS IN CAMEL (*CAMELUS DROMEDARIUS*)

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The camel has been a necessity of mankind in the desert and semi-desert areas since its domestication, about 2500 to 3000 years ago. The skin infections in camel are caused by bacteria, viruses, parasites and fungi. Among bacterial infections, staphylococcal dermatitis, contagious skin necrosis or skin wound and abscesses have been reported to occur the most. Ulcerative dermatitis is one of the important type of dermatitis found in skin of camel and in some instances, it cause a nuisance and cause debilitation in the animal. In the present study, a total of one hundred eighty seven skin biopsies showing frank macroscopic lesions were collected from camel. Histopathological aspects of these skin problems were studied alogwith haemato- biochemical parameters and isolation of organisms. These samples were processed mechanically for paraffin embedding by acetone and benzene technique. The ulcerative dermatitis was found in nine cases. Haemato- biochemical observations revealed significant increase in TEC, haemoglobin, MCHC, TLC, neutrophils, eosinophils, serum glucose, total protein, serum albumin and serum globulin. A decrease in MCV and lymphocyte count were also observed, however the PCV, basophil and monocyte count among haematological parameters and A:G ratio among biochemical parameters were not affected. Histopathological observations revealed grossly, the lesion represents a typical, solitary and situated at the neck and shoulder area. Microscopically, there is focal dermoepidermal separation, epidermal hyperplasia and parakeratosis were common feature of the ulcer edge. Layers of inflammatory exudate between collagen bundles and subjacent superficial perivascular lymphocytic infiltration in the dermis was frequently noticed.

II-P-21

THERAPEUTIC MANAGEMENT OF PNEUMONIA IN SHEEP-A CLINICAL APPROACH

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CSWRI AVIKANAGAR

Fifteen adult animals showing clinical signs of respiratory distress, nasal discharge (watery to mucus), depression, inappetance to anorexia, occasional high rise in temperature and lacrimation, rough and dull hair coat divided in to three equal groups were successfully treated with three different combinations of treatment (TI, TII, TIII). Nasal swabs recorded presence of E.Coli predominately .The superiority of T III regimen in terms of early easiness and restoration of clinical signs was discernible

III-P-1

MITOCHONDRIA PLAY ROLE IN CPV-2 INDUCED APOPTOSIS IN MDCK CELLS

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Canine parvovirus type 2 (CPV-2) an important pathogen of dogs causes acute enteritis, myocarditis and lymphopenia in particularly young dogs. The CPV-2 has been grouped along with B19, feline panleukopenia virus (FPLV), mink enteritis virus in the genus *Parvovirus* of family *Parvoviridae*. CPV-2 causes an acute lytic infection leading to cell death. Recent studies have shown that various species of parvovirus including CPV-2 can induce apoptosis *in vitro* in different cell types such as haematopoietic cells, lymphocytes, glioblastoma cells etc. The present study was undertaken to elucidate the molecular mechanism involved in CPV 2 induced apoptosis in cultured MDCK cells. For this, MDCK cells grown to around 30-40% confluency were infected with 0.1 m.o.i. of CPV-2. The infected MDCK cells were harvested at 24 h, 48 h and 72 h post infection along with mock controls. These cells were analysed for decrease in mitochondrial membrane potential (ÄØ) potential, a hallmarks of apoptosis, by flow cytometry after JC-1 dye staining. Our results indicated that there was significant decrease in the mitochondrial membrane potential, which had begun at 24 h p.i. which increased significantly with the time and was maximum at 72 h p.i. To further substantiate it, the efflux of cytochrome c was determined by western blot analysis which also showed that efflux was higher both 48 and 72 h p.i. in CPV-2 infected MDCK cells as compared to mock controls. Subsequently, activation of caspase 9, an initiator caspase of mitochondria mediated pathway, was also determined by western blot. Further, activation of effector caspase3 was also determined. The results indicated involvement of the activation of caspase 3 in CPV 2 infected MDCK. Thus, present study could demonstrate that CPV 2 induced apoptosis is mediated through intrinsic (mitochondria mediated) pathway. Further, work is in progress to delineate the role of various proteins and associated factors and pathways involved in CPV 2 induced apoptosis.

III-P-2

DETECTION OF ANTIBODY AGAINST STRUCTURAL AND NON STRUCTURAL PROTEIN OF FMD VIRUS IN DIFFERENT GROUPS OF YAK *(Poephagus grunneins)* AND MITHUN*(Bos frontalis)*

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The present study was undertaken with the objectives to detect the presence of antibody against structural proteins (SP) of different serotypes of FMDV in different groups of animals. Also to monitor the presence of antibody against NS proteins of FMDV in different groups of animals, to compare the occurrence of antibody against NS proteins as well as against different serotypes of FMDV in different groups of animals considering the significance of presence of antibody against NS proteins. A total 166 samples, 150samples from yak and 16 samples from mithun were collected from different part of the Arunachal Pradesh for the present study. The samples were subjected to both Liquid phase blocking ELISA (LPBE) and recombinant 3AB3 nonstructural protein based indirect ELISA (DIVA test). The tests were carried out in different groups of yak (2-6 days post infection-vaccinated, 40-45 days of post infection-vaccinated and random) and mithun (random and unvaccinated infected). The samples were tested for presence of antibody against SPs of different serotypes of FMD virus as well as antibody against NSP of FMDV. Further, sandwich ELISA was taken up for confirmation by seroptyping of clinical samples collected at the phase of FMD outbreaks in Yak and mithun. Comparison on the basis of all the tests results and the available history was taken up and accordingly interpreted. Out of 150 serum samples from yak, 122 (81.33%) could show LPBE titre of e"1.8 log$_{10}$ against different serotypes of FMDV. Among the serum samples exhibited LPBE titre e"1.8 log$_{10}$, 99 (81.15%) samples were found to be positive for NSP antibody of FMDV, while 23(18.85%) were negative. Likewise in case of mithun out of 16 serum samples only one was showing e"1.8 log$_{10}$ titre which also gave DIVA positive results and rest 15 were <1.8 log$_{10}$ titre and were DIVA negative. The outbreaks were attributed to the serotype O of FMDV in yak as well as in mithun, which were confirmed by sandwich ELISA. Indirect ELISA for DIVA revealed a high percentage of positive reactors for NSP antibody and it is indicative of circulation of FMD virus among the yak population and presence of large number of carrier animals. LPBE and ELISA for NSP can be applied for retrospective diagnosis of FMD outbreak from where no tissue samples could be collected, no antigen could be detected or no virus isolated in suspected clinical sample due to low antigenic content, delay in sample collection, mild nature of the infection, deterioration during transport. A direct correlation could be observed between the identification of FMDV serotype associated during the outbreaks and monitoring of antibody titre against different serotypes as well as the detection of antibodies against NSP of FMDV in infected or non-infected animals, irrespective of vaccination. For epidemiological study of FMD, combinations of the three tests were found to be useful.

III-P-3

IMMUNE RESPONSE TO HN PROTEIN OF NEWCASTLE DISEASE VIRUS IN DMBA INDUCED TUMOR MODEL OF RATS

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HN is a multifunctional protein of Newcastle disease virus (NDV) which is actively involved in virus infectivity. The protein is also reported to induce apoptosis in many cell lines *in vitro*. The HN gene encodes for proteins of 571, 577, 581 and 616 amino acids. The largest of all HN616 is synthesized as biologically inactive precursor and converted to biologically active form (HN) following the proteolytic cleavage of 45 residues from C-terminus. It is reported that HN gene of NDV is a potent inducer of interferons and capable of upregulating the TNF-related apoptosis inducing ligand (TRAIL). HN protein also activates human monocytes that kill various human cancer cell lines through the TRAIL mediated tumoricidal activity. In the present study, the HN gene of NDV was cloned with Kozak sequence for better expression in eukaryotic expression vector pcDNA3.1 (+). The recombinant clone containing HN gene (pcDNA.nd.hn-kozak) was confirmed by restriction enzyme digestion and PCR. The *in vitro* expression analysis was done by transfecting CEF cells with recombinant pcDNA.nd.hn-kozak and total RNA was extracted 72 h p.t. For the assessment of immunogenic response of HN protein, skin tumor were induced (Papillioma and squamous cell carcinoma) in 12 male Wistar rats (age 6-7 week) by applying 1% DMBA (7, 12-dimethyl benz(a) anthracene, M.W-256.3, sigma USA) in acetone solution topically on shaved skin of hind back region weekly. In 3 to 5 months all the rats developed benign tumor (Papillioma like growth) and squamous cell carcinoma. Many of the rats showed multiple growth that coalesced, forming a single large growth which continued to grow. The rats were grouped in two groups- I and II having 6 rats in each group. The recombinant pcDNA.nd.hn-kozak was treated intratumorally @100μg/rat in group I and pcDNA 3.1(+)

@ 100µg/rat in group II (vector control). Two rats from each group were sacrificed humanely at 3, 7 and 14th day post treatment. Spleen and peripheral blood was collected from these rats for the assessment of proliferation of CD4+, CD8+ and NK cells by flow cytometery (B.D. Bioscience).The result showed a significant increase in CD4+ and NK cells population on 3rd day p.t. in treated group than vector control but the difference in population of CD8+ T cells was relatively stangnant. On 14th day the CD4+, CD8+ T cells and NK cells populations increased significantly in treated group than vector control. Our results showed that recombinant pcDNA.nd.hn-kozak effectively induced innate and adaptive immune response in *invivo* tumor model. Further studies are being designed to assess the expression pattern of different cytokines in *invivo* tumor model after treatment with recombinant pcDNA.nd.hn-kozak vaccine.

III-P-4

STUDY OF APOPTOTIC POTENTIAL OF HN PROTEIN OF NEWCASTLE DISEASE VIRUS IN CULTURED HELA CELLS

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Newcastle disease virus is one of the most important avian pathogen which causes a highly contagious and fatal disease in birds of various species. The virus has been classified as a member of the newly defined genus *Avulavirus* in the family Paramyxoviridae. NDV has a single stranded negative sense RNA genome. The genomic RNA contains six genes encoding at least eight proteins. Among these, the hemagglutinin-neuraminidase (HN) and fusion protein (F) constitute the external envelope proteins. . HN is such a multifunctional protein which is also actively involved in virus infectivity. It has been reported that NDV has inherent oncolytic potential that can be harnessed for treatment of various types of cancer. Virotherapy is a new strategy to treat cancer by selectively infecting and killing tumor cells by using viruses. It has been reported that HN protein is responsible for induction of apoptosis in many mammalian cell lines *invitro by* up-regulating the expression of IFN-á and TRAIL in the target cells. This study was undertaken to determine the apoptotic potential of the HN gene in cultured human cervical cancer cell line (HeLa cell). The HN gene of NDV (with Kozak sequence) was amplified by PCR and cloned in eukaryotic expression vector pcDNA 3.1(+). The recombinant clone containing HN gene (pcDNA.nd.hn-kozak) was confirmed by restriction enzyme digestion and PCR. The in vitro expression analysis of recombinant pcDNA.nd.hn-kozak was done by transfecting CEF cells and isolation of total mRNA followed by RT-PCR. To study the apoptotic potential of HN protein, HeLa cells grown to 60-70% confluency were transfected with recombinant pcDNA.nd.hn-kozak and cells were harvested at 24, 48 and 72 hours post transfection. Genomic DNA of cells was extracted by QIAGEN blood and tissue extraction kit to detect inter nucleosomal fragmentation by ladder assay. A typical ladder pattern was observed on agarose gel electrophoresis. The transfected cells were also checked for other apoptotic changes by flow-cytometry such as PARP (Poly-(ADP-ribose) polymerase) cleavage activity, propidium iodide staining, TUNEL assay and by Annexin V binding assay. Results of these assays clearly showed that the recombinant pcDNA.nd.hn-kozak causes apoptosis of HeLa cells. Further studies are being carried out to elucidate the molecular mechanisms and pathways involved in HN protein induced apoptosis.

III-P-5

EFFECT OF TREADMILL EXERCISE ON PLASMA ENZYMES LEVEL IN GERMAN SHEPHERD DOGS

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The present paper highlighted the influence of treadmill exercise on plasma enzymes level with different stages of exercise in both male and female of German shepherd dog. Twelve dogs of either sex were included in present study and subjected to treadmill exercise at the speed of 9 Km/hour. The blood samples were collected at control or per-exercise condition and at 20 minutes, 40 minutes and 60 minutes during the course of exercise. The mean values of plasma enzymes (AST, ALT, ALP, CK and LDH) showed significantly (P £ 0.01) increasing pattern with advancement of exercise, with the highest concentrations at 60 minutes of exercise and lowest concentrations at control or pre-exercise conditions in present investigation. The concentrations of AST increased 38.19%, 72.59% and 109.04%; ALT increased 66.97%, 118.79% and 176.97%; ALP increased 8.98%, 19.70% and 31.67%; CK increased 90.38%, 158.80% and 221.96% and the concentrations of LDH increased 30.47%, 64.22% and 103.59%, during the course of study at 20 minutes, 40 minutes and 60 minutes of exercise, respectively. According to sex, significantly (P £ 0.01) higher creatine kinase activity was measured in male animals than that of female animals, while other enzymes revealed non significant (P > 0.05) sex related variations.

III-P-6

SERUM PROTEIN PROFILES OF CATTLE AND BUFFALOES VACCINATED AGAINST HAEMORRHAGIC SEPTICAEMIA

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The aim of this study was to investigate serum protein levels and profiles in pre- and post vaccinated cattle and buffaloes of 3-4 months age vaccinated with alum precipitated vaccine against haemorrhagic septicemia. The levels and profiles were determined at monthly intervals up to 4 months post-vaccination. The mean values of total protein (g/dl) in cattle at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 6.7±0.55, 6.78±0.39, 6.92±0.67, 6.893±0.31 and 7.01 ± 0.61, respectively and those in buffaloes were 6.27±0.58, 6.36±0.36, 6.13±0.66, 6.41±0.27 and 6.59±0.44, respectively. The mean values of albumin concentrations (g/dl) in cattle at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 2.62±0.28, 2.63±0.38, 2.88±0.53, 2.87±0.29 and 3±0.51, respectively. Similarly in buffaloes, mean albumin values (g/dl) at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 2.52±0.34, 2.45±0.23, 2.27±0.33, 2.7±0.21 and 2.88±0.34. The mean values of globulin concentrations (g/dl) in cattle at 0 day, 30 days, 60 days, 90 days and 120 days post vaccination were 3.75±0.35, 3.85±0.33, 3.96±0.41, 3.71±0.23 and 3.77±0.315, respectively. Similarly in buffaloes, mean globulin values (g/dl) were 3.75±0.35, 3.85±0.33, 3.96±0.41, 3.71±0.23 and 3.77±0.315, respectively. Total protein, albumin and globulin levels did not show significant variation between prevaccinated and post vaccinated cattle and buffaloes at different time intervals (30 day, 60 day, 90 days and 120 days). The electrophoretic profile of serum proteins by SDS-PAGE, revealed more than 11 protein bands of sizes >116.00, 70.99, 64.65, 57.24, 41.24, 37.92, 28.63, 25.35, 24.24, 11.84 and 10.71 KDa, respectively. The same protein bands were observed in the profiles at various intervals. There was no variation in electrophoretic protein profile in prevaccinated and post vaccinated cattle and buffaloes at different time intervals (30 day, 60 day, 90 days and 120 days). So the serum protein profile did not clearly indicate any qualitative and quantitative variation due to the vaccine induced immune responses.

III-P-7

Poster Num
2

STUDIES ON THE PROTECTIVE EFFICACY OF OUTER MEMBRANE PROTEINS OF *PASTEURELLA MULTOCIDA* B: 2 IN RABBITS

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The study was carried out to elucidate the protective efficacy of outer membrane proteins extracted from *Pasteurella multocida* B:2 by different procedures. The OMP's were extracted by sodium lauryl sarcosinate with ultracentrifuge and without ultracentrifuge and by lithium chloride-lithium acetate (LCA) procedure. Outer membrane protein profile of *Pasteurella multocida* revealed the presence of several protein bands in the range of 16 to 100 kDa. Some higher molecular weight protein bands were missing when protein profile by LCA method was observed and some additional higher protein bands were observed in sonicated antigen treated with SLS. A total of 30 rabbits were divided into 5 groups of 6 rabbits each. The rabbits in each of group I, II and III were given OMP's extracted by above procedures respectively @ 200mg after mixing with equal volume of indirect Freund's adjuvant. One of the group (IV) were injected whole cell formalin killed suspension. A group of 6 rabbits was kept as unvaccinated control. A booster dose of respective vaccine was administered after 4 weeks. All the animals were challenged 4 wk. after booster vaccination. The sera samples were collected at weekly interval and antibody titres measured using IHA, ELISA & MAT. The highest ELISA ($\log_{10}$) titres were observed in group I (conventional procedure) and similar was the result found in MAT. The highest IHA ($\log_{10}$) titres were observed in group III (sonicated antigen treated with SLS.)& group IV (Formalin filled whole cell). However, there was no significant variation in titre among different groups as revealed by statistical analysis using analysis of variance (5%). The protection against homologous challenge with *Pasteurella multocida* (P_{52}) was observed in mice passively immunized with pooled rabbits sera. The increase in protection in mice, in relation to corresponding increase in antibody titre at day 7 and 14, 21, 28 & 56 day post vaccination indicated a direct relationship between antibody titres and protection efficacy. The rabbits in group I (Conventional method) & in group IV (formalin killed whole cell) showed 100% survival except one morality each in group II (LCA) and group III (SS).

III-P-8

EFFECT OF GLUCOSE ON ASCORBIC ACID IN *ZIZIPHUS NUMMULARIA* TISSUE CULTURES

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Ascorbic acid is essential for the normal function of living cells and many enzymatic reactions in humans and is obtained from dietary sources (such as vegetables and fruits) and synthetic vitamin C. Its deficiency typically causes abnormalities in bones, teeth and scurvy. Apart from its role in nutrition, ascorbic acid acts as an antioxidant to protect the natural flavour and colour of many foods. Many of desert plant fruits appear to be food sources of ascorbic acid. *Z. nummularia* is among widely consumed vegetables in arid zone of Rajasthan and to our knowledge there has been no systematic study on the antioxidant constituents of ber, the aim of the present study was to quantify the amount of ascorbic acid content in callus cultures of *Z. nummularia*. Fresh young leaves collected and sterilized for 2-3 min in 0.1% $HgCl_2$ solution, rinsed with sterile distilled water. Sterilized leaves were taken as explants and cultured on Murashige and Skoog's (1962) medium supplemented with 2mg/l 2,4-D, 2mg/l IAA and 2mg/l Kn. The callus tissues were maintained for 8 months by frequent subculturing at 26 ± 2°C, 55 % relative humidity under light condition (3000 Lux). All the media used throughout this study were supplemented with 3% sucrose and 0.8% agar. The pH was adjusted to 5.80 ± 0.02 with 1N NaOH or 0.1 N HCl before autoclaving at 121°C and 15 lb psi for 20 min. Further, glucose (0.5%, 1%, 1.5% and 2%) was singly supplemented in to medium and the callus tissues were harvested regularly at their transfer ages of 2, 4, 6 and 8 weeks and growth indices (GI) were calculated in each case on wet weight basis.The cultures were weighed, crushed in ice cold CO_2 saturated water and the extract was made to a definite volume. 3 ml of extract was mixed with an equal volume of buffered metaphosphoric acid pH 3.6. 2 ml aliquot of this solution was mixed with 5ml distilled water and the turbidity produced was adjusted to zero with UV-VLS spectrophotometer-119. Another 2 ml aliquot was then mixed with 5ml of 2,6 dichlorophenol indophenol, prepared by dissolving 5 mg in 100 ml of distilled water at 80°C and the optical density measured by UV-VLS spectrophotometer-119 set at 546 nm against blank (Chinoy 1962). Five replicates have been taken for each plant part. The explants first showed swellings at the cut ends within 7-8 days of inoculation which bursted out to release the undifferentiated callus mass after 12-15 day. Maximum growth indices (15.73) were observed in 8 weeks old tissues of 0.5% glucose concentration where as it was minimum (0.30) in 2 weeks old cultures supplemented with 1% glucose. Growth indices up to weeks old culture up to 3-4 times where as it was slightly decrease in 8 weeks old cultures except 0.5% fed concentration. The growth indices and ascorbic acid contents in tissues were determined at time interval of two, four, six and eight weeks. Maximum amount of ascorbic acid was observed in 4 weeks old cultures (67.41 mg/gfw) fed with 1.5% glucose. Where it was minimum (1.48 mg/gfw) in 2 weeks old cultures of 1% glucose fed concentration. Four weeks old tissue cultures showed an increase in the amount of ascorbic acid content as well as growth indices in all the sample tested. The data also reveals that the incorporation of the glucose into the medium might augment the synthesis of ascorbic acid in *Z. nummularia* callus tissues and supports the previous findings that glucose acts as one of the precursors of ascorbic acid (Loewus and Kelly 1961). The objectives of this study were to determine the variability in ascorbic acid content of *Z. nummularia* and the biosynthetic potentialities of the callus tissues to produce ascorbic acid and promote the application of plant tissue culture technology in the area of metabolites.

III-P-9

SDS-PAGE OF GRANULOSA CELL PROTEINS OF BUFFALO AT DIFFERENT STAGES OF ESTRUS CYCLE

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The present study was conducted to ascertain the comparative protein profile of granulosa cells at different stages of estrus cycle of buffaloes. The ovaries of buffaloes were obtained from local abattoir and ovarian stages were determined on the basis of corpus luteum morphology. The follicular fluid was aspirated from the follicles by syringe and the cells were separated by centrifugation at low temperature (4°C) in PBS (0.1M pH 7.4). Supernatant discarded and the pellets of granulosa cells were suspended in 1ml of PBS and washed by repeated centrifugation. The granulose cells were lysed first with repeated freezing and thawing and then by sonication. The proteins of isolated granulosa cells of stage III and IV of ovary were analyzed by SDS – PAGE. The proteins of granulose cells of ovarian stage III revealed eleven bands of molecular weight (202.31, 153.63, 125.86, 94.49, 68.55, 48.25, 41.44, 24.36, 17.15, 14.45 and 6.45 kDa) and ovarian stage IV revealed protein bands of molecular weight (183.99, 145.12, 112.31, 96.41, 58.90, 50.68, 50.60, 41.45, 33.01 and 17.16 kDa) on the silver stained gels. The band of molecular weight 24.36kDa, 4.45 kDa and 6.45 kDa were detected only in stage III and were absent in stage IV.

III-P-10

HEMATO-BIOCHEMICAL INDICES OF ZANSAKARI PONIES AT BIKANER

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Zanskari ponies belong to Zanskar region in Kargil district of Ladakh region of Jammu and Kashmir State of India. These ponies are bred in the high altitude cold desert. National Research Centre on Equines procurred these ponies from their hometract for *ex-situ* conservation at Bikaner. The present study was planned to understand the thermoregulatory processes involved in adaptation of the horses from high altitude to desert plains. Pulse rate (PR), respiration rate (RR) and rectal temperature (RT) were recorded during winter (November & December), summer (May & June) and rainy (July & August) seasons. Blood and serum was also collected during this period for hematology and biochemical indices, respectively. No effect of season was observed on PR and RT while RR was significantly low during winter. Most of the haematological parameters were comparatively low during winter season. Mean total protein (TP), albumin, globulin, cholesterol, glucose, triglyceride, phosphorus, bilirubin (total) and bilirubin (direct) were 6.39 ± 0.22, 3.45 ± 0.76, 2.93 ± 0.17, 83.89 ± 3.64, 105.10 ± 9.09, 27.87 ± 1.6, 4.27 ± 0.14, 2.05 ± 0.09, 0.99 ± 0.04 (g/dl), respectively. Mean GGT, LDH and CK were 10.47 ± 0.37, 1433.23 ± 57.74 and 157.52 ± 10.62 IU/1, respectively. Overall mean irrespective of sex of Hb (g/dl), PCV (%), TLC (cumm^{-1}), TEC (million/ cumm), MCV (fl), MCH (pg) and MCHC (%) were 11.77±0.11, 37.54±0.47, 8782±233, 8.23±0.05, 49.12±1.13, 14.98±0.02 and 31.63±0.54, respectively. Hb was observed low in the ponies and probable reason may be the low nutritional status of these ponies in the field. Haematological indices reported during this study were comparable with the earlier reports in literature. The hemato-biochemical estimates may be used as base line data for Zanskari ponies. All the physiological, hematological and biochemical indices were within normal physiological range indicating the adaptation of Zanskari ponies at Bikaner.

III-P-11

MOLECULAR CHARACTERIZATION OF TOLL-LIKE RECEPTOR 9 IN MARWARI HORSE, POITU AND INDIGENOUS DONKEY

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Toll-like receptor 9 (TLR9) has been characterized as a receptor that can recognize unmethylated CpG motif within bacterial DNA and signalling by this receptor triggers pro-inflammatory cytokine response that influences both innate and adaptive immune responses. Although TLR9 is extensively studied in human, mice, swine, canine, bovine, ovine and feline species, there is no report on characterization of TLR9 of Indian breeds of horses, donkeys and Poitus. Expression of TLR9 gene was studied in PBMCs of Marwari horses, Poitu and indigenous donkeys. Partial TLR9 gene amplicons from mRNA were cloned into pGEMT vector. Sequences were submitted to GenBank database. Deduced amino acid sequences of TLR9 proteins were aligned and analysed using MEGA 4. Estimates of evolutionary divergence between the sequences of TLR9 revealed that *Equus caballus* (Marwari breed) and Equus asinus differ from human by 19% and 16%, respectively. Phylogenetic tree was constructed by the Neighbour-Joining method. The TLR9 proteins from the present study clustered with *Equus caballus* protein sequences, while human, cattle, dog, sheep, mice, and buffalo formed separate clades. The analysis shows conserved sequences and close association of TLR9 proteins within species and high divergence between species of animals. An understanding of sequence variability of TLR9 amongst different species of equines would help to better understand the biological responses to CpG DNA. The information can be used to devise new vaccine adjuvants and enhance the immune responses.

IV-P-1

PCR-RFLP BASED MEAT SPECIATION

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Mitochondrial DNA sequence is highly conserved in different species of animals and it has enabled designing of universal primers for mitochondrial genes, which can amplify corresponding fragments in wide variety of species. Using universal primers for PCR amplification obviates the requirement for an internal control, which is otherwise used to monitor the success of DNA amplification. As vertebrates contain about 1,000-10,000 copies of mitochondrial DNA per cell, PCR assays based on its amplification are more sensitive in comparison to single or low copy nuclear DNA targets. Since quantity of PCR product relates to the copy number of target DNA sequence when a very small amount of DNA is used as a template, high copy number of mitochondrial DNA ensures sufficiently high quantity of PCR product. The nucleotide variability has been reported in different mitochondrial genes between the species. This nucleotide sequence variability creates or abolishes the restriction sites in a given region between species. Therefore, amplifying a common region of the mitochondrial genes using universal primers in different species and its subsequent different restriction enzyme profile in these species provides an easy, fast and effective method of species identification. The universal primers (Forward : 5'- CGC CTG TTT ACC AAA ACA T -3' and (Reverse : 5'- CCG GTC TGA ACT CAG ATC ACG T -3') were designed for amplification of ~ 590 bp partial mt 16S rRNA. These primers were synthesized commercially and diluted to the final concentration of 10 pmole/ ml. The genomic DNA may be isolated from raw/cooked/ processed meat sample using any commercial kit. The extracted DNA was quantified by using spectrophotometer and the OD at 260 nm wavelength was recorded. The concentration of DNA samples was calculated by the following formula: OD at 260 nm x dilution ratio x 50. The genomic DNA was diluted to the final concentration of 25-50 ng/ ml. The PCR was set up in 25 µl reaction volume. The reaction mixture comprised of 2.5 µl of 10 X Assay buffer (100 mM Tris-HCl, pH 9.0, 15 mM $MgC1_2$, 500mM KCl and 0.1% gelatin), 0.20 mM of dNTPs mix, 10 pmole each of forward as well as reverse primer, 1U Taq DNA polymerase, 50 ng of purified DNA and autoclaved milliQ water to make up the volume. The PCR tube containing the reaction mixture was to be spun on a microcentrifuge and was placed in a Thermocycler. The cycling conditions of an initial denaturation at 94 °C for 5 min followed by 30 cycles of 45 sec denaturation at 94 °C, 45 sec annealing at 60 °C and 1 min elongation at 72°C were used. After the reaction, small aliquot of amplified product may be resolved on 1.4 % agarose gel. PCR amplified product was subjected with each of the restriction enzymes identified for each species, separately, following the instruction of the restriction enzyme manufacturer. The digested products were run on 3 % agarose gel along with the suitable size marker. The source of meat was identified on the basis of the restriction enzyme profile. The PCR-RFLP based assay will be used in meat speciation in case of adulteration and in forensic cases.

IV-P-2

COA GENE CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CATTLE AND GOATS WITH CLINICAL MASTITIS

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Staphylococcus aureus has been identified to one of the most important pathogens in causation of different types of mastitis in various species of animals and man and is a significantly economical disease in dairy animals. In the present investigation *S. aureus* isolates from milk of cattle and goats with clinical mastitis were subjected to phenotypic and genotypic identification. All the isolated produced coagulase in tube test and a 1250 bp species-specific amplicon by PCR using specific primers. There was limited strain variations in the isolates of region under study, isolates producing *coa* gene amplicons of three different sizes (600, 680 or 850 bp), one specific to each isolate. No difference in the RFLP patterns of *coa* amplicons with *Alu*I endonuclease was obtained in the isolates from cattle and goats. All patterns were common to both cattle and goat but predominance of *S. aureus* isolates with *coa* gene amplicon of 680 bp was observed in cattle and that with 600 bp *coa* gene amplicon in goats.

IV-P-3

PCR BASED SEX DIFFERENTIATION IN GUINEA FOWL

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In avian species, females are the heterogametic sex (ZW) while males are the homogametic sex (ZZ), hence, the W chromosome specific sequences/ genes may provide an effective way for sex differentiation using PCR-based methods. The XhoI and EcoRI family repetitive sequences on the chicken W chromosome are useful targets of PCR-based sex identification in different avian species. A unique sequence, EE0.6 (0.6 kb EcoRI fragment) is found on the long arm of the chicken W chromosome and has been utilized for the purpose of sex identification. This sequence is widely conserved on the W chromosome in avian species, however, EE0.6 sequences have their counterpart sequences on the Z chromosome in some species. Major problem faced with W chromosome specific sequence-based PCR methods is to differentiate between the no amplification in males with PCR failure. Hence to amplify a common band in both the sexes and female-specific band only in female simultaneously is the best method to overcome this problem. A set of primers i.e. USP1 and USP3 specific to W chromosome 0.6 kb EcoRI fragment were used. These primers were expected to amplify a 370 bp band in female only. The primers USP1 and USP2, specific to partial 16S rRNA gene were used as control and were expected to amplify a ~ 590 bp fragment in both the sexes. The genomic DNA may be isolated from any tissue like blood/ feather-follicle using any commercial kit. The extracted DNA was quantified and diluted to the final concentration of 25-50 ng/ ml. The PCR reactions were set up in 25 µl reaction volume containing 2.5 µl of 10 X Assay buffer (100 mM Tris-HCl, pH 9.0, 500mM KCl and 0.1% gelatin), 1.5 mM MgCl$_2$, 200 mM of dNTPs (dATP, dGTP, dCTP and dTTP), 0.4 mM each of primers (DS1 and DS23) and 10 pmole of 16S rRNA specific primers, 1 U Taq DNA polymerase (Promega), 50 ng of genomic DNA and autoclaved milliQ water to make up the volume. The amplification was carried out using the amplification programme: initial denaturation at 94° C for 4 min, followed by 35 cycles of PCR, each cycle consisting of 80 s at 95° C, 90 s at 56 °C and 60 s at 72 °C; and followed by a final extension step of 5 min at 72 °C. After the completion of PCR reaction, the tubes were spun briefly, 5 ml of gel loading dye was added to stop the reaction and the amplified product was resolved in 3 % agarose gel for 45-60 min at 7-10 V/cm. The gel was stained with ethidium bromide and the resolved gel was visualized under U-V light. In guinea fowl, females showed 2 bands i.e. 370 bp female specific and 590 bp common band, while males showed only 590 bp band. This method will be used in sex differentiation in guinea fowl at day-old stage, as sexual dimorphism is very poorly defined in this species.

IV-P-4

RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF PROLACTIN GENE IN MARATHWADI BUFFALOES

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Blood samples were collected aseptically from fifty Marathwadi buffaloes belonging to Parbhani Jalna and Beed districts of Marathwada region (Maharashtra state). From these samples DNA was extracted by using standard methods. The quality and quantity of DNA was checked with agarose gel electrophoresis and Biophotometer, respectively. The DNA samples along with bovine prolactin gene specific primers were amplified by polymerase chain reaction. The PCR amplification was confirmed by running the PCR product on agarose gel electrophoresis.Amplified PCR products of prolactin gene (exon III) were digested with restriction enzyme Rsa I for 14-16 hrs at 37°c. Digested products were electrophoresed on the agarose gel. Digestion with Rsa I has revealed intact product of 156 bp prolactin gene. Further it is observed the monomorphic nature of prolactin gene (exon III) locus showing AA genotype in the population of Marathwadi Buffaloes under the study. It indicated that there was no restriction site for Rsa I in amplified Prolactin gene (exon III) of Marathwadi Buffaloes.

IV-P-5

MOLECULAR CHARACTERIZATION OF TH2 CYTOKINE GENE (IL-10) OF CAMEL (*Camelus dromedarius*)

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The present study was carried out to understand determinant of cell mediated immunity of *Camelus dromedarius i.e.* Interleukin-10 produced by Th2 cells mainly B cells, T cells, macrophages, Th2 subset of CD4+ cells. For this investigation peripheral blood mononuclear cells were separated from *Camelus dromedarius* using Histopaque®-1077 and cultured in RPMI 1640 medium. PBMCs were stimulated with *Concavalin-A* to maximize the expression of genes. Total RNA was separated from stimulated cells using TRIZOL reagent. cDNA was synthesized from total RNA using reverse transcriptase (Superscript III). Using base sequence of *Camelus bactrianus,* primers were designed for specific amplification of interleukin-10 gene of *Camelus dromedarius.* The IL-10 gene was successfully amplified from cDNA using Taq polymerase and was identified on the basis of its size homology with that of *Camelus bactrianus* in agarose gel electrophoresis i.e. 600 bp. The IL-10 gene of *Camelus dromedarius* was cloned in pGEM-T easy vector at its 3' T overhangs and recombinant plasmid was named as pGEM-TILT and transformed in *Escherichia coli* DH5á strains. The cells containing recombinant plasmid could be identified on the basis of blue/white colony selection on LB agar containing X-Gal and ampicillin. The interleukin-10 gene was isolated from recombinant plasmids on ligation with restriction enzyme EcoRI and identified on the basis of its size. Using the ideal Camel model, further study of IL-10 characterization and function may provide insight on the understanding of the cell mediated immune system.

IV-P-6

AMPLIFICATION AND CLONING OF SCHLAFEN-LIKE PROTEIN GENE OF INDIAN CAMELPOXVIRUS (CMLV) ISOLATES

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Camelpox is a wide-spread infectious viral disease of camelids. Camelpox is caused by *Orthopoxvirus cameli* virus which belongs to genus *Orthopoxvirus* within family *Poxviridae.* Based on sequence analysis, it has been determined that the camelpox virus is the most closely related to variola virus, the aetiological agent for small pox. In January 2007, a sporadic outbreak of camelpox occurred in camel herds of Rajasthan. Since then, the incidence of camelpox is continuing among the dromedarian camels. Sequence analysis of the most important viral genes will be useful for the molecular epidemiological studies. Camelpox virus (CMLV) gene *176R* encodes a protein Schlafen-like protein (v-slfn). v-slfn is a predominantly cytoplasmic 57 kDa protein that is expressed throughout infection. v-slfn did not prevent establishment of infection or virus replication, but probably accelerate virus clearance by the immune system and this play a role in the modulation of the innate and adaptive immune responses against pathogens. The exact role of v-slfn within the context of a viral infection remains to be elucidated. Keeping this in view, the v-slfn gene of the camelpoxvirus isolates was amplified from the infected scab materials by PCR and subsequently the amplicon was cloned into P^{GEM-T} vector for sequence analysis.

IV-P-7

PCR AMPLIFICATION OF PROTEASE GENE OF *TRYPANOSOMA EVANSI* ISOLATED FROM CAMEL

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Proteases play vital role in number of physiological process like virulence factor of trypanosome infections. Thus,these endogenous enzyme may be targeted for developing anti trypanosomal drugs. In the present study, the DNA was isolated from the lysates of *Trypanosoma evansi* from infected camels by Proteinase K digestion coupled phenol-chloroform extraction. The PCR reaction for the successful amplification of one of the proteases gene, Cysteine protease (Cp) of *T. evansi* was optimized with $MgCl_2$ concentration needed for the Taq DNA polymerase activity and the annealing temperature for the proper primer binding by gradient PCR. The ideal $MgCl_2$ concentration and the annealing temperature were 2mM and 55 °C, respectively. An amplicon of size about 530bp specific to the Cp gene of *T. evansi* was successfully cloned into the plasmid vector for the subsequent expression and characterization of the recombinant proteins.

IV-P-8

AMPLIFICATION AND CLONING OF THE TOPOISOMERASE GENE OF PSEUDOCOWPOXVIRUS ISOLATES FROM INDIAN DROMEDARIAN CAMELS

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Camel Contagious Ecthyma (CCE) is characterized by localized lesions, mainly around the mouth, nares and other sites may also become affected with high morbidity but without mortality. The etiological agent of CCE in Indian dromedaries is found to be pseudocowpoxvirus (PCPV).In the year 2010, incidences of contagious ecthyma in dromedarian camels of less than one year of age were observed in two districts of Rajasthan viz., Udaipur and Bikaner. Viral DNA was extracted from the skin lesions of dromedarian Camels(*Camelus dromedarius*) presenting with Contagious ecthyma .and amplified using the sequences of the topoisomerase gene. DNA fragments of expected size (around 1000 bp) observed on agarose gel electrophoresis were subsequently purified and cloned into pGEM-T vector for sequence analysis.

IV-P-9

POLYMORPHISM ANALYSIS OF MHC CLASS II GENES IN MARWARI HORSES

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Major histocompatibility complex (MHC) is one of the few identified gene systems in domestic animals, which is associated with immune response, resistance or susceptibility to infectious diseases and is termed as equine leukocyte antigen (ELA) in horses. Using standard method of Phenol-Chloroform extraction, sixty six DNA were isolated from the blood samples of Marwari horses belonging to NRCE farm and Bikaner area of Rajasthan. The quality of all the samples of DNA was checked in agarose gel and at 260 & 280 nm wavelength using UV spectrophotometer. The PCR amplification of DRA and DQA genes was carried out using published primers and fragments of 229 bp and 246 bp were successfully amplified. The PCR- RFLP analysis using different restriction enzymes in case of DRA and DQA loci was carried out. Digestion of DRA fragment (229 bp) with *Nsi* I, *Hind* III, *Msp* I, *Pvu* II, *Eco* RI enzymes resolved no RE site. Whereas, digestion of above fragment with *Hae* III enzyme resolved two bands of 165 bp & 64 bp and with *Hinf* I enzyme resolved 200 bp & 29 bp. Digestion of MHC-DQA fragment was carried out with *Msp* I, *Nsi* I, *Pvu* II and *Hinf* I enzymes but no RE site was found. Sequencing of selected genotypes in respect of DQA, DRA, DRB3 and DRB2 loci was performed to ascertain polymorphism of said loci and the results obtained through PCR-RFLP using different restriction enzymes could be verified. Sequencing data were analyzed with clustal X and MEGA 4 soft wares which revealed that polymorphism existed in the Marwari horses on the basis of analysis of DQA, DRB3 and DRB2 genes. The same was confirmed by phylogenetic analysis also. However, no polymorphism was observed in case of MHC-DRA gene.

IV-P-10

TRANSCRIPTIONAL EXPRESSION AND PHYLOGENETIC ANALYSIS OF MX GENE SEQUENCE IN MARWARI HORSE (*EQUUS CABALLUS*)

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Mx protein confers resistance to *Orthomyxo* virus infection by modifying cellular functions needed for the viral replication. Differential antiviral activity has been observed in chicken due to amino acid substitution in Mx gene. Mx protein sequences have been well characterized in mice, human and chicken but still unravelled in equines. In the present study transcriptional expression of Mx gene was studied in interferon induced peripheral blood mononuclear cells (PBMC) of Marwari horse (*Equus Caballus*). The partial cDNA amplicon (660 bp) was sequenced and analysed. These nucleotides coded for 219 amino acids out of which 34 were negatively charged and 29 positively charged. Blast analysis revealed 99% sequence homology with *Equus Caballus* (ECU55216), 81% with *Homo sapiens* (AB527675) and *Pan troglodytes* (XM531569) and 80% with *Canis lupus familiaris* myxovirus (influenza virus) resistance genes. On phylogenetic analysis by neighbor joining method the sequence again formed separate clade with *Equus caballus* Mx protein homolog.

IV-P-11

GENETIC EVALUATION OF GROWTH OF MARWARI SHEEP

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The data on body weights of 3915 Marwari lambs from birth to yearling age spread over a period of 1996-2009 maintained at Central Sheep and Wool Research institute, Arid-Region Campus Bikaner were analyzed to assess the growth. The overall means of body weights at birth, 3, 6, 9 and 12 months of age were 3.08±0.02, 15.22±0.20, 21.13±0.30, and 25.49±0.33 and 28.02±0.38 kg, respectively. For gain in body weights were 12.13±0.19, 5.97±0.15, 4.37±0.18, 2.67±0.20 and 24.92±0.36 kg and for average daily gain in body weights were 134.80±2.14, 66.47±1.73, 48.96±2.02, 31.23±2.25 and 68.28±1.00 g/day, respectively during 0-3, 3-6, 6-9, 9-12 and 0-12 months of age. Effect of period of birth, sex and sire on body weights, weight gain and average daily gain were found to be highly significant in all the age groups. However, effect of sex on average daily gain was significant on 9-12 months of age. Effect of the parity was found to be highly significant on body weights at birth, 3 months, 9 months and 12 months of age and on average daily gain during 6-9 months of age whereas significant effect on body weight at 6 months of age, on weight gain and average daily gain during 0-12 months while on weight gain as non-significant during 0-3, 3-6, 6-9 and 9-12 months of age and on average daily gain during 0-3, 3-6 and 9-12 months of age. The interaction effect of sex with period on all age groups of body weights, weight gain and average daily gain was highly significant while non-significant on birth weight. The interaction effect of sex with parity was significant on birth weight and 12 months of body weight; during 0-12 months of age on weight gain and average daily gain while non-significant on 3, 6 and 9 months of body weights; during 0-3, 3-6, 6-9 and 9-12 months of age on weight gain and average daily gain.

IV-P-12

GENETIC PARAMETERS OF GROWTH TRAITS IN MARWARI SHEEP

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The data on body weights of 3915 Marwari lambs from birth to yearling age spread over a period of 1996-2009 maintained at Central Sheep and Wool Research institute, Arid-Region Campus Bikaner were analyzed to estimate the genetic parameters or improvement of the growth.. Heritability of birth, three, six, nine and twelve months body weight was 0.328±0.062, 0.735±0.086, 0.865±0.092, 0.825±0.090 and 0.792±0.089, respectively, indicating that the selection of the sires and dams on the basis of their own body weights at 3 months of age might prove quite effective for bringing about genetic improvement. The genetic correlation of birth weight with weights at subsequent ages and weight at 3 months with weights at subsequent ages ranged from 0.416 to 0.481 and 0.624 to 0.886, respectively and that of 6 months with weights at subsequent ages ranged between 0.650 to 0.889. The genetic correlation of weight at 9 months with 12 months weight was 0.831±0.031. The positive correlations indicate that lambs with heavier birth weight and weaning weight might be heavier at subsequent ages. The phenotypic correlations of birth weight with weights at subsequent ages and weight at 3 months with weights at subsequent ages ranged from 0.312 to 0.422 and 0.543 to 0.723, respectively and that of 6 months with weights at subsequent ages ranged from 0.639 to 0.781. The phenotypic correlation of weight at 9 months with 12 months weight was 0.774±0.026.

IV-P-13

EVALUATION OF REPRODUCTION IN MARWARI SHEEP

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The data on reproductive traits of 1277 Marwari ewes spread over a period of 1996-2009 maintained at Central Sheep and Wool Research institute, Arid-Region Campus Bikaner were analyzed to study the reproduction The overall least-squares mean for age at first service, weight of ewe at first service, age at first lambing, service period and lambing interval were observed to be 640.78±16.97 days, 28.18±0.26 kg, 794.15±21.84 days, 238.12±8.98 days and 384.21±10.10 days, respectively. The effect of sire and month on age at first service, weight of ewe at first service and age at first lambing was highly significant (Pd" 0.01) while non-significant on service period. The effect of sire on lambing interval was non-significant. The effect of month on lambing interval was significant (Pd"0.05). The effect of period on age at first service, weight of ewe at first service and lambing interval was highly significant (Pd"0.01) whereas significant (Pd"0.05) on service period and non-significant on age at first lambing.

IV-P-14

ASSOCIATION OF METEOROLOGICAL VARIABLES WITH HERD PRODUCTION TRAITS IN GIR PUREBRED AND CROSSBRED COWS

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The present study was undertaken to find association of meteorological variables with herd production traits in Gir purebred and crossbred cows. Data on herd production traits and meteorological variables maintained at Livestock Farm and Meteorological observatory, Rajasthan College of Agriculture, Udaipur during January 1995 to December 2004 were used for this study. Magnitude of correlation coefficient during early stage of lactation was higher than mid and late stage of lactation. All the correlation coefficients of meteorological variables included in the study and milk production in morning, evening and total milk yield of a day during early stage of lactation was negative and highly significant in both the purebred and crossbred cows. The correlation coefficients of morning milk yield (MMY), evening milk yield (EMY) and total milk yield (TMY) with maximum temperature (MAXT), Maximum Humidity (MAXH) and Evening Temperature Humidity Index (THIE) were negative and highly significant during mid lactation in Gir purebreds. The association of MMY, EMY and TMY with MAXT was positive and highly significant while with MAXH and Minimum Humidity (MINH) were negative and highly significant in the crossbred cows. During late stage of lactation, the coefficients of correlation of all the three components of milk yield with MAXT, minimum temperature (MINT) and THIE were positive and highly significant while with MAXH and MINH were negative and significant in Gir purebreds. On the other hand, all the correlation coefficients of MMY, EMY and TMY with MAXT, MINT, MAXH, MINH and THIE were negative and highly significant in crossbred cows during late stage of lactation.

V-P-1

ANTIGENECITY AND SAFETY EVALUATION OF *ESCHERICHIA COLI* WHOLE CELL AND PILI VACCINE FROM LOCAL STRAIN

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Escherichia coli is highly diverse bacteria as it contain many 'O' and 'H' antigens , therefore, it was found beneficial to produce vaccine from local strain . Serotype prevalence, Congo red dye test, Sereny test, serum resistance, enterotoxin and haemolysin were selected as main determinants to select an isolate for whole cell and fimbrial vaccine production, along with that MS-MR haemagglutination and antibiotic sensitivity were also taken into consideration. Isolate no 99, serotype O86 was selected upon these bases. This was though, Sereny test negative but CR test positive, serum resistant, enterotoxin and haemolysin producer, MR type of haemagglutinating for three species erythrocytes and resistance towards four antibiotics. Formalin killed and pili vaccines were prepared from the strain and compared for antigenecity and safety in mice . In experiment carried on mice somatic 'O' and pili titre was achieved at 64 and 1024 respectively for whole cell vaccine and 1024 for pili vaccine against bases titre of zero. In protection test, both vaccines provided protection to 5 mice out of 6 during homologous challenge, while whole cell vaccine protected 2 and fimbrial vaccine protected 3 animals upon challenge the animals with serotypes other than vaccine strain. In control unvaccinated group no animals could be protected upon both types of challenge hence both vaccines proved significantly superior to control group and pili was found equally superior to whole cell vaccine.

V-P-2

PCR BASED IDENTIFICATION OF BACILLUS ANTHRACIS ISOLATES OBTAINED FROM CLINICAL SAMPLE OF SUSPECTED ANTHRAX OUTBREAK IN SHEEP OF GUJARAT STATE

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In the present study, isolates obtained from culture of blood samples from suspected cases of anthrax of sheep were subjected to PCR based identification using specific primers for capsular and S-layer region of the organism. The amplicons of expected size of 846 and 639 bp were obtained by PCR using primers for capsular and S-layer regions, respectively. The results confirmed the isolate as *Bacillus anthracis*, the causative agent of mortality in the sheep flock. This also underlines the significance of PCR as a rapid and specific diagnostic test for the detection of *Bacillus anthracis* which is an important zoonotic agent.

V-P-3

DETECTION OF CANINE ADENOVIRUS TYPE 2 IN VACCINES BY PCR

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Canine adenovirus 1 (CAV-1) and CAV-2 respectively cause infectious canine hepatitis (ICH) and infectious canine laryngo-tracheitis (ICLT) in dogs. The infectious canine hepatitis is characterized by fever, anorexia, increased thirst and abdominal pain with swollen liver whereas CAV-2 infections is associated with respiratory symptoms. Further corneal opacity (blue eye) and interstitial nephritis may occur 1-3 weeks after the clinical recovery as a consequence of the deposition of circulating immune complexes after CAV-1 infections in dogs. CAV-1 is genetically and antigenically distinct from canine adenovirus 2 (CAV-2). Both viruses are shed in faeces and urine of the infected or recovered dogs, thus urine and faeces are important sources of infection to healthy dogs. A galaxy of immunoprophylactic agents based on CAV-2 are available in the market for use in dogs to control infectious canine hepatitis in dogs. In spite of vaccination against CAV infections in dogs, the outbreaks has been reported occasionally on dogs in India. In this study, PCR has been employed to amplify the genomic DNA of CAV in the vaccines and it has been found that CAV-2 strains are present in 4 vaccines out of 7 vaccines tested as revealed by the presence of 1030 bp amplicons in the agarose gel.

V-P-4

Poster Award I

DEVELOPMENT OF A MULTIPLEX PCR ASSAY FOR THE DETECTION OF MAJOR BACTERIAL PATHOGENS OF SUBCLINICAL BOVINE MASTITIS

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In the present study, multiplex PCR assay was developed to detect five major bacterial pathogens causing subclinical bovine mastitis. They include *Streptococcus agalactiae, S.dysgalactiae, S.uberis. Staphylococcus aureus* and *E.coli.* Primers were designed by targeting *tuf* gene with an amplicon size of 110bp for Streptococcus species and 235bp for Staphylococcus species at genus level. The target sequences were sip gene (281bp amplicon size) for *S.agalactiae*, 16s r RNA for *S.dysgalactiae* (429bp), *pauA* gene for *S.uberis* (534bp) , nuc gene for *S.aureus* (181bp) and *tra*T gene for *E.coli* (313bp). A two tube method multiplex PCR was standardized for simultaneous detection of these pathogens. The assay included, *S.agalactiae* and *S.uberis* in one tube and *S. dysgalactiae, S.aureus* and *E.coli* in the other tube for amplification. Screening of milk samples for subclinical cases of mastitis, primer designing, specificity and sensitivity of PCR and PCR conditions employed will be discussed in detail. The results suggested that the multiplex-PCR could be explored as an alternate method in routine diagnosis for rapid, simultaneous detection of these pathogens directly from milk samples.

V-P-5

ISOLATION AND IDENTIFICATION OF NEPHROPATHOGENIC AVIAN INFECTIOUS BRONCHITIS VIRUS FROM CASES OF VISCERAL GOUT IN BROILERS

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Infectious bronchitis (IB) is an extremely contagious respiratory disease of chickens, caused by avian infectious bronchitis virus (IBV) of family *Coronaviridae.* The nephropathogenic strains of IBV have been reported from India with incidence of M41 serotype. The present study was undertaken with the aim to find out the exact cause of IB in broilers. Tissue samples such as trachea, lung and kidney from dead birds (n=50) showing the lesions of visceral gout were collected in 50 percent glycerin saline and immediately transferred to the laboratory for isolation of avian IBV. For each sample, five; well developed, ten-day-old, healthy and IB free chicken embryos were inoculated with 0.2 ml of tissue homogenate via allantoic cavity route. Similarly, five embryos, each inoculated with 0.2 ml sterile phosphate buffered saline, were taken as control. Simultaneously, the Mass type vaccine strain was adapted and propagated in chicken embryos. Allantoic fluid (AF) from the inoculated embryos was collected at three to five days post inoculation and presence of IBV was confirmed by haemagglutination (HA), haemagglutination inhibition (HI) and virus neutralization (VN) tests. Nephropathogenic avian IBV was isolated in inoculated chicken embryos which showed typical curling and dwarfism, thickening of mesonephros with deposition of urate crystals, shrunkened yolk sac, and increased volume of AF along with high mortality in inoculated embryos. The isolated IBV showed HA activity after treatment of infected AF with 1.0 percent trypsin. In HI test, cross titration between field viral isolate and vaccine strain indicated minor antigenic variation between both the viruses. The field viral isolate was neutralized by hyperimmune serum against Mass type vaccine strain of IBV. The changes in chicken embryos and positive findings by HI and VN tests confirmed the presence of avian IBV that caused nephropathogenicity and visceral gout in broiler chickens.

V-P-6

MULTI DRUG RESISTANT ALPHA HAEMOLYTIC *E.COLI* IN WATER RESOURCES: A MAJOR HEALTH HAZARD

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The present study was taken to understand the status of water resources in the holy city of Mathura. As it is a tourist place and pilgrims come from all around the world, there are more chances of spread of pathogens through them particularly those which are excreted through urine and faeces and *E. Coli* is one of them which causes many severe diseases particularly in neonatal calves or neonates of human. As the city has its limitation of accommodation and river Yamuna is also shrinking, the pressure of millions of pilgrims has enormous, drastic and stipulated effect on water resources particularly river Yamuna. The samples collected showed the presence of *E. coli.* Out of total 100 samples 40 were found positive for *E. coli* presence. The 60 samples of different water resources showed the presence of *E. coli* in 26 samples while 14 were found positive out of 40 samples of Yamuna water. The mean of the $\log_{10}$ standard plate count for total coli form count was 3.4cfuml^{-1}. When these isolates were analyzed for antibiotic sensitivity pattern against some commonly used antibiotics as Kanamycin, Ampicillin, Amoxicillin, Cefotaxime, Tetracycline, Chloramphenical, Ceftriaxone, Amikacin and Ciprofloxain. Isolates showed multi drug resistant against them. The number of resistant isolate is significant enough to make an alert at the earliest to protect the water resources and also to secure better and brighter future of human population as increasing prevalence of resistance to antimicrobial agents such as fluoroquinolones and third-generation cephalosporins, which are important for the treatment of infections caused by enteric pathogens, has significant public health implications.

V-P-7

DETECTION AND ISOLATION OF ROTAVIRUS FROM DIARRHEIC PRE-WEANED PIGLETS

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Rotavirus causes significant mortality and morbidity in neonatal animals throughout the world especially in multiparous animals like pigs. An investigation was done to detect the viruses with segmented genome associated with diarrhea in pre-weaned piglets. In all, 164 diarrheic and 24 non-diarrheic fecal samples collected from 0-8 weeks aged piglets from 8 piggery units were screened. Electropherotyping has become a popular and widely used method for characterization of rotaviruses. Polyacrylamide gel electrophoresis (PAGE) was used as a rapid diagnostic technique for screening fecal samples for viruses with segmented genome. Diarrheic fecal samples, which were pooled from 2-4 piglets from the same litter and clarified, were negative by PAGE. Thirteen out of 78 unprocessed diarrheic samples , which were screened individually, were found positive for rotavirus by PAGE and 11 segments were demonstrable in all the positive samples which is characteristic for porcine rotaviruses . Rotavirus was isolated from seven of the ten PAGE positive samples in MA 104 cell lines wherein characteristic cytopathic effects were seen after 3-5 passages. Virus could not be isolated from the remaining three samples even after five passages. Presence of the virus was confirmed by demonstrating characteristic rotaviral RNA profile in cell culture fluid by discontinuous PAGE. All the seven samples showed identical electropherogram pattern of group-A rotaviruses except for slight variation in migration pattern of bands 5 and 6 in one isolate. Nearly all the isolates showed an identical RNA migration pattern with typical segment clusters 4:2:3:2 in regions 1,2, 3 and 4 respectively, which is characteristic of group-A rotaviruses. In this study, some minor variations in the migration pattern of bands were observed in the isolates obtained in one farm, when compared to that of the isolates obtained from the other farms. This indicated the genetic diversity among the porcine rotaviruses. Although the detection of differences in the genomic profiles by PAGE need not reflect antigenic differences, it helps in the formulation of additional prospective and retrospective studies on the molecular epidemiology of rot virus infections.

V-P-8

MICRO-BIOCHEMICAL STUDIES OF CANINE PARVOVIUS INFECTION IN PUPPIES

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The present study was conducted on eleven puppies naturally infected with parvorival gastroenteritis. Five puppies seronegative for Canine parvovirus (CPV) were taken as control. Disease diagnosis was done from dog faeces using various serological tests viz. Haemagglutination (HA) test using 0.8 % swine RBC and direct Fluorescent antibody technique. Viral antigen was further confirmed by performing Direct ELISA using monoclonal antibody (Mab) against CPV (VMRD,Pullman, U.S.A.). It was found that seven out of eleven samples were positive for presence of CPV antigen. Biochemical studies were further carried out on all the samples along with control. It was found that the puppies which were positive for CPV showed significant hypoglycemia *i.e.* 60.14±1.16 mg/dl ($p<0.05$) along with hypoprotinemia (5.11±0.061g/dl), hyponatremia (135.4±0.023mM/L), hypokalemia (3.89±0.36mM/L) and hypochloremia (104.81±0.053mM/L). Blood urea nitrogen (BUN) levels were also significantly decreased from 15.62±1.02 mg/dl in seronegative to 9.14±0.091 mg/dl in CPV positive puppies. Further, the levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were significantly increased ($p<0.05$) *i.e.* 75.55±0.028 IU/L and 140.24±0.021IU/L respectively. This study clearly indicates that ELISA is the most sensitive test to diagnose CPV infection and HA is the most rapid test. Biochemical studies revealed that there is increased extacellular leakage resulting from increase cell permeability and injured hepatocytes which further leads to dysfunction of liver to a great extent.

V-P-9

DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CANINE PARVOVIRUS (CPV) INFECTION.

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Canine Parvovirus (CPV) Infection is highly contagious & fatal disease of dogs, causing acute hemorrhagic enteritis & myocarditis. In the present study, the faecal sample of twelve dogs suffering from gastroenteritis of different breed, sex and age (below one year) were tested for CPV by using monoclonal antibody based canine parvovirus test kit (In-vitro) manufactured by VAL Vet All Laboratories to detect the antigans of CPV. As no specific treatment against parvovirus infection is available so an attempt was made to treat the animals on the basis of clinical signs. Out of these twelve dogs, four (33.33%) were found positive for canine parvovirus infection. In positive cases, only one was vaccinated against CPV. All the positive dogs were below six month of age and having hemorrhagic enteritis along with vomiting. The treatment Comprising of Ringer's lactate solution @ 5-10 ml/kg b.wt i.v for first three days to control metabolic acidosis followed by 5% dextrose normal saline i.v for three days to control hypoglycemia along with ceftriaxone @ 15-25 mg/kg b.wt i/m daily for five days to combat secondary bacterial infection, metacloprimide @ 0.2 mg/kg. b.wt. i/m for three days to check vomition, ranitidine @ 0.5 mg/kg. b.wt. as an antacid, haemocoagulase total dose 0.5-1ml i/m for two days as haemostatic and multivitamins to tone up the condition of the animal was found effective. One positive and one negative case was died after second day of treatment.

V-P-10

"PREVALENCE OF BLUETONGUE IN LARGE RUMINANTS OF MARATHWADA REGION"

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The present study was planned with objectives of Seroprevalence study of BT disease in Cattle and Buffaloes in Marathwada region as well as attempt to isolate BT virus from cattle and buffaloes on Embryonated chicken eggs (ECEs) and BHK-21 cell line and Collection and identification of *culicoides* from BT endemic areas.

A total of 246 serum samples (Cattle and Buffalo) were screened by cELISA from different slaughter houses for seroprevalence study. A high percentage of cattle showed BT antibodies are 92.53, 86 and 81.81percent respectively in Parbhani, Nanded and Aurangabad, Similarly in buffalo 70, 100, 100 and 87.5percent respectively in Parbhani, Nanded, Aurangabad and Hingoli. The overall percentage of BT positive cattle and buffalo was 89.80 and 80 percent respectively. A total of 55 blood samples (Cattle and Buffalo) were collected from different slaughter houses and inoculated into 10-12 days old ECEs and BHK-21 cell line to attempt isolation of BT virus. All the samples were negative for BTV as no specific death of embryo observed in case of ECE and in BHK-21 cell line no CPE was observed even after three blind passages. The s-ELISA was used to screen blood samples tested negative for BTV in ECEs and BHK-21 cell lines. All the blood samples tested negative for BTV in ECEs and BHK-21 cell lines were negative in s-ELISA for detection of BTV antigen. The *Culicoides* were collected and identified in the Parbhani was *Culicoides schultzei* and *Culicoides peregrinum* to attempt BTV isolation. All the lots were negative for bluetongue virus as no specific death of embryo observed in case of ECE and in BHK-21 cell line no CPE was observed even after three blind passages. The present study concluded that a high percentage of BT seroprevalnce was seen in cattle and buffaloes in BT endemic areas of Parbhani, Nanded and Aurangabad districts of Marathwada region of the state and cELISA were found to be sensitive and effective for screening of BT group specific antibodies. BTV could not be isolated from cattle and buffaloes in BT endemic districts in ECE and BHK-21 cell lines but BT is prevalent in inapparent from in cattle and buffaloes in the Parbhani, Nanded and Aurangabad districts. *Culicoides schultzei* and *Culicoides peregrinum* are prevalent in BT endemic districts of region.

V-P-11

STUDY OF DIFFERENT VIRULENCE FACTORS OF *ESCHERICHIA COLI* ISOLATED FROM COLIBACILLOSIS IN POULTRY

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Colibacillosis is an economically important disease, prevalent throughout the world. Avian pathogenic Escherichia coli (APEC) has been implicated in a variety of disease syndrome in poultry such as coli septicaemia, coli granuloma, air sacculitis, peritonitis, pericarditis, omphilitis and oophritis leading to 5-50% mortality in poultry flocks. The objectives of the present study was to identify and characterize the different virulence factors of *E. coli* isolated from extra intestinal infections in poultry. A total of 35 samples were collected from necropsied birds with different pathological lesions. Twenty isolates of *E. coli* were recovered from 35 cases of colibacillosis. Serotyping of these isolates revealed ten 'O' group serotypes including O60, O80, O84, O95, O102, O110, O114, O119, O120, O132. One of the isolate was rough type and one was untypable. These isolates were screened for virulence factors through Congo red dye agar test (CR test), haemolysin assay, motility test and mouse paw edema test (MPOT). Congo red dye agar test (CR test) was used to differentiate invasive and non invasive *E. coli*. Out of these 20 isolates tested 9 showed CR-positive reactions while 11 were CR negative. Plate haemolysis test was done for the detection of á-haemolysin produced by *E. coli*. All samples showed positive reaction to this test. Motility test was performed using mannitol motility medium to check for presence of flagella. 11 serotypes were motile while rest 9 were non motile. Mouse paw edema test was performed to determine the production of enterotoxins. 15 samples showed positive reaction while 5 were negative. From the present study, it can be concluded that presence of various avian pathogenic *Escherichia coli* (APEC) are significantly high in poultry, possessing various virulent determinants which need to be further studied for the development of immunoassay for quicker determination of disease.

V-P-12

MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* BY AMPLIFICATION OF 16S RRNA GENE FRAGMENT BY PCR

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The nucleotide sequences found in 16s rRNA vary in an orderly fashion throughout the phylogenetic tree and have been found useful for the study of molecular evolution. The present study involved the application of species specific primers for amplification of DNA employing PCR following the technique of Widjojoadmodjo *et.al* (1990). Out of 34 *Staphylococcus aureus* strains from clinical mastitis milk samples used in the present study showing polymorphism in some of the biochemical properties and presence of Protein A did not show any variation in amplification of nucleotides of the selected 16s rRNA gene fragments. All resulted in an amplicon of 305 bp size. Bacteria belonging to other genera namely *E. coli* and *Salmonella spp.* did not show the presence of this region as no amplification could be achieved under similar conditions of PCR.

V-P-13

EQUI FACTORS AN INDICATOR TEST FOR THE IDENTIFICATION OF RHODOCOCCUS EQUI

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Rhodococcus equi, an important gram's variable pathogen in horses that causes chronic bronchopneumonia with extensive lung abscess formation , is generally thought to be non-haemolytic. R. equi produces equi factors which interact with the beta-toxin of S. aureus, and give an area of complete hemolysis with sheep erythrocytes. In the present study, the production of equi factor(s) by 75 Rhodococcus species isolates was tested by streaking strains at right angles to a culture of Staphylococcus aureus on a sheep blood agar plate. Among them 29 Rhodococcus equi isolates produce equi factor(s). This characteristic was more specific than other tests used for the identification factor of *Rhodococcus equi.*

V-P-14

ANTIBIOGRAM OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM POOLED RAW MILK OF LOCAL DAIRIES AND VENDORS OF BIKANER CITY

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Raw (unpasteurized) milk can be a source of food-borne pathogens. Pasteurization is designed to destroy all bacterial pathogens common to raw milk, but some people continue to consume raw milk, believing it to be safe. Milk and other dairy products have been reported to be main source of *S. aureus* of animal origin (Gilmour and Harvey, 1990). The study showed that 22 per cent of food handlers were carrier of enterotoxigenic *S. aureus* (Mori *et al*., 1977). In many countries, *Staphylococcus aureus* is considered the second or third most common pathogen responsible for outbreaks of food poisoning (Atanassova *et. al.*, 2001). In present study of antibiotic susceptibility test by agar disc diffusion method (CLSI, 2005) against twenty *S. aureus* isolates from pooled raw milk of local dairies and vendors of Bikaner city. Fourteen antibiotics were chosen for the study according to their common use in research, human medicine and veterinary practice. They belonged to the following groups: penicillin (ampicillin, cloxacillin); aminoglycosides (gentamicin, kanamycin and neomycin); tetracycline's (chlortetracycline, doxycycline and tetracycline); macrolides (erythromycin); quinolones (ciprofloxacin) and miscellaneous (chloramphenicol and streptomycin). Investigation had shown that antibacterial susceptibility against *S. aureus* in following order, of sensitivity ciprofloxacin (100%), gentamicin (75%), doxycycline and chlortetracycline (70%), norfloxacine (65%), neomycin (60%), kanamycin and streptomycine (50%), ofloxacine and erythromycin (20%), bacitracin (10%), ampicillin and tetracycline (5%) and cloxacillin (0%). Chlortetracycline and gentamicin were more sensitive than other antibiotics in their same group.

V-P-15

MONOCLONAL ANTIBODY C-ELISA BASED SEROPREVALENCE OF PESTES DES PETITS RUMININTS IN UNVACCINATED SHEEP FLOCK

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Peste des petits ruminants (PPR) is a highly contagious animal disease characterized by pyrexia, occulo-nasal discharge, stomatitis, pneumonia and diarrohoea. The disease is endemic in many regions of world and responsible for significant economic losses in goats and sheep due to high morbidity and mortality rates. The huge number of small ruminants, which are reared in the endemic areas, makes PPR a serious disease threatening the livelihood of poor farmers. A tissue culture live attenuated vaccine is being used in Karnataka against PPR in small ruminants. In present study, an organized farm, located in an isolated place, was selected for seroprevalence of PPR, where sheep were not vaccinated against PPR since four years. A total of 120 serum samples were collected from sheep in the age group of 4 months to 5 years, maintained in the farm. A monoclonal antibody based Competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA) kit, procured from IVRI, Muktheshwar, was used to detect the antibodies in sera against PPR virus. AS per the instructions of the kit manual, Percentage Inhibition more than 40% were considered positive for the presence of PPRV antibodies. The seropositivity of sheep for PPR was found to be 1.6 % (2/120), indicating low seroprevalence of PPR antibodies in sheep in the farm. Since the sheep in the farm were not vaccinated against PPR for the last four years, the prevalence of the anti PPRV antibodies could be purely attributed to the low level lurking of viral infection in the farm. Further, clinical examination of the two sero-positive sheep showed that apparently they were healthy without exhibiting the clinical manifestation of the disease and indicated their possible sub clinical status of the disease. However, suitable control strategies were recommended to check the further spread of the disease.

V-P-16

MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS*

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Molecular characterization of thirty four *Staphylococcus aureus* strains from clinical mastitis milk was performed. These isolates showed polymorphism in terms of biochemical properties and presence of protein A. But all these isolates gave an amplicon of 305bp when subjected to PCR using primers as reported by Widjojoadmodjo *et.al* (1990) confirming them to be of *Staphylococcus aureus.* Whole chromosomal DNA was then subjected to restriction enzyme digestion using Bgl II, Bam HI, Eco RI and Kpn I. Of the four enzymes used Kpn I restriction digestion yielded polymorphism amongst the isolates. But other three restriction enzymes did not show any polymorphism. Therefore, Kpn I canbe used to compare various isolates of *Staphylococcus aureus* causing mastitis.

V-P-17

ISOLATION OF PPR VIRUS FROM SHEEP AND GOATS OF GUJARAT

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Seven postmortem samples (i.e. including Lymph node, Spleen, Lung, Intestine and one blood sample) were collected from Sheep and Goats from local abattoir of Banaskantha district in Gujarat. After primary screening with PPR s-ELISA for the presence of PPR Viral antigen, all the samples were subjected for isolation of PPR Virus in Vero cell. PPR Virus was isolated following second passage in Vero cells. Cytopathic Effects (CPE) produced were characterized initially by rounding and ballooning of cells, later on aggregation of cells followed by formation of fusion mass and syncytia. Cell lysis was also observed in some cases. Virus isolates were identified using s-ELISA and read at 492 nm. PPR viral antigen load as measured by Optical density value was higher in third passage as compared to second passage. Also, the RNA was isolated from PPRV isolates following second passage using Trizol method and further processed for Reverse Transcriptase-PCR (RT-PCR).Reference vaccine virus (Sungri isolate) as well as all 7 isolates produced approximately 351 bp amplicons with primer pair NP3a and NP4 by RT-PCR. In the present study, isolation of virus just after first passage indicated the quick adaptation of virus in the Vero cells and might be attributed to the quality of sample, proper collection and storage till inoculation and the quantity of inoculums (Which greatly affect multiplicity of infection).

Key words: Vero, s-ELISA, RT-PCR, CPE

V-P-18

ISOLATION AND MOLECULAR CHARACTERIZATION OF SHIGA TOXIN-PRODUCING, AND ENTEROTOXIGENIC *ESCHERICHIA COLI* ASSOCIATED WITH CALF DIARRHEA IN NORTH GUJARAT, INDIA

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Neonatal calf diarrhoea is a major threat to dairy industry due to heavy mortality, treatment cost and poor growth of calves. The syndrome has great complexity due to its multifactorial etiology. The present study was undertaken to find out the prevalence of *E. coli* associated with neonatal calf diarrhea and to characterize them for different pathotypes of diarrhegenic *E. coli* viz. Shiga toxin- producing, enteropathogenic, enterotoxigenic, enteroaggregative and diffusely adherent *E. coli* by PCR. A total of 45 *E. coli* isolates (16 from cow calves and 29 from buffalo calves) were obtained from 76 faecal samples collected from diarrhoeic calves (cow 32 and buffalo 44) upto 4 wks of age. The isolates were confirmed to be *E. coli* on the basis of standard biochemical tests and 16S rRNA specific PCR. Five isolates (11.11%) from cow calves were found to be STEC and all of them carried stx_1, stx_2 and *ehxA* genes. Three isolates (two from cow calves and one from buffalo calves) were ETEC and they possessed *est* gene only.

V-P-19

SEROTYPING AND ANTIBIOGRAM PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM DIARRHOEIC CALVES, LAMBS AND KIDS

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The present study was carried out to investigate serotypes and drug resistance pattern of *E.coli* isolated from faecal samples of diarrhoeic calves, lambs and kids. One hundred and twenty five faecal samples were collected from diarrhoeic calves, lambs and kids from various locations in Bikaner. All 125 isolates obtained from diarrhoeic faecal samples revealed typical cultural characters of *E.coli*. All isolates were sent to National Salmonella and *Escherichia coli* Research Centre, Kasauli, H.P for serotyping. Samples were sent in transport media. Seven isolates were repotered to be non *E.coli* and three were lost in transportation hence, 115 were got serotyped. Out of total 115 isolates, 74 typable isolates belonged to 19 different 'O' serogroups, while 37 isolates were untypable and 4 were rough isolates. Serogroups obtained from calf, lamb and kid isolates were O2, O3, O5, O17, O21, O22, O25, O41, O45, O55, O60, O65, O70, O76, O114, O116, O147, O152 and O158. Out of 19 different 'O' serogroups O5 and O152 were common in calves, lambs and kids. *E.coli* isolated from lambs and kids revealed three common serogroups viz. O5, O55 and O147, lambs and calves shared five common serogroups viz. O5, O22, O25, O116 and O147 and seven common serogroups viz. O5, O17, O55, O60, O76, O147 and O152 were shared by calves and kids. Among all serogroups obtained, O22 (12.17%) revealed highest prevalence followed by O76 (10.43%), O147 (8.69%) and O60 (6.08%) while rest of all serotypes revealed low prevalence (less than 5%). Some serogroups obtained in present study viz. O2, O5, O45 and O55 were of zoonotic importance.

In vitro antibiotic sensitivity pattern against 14 antibiotics viz. ampicillin, amoxyclav, azithromycin, cephotaxime, chloramphenicol, chlortetracycline, ciprofloxacin, co-trimoxazole, gentamicin, nalidixic acid, nitrofurantoin, polymixin-B, sparfloxcin and triple sulpha were studied. All isolates revealed 100% sensitivity for ciprofloxacin; sparfloxacin and triple sulpha while 100% resistance for ampicillin and cephotaxime. Some antibiotics revealed variation in resistance pattern in three species. All *E.coli* isolates obtained from diarrhoeic lambs and kids showed resistance to azithromycin while only 15% isolates obtained from diarrhoeic calves were found resistant and rest of all were sensitive to azithromycin. Likewise all lamb and kid isolates were sensitive to amoxyclav and chloramphenicol while three percent and 19% of calf isolates were found resistant to amoxyclav and chloramphenicol respectively. Different sensitivity pattern of a single antibiotic at different locations were significant. This is due to frequency of particular antibiotic used in treating enteric infections at particular location.

V-P-20

CONGO-RED BINDING ABILITY, HEMOLYSIN PRODUCTION OF *E.COLI* RECOVERED FROM PIGEONS

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Escherichia coli commonly infects pigeons. A total of 60 fecal samples were collected aseptically from apparently healthy pigeons. They samples were processed for the isolation of *E.coli* by inoculating into broth for 6 hrs followed by plating onto MacConkey's and Eosin methylene blue agar. The isolates recovered were identified by morphological cultural and biochemical characterization using standard procedures. A total of 44 isolates of *E.coli* were recovered. All the isolates were inoculated onto blood agar to study their hemolysin production ability. Of 44 isolates 21 could show hemolysin production. To study presence of pathogenicity marker congo red binding ability was studied by inoculating isolates onto congo red medium. Of 44 isolates inoculated 24 could show congo red binding ability.

From the present investigation it could be concluded that though *E.coli* is considered a normal constituent of pigeon feces, but certain strains of E. coli can be severely pathogenic. Further studies on serotyping and antibiotic resistance pattern are required to be undertaken.

VI-P-1

EFFICACY OF *DALBERGIA SISSOO* LEAVES AS A SOURCE OF PLANT SECONDARY METABOLITES ON *IN VITRO* DIGESTIBILITY

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The present experiment was determined the efficacy of plant secondary metabolites estimated in terms of total phenol and condensed tannins present in *Dalbergia sissoo* leaves on *in vitro* digestibility. *Dalbergia sissoo* tree are commonly found in the arid region and known to contain plant secondary metabolites i.e. phenols and tannins. The leaves samples were dried, ground and stored for chemical analysis. For in vitro digestibility of complete feed containing wheat straw (2.65% CP) 60 parts and concentrate (19.60% CP) 40 parts was prepared and supplemented with 1, 2.5, 5, 7.5 and 10 per cent levels of leaves supplementation. The dietary combinations were subjected to *in vitro* evaluation by following the method of Tilley and Terry (1963). *In vitro* digestion was carried out in 100 ml conical flask fitted with Bunsen valve after incubation at 39°C for 48 hours. The rumen liquor was collected from adult rams kept on standard maintenance diet. Following incubation for stipulated period, the substrate were filtered through GI crucible to determine the *in vitro* digestibility of dry matter, organic matter, neutral detergent fibre, acid detergent fibre, hemicellulose, and cellulose. The *Dalbergia sissoo* leaves were rich sources of organic matter (88.40%) and crude protein (21.52%). The total phenol and condensed tannins content were found to be 3.19% and 1.44% respectively. The *in vitro* dry matter digestibility was (IVDMD) was 64.35% in T_1, 63.62% in T_2 64.88% in T_3, 64.07% in T_4 and 62.56% in T_5. The *in vitro* organic matter digestibility were 59.87%, 60.15%, 61.27%, 62.00% and 62.33% in T_1, T_2, T_3, T_4 and T_5 respectively, reflecting the improvement of digestibility with increasing levels of *Dalbergia sissoo* leaves. Similarly, the *in vitro* digestibility of various fibre fractions viz., IVNDFD, IVADFD, IVHCD and IVCD also increased with the corresponding increase in the levels of *Dalbergia sissoo* leaves in complete feed. At 1 and 10% level of supplementation, *in vitro* digestibility of NDF, ADF, hemicellulose and cellulose increased were 43.18 and 46.57, 39.67 and 40.95, 51.02 and 51.75 and 37.25 and 37.63 per cent respectively. The high total phenols and condensed tannins containing *Dalbergia sissoo* leaves can be incorporated up to 10% level in the compete feed without adversely affecting digestibility of dry matter, organic matter and fibre fractions.

VI-P-2

ANAPHYLACTIC SHOCK DUE TO UNKNOWN CAUSE IN A ZANSKARI MARE

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A Zanskari mare 12 years of age suddenly showed signs of acute Respiratory distress with Respiration rate and pulse rate 40 and 70 per minute, respectively. Animal was treated with inj adrenalin 1/1000, 3ml i/m and Dexamethasone 40mg i/v and inj Pheniramine maleate 227.5 mg i/v. On hematological examination Hb, PCV, TLC, TEC and DLC were in normal range. After 15 minutes of treatment animal was quite normal. Animals showed three episodes on interval of 5 to 7 days, with same symptoms with increasing severity. All episodes occurred at night hours during winter. In fourth episode the reaction was so fast that the animal died before any medication. On post-mortem examination nothing abnormalities detected except frothing in huge amounts in trachea, bronchi and bronchioles.

VI-P-3

IMMUNOMODULATORY EFFECT OF *TINOSPORA CORDIFOLIA* AND *WITHANIA SOMNIFERA* ON BROILERS IMMUNIZED WITH INCLUSION BODY HEPATITIS-HYDROPERICARDIUM SYNDROME VACCINE

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Hydropericardium syndrome (HPS) is an emerging disease of broiler chickens, caused by group-I Aviadenovirus serotype-4 belonging to family *Adenoviridae* and characterized by high mortality with typical hydropericardium. The HPS virus causes immunosuppression by damaging the lymphoid tissues. Immunopotentiation helps to regain or revitalize the suppressed immune system to its normal status by immunomodulating effect of certain medicinal plants. The immunomodulatory effect of *Tinospora cordifolia* and *Withania somnifera* on cell mediated immune (CMI) response and humoral immune (HI) response in broiler chickens immunized with inactivated inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) vaccine was studied. Three-day-old broiler chicks (n=48) were randomly divided into four groups viz. T_1 (control), T_2 (immunized with inactivated IBH-HPS vaccine), T_3 (immunized with inactivated IBH-HPS vaccine along with *T. cordifolia* feed supplementation) and T_4 (immunized with inactivated IBH-HPS vaccine along with *W. somnifera* feed supplementation). For HI response, antibody level measured by enzyme linked immunosorbent assay showed significantly ($P<0.05$) increased antibody titre in T_3 and T_4 groups compared to T_2 and T_1 groups. The CMI response was assessed by measuring the skin thickness at 24, 48 and 72 hr post challenge with allergen Dinitro chlorobenzene. The T_3 and T_4 groups showed significantly ($P<0.05$) increased skin thickness compared to the T_2 and T_1 groups. The present study indicated that both *T. cordifolia* and *W. somnifera* supplementation in feed had augmented HI and CMI responses in IBH-HPS vaccinated broiler chickens.

VI-P-4

SCREENING OF ANTIMICROBIAL ACTIVITIES OF ALCOHOLIC EXTRACT OF TRIBULUS TERRESTRIS FRUITS AGAINST BACTERIAL ISOLATES

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In vitro antibacterial sensitivity of the alcoholic extract of *Tribulus terrestris* fruits extract was tested. Microbial sensitivity test disc of known concentration were used. Extract disc were prepared by impregnating filter pare discs with extract. Gram positive and gram negative organisms isolated in pure cultures were used. These were considered adequate to represent a wide range of pathogenic organisms varying in their susceptibility to antibacterial agent. The antibiotic discs were put onto the surface of plates along with extract discs. The antibiotic discs commercially available were used against all organisms to maintain the uniformity of disc. The plates were incubated for a period of 18 hrs at 37 ^{0}C and were observed for the growth and the zone of inhibition around the discs for the antibacterial activity along with extract. The zone around disc were measured in mm and compared with control (blank disc) and standard. The *in vitro* antibacterial sensitivity of alcoholic extracts of *fruits of Tribulus terrestris* against different bacteria both gram positive and gram negative bacteria. The result showed that the alcoholic extract of *Fruits of Tribulus terrestris* possessed antibacterial activity which was demonstrated by the zones of inhibition against the organisms tested. Among gram positive organisms, *Staphylococcus spp.* was found to be highly sensitive to the alcoholic extract @ 200mg/ml of concentration followed by *Bacillus spp* and *Streptococcous spp*. Among gram negative organisms *Salmonella spp.* was found to be sensitive to the alcoholic extract @ 200mg/ml followed by *Escherichia coli* and *Proteus spp.* Thus, the antibacterial effect of alcoholic extract was comparable to the effect of standard antibiotics.

VI-P-5

Isolation and characterization of bacteriophages recovered from animal waste

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In present study, 186 waste water samples were collected from waste water collection tanks located in livestock farms of different species (Cattle, pig, goat and poultry). These samples were subjected to streak plate method for the detection of lytic activity followed by primary isolation of phage against two most common bacteria of environment, namely, *B. subtilis* and *E. coli* by Double agar layer (DAL) method. Recovery of phages was maximum from pig feces (67%) followed by dairy cattle farm waste (63%), buffalo farm waste (50%), goat farm waste (13%). Recovered phage isolates were subjected to physical and biological characterization based on plaque morphology, host range determination, effect of temperature on their viability. Phage isolated against *B. subtilis* (BsHR$_1$, BsHR$_2$, BsHR$_3$ and BsHR$_4$) and isolated against *E. coli* (EHR$_1$ and EHR$_2$) were categorized by plaque morphology. For host range determination, phage isolates were tested for lytic activity against pathogenic bacteria. Phage Lysate (EHR$_1$) had widest hosts range followed by EHR$_2$, BsHR$_1$, and BsHR$_2$. These isolates were also investigated for their temperature sensitivity. Phage Lysate BsHR$_1$, BsHR$_2$ and EHR$_2$ remain viable at 70^{0}C up to 3 min while BsHR$_3$, BsHR$_4$ and EHR$_1$ showed a definite decrease in the percent survivors at 70^{0}C for 2 min and no viability was seen after exposure of 3 min for these isolates.

VI-P-6

ANTIBIOTIC SENSITIVITY PATTERN OF ENTERIC AND EAR BACTERIAL PATHOGENS ISOLATED FROM DOG

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Antibiotic sensitivity pattern of enteric and ear bacterial pathogens isolated from dog was studied in 23 ear swabs and 30 rectal swabs. Nine bacterial field isolates were studied which includes *Escherichia coli* (23 isolate), *Staphylococcus* (19 isolates), *Pseudomonas aeruginosa* (14 isolates), *Proteus mirabilis* (16 isolates), *Proteus vulgaris* (7 isolates), *Klebsiella pneumoniae* (11 isolates), *Klebsiella oxytoca* (6 isolates isolates), *Salmonella* spp (4 isolates) and *Micrococcus* (5 isolates).Sensitivity pattern reported in ear infection was as follows: Ciprofloxacin (67.27%) followed by Gentamicin (62.18%), Cephalaxin (40%), Chloramphenical (32.72%), but moderate sensitive to Amoxycillin (21.81%), Ampicillin (20%), Doxycycline (16.36%) and least sensitive to Penicillin (12.72%) and Erythromycin (3.63%). Ciprofloxacin was reported as the most effective antibiotic for chronic ear infection. Similarly , Chloramphenical was found to be the most effective drug in enteric infection (showing sensitivity of 72%), followed by Ciprofloxacin (70%), Gentamicin (52%), Cephalixin (40%), Doxycycline (30%), Ampicillin (14%), Amoxycillin (6%) and Eythromycin (2%).

VI-P-7

EFFECT OF NEW CASTLE DISEASE VIRUS SPRAY VACCINE ON HARDERIAN GLAND

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An experiment was conducted on 100,day old broiler chicks which were vaccinated with Newcastle disease vaccine administered by aerosol route.Group T$_1$ was kept as unvaccinated control group while T$_2$,T$_3$ and T$_4$ with intraocular vaccination,spray vaccination and spray vaccination with 1 % *Tinosporia cordifoila* in feed as herbal immunomodulator,respectively. Birds were primed at day 7 by Live B$_1$ strain of NDV and booster was given on day 28 with Lasota strain.After decapitation and removal of both the eye balls,the Harderian gland(HG) was separated. HG located at ventral and posteromedial to the eye ball was separated from fascia and intact muscle..Effect of aerosol vaccination on harderian gland was studied by measuring weight (gm),geometric changes (mm) per 1000 gm body weight. Histopathological observations were recorded 72 hours after vaccine administration. Increase in number of plasma cells and enlargement of lymphoid foci in all vaccinated groups ,particularly in T$_2$ group,was observed.There were no gross pathological changes in other lymphoid organ. Changes in Harderian gland were almost similar in both intraoccular and spray vaccination in which increase in plasma cells and enlargement of lymphoid foci was noticed .

Key words: Spray vaccine,Harderian gland

VI-P-8

DEVELOPMENT OF TRANSGENIC SPERMATOZOA IN CHICKEN

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Sperm cells under certain conditions can take up the foreign DNA. The exogenous DNA could be incorporated into spermatozoa by simple incubation, however, type, size and amount of exogenous DNA, time and temperature of incubation and quality of spermatozoa are important factors, which influence the efficiency of internalization. There are some factors which can improve the efficiency of internalization of the exogenous DNA. These are liposome transfection and Restriction Enzyme Mediated Integration (REMI). Liposomes are the tiny bubbles (vesicles), made out of the same material as a cell membrane (lipid bilayer) and are composed of naturally-derived phospholipids with mixed lipid chains. Liposome preparations efficiently encapsulate exogenous DNA and transfer DNA to chicken sperm cells while still maintaining the fertility of the sperm. Lipofection does not only increase the rate of integration, but makes it possible to incorporate higher size of exogenous DNA. Semen was collected from the identified cocks yielding good quality semen. Soon after collection, semen was evaluated for sperm concentration. Subsequently, semen was centrifuged at 3000 rpm for 5 minutes at 4 °C. The sperm cells were then washed in phosphate buffer saline (PBS) by dispersing them in PBS, centrifuging at 3000 rpm for 5 min and discarding the supernatant, three times. Finally the sperm cells were left in the PBS to get a concentration of 100 million cells in 100 µl volume. Every 100 million washed cells re-suspended in 100 µl volume was considered as one unit of sperm or a single dose for insemination. The exogenous DNA (upto 14 Kb) was diluted or concentrated to get 100 ng per µl and stored at -20 °C for their further use in transfection. The mixture of exogenous DNA and liposome was prepared by adding 10 µg of purified linearized exogenous DNA slowly to 30 µl of lipofectin® (Invitrogen, USA). This mixture was left for 20 minutes at room temperature. At the same time, 100 units of restriction enzyme *Nco*Ii were gently mixed with 10 µl of lipofectin and this liposome-restriction enzyme mixture was incubated for 10 min at room temperature. This enzyme mixture was later gently added to the DNA-liposome complex and the incubation was continued for another 10 minutes. This exogenous DNA-liposome-restriction enzyme mixture was gently added to one unit of spermatozoa containing 100 million cells in 100 µl semen extender, mixed thoroughly and incubated for 1hr at 37° C to facilitate DNA uptake. After the incubation, the semen was ready for insemination. The transgenic spermatozoa will be used in making of transgenic chicken by simply inseminating the laying hen through artificial insemination. The integration rate of exogenous DNA was 15-20 %.

VI-P-9

IN-VITRO ANTIVIRAL ACTIVITY OF HOT AQUEOUS *ADHATODA VASICA* LEAVES EXTRACT AGAINST IBR VIRUS USING MDBK CELL LINE

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Adhatoda vasica is a primarily herb of ayurvedic system used in treatment of coughs, bronchitis, asthma, and symptoms of common cold. These are the affections which are mainly has the viral etiology. Thus the present study was conducted to get an idea about the antiviral activity Moreover, *Adhatoda vasica* is mainly used as an expectorant and antispasmodic. Cellular toxicity of plant extract on MDBK cell line was carried by 3-(4, 5-Dimethylthiazolyl)-2, 5-diphenyl tetrazolium bromide (MTT) and the concentration at which there was no reduction of viable cells was recorded as maximum non toxic dose of the extract. On the basis of observation of cytotoxic effect of HAE of *Adhatoda vasica* in MDBK cells and absorbance, 1.25 mg/ml aqueous extract was determined as non toxic dose. For further investigation, 1.25, 0.625 and 0.3125 mg/ml were used to study the antiviral activity of HAE of *Adhatoda vasica* against IBR virus. Non toxic concentrations of aqueous extracts were screened for antiviral property by determining cytopathic inhibition effect in MDBK cell lines and by measuring optical densities. Reduction in virus induced cytotoxicity was measured by using MTT dye uptake technique in MDBK cell lines infected with $TCID_{50}$ virus challenge dose. Cell viability was evaluated by adding 10µl MTT dye (5mg/ml) and then extracting the dye with DMSO and measuring optical density at 560nm-670nm and the percentage protection was calculated. HAE of *Adhatoda vasica* leaves extract showed antiviral property against IBR virus with the protection at 0.625 and 1.25 mg/ml were found 15.02 and 2.13 %, respectively. These results of antiviral activity of the HAE of *Adhatoda vasica* leaves validate the use of plant extract in disease conditions like common cold, bronchitis and coughs.

VI-P-10

EFFECT OF SPRAY VACCINATION ALONG WITH HERBAL IMMUNOMODULATOR ON HUMORAL IMMUNE RESPONSE AGAINST NEW CASTLE DISEASE VIRUS

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An experiment was conducted on 75,day old broiler chicks to assess immune response against Newcastle disease vaccine administered by aerosol route in 3 groups *viz*. T_1(unvaccinated control group),T_2(Spray vaccination alone) and T_3(Spray vaccination with 1 % *Tinosporia cordifoila* in feed as herbal immunomodulator).Live B1 and Lasota strains were used at 7th day for priming and 28th day of age as booster respectively.Commercial Vaccine(Ventri Biologicals,India) was reconstitued to get aerosol containing droplets of 150-160 μm sprayed on chicks of group T_2 and T_3 by aerosol vaccinator(Desvac,France).The specific antibody level was measured by Micro Haemagglutination inhibition(HI) and virus neutralization test (VNT) at weekly interval after vaccination.The maximum antibody level was recorded in T_3 that received spray vaccine along with immunomodulator.Comparision of results between group T_2 and T_3 have indicated immunomodulatory effect of *T.cordifolia(Giloe)*. The protein profile of serum sample collected periodically revealed that total protein and globulin was increased as immunity increases.Significant increase in total protein and globulin values were observed in T_3 perhaps due to hepatoprotective activity of *T.Cordifolia*.It was also concluded that supplementation with *T.cordifolia* improve performance of chicks besides immunomodulation. Hence ,Spray vaccination can be practised to reduce the vaccination and managemental stress during early life of chicks.

VI-P-11

SEROPREVALENCE OF HIV, HBV, MALARIA AND SYPHILIS INFECTIONS AMONGST BLOOD DONORS

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Transfusions related diseases are a major concern to the Transfusion medicine community as well as other medical areas. According to the report a total of 30 million units of blood or its various components are transfused each year in India. However, blood safety remains an issue of major concern in transfusion medicine. Most of the donated blood is screened for HIV alone.We determined among blood donors at B.M.C.H.R.C Hospital, the seroprevalence of human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), Malaria and syphilis. The samples were screened using Enzymatic Immunoassays (EIA), Chemiluminescent (CLIA) Immunoassays, Hemaglutination assays and Immunochromatographic tests.. Most of them were voluntary donors. Their age (in years) ranged from 20 to 60 and most (70%) were between 20-39 years.60% of the donated blood had serological evidence of infection with at least one pathogen and 10% had multiple infections. The current seroprevalence of HIV, HBsAg, Malaria and syphilis among blood donors at B.M.C.H.R.C was found to be 10%, 25%, 25% and 15%, respectively. There was no association found in the prevalence of HIV infections in patients found to be positive for syphilis. There were no significant age differences in the occurrence of pathogens. The high (70%) seroprevalence of blood-borne infections in blood donated at B.M.C.H.R.C calls for routine screening of blood donors for HBV, Malaria, HIV and syphilis and for strict selection criteria of donors, with emphasis on getting young voluntary donors and for establishment of strict guidelines for blood transfusions.

VI-P-12

MORPHOLOGICAL SURVIVAL AND SUBSEQUENT IN VITRO MATURATION OF DENUDED AND CUMULUS COMPACT BUBALINE OOCYTE CRYOPRESERVED BY ULTRA RAPID COOLING

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Culturable grade oocytes(n=380) recovered by aspiration of surface follicles from buffalo ovaries (n=97) were either mechanically denuded (DN) or kept cumulus intact(CC) and were vitrified in DPBS+0.4% sucrose,0.4% BSA and 6M concentrations of either ethylene glycol(EG) or propylene glycol (PG). Oocyte were randomly allocated to four groups of EGCC, EGDN, PGCC and PGDN respectively, of vitrificaton and cryostorage for 7-10 days in LN_2 They were then warmed to record morphologic survival and morphologically normal oocytes were matured in vitro along with fresh oocytes (control)for 24h in TCM -199 containing hormone (LH + FSH + estradiol) at 38± 1°C and $5\%CO_2$ in vitrified air in a CO2 incubator . The arcsine transferred data of the proportion of morphologic survival of oocytes and in vitro maturation of oocytes was compared by DNMR-test. The morphologically normal oocytes were significantly higher ($P<0.05$) for cumulus compact oocytes compared to denuded oocytes for both cryoprotctants EG and PG. The in vitro maturation was significantly higher ($P<.05$) for non vitrified oocytes (control) compared to vitrified oocytes. Significantly higher ($P<0.05$) proportion of cumulus compact oocytes matured in vitro (as evident by he proportion at M-11 stage and the proportion arrested) compared to denuded oocytes for both cryoprotectent EG and PG. The differences between the cryoprotectants were non significant. It was concluded that vitrification brings about damage to the oocytes which can be minimized by the presence of cumulus cells with the oocytes,whereas the two cryoprotectants EG and PG are equally effective in preventing cryodamage.

VI-P-13

LICE INFESTATION AND ITS EFFECT ON RESISTANCE IN RUMINANTS OF MARATHWADA REGION WITH SPECIAL REFERENCE TO HERBAL CONTROL

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An epidemiological study pertaining to prevalence of lice species in ruminants of Marathwada region of Maharashtra state was conducted by collecting the lice from 846 cattle, 883 buffalo, 1045 sheep and 866 goats. The lice were brought in the laboratory and specific identification was made which yielded Linognathus vituli, Haematopinus eurysternus and Damalinia bovis in cattle, Haematopinus tuberculatus In Buffalo, Damalinia ovis in Sheep and Linognathus stenopsis and Damalinia caprae in Goats. The prevalence of lice infestation on various host animals was 11.22, 32.16, 30.81 and 29.33 per cent on cattle, buffalo, sheep and goat respectively. The prevalence of lice was studied on the various body parts of host on which the lice population was predominantly occurring. In present study, the predilection sites noted were at neck and crest followed by dewlap, shoulder, loin and back, inguinal regions, tail region and rib and belly region in the descending order of prevalence in cattle, while in case of buffalo the most predominant body part bearing lice population was neck and crest, shoulder, belly, and head region. Neck region followed by belly, loin, shoulder and inguinal region were the predominant sites in case of sheep and more or less similar sites were noted for the prevalence of lice on goats. The lice population was also found scattered over the body in 14.73, 25.35, 30.12 and 25.59 per cent in cattle, buffalo, sheep and goat respectively. In the present study four herbal and one standard chemical drug were tested against lice at In vitro and In vivo level. The herbal pesticides like Karanj oil, Eucalyptus oil, Neem oil were diluted in 1 per cent Teepol solution as emulsifier. Topicure were used as such spray without any dilution. All the four herbal pesticides showed the results as Karanj oil 83-100 per cent, Eucalyptus oil 85-92 per cent, Neemazal 86-100 per cent and Topicure 100 per cent. All the pesticides showed significantly good results and seem to be promising tools as an alternative to the chemical pesticides.

VI-P-14

DIVERSITY OF PHYLLOSPHERE AND RHIZOSPHERE BACTERIAL FLORA OF *Ziziphus spp.*

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The genus *Ziziphus* belongs to the family Rhamnaceae.This genus comprises of about 100 species of deciduousor evergreen trees and shrubs distributed in the tropicaland subtropical regions of the orld. *Ziziphus* speciescan grow either as shrublets, shrubs or trees with thornybranches and are used as a hedge to form defensivefences for animals. Some species, like*Ziziphus mauritiana* Lam. and *Ziziphus numularia* (L.)wild occur on nearly every continent. *Z. mauritiana* and *Z. numularia* have very nutritious fruits and are usuallyeaten fresh. The fruits are applied on cuts and ulcers.They are lso used to treat pulmonary ailments andfevers and to promote the healing of fresh wounds, fordysentery. The leaves are appliedlocally to sores, and the roots are used to cure andprevent skin diseases. To expose the bacterial flora of phyllosphere and Rhizosphere bacterial flora the leaves and root soil with root were collected in natural environment in a sterile poly bag and transported to Department of Microbiology, M. N. Institute of applied sciences and isolate the organisms with basic and special media with basic microbiological techniques and identified as per biochemical key test of Bergey's manual of systematic bacteriology. In overall, comparative analysis of phyllosphere and rhizosphere bacterial flora of Ziziphus spp. both species harbor many types of bacteria where Z. mauritiana having much bacterial flora than the Z. numularia. In phyllosphere of *Z. mauritiana,* various types of aerobic and common bacteria like *Staphylococcus aureus, Enterobacter aeruginosa, Micrococcus spp., Pseudomonas spp.,* and *Klebsiella spp.* were identified where in same region of *Z. numularia* various species of *Bacillus* like *Bacillus cereus, Bacillus megaterium* were abundantly present with most common Gram positive aerobic *Staphylococcus aureus.* In Rhizosphere of Z. mauriatiana region *contain E.coli, Bacillus cereus, Bacillus polymexa and Bacillus megaterium and Z. numularia Bacillus cereus and Bacillus polymexa.* Presence of Gram negative fermenting organisms on phyllospheric region showing that plant complete there all the respiration activity very easily in this extreme environment and combination of *Staphylococcus aureus* and *Micrococcus leuteus* indicate the high content of mineral and mineral-acid-transformation reaction in plant. Where the Rhizospheric region the abundant growth of *Bacillus spp.* with minute growth of fermenting bacteria indicating the preliminary antagonism and commensalism between plant and microbes.

VI-P-15

ISOLATION AND IDENTIFICATION OF THERMOSTABLE á- AMYLASE PRODUCING BACTERIUM IN SOIL SAMPLES OF BIKANER REGION

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Recent discoveries of starch degrading enzymes have led to increased application of amylases in various industrial processes. The á- amylase (1,4-á-D glucan glucanhydrolase, EC 3.2.1.1.) hydrolyses á-1,4 glycosydic linkage in starch related substrate. This enzyme has extensive commercial applications in starch liquification, brewing, Sizing in textile industries etc. Thermostability, it a feature of most of the enzymes sold for bulk industrial uses and thermophillic organisms are therefore of special interest as a source of novel thermostable enzymes.The present study deals with the isolation and identification of thermostable á-amylase producing bacterium in Bikaner region. To isolate and identify the bacterium, in summer app. 20 soil samples were collected in sterile polythene bags from various location of Bikaner region and transported to Department of Microbiology, M. N. Institute of Applied sciences for further processing. In lab, soil suspension in sterilized water were poured and spread on agar plate. These plates then incubated at 55 C for 2 days. Several amylase producing bacterial colonies were produced and then selected after flooding the plate with iodine solution. The strain that yielded high level of alpha amylase was selected for further identification.The strain was Gram positive Bacillus, negative on voges-praskauer test and facultative anaerobic. The strain possessed the ability to hydrolyze starch and gelatin. Catalase was positive. Indole was not formed and acetoin formation was positive.From these results and as per molecular and biochemical key test of Bergey's manual of systematic bacteriology, the strain was identified as *Bacillus licheniformis* in Bikaner soil samples as maximum yield producing bacterium.

VI-P-16

ISOLATION AND QUANTIFICATION OF PLANT SECONDARY METABOLITES IN TERMS OF PHENOL AND CONDENSED TANNIN IN SHRUBS OF ARID ZONE

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Leaves of 5 plant species viz., *Capparis aphylla* , *Aeura tomentosa, Leptadenia reticulata, Calligonum polygonoides* and *Calotropis gigantea* collected from arid zone of Rajasthan have been analyzed for their chemical composition like nutritional parameters (crude protein, cell wall fractions) and antinutritional factors (total phenol and condensed tannin). The samples collected were dried, ground and subjected to analysis for dry matter, gross nutrients and cell wall fractions (Van Soest et al.,1991).The condensed tannin and total phenol were estimated by Vanillin-HCl (Burns,1971) and Prussian blue method (Price & Butler,1977) respectively. The chemical composition of the plants showed that crude protein content varied between 10.32-16.8%, ether extract between 1.27-4.05%, crude fibre between 13.33-39.4% & ash varied between 2-10%. Among the cell wall fractions the NDF and ADF content varied between 50.49-75.58% and 17.23-24.9% respectively. The hemicellulose content varied from 27.14-47.2%. The contents of total phenol (g/kg) and condensed tannin (g/kg) in different samples were estimated to be 72.41 & 4.32 in *Capparis aphylla*, 53.23 & 1.42 in *Aeura tomentosa*, 121 & 11.46 in *Leptadenia reticulata*, 56.21 & 1.08 in *Calligonum polygonoides*, and 171.58 & 15.42 in *Calotropis gigantea*.

VI-P-17

VITRIFICATION OF IMMATURE AND IN VITRO MATURED CUMULUS COMPACT AND DENUDED GOAT OOCYTES AND THEIR SUBSEQUENT FERTILIZATION IN VITRO

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The morphological survival, *in vitro* maturation and *in vitro* fertilization of cumulus compact immature (ImCC), and in vitro matured (MCC) and, denuded immature (ImDN) and denuded in vitro matured (MDN) goat oocytes vitrified using DPBS + 0.5% sucrose + 0.4% BSA with 8 M propylene glycol was studied in two separate experiments (Experiment 1 and 2) in order to evaluate the effect of cumulus cells and the meiotic stage at cryopreservation on subsequent oocytes development. After 7-10 days of cryostorage oocytes were rewarmed to record i) morphological survival (Experiment 1 and 2) ii) nuclear maturation (Experiment 1, 10 replicates) and iii) fertilization 24 h after inseminatioin with prepared epididymal buck sperms (Experiment 2, 8 replicates per group). The arcsin transformed data of the proportion of oocytes survived, matured or fertilized was compared by DNMR - test. The proportion of oocytes that were recovered in morphologically normal form were highest for cumulus compact mature (MCC) oocytes in Experiment 1 and cumulus compact immature (ImCC) oocytes in Experiment 2. The presence of cumulus cells had a significant effect in preventing morphologic damage whereas the meiotic stage at maturation had little effect. At the end of experiment 1 and 2, the nuclear maturation and fertilizationf of fresh oocytes was significantly higher ($P < 0.05$) compared to vitrified oocytes. The nuclear maturation was significantly affected by the presence or absence of cumulus cells but not by the meiotic stage at maturation however, subsequent fertilization of oocytes was significantly higher ($P < 0.05$) for immature vitrified oocytes compared to *in vitro* mature vitrified goat oocytes and not different for denuded and cumulus compact oocytes. It was concluded that cumulus cells attached to oocytes decrease morphologic damage during vitrification and support their nuclear maturation, however ,the subsequent fertilization of vitrified goat oocytes is more dependent on the meiotic stage at vitrification, with immature oocytes being better compared to matured oocytes.

VI-P-18

IDENTIFICATION OF EFFECTIVE ANTIBIOTICS AGAINST PRESENT SCENARIO OF URINARY TRACT INFECTION CAUSING BACTERIA IN BIKANER REGION

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The urinary tract is the body's filtering system for removal of liquid wastes. Because we have a shorter urinary tract, women are especially susceptible to bacteria that may invade the urinary tract and multiply — resulting in infection known as a urinary tract infection, or UTI. The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Although they cause discomfort, urinary tract infections can usually be easily treated with a short course of antibiotics. Symptoms include frequent feeling and/or need to urinate, pain during urination, and cloudy urine.Although antibiotics are the first treatment choice for urinary tract infections, antibiotic-resistant strains of *E. coli*, the most common cause of UTIs, are increasing worldwide. As more bacteria have become resistant to the standard UTI treatment trimethoprim-sulfamethoxazole (TMP-SMX), more doctors have prescribed quinolone antibiotics to treat UTIs. In some areas, quinolones have now overtaken TMP-SMX as the most commonly prescribed antibiotic for UTIs. Researchers are concerned that resistance may develop to these drugs as well. To develop complete antibiogram against urinary tract infection causing bacteria, already isolated & identified cultures of *E.coli, Klebsiella spp., Pseudomonas spp. & Enterobacter spp.* were collected from government hospital laboratory and from some multinational diagnostic centers branches like Pathnet india, Piramal health care and Metropolisindia health care services. All the cultures then transported to Department of Microbiology, M. N. Institute of applied sciences and re-identified through selective media and rapid diagnostic kits. After complete identification process, antibiotic sensitivity test performed on Muller Hinton Agar by Disk plate method with present line and traditional antibiotics i.e. Gentamycin, Amikacin, Levofloxacin, Cefuroxime, Cefexime, Cephalothin, Cefeperazone, Cefepime, Meropenem, Imipenem, Pipericilline/Tazobactem, Co-trimaxazole, Chloremphenicol, Tobaromycin, Netilimycin, Prulifloxacin, polymixin-B, Gatifloxacin, Amoxycilline, Ampicilline, Penicilline, Ciprofloxacin, Norfloxacin, Nitrofurentoin and Cefetezimide. After analysis of zone of inhibition, maximum sensitivity measured in Meropenem, Pipericilline/Tazobactem, Cefexime, Ceftriaxone, Gatifloxacin and rest antibiotics have vary the effect as per bacterial strain where present line Cefuroxime & Norfloxacin resist almost by all bacteria. According to result the more suitable antibiotics in UTIs are Meropenem, Pipercilline/ Tazobactem & Cefexime where rest of the antibiotics beside cefuroxime and norfloxacin, can be used in routine treatment of urinary tract infections.

VI-P-19

HUMAN HEALTH HAZARDS DUE TO MICROBIAL CONTAMINATION IN SAMPLES OF SPICES COLLECTED FROM DIFFERENT AREAS OF UDAIPUR CITY OF SOUTH RAJASTHAN

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Spices are the essential part of every food item cooked in Indian kitchen and is used by every common man in his daily life. However unhygienic conditions of handling and improper storage techniques, lack of knowledge & awareness and lack of strict rules have emerged to be the main reasons for such contaminations causing severe health hazards due to heavy microbial contaminations in spices samples. Samples of chilli, coriander and turmeric were collected from three different areas of Udaipur city ie, Surajpole (heart of city),Udaipole (main bus stand and hotels),and Chetak Circle(Hospital area),and brought to laboratory for microbial contamination studies by adopting slandered protocol. The extents of microbial contaminations were alarmingly high in all the samples at all the sampling sites.The mean colony count for *Staphylocococcus aureus* was 7.11,7.44 and 8.00 x10^5CFU/gm for chilli samples ,7.56,8.78 and 9.61 x10^5CFU/gm for coriander samples and 6.72,8.78 and 5.94 x10^5CFU/gm for turmeric samples from site i ii and iii respectively. Similarly when all the three spices were collected from three different sites and analysed for yeast and mould, the situation was really alarming with 12.50,98.44 &11.83,11.00,11.22 & 10.89,12.11,12.06 &10.22x10^5CFU /gm for chill ,coriander and turmeric respectively for all the three sites respectively. Microbiological analysis is the useful ways to assess the safety and quality of food .Substantial number of micro-organisms suggest a general lack of cleanliness in handling and improper storage practices. Udaipur a city of tourists is also not untouched from the supply of contaminated important edible items like spices. Hence an immediate step should be taken to educate shopkeepers for proper handling and storage and strict food safety rules should be made and implemented strictly too. Microbial contamination, spices , *Staphylococcus aureus* ,yeast &mould

VI-P-20

STUDIES ON SEMEN COLLECTION BEHAVIOUR AND SEMINAL CHARACTERISTICS IN THE DOG

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Studies on sex behaviour during digital semen collection and on seminal evaluation parameters were conducted in dogs of different breeds. Thirty six semen samples (six ejaculates/dog) from six sexually mature dogs, three each of German shepherd and Labrador breeds, aged from 2 to 6 years were collected by digital manipulation and subjected to macroscopic, microscopic semen evaluation test and stored the II fraction of ejaculates at 5^0C for 3 days in EYC and TFC semen extenders for keeping quality determination. The parameters like reaction time, ejaculation time and response to simulating locking mode of penis were also recorded. The overall mean value of I, II and III fraction of ejaculates was 7.51, 1.56 and 27.16 ml, respectively. The overall mean value for sperm motility, sperm concentration, live sperm, sperm abnormalities, damaged acrosome, hypo osmotic swelling and cold shock resistant sperm was 64.44%, 394.25 million/ml, 79.83%, 15.00%, 7.39%, 68.97% and 31.86%, respectively. EYC proves better to TFC subsequent to first day.

VI-P-21

ANTIBACTERIAL ACTIVITY OF ZIZIPHUS NUMMULARIA AND PROSOPIS CINERARIA LEAVES EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI.

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About 80% of world's rural population relies on herbal traditional medicines as their primary health care. *Ziziphus spp.* and *Prospis spp* are common plants of arid region which are used as feed as well as have been reported to have medicinal properties. *Ziziphus spp.* is used as astringents, demulcent, anodyne, etc. (Nazif, 2002). *Prosopis juliflora* has been reported to have antibacterial activities (Sathiya and Muthuchelian, 2008). Since both these plants are commonly found in this region the present study was planned to study antibacterial activities of these plants the antibacterial effect of aqueous, acetone, benzene, chloroform and ether extracts of leaves of *Prosopis cineraria* and *Ziziphus nummularia* on *S. aureus.* & *E. coli* by the agar disc diffusion method (CLSI, 2005). All the assays were carried out in duplicates and the average values of the two experiments were recorded. It was found that for *S. aureus, Prosopis cineraria* extract in benzene had the best antibacterial properties followed by ether, aqueous, *chloroform and* acetone extracts, respectively and in case of *Ziziphus nummularia* extract, ether had the best antibacterial properties followed in order by benzene, chloroform, aqueous and acetone, respectively. The *benzene leaves extract of Prosopis cineraria had the maximum antibacterial activity against S. aureus. No antibacterial activity was obtained against E.coli by* both plants.

VI-P-22

ISOLATION AND IDENTIFICATION OF URINARY TRACT INFECTION CAUSING BACTERIA IN BIKANER REGION

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Urinary tract infections are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body and it become more sever in later form of renal failure. Women are especially prone to UTIs for reasons that are not yet well understood. UTIs in men are not as common as in women but can be very serious when they do occur. The urinary system consists of the kidneys, ureters, bladder, and urethra. The key elements in the system are the kidneys and it removes excess liquid and wastes from the blood in the form of urine. Narrow tubes called ureters carry urine from the kidneys to the bladder. Urine is stored in the bladder and emptied through the urethra. To expose the diversity of urinary tract infection bacteria, all the samples were collected in screw capped sterile container from Government hospital laboratories, some private hospital laboratory, some private multinational diagnostic centre like Metropolisindia health services, Piramal health care, Pathnet india etc. and from individual patients too. All the samples transported to department of Microbiology, M. N. Institute of applied sciences and isolation process were completed with serial dilution agar plating and spread plate method and colonies were further purified and separately isolated by Quadrate streaking method and continue microscopic observation with Gram staining, after that all the isolates were identified on the basis of various biochemical reaction as per mentioned in Bergey's manual of systematic of Bacteriology. All the data's showed much diversity with dominant infection of *Enterobacteriaceae* family in urinary tract infection in Bikaner region where *E.coli* (65%), *Klebsiella spp.* (20%), *Pseudomonas aeruginosa* (10%) and *Enterobacter spp.* (05%) found as main causal organisms. In view of sex , females have more infection (45%) as compare to male (30%) and in view of age group 12 yr -25 yr and 45 yr and above are normally showing maximum infection as compare to mid age group. There is one main finding that 15% of pediatrics cases are suffering from this kind of severe infection with *Klebsiella spp.* & *Pseudomonas aeruginosa* in Bikaner region which is not reported till date as per best of our knowledge.

VI-P-23

ISOLATION, CULTIVATION AND IDENTIFICATION OF UPPER RESPIRATORY TRACT INFECTION CAUSING BACTERIA IN BIKANER ZONE.

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Animal and human bear microflora on their body surface and other parts exposed through various tracts to the external environment and show a great diversity. The availability of bacterial flora varies according various environmental conditions.Present study carried out in Bikaner and showing much diversity of human upper respiratory tract infection causing bacterial flora in various parameters. For isolation, cultivation and identification purpose, the human sputum samples were collected in sterile capped container and transported to Department of Microbiology, M. N. Institute of applied sciences and then pure cultures were isolated with various selective / special media like MSA, PsA, EMB, MacConkey etc. and techniques and all the isolates then identified as per biochemical key of Bergey's manual of systematic bacteriology. In samples most common bacteria are *Staphylococcus aureus* (38%), *Corynebacterium spp.* (20%), *E.coli* (10%), *Klebsiella pneumonia* (8%), *Streptococcus pyogens* (10%), *Pseudomonas aeruginosa* (8%), *Haemophillus spp.* (4%), and rare are *Nesseria spp.*(2%). All the data's showing that most of the persons in Bikaner region suffering from *Staphylococcus aureus* but the presence of *Nessria spp.* indicate the seriousness of in Bikaner too.

VI-P-24

ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA ASSOCIATED WITH OTITIS EXTERNA AND DIARRHOEA IN DOGS

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Chronic ear infection (*Otitis externa*) and diarrhoea in dogs are common presenting problems to the dog owners as well as pet practitioners. Dogs were clinically examined and bacterial pathogens were isolated from 23 ear swabs and 30 rectal swabs. Isolation and identification by morphology, cultural character and biochemical tests of fresh isolates resulted a total of 105 isolates namely *Escherichia coli* (23), *Staphylococcus* (19), *Pseudomonas aeruginosa* (14), *Proteus mirabilis* (16), *Proteus vulgaris* (7), *Klebsiella pneumoniae* (11), *Klebsiella oxytoca* (6), *Salmonella* spp (4) and *Micrococcus* (5). The results on microbial investigation of ear infection indicated that the highest prevalence rate was of *Staphylococcus* spp (34.53%) . 7 out of 19 isolates of *Staphylococcus aureus* were coagulase positive (12.72%). Incidence of other pathogenic bacteria namely *Pseudomonas aeruginosa* , *Proteus mirabilis, Proteus vulgaris Klebsiella pneumoniae, Klebsiella oxytoca, Micrococcus* was 25.45%, 16.36%, 5.45%, 9.09%, 5.45% and 2.36% respectively. However, pathogenic bacteria isolated from diarrhoeic dogs were *Escherichia coli* (46%), *Proteus mirabilis* (14%), *Proteus vulgaris* (8%), *Klebsiella pneumoniae* (12%), *Klebsiella oxytoca* (6%), *Salmonella*(8%) and *Micrococcus* (6%). The present study is helpful for the characterization of common bacteria associated with ear and intestinal infection in dogs.

VI-P-25

ANTIBIOTIC RESISTANCE AMONG *PSEUDOMONAS AERUGINOSA* ISOLATES OBTAINED FROM DIFFERENT ECOLOGICAL NICHES

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Present study was conducted to obtain antibiogram of *Pseudomonas aeruginosa* isolates collected from different ecological niches. Out of total 296 samples collected from clinical, non-clinical and environmental niches, 64 were preliminarily identified as *P.aeruginosa.* Upon further confirmation with other phenotypic (biochemical tests) and genotypic (16S rRNA gene based ribotyping) methods, 61 isolates could be typed as *Pseudomonas* and 43 isolates could be typed as *P.aeruginosa.* The confirmed 43 *P.aeruginosa* isolates were subjected for resistotyping with 21 antibiotics of different groups. All *P.aeruginosa* isolates from the three ecological niches were found to be resistant to ampicillin and vancomycin (resistance-100%) whereas most of the isolates were resistant to minocycline (resistance-97.67%), rifampicin and nitrofurantoin (resistance-95.35%), cephalothin (resistance-93.02%) tetracycline (resistance-90.70%), co-trimoxazole (resistance-76.70%), chloramphenicol (resistance-53.49%) and cephotaxime (resistance-51.16%). All the isolates of three niches were sensitive to polymyxin (resistance-0%). On the other hand all the isolates were proved to be lesser resistant to colistin (resistance-20.93%), ticarcillin and ceftazidime (resistance-18.60%), amikacin (resistance-9.30%), imepenem (resistance-6.98%), gentamicin, tobramicin and ofloxacin (resistance-4.65%), ciprofloxacin and carbanicillin (resistance- 2.32%). Rate of resistance was almost similar in environmental isolates to that of isolates of other two niches for most of the antibiotics except for colistin and cephotaxime to which comparatively less resistance was observed among environmental isolates. This increasing trend of resistance in isolates from environmental niches is alarming signal and indicative of antibiotic abuse which needs to be sorted out by preventing the indiscriminate use of the antibiotics.

VI-P-26

STUDIES ON HAEMATO-BIOCHEMICAL AND MINERAL STATUS OF KETOTIC COWS

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The study was conducted on ketosis in and around Bikaner. The prevalence was higher in 6 to 9 years of age, 0 to 1 months post-partum, between second and forth lactation and particularly in November-December months. The major clinical manifestation were abrupt decrease in milk yield, cessation of feeding of concentrate, decreased fodder intake, firm mucous-coated faeces, constipation and acetone like odour in urine of severe cases of ketosis. The temperature, pulse and respiration rate were in normal range in ketotic cows. Ruminal movements decreased significantly. There was no significant difference in haematological parameter viz- Hb, PCV, TEC, TLC and DLC except eosinophils in ketotic cows as compared to apparently healthy cows. Serum glucose was significantly lower in ketotic cows and other biochemical parameters like serum ketone, serum total protein, serum albumin, serum globulin, serum cholesterol, serum triglycerids, serum total lipids, serum enzyme i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated significantly in ketotic cows as compared to apparently healthy cows. Mineral parameters of ketotic cows showed non- significant difference in serum Phosphorus, Iron, Manganse and Molybdenum. While a significant increased level of serum Magnesium was recorded. There was significant decrease in serum calcium, copper, cobalt and zinc level in ketotic cows.

Vi-P-27

EFFICACY OF SPRAY VACCINATION AGAINST NEWCASTLE DISEASE VIRUS IN EXPERIMENTAL FIELD TRIAL ON BACKYARD BROILER CHICKS

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A comparitive study was undertaken in two different groups of 25 (day-old broiler chicks) each. The first group was kept in intensive system and other in backyard system. Commercial vaccine (Ventri Biologicals, India) of Live B1 and Lasota strains were used at 7th day for priming and 28th day of age as booster, respectively . Each vaccine was reconstitued to get aerosol containing droplets of 150-160 μm sprayed on chicks of both groups by aerosol vaccinator (Desvac, France). The specific antibody level was measured by micro Haemagglutination inhibition (HI) and virus neutralization index (VNI) at weekly interval up to five weeks. On day 36, the HI titre did not differ significantly, although it was higher (5.77 ± 0.29)in first group then in second (5.08 ± 0.29). Similair results were obtained in Virus neutralization test in which VNI of first and second group was 2.90 and 2.15, respectively. The chicks in both the group showed a increasing trend in the HI antibody titre from 7th day to 36th day. The low level of HI antibody titre in second group reared by backyard system may be due low nutritional status of the chicks. Hence, it may be concluded that backyard poultry can be effectively and conveviently vaccinated by aerosol route.

VI-P-28

MICROBIOLOGICAL EVALUATION OF CLINICAL WOUNDS IN CAMEL

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Cutaneous wound with infection or abscessation is common in camel. A microbiological study of clinical cases of wounds was conducted to indentify the microorganism causing wound infection and their sensitivity pattern in camel.The microorganism isolated from varieties of septic wound in camels were identified in to 21 different bacterial species belonged to the genera Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Bacillus, Pseudomonas, Escherichia, Proteus, Klebsiella and Actinobacillus. The bacterial isolated from the camel wounds showed high sensitivity to gentamycin, triple sulpha, chloramphenicol and trimethoprim. A moderate sensitivity was shown to ampicillin, erythromycin, nitrofurazone and penicillin –G and low sensitivity was revealed for furazolidone, oxytetracycline, nalidixic acid and streptomycin.

VI-P-29

ANTIBACTERIAL ACTIVITY IN LEAVES AND CALLUS CULTURE OF *OCIMUM SANCTUM* (TULSI)

Nag T N[1], Tyagi Sapna[2], MathurKavita[3], Vyas Man Mohan[4], Kumar Sunil[2].

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Ocimum sanctum, the holi basil with its wonderful characters, is one of the most popular & widely grown herbs in the world. *Ocimum* sanctum & other *ocimum* species are members of the family *Lamiaceae*. The plants as well as their oil have received lots of attention for their potential medicinal properties of these plants; *ocimum* sanctum is the most widely used in cosmetic, liqueur medicine & perfumes. Present study has been carried out to expose the antibacterial activity in *Ocimum sanctum* of Bikaner region against present Gram +ve& Gram –ve bacteria. Plant sample of *ocimum* were collected from various region of Bikaner in a sterile polybag & identified the *ocimum* species from Plant herbaria Centre of Govt. Dungar College and the transported to Department of Plant Tissue Culture, M.N. Institute of Applied Sciences, for further processing. To evaluate the antimicrobial activity, the test organism, *E. coli, Proteous vulgaris, Bacillus cerus* and *Mycobacterium Smegmatis* ATCC 14468 were obtained from culture collection of NCL Pune. After Callus formation of plant leaves on M S media, the Ethanol and Aqueous extracts of both natural leaves and callus were prepared and applied against test organism with various concentration and calculate the affectivity per day by SPC method. The results showed, Alcoholic extract is more effective as compare to aqueous extract against Gram +ve & Gram –ve bacteria. Where in view of *Mycobacterium smegmatis* ATCC 14468 , leaf alcoholic extract show much effectively & we got a high definition curve showing great inhibitory effectively during the whole life of organism , where the aqueous extract effects on the earliest stage of stationary phase then it became ineffective. According to all results, it opens the gate for discovering new effective and efficient drugs for Tuberculosis & routine side effect free drugs for Common Cold, Dyspepsia Colitis, Enterogastritis Etc.

VI-P-30

ANTIMICROBIAL SCREENING OF SEED PART OF *ZIZIPHUS NUMMULARIA* AGAINST SOME BACTERIAL PATHOGENS

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Antibiotics are essential parts for combating harmful infections *in vivo*. During the last decade, infectious disease have played a significant role in death of millions around the world especially in developing countries like India. Ziziphus nummularia is among widely consumed fruit in arid zone of Rajasthan. The aim of present study was screening of antimicrobial activity in leaf part of Ziziphus nummularia. Present studies entitled "**ANTIMICROBIAL SCREENING OF SEED PART ZIZIPHUS NUMMULARIA AGAINST SOME BACTERIAL PATHOGENS**" was undertaken to determine antimicrobial activity of leaf extracts prepaired in water and ethanol against some bacterial and fungal pathogens of clinical origin. In the present study water and ethanol extract of leaves were screened against gram positive and gram negative bacteria of clinical origin for their antimicrobial activity. Antimicrobial assay was performed by agar disc diffusion method. 15ml of molten nutrient agar medium was inoculated with 1 ml of the innoculumn and poured into sterile petriplates. A 6mm sterilized filter paper disc was saturated with each extract, allowed to dried and introduced on the surface of the seeded of the agar plates. The plates were incubated over night at $37^{\pm\%}$ C in incubator and observed after 24 Hr for bacterial strain. Antimicrobial activity was determined by measuring the diameter of zone of inhibition (in mm)produced by the respective extract. The water extract of seed showed activity against S.epidermidis and P.aeruginosa whereas ethanolic extract showed activity against B.cerus, S.aureus,S.epidermidis P.aeruginosa and P.vulgaris In the present study it has been observed that ethanolic extract was found to be more active than water extract against the micro organisms tested but none of the extract showed activity against E.coli .The result showed that the use of organic solvent in the preparation of leaf extract provides more consistent antibacterial and anticandidal activity as compaired to water extracts.

VI-P-31

OVULATORY RESPONSES OF DIFFERENT DOSES OF FOLLITROPIN-V AND THE EMBRYO RECOVERIES IN RATHI CATTLE

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Two different doses of Follitropin-V were evaluated for superovulation in Rathi cows. Cows were implanted with a progestagen vaginal implant (CIDR-B) and superovulatory protocol started on day 8-10 of the implant. Cows were treated with either 20 mg of Follitropin-V administered in 8 divided doses given IM,12 h apart (Group I, n=5) or 10 mg of Follitropin-V given in a similar pattern of 8 divided doses (Group II, n=5). Prostaglandin was injected at the time of last injection of Follitropin-V and cows were mated to fertile bulls when in estrus (day 0). Seven days after mating cows were examined to record the ovulatory response and embryos were recovered by non-surgical methods. The mean superovulatory responses were 5.8 ± 1.83 and 9.8 ± 3.16 in Group I and II and total embryo recovery rates were 3 ± 0.45 and 6.4 ± 3.45 respectively.Differences between the groups were significant ($P<0.05$) . It was concluded that Rathi cows can be successfully superouvlated by using Follitropin-V with a dose of 10 mg being better.

VI-P-32

EXPRESSION OF BOVINE (BOS TAURUS INDICUS) INTERFERON Á IN E.COLI AND EVALUATION OF IT'S BIOLOGICAL ACTIVITY

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Interferon á (IFN-á) a member of type I Interferon family play important role in the regulation of innate and adaptive immune responses. Present study is envisaged to clone and express bovine IFN-á and evaluate it's antiviral and biological activitties with a objective to use it as genetic adjuvant for protein/DNA vaccines. PBMC isolated from cattle blood, were stimulated with LPS and the total RNA was extracted, cDNA synthesized using Oligo dT primer and IFN-á full length coding sequence was amplified by PCR using specific primers. Amplified product was cloned in pcDNA vector. The sequence analysis has shown 96% and 94% similarity with buffalo and goat IFN-á sequences respectively. To express the mature protein sequence coding for matured protein was amplified from IFN-á pcDNA plasmid and cloned in pET32a vector. 35kDa protein expressed in E.coli BL21 cells on induction with IPTG and the expressed protein was purified by NI-NTA column. Specificity of the expressed IFN-á was confirmed by western blotting using monoclonal abs for mouse IFN-á. Pre treatment of BHK cells with IFN-á inhibited the FMD viral replication which was measured by ELISA and qPCR. Biological activity of IFN-á was evaluated by measuring the stimulation of interferon stimulating genes (ISGs) like PKR, OAS, Mx1 proteins, STAT1, STAT2 by qPCR. Similarly, up regulation of IL-2, IL-4, IL12, IL-18 and IFN-ã in IFN-á treated PBMCs was observed. Thus present study has shown the expression of biologically active IFN-á which can be used as a potent adjuvant to Protein/DNA vaccines.

VI-P-33

FORMATION OF ANURAN LENS *IN VITRO* BY TRANS-DIFFERENTIATION OF PIGMENTED EPITHELIAL CELLS

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2 Prof. & Head, Department of Zoology, Dungar College, Bikaner (334001), Rajasthan, India

Cultures of anuran lens epithelial cells have been established using the homogenized retinal iris tissue of young tadpoles (3-toe stage tadpoles) of *E. cyanophlyctis*. These tissues undergo dedifferentiation and subsequently transdifferentiate into lens in culture medium. Trans-differentiation is the process by which differentiated cells alter their identity to become other, distinct cell types. The conversion of iris pigmented epithelial cells (PECs) into lens is well known not only in Urodele amphibians but also widely conserved in vertebrates. When culture became confluent 3-4 weeks after seeding tiny lens like structures differentiated from foci of cultured pigmented iris epithelium cells. The percentage of lens formation was higher in vitamin A treated cases. The culture system appears to be a suitable one for investigating the changes occurred during trans-differentiation of pigmented epithelial cells into lens.

VI-P-34

ATTEMPTS TO STUDY THE ANTIBIOTIC PRODUCING THERMOPHILLIC BACTERIA

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Micro organisms function in eco system that develops under a wide range of environmental condition. some micro organism are thermophillic,they can grow at temperature of 55*c or higher. The minimum growth is usually around 45*c and they often have optimum between 55*c and 65*c. Almost all of the thermophillic aerobic spore forming bacteria can produce more or less acid from a wide variety of carbohydrate and related compounds. Thermophillic bacteria were found in sand dunes of Bikaner. Cultivated actinobacteria are a group of gram positive with high G+C ratio. They include some of the most common soil life, fresh water life & of sand dunes. These organism play an important role in decomposition of organic matter, such as cellulose, chitin e.t.c. Most of actinobacteria are of medical or economic importance. Streptomyces notable as a source of antibiotic. Capacity of any micro organism to produce substance which inhibit the growth of any micro organism are called antibiotics. In the present study organism thermoactinomycities from sand was isolated and its capacity to produce the antibiotics material was studied. The organism to be not distinguishable for thermoactinomicities species. Its activity against staphylococcus was studied and zone of 4 to 5 mm surrounding the colonies was seen. Preliminary inoculation was done on nutrient agar media & incubated at 61*c & the growth was observed for one week. The colony started appearing short circular, white colony. The organism is gram positive, rod shaped, spore forming and filamentous.

VI-P-35

NUTRITIVE STATUS OF PLANT PARTS AND CALLUS CULTURES OF ARID ZONE SPECIES-ZIZIPHUS NUMMULARIA

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M.N Institute of Applied Sciences, Bikaner-334001, Rajasthan, India

Terrestrial plants growing in desert of Rajasthan may contain sufficient amount of nutrients to be considered as livestock feed. Callus culture of these plants also have sufficient amount of products of nutritional values. *Fully developed and healthy plant parts as leaves and fruits were collected and identified. Unorganized tissue cultures of* Ziziphus nummularia *were raised from leaves and maintained on MS media supplemented with 2mg/L, 2,4-D, IAA and Kinetin.*Plant parts as well as callus cultures were dried and analyzed for their nutritive contents as crude protein, ether extract, crude fiber, nitrogen free extract, phosphorus and calcium. Callus cultures have more crude protein (26.6%) contentment when compared with leaves (12.68%) where as ether extract (6.21%) is more in leaves then callus (1.28%). In vivo *studies showed more crude fiber (15.97%) when compared with cultures (5.55%). Minerals were however reported to be high in plant parts. Fruits which are locally called as "Ber" or "Bordi" have fare amount of vitamin C and minerals. Leaves, fruits and callus cultures of* Z.nummularia *contain sufficient amount of nutrients to be considered as livestock feed and edible fruits for local people.*

VI-P-36

UPGRADING OF THARPARKER CATTLE BREED THROUGH SELECTIVE BREEDING

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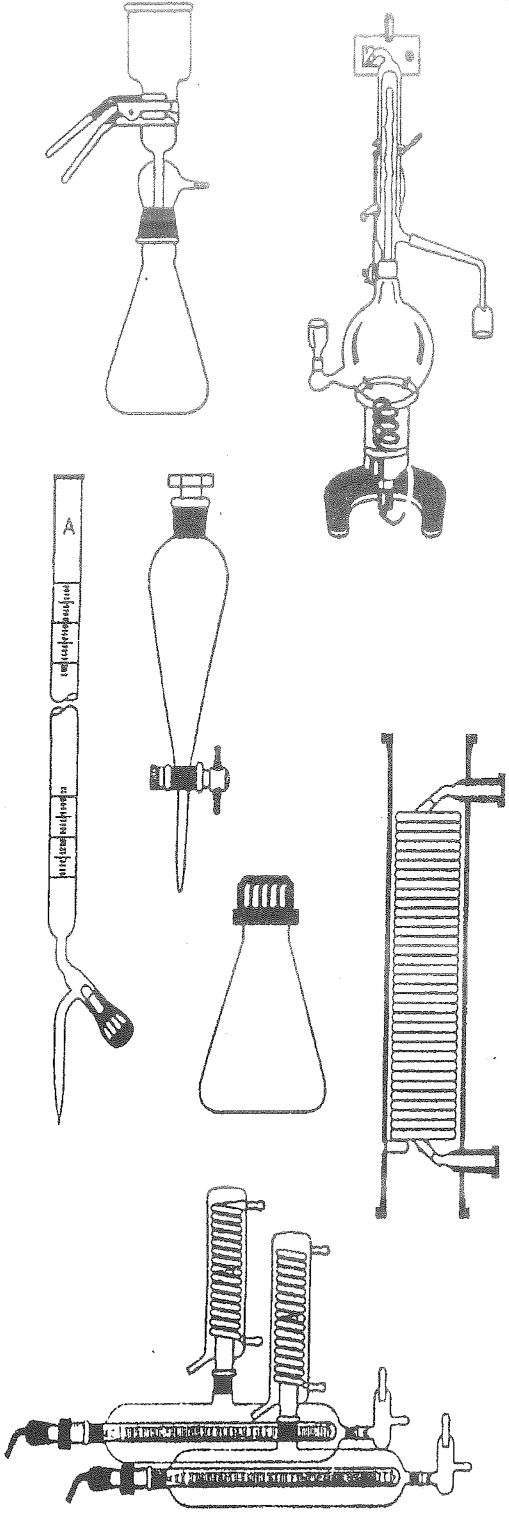
Directorate of Veterinary and Animal Science
Rajasthan University of Veterinary & Animal Sciences, Bikaner

The tharparker breed evolved as a drought resistant, heat tolerant and disease resistant breed of cattle, most suitable for hostile climatic conditions and scare feed resources. The breeding tract of tharparker breed are Jaisalmer, Barmer, Jodhpur, Pali and some parts of Nagaur, Bikaner & Srigangannagar Districts. The selected male bulls are used for breeding purposes. These male bulls are used continuously for 3 years; after 3 years these male bulls are changed due to avoid inbreeding.

From the last 8 years studies, the following parameters are obtained from these animals.

1.	The total average milk production per lactation per cow	-	2400 Kg	±	50 K.g.
2.	Total average lactation length per lactation per cow	-	330 Kg	±	15 days
3.	Average milk production in 305 days per cow	-	2250	±	100 Kg
4.	Average wet milk production per cow per days	-	7.80	±	0.18 Kg
5.	Average herd milk production per cow per days	-	5.25	±	0.2 Kg
6.	Average dry period per cow	-	125	±	15 days
7.	Average calving interval	-	450	±	30 days
8.	Average age at first calving	-	60	±	6 months
9.	Average mortality rate (%)	-	2.20	±	0.4%
10.	Average calving in the life per animal	-	15	±	2 No.
11.	Average age of the animal	-	21	±	2 years

This breed is very useful for western parts of the Rajasthan specially Jaisalmer and Barmer districts peoples. These areas only the Animal Husbandry is the sustainable, most of the people are dependent on animal farming, these farming are the only source of Income for alleviation of poverty and food security.



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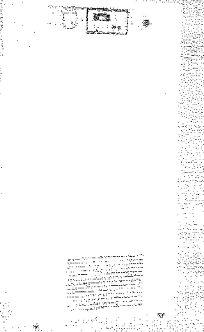
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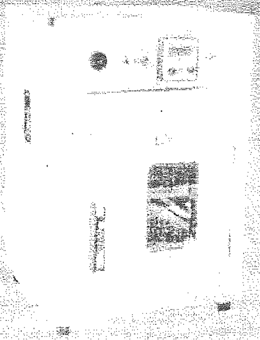
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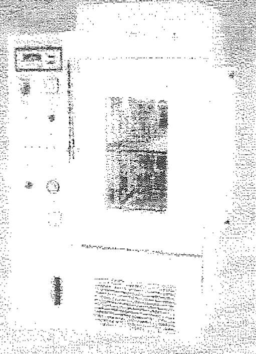
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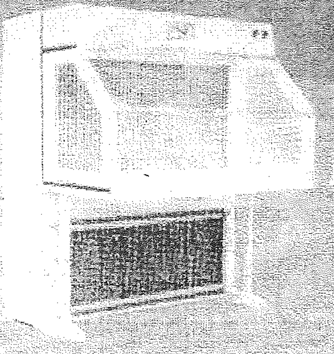
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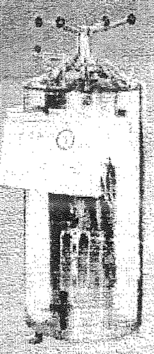
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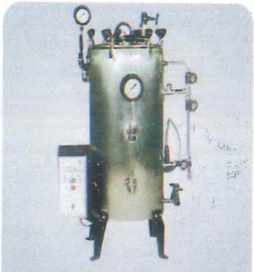

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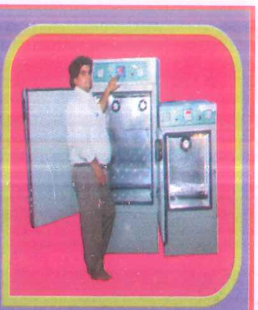

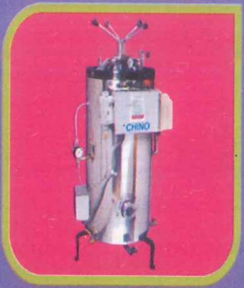
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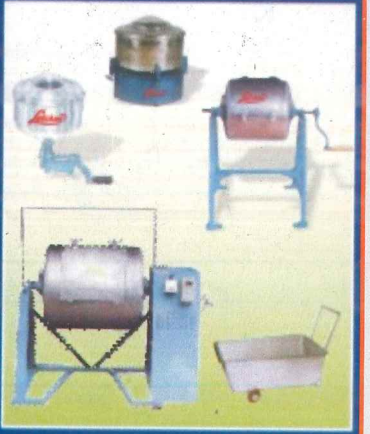

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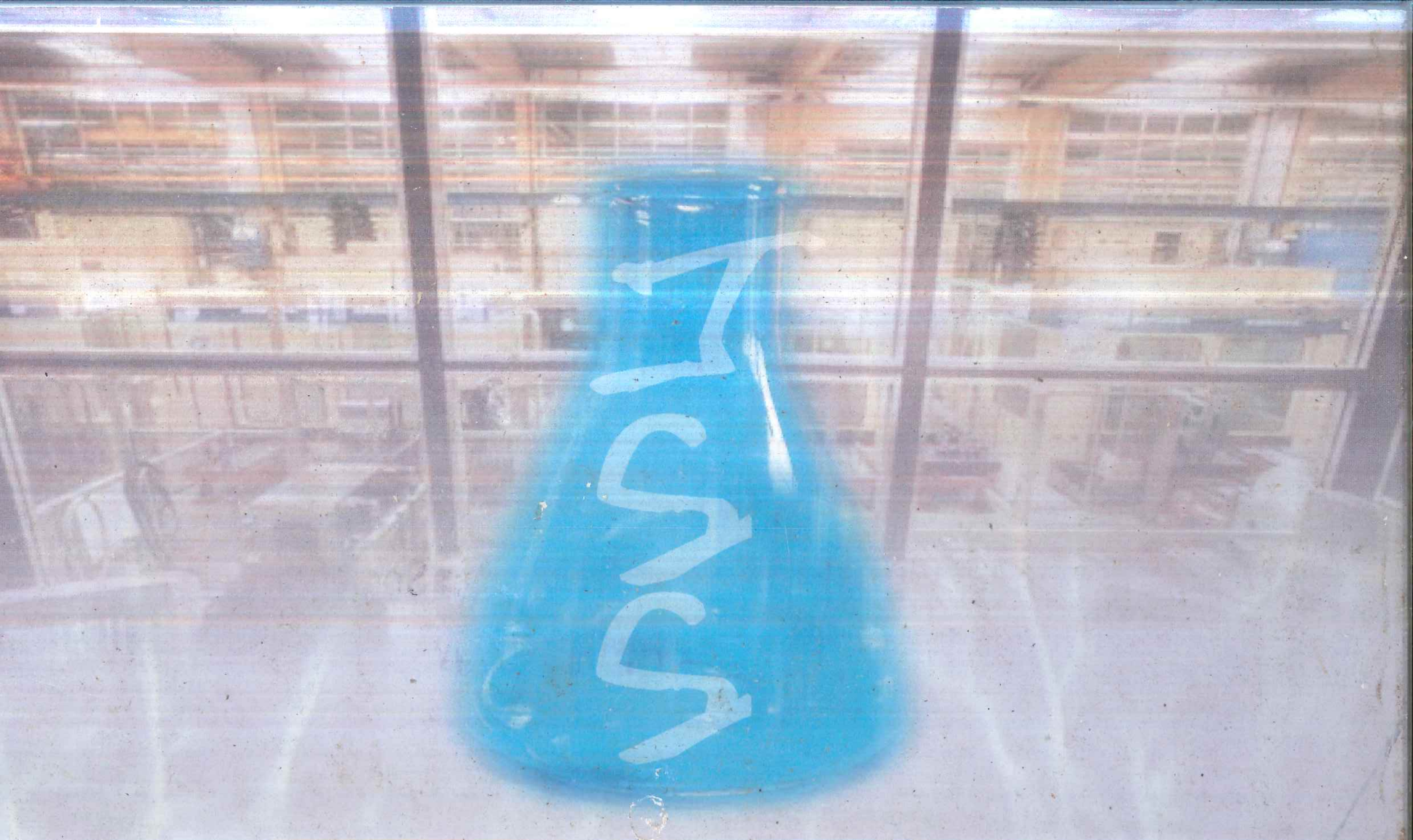

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