Prevention and Control of Leptospirosis

Proceedings

Stakeholder Meeting and Workshop on Laboratory Capacity Building for Leptospirosis

11th to 15th September, 2017

Venue: ICAR-NIVEDI, Bengaluru

Organised by:

Indian Council of Agricultural Research -National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI)

And

Centers for Disease Control and Prevention (CDC), Atlanta, USA

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ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Post Box No. 6450, Ramagondanahalli, Yelahanka, Bengaluru-560064, India

About ICAR-NIVEDI

Convergence of Animal Health and Research Par Excellence

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), (Formerly, Project Directorate on Animal Disease Monitoring and Surveillance, PD_ADMAS) under the Indian Council of Agricultural Research (ICAR), a pioneer research institute in veterinary epidemiology is carrying out disease surveillance, monitoring and analysis of livestock diseases in India through 32 collaborative centers of AICRP_ADMAS located in different states of the country.

The AICRP on animal disease monitoring and surveillance (AICRP-ADMAS) initiated by the ICAR, made a humble beginning during the VII five-year plan and became fully functional in 1987 with establishment of four Regional Research Units (RRUs) at Bengaluru, Hyderabad, Pune and Ludhiana. The Central Coordinating Unit (CCU) was established at the Institute of Animal Health and Veterinary Biologicals, Bengaluru to co-ordinate research activities of the regional units. In the VIII plan, the institute was strengthened with support of ICAR and European Union by taking up the major responsibility under National Project on Rinderpest Eradication (NPRE) involving 32 state level diagnostic/disease investigation laboratories in the country. On 1st April 2000 (during the IX plan), the CCU was given the status of Project Directorate and named as 'Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS)' with ten collaborating units under AICRP_ADMAS component. In the X and XIFive year plan period, five more collaborating units were added for providing impetus to a nationwide animal disease monitoring and surveillance.

Appreciating the contributions made by the Directorate to country's livestock health sector and the need to strengthen the effort, the council rechristened PD_ADMAS as 'National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)' on 25th October 2013 (XII plan period) with its exclusive campus at Bengaluru. Further, during the same plan period, 17 additional collaborating units covering other states were added under AICRP_ADMAS component totaling to 32 collaborating units for providing the needed impetus to a strong nationwide animal disease monitoring and surveillance network.

On 9thJanuary,2015, NIVEDI's newly constructed administrative building and Biosafety Laboratory (BSL-2) was dedicated to the nation by ShriRadha Mohan Singh, Hon'ble Union Minister for Agriculture, New Delhi in the presence of Shri D.V. SadanandaGowda, Hon'bleMinister of Law and Justice, GOI and Shri T. B. Jayachandra**,** Hon'ble Minister for Law, Justice & Human Rights, Parliamentary Affairs and Legislation and Animal Husbandry, Govt. of Karnataka and Dr. S. Ayyappan, Secretary DARE and Director General, ICAR. The centralized administrative and laboratory complex of the institute is located in a sprawling campus at Yelahanka, Bengaluru.

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), a pioneer research institute under Indian Council of Agricultural Research (ICAR) has been entrusted to conduct R&D in the field of veterinary epidemiology and surveillance of economically important livestock diseases in the entire country, its role is extremely pivotal for developing models for animal disease forewarning, forecasting, economic impact, risk assessment, and need based animal disease diagnostics. The institute has developed various technologies covering both products and processes and some of them are marketed and/or patented/copyright protected, which are being utilized by various institutes/organizations and different stakeholders in the country. The role of this institute in the eradication of Rinderpest disease in India and development of National Animal Disease Referral Expert System (NADRES) - interactive software for forecasting are noteworthy. The institute conducts various training programmes related to basic epidemiology, sampling frame and sampling techniques, outbreak investigation, research methodologies, disease diagnosis protocols for various

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stakeholders associated with animal healthcare. Overall, NIVEDI has been proving its worthiness to the Indian animal health sub-sector covering critical gaps indiagnostic techniques, animal disease modelling, economic impact assessment and analysis of animal diseases, human resource development in the form of skill development and empowerment, capacity building programmes etc. Further, NIVEDI envisions to provide newer direction to undertake in-depth R & D activities on epidemiology of emerging and re-emerging, transboundary animal diseases to others involved in the sub-sector in the country, leading finally to prevention, control and eradication of the diseases for achieving animal welfare and safer animal - human interface under one health approach.

Vision

Achieving freedom from animal diseases, animal welfare, food and nutritional security through healthy foods of animal origin, poverty alleviation and economic growth of rural India.

Mission

Capacity building in frontier areas of Veterinary Epidemiology: dynamics of animal diseases including zoonosis and animal healthcare intelligence.

Focus

- Improving disease monitoring and surveillancethrough development of population assays and pen side diagnostics
- Risk assessment for occurrence of economically important animal diseases
- Adapting strategies to improve animal disease data quality
- Understanding the threat from animal diseases in the background of climate change and globalization
- Developing early warning system and disease modeling/ forecasting
- Understanding economic impacts of animal diseases and the management strategies
- Promoting innovations and improving human resource capacity
- Fostering linkages and collaborations with public and private, national and international organizations
- Improving knowledge management system

Mandate of Institute

- Epidemiology, informatics and economics of animal diseases including zoonosis
- Surveillance, forecasting and forewarning for management of animal diseases including Zoonosis
- Repository and Capacity Development

AICRP on ADMAS

All AICRP collaborating units are extensively working on animal disease diagnosis, outbreak investigation, disease reporting, pathogen characterization and mapping etc., with major focus on bacterial (Brucellosis, Leptospirosis, Mastitis, HaemorragicSepticaemia, Anthrax, Black Quarter, Enterotoxaemia,), viral (Infectious Bovine Rhinotracheitis, Bluetongue, Classical Swine Fever, *Peste des Petits Ruminants* and Sheep and Goat Pox) and parasitic (Trypanosomosis, Theileriosis, Babesiosis, Fascioliosis and Amphistomiosis) diseases of economic importancewith the following mandates.

Mandates of AICRP on ADMAS

- Sero-monitoring of animal diseases based on sample frame,
- Investigation of endemic, emerging and re-emerging animal disease outbreaks using innovative technologies,
- Strengthening of National Livestock Serum Repository,
- Effective updating of NADRES with active disease data and climatic and non-climatic risk-factors,
- Utilization of forecasting models through NADRES for forecasting and forewarning of animal diseases,

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- Analysis on economic losses due to animal diseases and the control measures adopted for their management, and
- Surveillance of diseases/pathogens of companion, laboratory and wild animals.

Acknowledgement

The constant support, encouragement and financial assistance benevolently from Indian Council of Agricultural Research, KrishiBhawan, New Delhi for ICAR-NIVEDI. Centers for Disease Control and Prevention (CDC), Atlanta, USA and American Society for Microbiology (ASM) for sponsoring capacity building programme on "Leptospirosis" usingGlobal Health Security Funds (GHSF)and providing opportunity to ICAR-NIVEDI for conducting such a workshop and stakeholder meeting in the field of "Leptospoirosis"are gratefully acknowledged.

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Dr. Avijit Roy, Joint Secretary, Integrated Disease Surveillance Programme, Andaman & Nicobar Islands

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Dr. Neeta Khandelwal, Department of Microbiology, Government Medical College Surat, Gujarat.

7. Leptospira Research activities at NIMHANS, Bengaluru
Dr. Nagarathna S,Department of Neuromicrobiology, NIMHANS, Bangalore.

8. Leptospira Research activities at TANUVAS, Chennai
Dr. T.M.A. Senthil Kumar,Department of Animal Biotechnology, Madras Veterinary College,Chennai,Tamil Nadu.

9. Leptospirosis situation in Maharashtra
Dr. Sunil Lahane, Assistant Commissioner of Animal Husbandry,Western Regional Disease Diagnostic Laboratory, Pune, Maharashtra

10. Leptospira Research activities at SVVS, Tirupati
Dr. Raniprameela,State Disease Investigation Laboratory,Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh.

11. Leptospira Research activities at ICAR-Indian Veterinary Research Institute
Dr. Sabarinath,Scientist, IVRI Deemed University, Izatnagar, Bareilly,Uttar Pradesh.

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Dr.Manish Kumar,Department of Biosciences and Bioengineering, IIT, Guwahati, Assam.

13. Leptospira Research activities at ICAR-NIVEDI, Bengaluru
Dr. V. Balamurugan, ICAR-NIVEDI, Bengaluru. Karnataka.

14. Leptospira Research activities at MCVR, **Manipal University**
Dr. G. Arun Kumar,MCVR, Manipal University, Manipal, Karnataka

BrainstormingSession: 4.45 -6.00 PM

Session was chaired by Dr. Parimal Roy, Director, ICAR-NIVEDI, Bengaluru, and Dr. Daniel L. Garcia, Senior Laboratory Advisor, Division of Global Health Protection,CDC-India, New Delhi.

Deliberations and Brainstorming by different experts and resource persons on Identifying collaborative research issues and preparing roadmap for control of leptospirosis under one health approach

Workshop on Laboratory Capacity building for leptospirosis(12th-15th Sep, 2017)

Hands-on training on different diagnostic techniques for diagnosis of leptospirosis jointly conducted by experts from ICAR-NIVEDI, Bengaluru, India and CDC, Atlanta, USA.

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Day 2: 12.09.2017

- Glimpse of NIVEDI, Overview of the course and Pre-training evaluation.
- Laboratory Biosafety: Principles and Practices. BSL 2+ visit.
- Preparation of culture media (EMJH); Culturing, and examination of Leptospira.
- Maintenance of culture in liquid and semi-solid media.
- Dark field examination and staining of leptospira

Day 3: 13.09.2017

- Live leptospira culture antigen preparation for MAT.
- Screening of the human and animals serum samples for leptospira antibodies by MAT
- Diagnosis of human and animal leptospirosis by SYBR green RT-PCR.
- Sero-screening of the samples by MAT

Day 4: 14.09.2017

- Molecular diagnosis: Extraction of DNA from Leptospira cultural or clinical samples (Blood/Plasma/ serum/urine).
- Diagnosis of leptospirosis by PCR techniques, duplex/multiplex PCR, etc.,
- Diagnosis of human leptospirosis by human IgM based ELISA (Pan Bio kit)/ Lateral flow assay (LFA) /Latex agglutination test (LAT)

Day 5: 15.09.2017

- Economic impact of leptospirosis in animals and human and KAP studies
- Application of GIS in understanding the Spatial Epidemiology of Leptospirosis
- Discussion with Participants, Post-training evaluation and Feedback
- Valedictory and Certificate Distribution

Annexure 1 List of Participants

Annexure 2 Photographs of Stakeholders meeting and Workshop

Prepared and Edited by: Dr.V.Balamurugan, Dr. R.Sridevi, Dr. G. Govindaraj, Dr. G.B. ManjunathaReddy and Dr. M. Nagalingam

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Preface

Leptospirosis is one of the emerging zoonosis leading to significant morbidity and mortality in human as well as economic loss in livestock. It is known to be endemic in several states of India primarily Andaman & Nicobar, Gujarat, Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra. To combat this disease, it is necessary to improve capacity of personnel on leptospirosis diagnosis and surveillance. The proposed plan was to establish a scientific stakeholder meeting to ensure interaction among all the stakeholders from both human and Veterinary sectors, for identifying the challenges and to find suitable solutions under one health approach; to facilitate and strengthen inter sectoral co-ordination efforts;to train district level veterinary and public health laboratories along with apex laboratories to perform tests at different levels /tier (peripheral and reference level); linking the district and apex laboratories and to develop the uninterrupted sample transportation system within the network. To achieve the afore mentioned objective, Centers for Disease Control and Prevention (CDC) and ICAR-NIVEDI jointly organized stakeholder meeting and workshop on laboratory capacity building for leptospirosis at ICAR-NIVEDI, Bengaluru. The main focus of the programme was organizing a stakeholder meeting of apex laboratories to layout the roadmap for laboratory capacity building within the GOI's surveillance network of Leptospirosis (spearheaded by National Centre for disease control -NCDC) involving Veterinary sector and also to conduct wet laboratory training for different levels/tiers of laboratories: training of apex/reference labs on Microscopic Agglutination Test (MAT) (including maintenance of live strains of leptospira) and Molecular assays (PCR) and training of personal in district level Veterinary and public health laboratories on ELISA and rapid diagnostic tests (Latex agglutination test/ Lateral Flow Assays).

As a first step, stakeholder meeting and workshop on laboratory capacity building for leptospirosis was held at ICAR-NIVEDI, Bengaluru, India during 11th to 15th September, 2017 with technical supports from Bacterial Special Pathogens Branch, Division of High Consequence Pathogens, CDC, Atlanta, USA. The scientific personnel from the different laboratories in endemic states of India working on leptospirosis were invited to participate in the above said programme as per decision with CDC officials. The programme was approved by the Indian Council of Agricultural Research, New Delhi and sponsored by American Society for Microbiology (ASM), India using Global Health Security Funds (GHSF).

The meeting and workshop provided an opportunity to various stakeholders to share their problems and experiences to evolve a suitable strategy for surveillance of leptospirosis in endemic areas of India, and laboratory capacity building for diagnosis at district level. The stateholder meeting was attended by Experts from International organisations representing CDC, Atlanta, USA and CDC-India and ASM, India officials, Scientists and officials from the Indian Council of Agricultural Research- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), experts from different organization /laboratories working in leptospirosis in India, namely Regional Medical Research Centre (RMRC), Portblair, NCDC, New Delhi, National Institute of Mental Health and Neuro-Sciences (NIMHANS), Bengaluru, Manipal university, Karnataka, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh. Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Indian Institute of Technology, Guwahati, Govt. Medical College Surat, Gujarat, Western Regional Disease Diagnostic Laboratory (WRDDL), Pune, Maharashtra and State Disease Investigation Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh. The state nodal officers from Andaman and Nicobar Islands, Karnataka, Gujarat, under national programme on prevention and control of leptospirosis and many professors, laboratory technicians, research scholars, research manager, microbiologist, from apex leptospirosis laboratories of aforesaid organizations in India were participated.

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The experts focused their discussions on the nature of disease, case definition, extent of research activities to be undertaken including various initiatives taken by the government and their impact on the disease prevention and control, management and their status in endemic states of India including awareness of the disease. The important recommendations emerged were in the brainstorming session for effective initiation of the important activities towards surveillance, prevention and control of leptospirosis under one health approach.

We are grateful to the Indian Council of Agricultural Research (ICAR), Government of India, CDC, Atlanta, USA, CDC-India and ASM-India and all the experts for their valuable contribution and support. We hope this initiative will help for prevention and control of Leptospirosis in India.

Programme Director and
Coordinator,
ICAR-NIVEDI

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Executive Summary and Recommendations

A stakeholders meeting and workshop on laboratory capacity building for leptospirosis was jointly organizedby ICAR-NIVEDI and Centers for Disease Control and Prevention (CDC), Atlanta, USA during 11th to 15th September, 2017 at ICAR-NIVEDI, Bengaluru, India. The programme was approved by the Indian Council of Agricultural Research (ICAR), New Delhi and sponsored by American Society for Microbiology (ASM), India using Global Health Security Funds (GHSF).

The intended plan was to establish a scientific stakeholder meeting to ensure interaction among all the stakeholders from both human and Veterinary sectors, for identifying the challenges and to find suitable solutions under one health approach; to facilitate and strengthen inter sectoral co-ordination efforts;to train district level veterinary and public health laboratories along with apex laboratories to perform tests at different levels (peripheral and reference level); linking the district and apex laboratories and to develop the uninterrupted sample transportation system within the network.

The main focus of the programme was to layout the roadmap for laboratory capacity building within the GOI's surveillance network of Leptospirosis (spearheaded by National Centre for disease control -NCDC) involving Veterinary sector and also to conduct wet laboratory training for different levels of laboratories: training of apex/reference labs on Microscopic Agglutination Test (MAT) (including maintenance of live strains of leptospira), molecular assays (PCR) and training of personal in district level veterinary and public health laboratories on ELISA and rapid diagnostic tests (Latex agglutination test/ Lateral Flow Assays).

The meeting and workshop provided an opportunity to various stakeholders to share their problems and experiences to evolve a suitable strategy for surveillance of leptospirosis in endemic areas of India, and laboratory capacity building for diagnosis at district level. The stakeholders meeting was attended by experts from international organisations representing CDC, Atlanta, USA and CDC-India and ASM-India officials, Scientists and officials from the Indian Council of Agricultural Research- National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), experts from different organization /laboratories working in leptospirosis in India, namely Regional Medical Research Centre (RMRC), Portblair; NCDC New Delhi; National Institute of Mental Health and Neuro-Sciences (NIMHANS), Bengaluru;Manipal university, Karnataka; Indian Veterinary Research Institute, Bareilly, Uttar Pradesh;Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai; Indian Institute of Technology, Guwahati; Govt. Medical College Surat, Gujarat; Western Regional Disease Diagnostic Laboratory (WRDDL), Pune, Maharashtra and State Disease Investigation Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh. The state nodal officers under national programme on prevention and control of leptospirosis from Andaman and Nicobar Islands, Karnataka, Gujarat, and many professors, laboratory technicians, research scholars, research manager, microbiologist from India participated in the workshop.

The experts focused their discussions on the nature of disease, case definition, extent of research activities to be undertaken including various initiatives taken by the government and their impact on the disease prevention and control, management and their status in endemic states of India including awareness of the disease. The important recommendations emerged in the brainstorming session were mentioned below for effective initiation of the important activities towards surveillance, prevention and control of leptospirosis under one health approach

- Leptospirosis is endemic throughout the country and to understand entire status and plan for a road map, inter sectoral participation for surveillance is of paramount importance. The importance of capacity building among various stake holders was also stressed.

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- The need for working together in leptospirosis to understand and control the disease in the country was discussed in the meeting. Further geographic genomics, pathogenomicsand pharmacogenomics studies for understanding the leptospirosis epidemiology and control were stressed.
- The meeting highlighted the importance of surveillance and capacity building and ICAR-NIVEDI was identified to collaborate in all the aspects as a lead centre for animal surveillance and RMRC, Port Blair for human surveillance.
- During the meeting, the need for uniform and quality diagnosis and availability of diagnostics at various centres was felt by various stake holders.
- Handling of human samples at veterinary institutes and their ethical modality were discussed and it was recommended to write to heads of ICAR and ICMR to seek permission and approval for the same.

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Inaugural Session

Welcome Address: Dr.V.Balamurugan, Principal Scientist, ICAR-NIVEDI, Bengaluru

At the outset, heheartily welcomedthe officials present for the stakeholders meeting and workshop on laboratory capacity building for leptospirosis programme held at ICAR-NIVEDI, Bengaluru.He welcomed CDC and ASM officials for bringing the animal health and human specialists under one umbrella. He said, main purpose of this stakeholder meeting is to draw road map and to build laboratory capacity building within the Government of India's surveillance network of Leptospirosis involving Veterinary sector under one health approach. He stressed on important of the laboratory training for different levels/tiers of Laboratories in the network of the country in order to facilitate and strengthen inter-sectoral co-ordination efforts to control leptospirosis.Dr. V. Balamurugan welcomed the honorable Chief Guest for the stakeholders meeting Dr.Vijayachari sir, Director, Regional Medical Research Centre (RMRC), WHO collaborating centre for Diagnosis, Research, Reference and Training in Leptospirosis, Portblair. Andaman & Nicobar Islands, India and Dr. Naveen Gupta, Joint Director and Head of zoonosis Division, National Centre for Disease Control (NCDC), New Delhi for his participation in the stakeholders meeting. He extended the warm welcome to Dr.Renee L.Galloway, Bacterial Special Pathogens Branch, Division of High Consequence Pathogens, Centers for disease control and prevention (CDC), Atlanta, Georgia, United States of America (USA), for taking interest in attending this stakeholders meeting and imparting training to the participants and to share her knowledge and skills and Dr.Daniel L.Garcia, Senior Lab Advisor,Division of Global Health Protection, CDC, India for participating in this inaugural function. He welcomed Dr. Rekha Jain, Senior Consultant, Lab Strengthening ASM, India. He also welcomed Director, ICAR-NIVEDI, Dr. Parimal Royfor his kind help in planning the stakeholder meeting and Workshop. He also remembered and welcomed Country Director, CDC-India, Dr. Kayla Laserson,who could not attend the meeting due to other official engagement. He welcomed all the experts from different parts of the country and staff from CDC &ASM especiallyDr. MayankDwivedi,Dr, Mohan Papannaand experts and trainees working in various laboratoriesin different states of India for participating in the stakeholders meeting. Last but not the least, he welcomed all the Scientists, technical, administrative and supporting staff of ICAR-NIVEDI, for the inaugural function.

Opening Remarks: Dr. Parimal Roy, Director, ICAR-NIVEDI, Bengaluru

Leptospirosis is a serious disease causing considerable loss to farmers as well as human beings, but the level of awareness among the public and the farming community is low. There is a programme in place in human health sector – Integrated Disease Surveillance Programme (IDSP) for regular detection and reporting.There are different countrywide ongoing surveillance programmes in place but for leptospirosis in animals not yet adopted by the government. But different research studies or projects are being undertaken by various groups in the country and doing surveillance. There has to be collaborative approach between the human and animal health institutes on this aspect. He also thanked CDC for conducting or supporting effective capacity .

building programmes throughout India in onehealth approach. ICAR-NIVEDI being epidemiology Institute does work on forewarning of economically important animal diseases and also ready to take a lead role and collaboration with CDC /IDSP in Leptospirosis also.

Special Remarks:Dr. Renee L. Galloway, Bacterial Special Pathogens Branch, CDC, Atlanta, USA.

Leptospirosis is a global problem and considered one of the most common zoonotic infections in the world. In India, CDC-supported many programs, found Leptospirosis is important problem.This workshop and additional activities comprising partnership with India on Global Health Security, CDC is supporting NCDC and NIVEDI tobuild capacity for laboratory based leptospirosis surveillance within the national surveillance network of leptospirosis, using a One Health.This workshop will be useful for all the participants from both the human health and veterinary sectors. This will give them an opportunity to learn standard testing protocols for leptospirosis as well as exchange ideas and discuss challenges with national and international experts.

Special Remarks:Dr. Daniel L. Garcia, Senior Lab Advisor, Division of Global Health Protection, CDC-India, New Delhi.

He highlighted the principle objectives of this meeting; to build awareness of what each network is doing; to share best practices (field investigation, laboratory testing, diagnostics); to create linkages or strengthen inter-sectoral coordination of both animal husbandry health sectors by leveraging existing capacity for diagnosis of leptospirosis; To learn how to best handle outbreak investigations especially at the A/H health interface; To discuss ways to strengthen H/A labs at district & state levels and to plan best way forward for strengthening networks through continued coordination & training a. Include venues, periodicity, scope, timelines, designated leads, etc.

Additionally, he read out the message conveyed by Dr. Kayla Laserson,Country Director, CDC- India, New Delhi for the experts and participants.

Message:Dr. Kayla Laserson, Country Director, CDC- India, New Delhi

Leptospirosis is a global problem and considered one of the most common zoonotic infections in the world. In India, CDC-supported Acute Febrile Illness (AFI) and Acute Encephalitis Syndrome (AES) surveillance sites from different states have revealed that leptospirosis is a significant cause of undifferentiated fever, including fever characterized as acute encephalitis, and is not limited toonly a few endemic states of the country.Early diagnosis of leptospirosis is challenging but crucial as appropriate treatment with antimicrobial drugs can reduce illness and death. Clinical diagnosis is difficult as the clinical signs and symptoms range from a self-limiting mild influenza-like illness to a much more serious disease with multi-organ failure and the potential for death. Further these signs and symptoms are characteristic of numerus fever-causing pathogens. Laboratory diagnosis of leptospirosis is also challenging; most of the laboratories are dependent upon serologic tests. The capacity for performing the gold standard serologic test (Microscopic Agglutination Test, MAT) is generally lackingas itrequires

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maintenance of live serotypes of leptospira. Rapid serologic tests are non-reliablebecause of the high false positivity and cross reactivity with other pathogens, e.g. dengue. Moreover, serologic testing for leptospirosis detects infections only after the second week of illness. Molecular testing using PCR allows the identification of acute infectionas it provides rapid and reliable diagnosis during the bacteremic phase. But, the capacity for molecular and the gold standard serologic testing is limited to only a few laboratories. More regional laboratory capacity is needed to combat the nationwide problem.Control and prevention activities of leptospirosis are often limited due to the diagnostic challenges identifying leptospirosis, wide variation in the host's symptomatology, multiple serotypes of the agent and complexity of interactions among humans and animals enabling the disease transmission in local settings.The Government of India, through the National Program on Prevention and Control of Leptospirosis spearhead by NCDC, is trying to build surveillance capacity in several endemic states of India e.g. Kerala, Karnataka, Tamil Nadu, Gujarat, Maharashtra and Andaman & Nicobar.With respect to the Veterinary sector,NIVEDI is a leading Government institution with state of the art laboratory capacity and highly experienced scientists involved in leptospirosis surveillance and is the most appropriate organization to work as a collaborative partner with NCDC forinter-sectoral coordination.

Through this workshop and additional activities comprising partnership with India on Global Health Security, CDC is supporting NCDC and NIVEDI tobuild capacity for lab based leptospirosis surveillance within the national surveillance network of leptospirosis, using a One Health approach.I strongly believe that this workshop will be useful for all the participants from both the human health and veterinary sectors. This will give them an opportunity to learn standard testing protocols for leptospirosis as well as exchange ideas and discuss challenges with national and international experts.

Special Address: **Dr. Naveen Gupta, Joint Director and Head of Zoonosis Division, NCDC, New Delhi, India**

Leptospirosis is public health problem in Gujarat, Kerala, Karnataka, Tamil Nadu, Maharashtra and Andaman. Frequent outbreaks of leptospirosis are being reported, predominantly affecting young adult males. The disease is easily treatable and the mortality is preventable if detected and treated early. Under XII plan, Programme for Prevention and Control of Leptospirosis has been approved and is being implemented in six endemic states as mentioned above. The strategy includes-

- Strengthening of diagnostics laboratories for early diagnosis
- Strengthening of patient management facilities
- Trained manpower development,
- Strengthening of inter sectoral coordination
- Create awareness in general community.

Presidential Address: **Chief Guest-Dr. P. Vijayachari, Director, RMRC (ICMR), Portblair, A &N Islands, India**

Leptospirosis, is a direct zoonoses of global public health importance. Majority of infections are either sub clinical or result in very mild illness and recover without any complications. A small proportion develops various complications due to multiple organ injury, the clinical presentation depends upon the predominant organs involved and the case fatality ratio could be about 40%.

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The natural history or biologic spectrum of the disease changes frequently. This probably could be due to evolution of pathogen over time.Pathogens, evolve through genetic changes in the form accumulation in the genome as a repertoire of gene acquisition and loss on an evolutionary time-scale, this phenomenon is known as geographic genomics. These changes contribute towards flexibility in gene content, gene order and gene regulation which makes the pathogen gain more virulence. On this analogy, studies have shown this phenomenon is evident in leptospires– non-virulent strains or less virulent strains evolving in to pathogenic or more virulent ones on evolutionary time-scale. Such a phenomenon is being observed as a dynamic process, which facilitates survival mechanisms to tide over adverse conditions and gain virulence, infects an array of animals and humans and responsible for the multiple syndromes, associated with high case fatality.Quite recently, *Leptospira* have been shown to be capable of forming biofilms by themselves or in combination with other environmental bacteria.Bacteria in biofilms are more resistant to physical stress such as temperature, pH, UV radiation and also to antibiotics.These biofilms are more frequently seen on various surfaces such as the walls of sewage canals, paddy fields and water bodies.Therefore, these biofilms perennially serve as a source of infection. Probably, this unravels the mystery of the transmission dynamics of urban leptospirosis.The measures needs be under taken at different levels for control include 1)Development of algorithms for clinical and laboratory diagnosis of the disease in humans and animals. 2)Sero and molecular epidemiological studies to resolve genetic differences among the strains linked with epidemics/epizootics as well as sporadic cases which could facilitate in tracking of potential animal vectors harbouring virulent strains for the prediction of future epidemics. 3)Geographic genomics to identify circulating new serovars or emerging virulent strains if any and to find out dissemination dynamics.4)Promoting early diagnosis and prompt treatment in humans and animals to reduce morbidity and mortality. 5)Monitoring antibiotic susceptibility pattern of circulating serovars/strains and 6)Promote hygienic animal rearing practices for farm and working backyard animals and animal work force in agriculture, coupled with adopting personal protection while handling animal excreta.

Vote of thanks:Dr.G.Govindaraj, Senior Scientist, ICAR-NIVEDI, Bengaluru

At the outset, he sincerely thanked ICAR and Hon'ble Secretary, DARE and Director General, ICAR, and DDG (AS) for permitting ICAR-NIVEDI to organize the stakeholders meeting and Workshop on laboratory capacity building for Leptospirosis. He thanked Honorable Chief guest Dr. P. Vijayachari, Director, RMRC, Port Blair for consenting to participate in this stakeholders meeting and providing valuable inputs for planning the road map to control leptospirosis in the country. He extended thanks to Dr. Naveen Gupta, Joint Director, NCDC, New Delhi. He thanked Dr. Renee L. Galloway, scientist from CDC, Atlanta and Dr. Daniel L. Garcia, CDC, India for participating in the stakeholders meeting and to share their knowledge and experience with the trainees in the due course. He thanked Dr. Parimal Roy, Director, ICAR-NIVEDI, for his constant support and guidance in organizing the stakeholders cum training workshop. Finally, he thanked all the experts working in different states in different

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capacities for participating in the one day deliberation. The initiatives of CDC & ASM officials bringing the animal health and human specialists under one umbrella was highly appreciated. He also thanked the sponsors CDC and ASM officials for their financial support and their constant support and guidance in organizing the stakeholders cum training workshop. Last but not least,he thanked scientists, technical, administrative and supporting staff of ICAR-NIVEDI and members of various committees constituted for their participation in the inaugural session.

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TECHNICAL SESSION 1:

Chairman:Dr.Naveen Gupta,Joint Director,NCDC,New Delhi.

1. Eco-system Interfacesinter sectoral Co-ordination-control of Leptospirosis

Dr. PaluruVijayachari,Director, RMRC (ICMR), Port Blair, A & N Islands, India.

Leptospirosis, is a direct zoonoses of global public health importance. Majority of infections are either sub clinical or result in very mild illness and recover without any complications. A small proportion develops various complications due to multiple organ injury, the clinical presentation depends upon the predominant organs involved and the case fatality ratio could be about 40%.The natural history or biologic spectrum of the disease changes frequently. This probably could be due to evolution of pathogen over time.Pathogens, evolve through genetic changes in the form accumulation in the genome as a repertoire of gene acquisition and loss on an evolutionary time-scale, this phenomenon is known asgeographic genomics. These changes contribute towards flexibility in gene content, gene order and gene regulation which makes the pathogen gain more virulence. On this analogy, studies have shown this phenomenon is evident in leptospires–non-virulent strains or less virulent strains evolving in to pathogenic or more virulent ones on evolutionary time-scale. Such a phenomenon is being observed as a dynamic process, which facilitates survival mechanisms to tide over adverse conditions and gain virulence, infects an array of animals and humans and responsible for the multiple syndromes, associated with high case fatality.

Quite recently, *Leptospira* have been shown to be capable of forming biofilms by themselves or in combination with other environmental bacteria.Bacteria in biofilms are more resistant to physical stress such as temperature, pH, UV radiation and also to antibiotics. These biofilms are more frequently seen on various surfaces such as the walls of sewage canals, paddy fields and water bodies.Therefore, these biofilms perennially serve as a source of infection. Probably, this unravels the mystery of the transmission dynamics of urban leptospirosis. Although direct transmission of leptospirosis occasionally occurs between animals and humans, majority of human infections are acquired from the environment. The survival of *Leptospira* in the environment is a crucial factor in the successful transmission of the infection. This implies that the environmental niches such as sewage canals and wet rice fields, water bodies once contaminated with pathogenic *Leptospira* excreted once by carrier animals, may remain infectious for prolonged period of time or at times indefinitely.

Therefore, a new paradigm in the environmental transmission models of leptospirosis emerges, in which the stronger determinant is the supportive ecosystem with human and animal interface.The risk reduction must consider the complexity of interactions among humans, animals, and the various environments they live in. This requires cooperation among the multiple sectors/stake holdersviz. public health, animal health, agriculture, environmental management,NGOs, policymakers and also involvement of the community at risk. In toto this is known as One Health Vision approach Or *Veterinary public health*(VPH). Therefore, there is a need for convergence of various stake holders towards achieving the goal of one health. This vision needs to be translated in to action in a sustainable way.The measures needs be under taken at different levels for control include 1)Development of algorithms for clinical and laboratory diagnosis of the disease in humans and animals. 2)Sero and molecular epidemiological studies to resolve genetic differences among the strains linked with epidemics/epizootics as well as

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sporadic cases which could facilitate in tracking of potential animal vectors harbouring virulent strains for the prediction of future epidemics. 3)Geographic genomics to identify circulating new serovars or emerging virulent strains if any and to find out dissemination dynamics.4)Promoting early diagnosis and prompt treatment in humans and animals to reduce morbidity and mortality. 5)Monitoring antibiotic susceptibility pattern of circulating serovars/strains and 6)Promote hygienic animal rearing practices for farm and working backyard animals and animal work force in agriculture, coupled with adopting personal protection while handling animal excreta.

Although, there is no systematic implementation of one Health Vision approach, an inter-sectoral collaborative efforts have been made in the Andaman &Nicobar Islands, which improved the leptospirosis situation. Studies in the 1990s conducted at Primary Health Care facilities showed an incidence as high as 747 cases/100,000 population and case fatality ratio of 2.8% with disability adjusted life years lost (DALY) of 853.66/100,000, whereas incidence of severe cases requiring tertiary care treatment was about 30/100,000 and the specific mortality was 7.5/100,000 with DALY of 312.063/100,000. In the recent times adrop has been observed in the incidence of leptospirosis as well as severe leptospirosis and specific mortality due to leptospirosis. The incidence at Primary Health Care facility dropped from 747/100,000 to 377/100,000 with DALY of 4.3/100,000 and incidence of severe cases at tertiary care level from 31/100,000 to around 15.2/100,000 and specific mortality from 7.5/100,000 to around 1/100,000 with DALY of 42.37/100,000.

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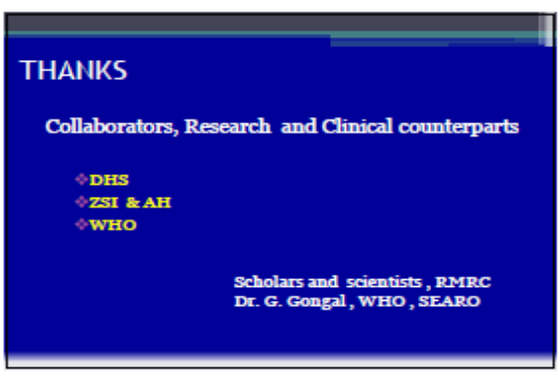

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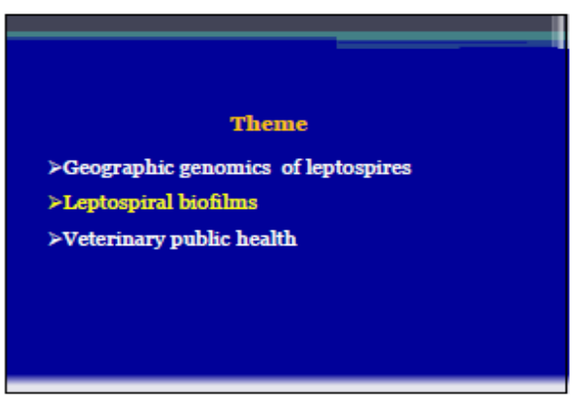

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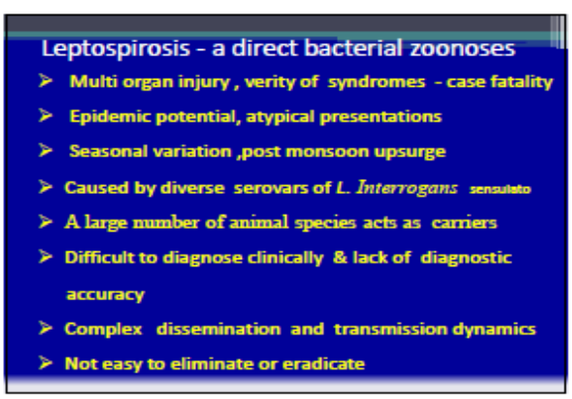

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Geographic genomics of leptospires

Genetic changes accumulate in the genome as a repertoire of gene acquisition and loss, on an evolutionary time-scale

These changes contribute towards flexibility in gene content, gene order , gene regulation and expression

Many human pathogens including leptospira have such changes to rigorous selection against the host defenses and adaptation to different niches

Susceptible Animal | Supportive Ecosystems | Susceptible Human

5

Transformation of saprophytic or less virulent leptospires to pathogenic ones highly virulent

L.bifexa

CI – 3603977bp
CII- 277995bp
P- 74117kb
3.9mb

Intermediate *Phenotypes*

CI – 4,122,216bp
CII- 296,544bp
P- Nil
(integrated)
4.3 mb

L.Interrogans

CI – 4,332,241bp
CII- 358,943bp
P- Nil
4.6mb

Tide over adverse conditions
Gain virulence
Infects an array of animals
Multiple syndromes - high case fatality

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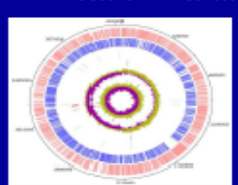
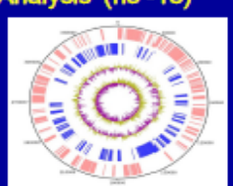
Whole Genome Analysis (no -18)





CI *Circular representation of MG19* CII

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Number of genes gained by assembled strains when compared with reference (1920s vs 1990s -2010s)

	MG19	MG392	MG17	H22	AHF401	MG333	MG371
Total Gained	548	561	621	550	604	670	639
Characterized	298	307	338	303	327	315	338
Hypothetical	250	254	283	247	277	355	301
Known Pathogenic	43	47	42	42	44	32	48

1920s - Renal, *1990s -2010s –, Renal, Pulmonary, Renal_Pulomonary, Gatro-Intestinal and Renal*

8

Number of genes loss by assembled strains when compared with reference (1920s vs 1990s -2010s)

	MG19	MG392	MG17	H22	AHF401	MG333	MG371
Total Lost	397	421	369	424	393	427	383
Characterized	237	250	225	252	233	240	233
Hypothetical	160	171	144	172	160	187	150
Known Pathogenic	24	30	25	29	24	28	24

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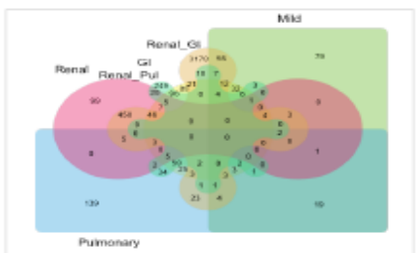
Expression Microarray



Venn diagram showing the common, shared and unique up-regulated genes in various groups (Mild, Renal, Pulmonary, Renal_Pulomonary, Gatro-Intestinal and Renal_GI)

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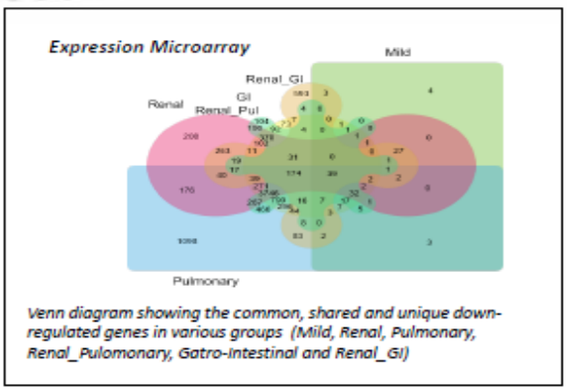

Venn diagram showing the common, shared and unique down-regulated genes in various groups (Mild, Renal, Pulmonary, Renal_Pulomonary, Gatro-Intestinal and Renal_GI)

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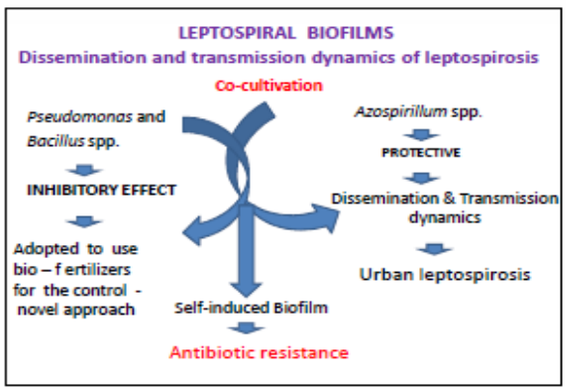

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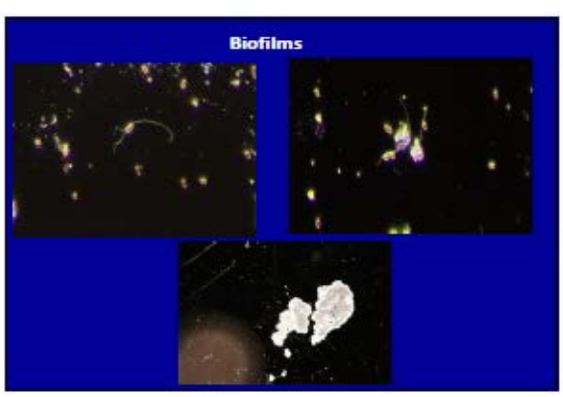

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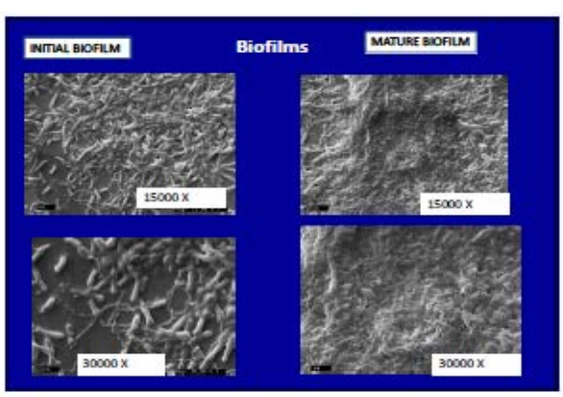

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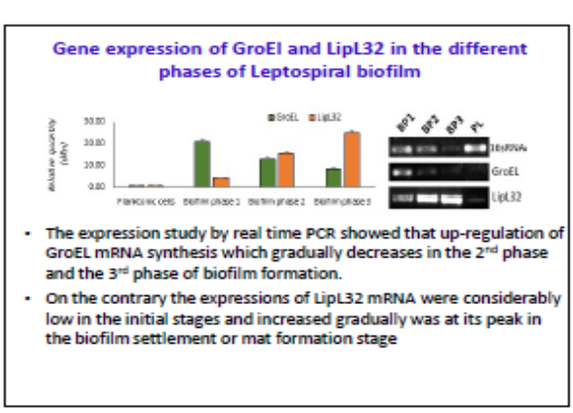

- The expression study by real time PCR showed that up-regulation of GroEL mRNA synthesis which gradually decreases in the 2nd phase and the 3rd phase of biofilm formation.
- On the contrary the expressions of LipL32 mRNA were considerably low in the initial stages and increased gradually was at its peak in the biofilm settlement or mat formation stage

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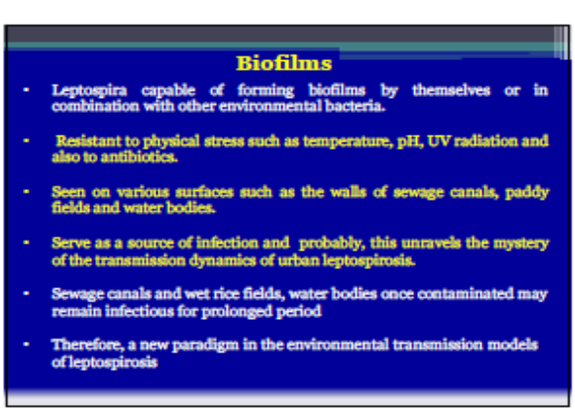

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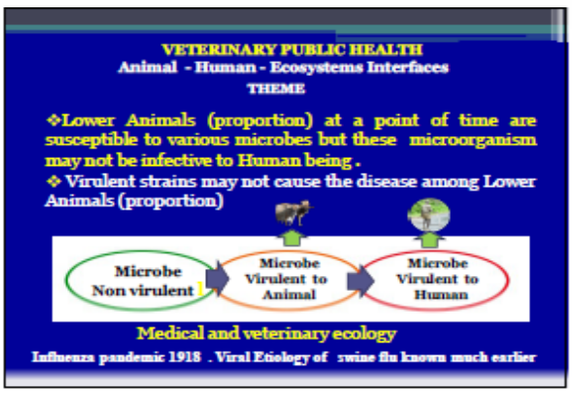
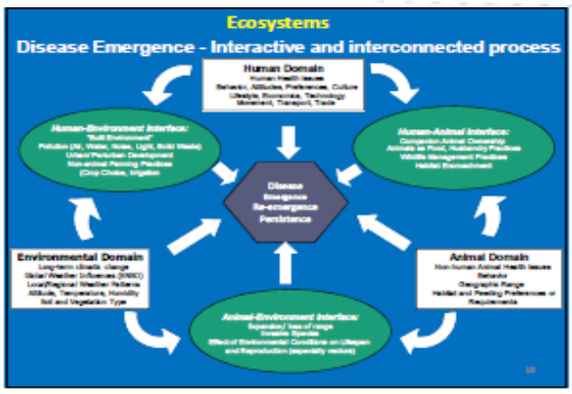
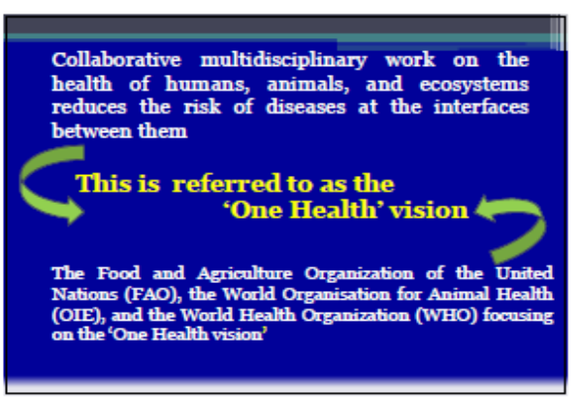

VETERINARY PUBLIC HEALTH
Animal - Human - Ecosystems Interfaces
THEME
❖Lower Animals (proportion) at a point of time are susceptible to various microbes but these microorganism may not be infective to Human being .
❖ Virulent strains may not cause the disease among Lower Animals (proportion)
Microbe Non virulent
Microbe Virulent to Animal
Microbe Virulent to Human
Medical and veterinary ecology
Influenza pandemic 1918 . Viral Etiology of swine flu known much earlier

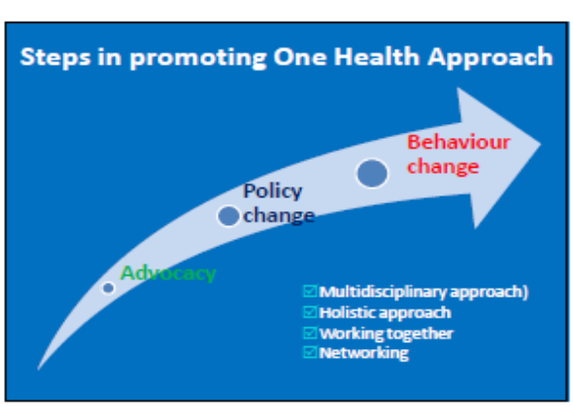
17


Ecosystems
Disease Emergence - Interactive and interconnected process
Human Domain
Environmental Domain
Animal Domain

18


The 'One Health' vision
Veterinary public health
Intersectoral coordination between public health, veterinary medicine, agriculture departments and involvement of community


Collaborative multidisciplinary work on the health of humans, animals, and ecosystems reduces the risk of diseases at the interfaces between them
This is referred to as the 'One Health' vision
The Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), and the World Health Organization (WHO) focusing on the 'One Health vision'


Steps in promoting One Health Approach
Behaviour change
Policy change
Advocacy
Multidisciplinary approach)
Holistic approach
Working together
Networking


Five Critical Steps in establishing One Health System
Cooperation among interdisciplinary groups
Commitment (Political and financial)
Coordination among partners
Collaboration among professionals
Concensus among stakeholders
involvement of the community at risk

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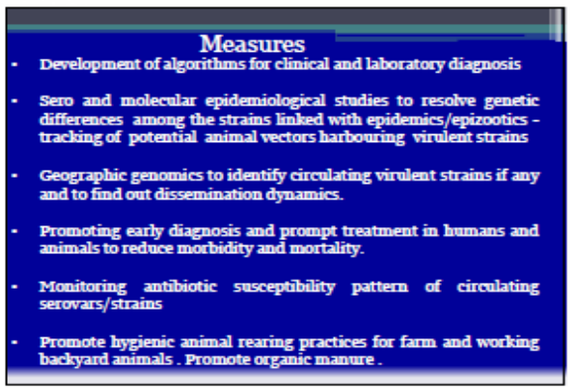

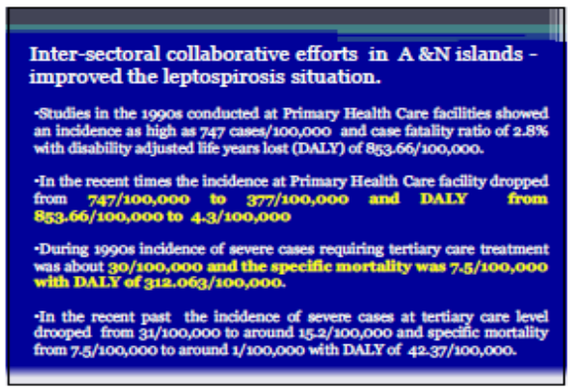

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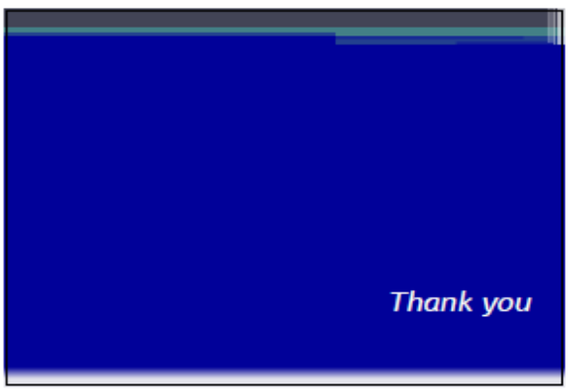

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2. Overview of National Program on Prevention and Control of Leptospirosis

Dr. Naveen Gupta, Joint Director, and HOD (Zoonosis), NCDC, New Delhi, India

Under XII plan, Programme for Prevention and Control of Leptospirosis has been approved and is being implemented in six endemic states as mentioned above. The strategy includes-

- Strengthening of diagnostics laboratories for early diagnosis
- Strengthening of patient management facilities
- Trained manpower development,
- Strengthening of inter sectoral coordination
- Create awareness in general community.

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Overview of Programme On Prevention and Control of Leptospirosis under XIIth five year plan (2012-17)

Dr Naveen Kumar Gupta, MD

MBBS , MD Medical Microbiology
Fellowship Infectious Diseases

Joint Director & Head , Zoonosis Division
National Centre For Disease Control , Delhi

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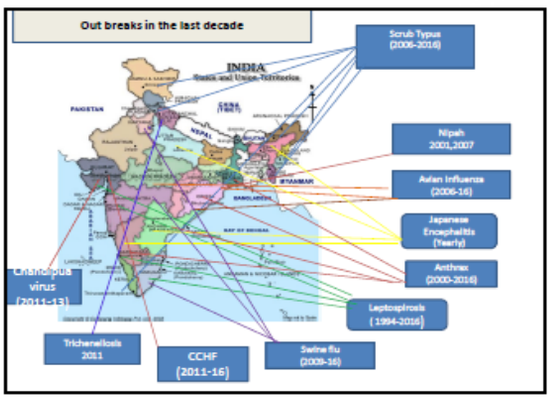

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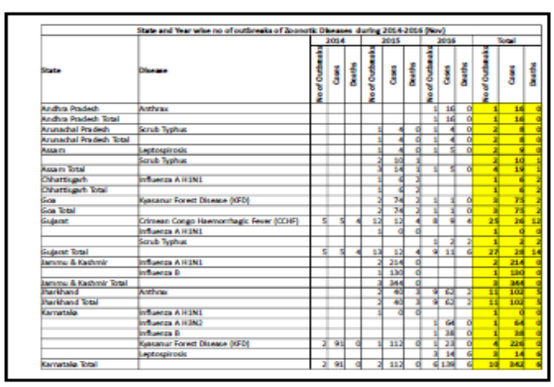

State and Year wise no of outbreaks of Zoonotic Diseases during 2014-2016 (Nov)

State	Disease	2014			2015			2016			Total		
		No of Outbreaks	Cases	Deaths	No of Outbreaks	Cases	Deaths	No of Outbreaks	Cases	Deaths	No of Outbreaks	Cases	Deaths
Andhra Pradesh	Anthrax							1	16	0	1	16	0
Andhra Pradesh Total								1	16	0	1	16	0
Arunachal Pradesh	Scrub Typhus				1	4	0	1	4	0	2	8	0
Arunachal Pradesh Total					1	4	0	1	4	0	2	8	0
Assam	Leptospirosis				1	4	0	1	5	0	2	9	0
	Scrub Typhus				2	10	1				2	10	1
Assam Total					3	14	1	1	5	0	4	19	1
Chhattisgarh	Influenza A H1N1				1	6	2				1	6	2
Chhattisgarh Total					1	6	2				1	6	2
Goa	Kyasanur Forest Disease (KFD)				2	74	2	1	1	0	3	75	2
Goa Total					2	74	2	1	1	0	3	75	2
Gujarat	Crimean Congo Haemorrhagic Fever (CCHF)	5	5	4	12	12	4	9	9	6	26	26	14
	Influenza A H1N1				1	0	0				1	0	0
	Scrub Typhus							1	2	2	1	2	2
Gujarat Total		5	5	4	13	12	4	9	11	6	27	28	16
Jammu & Kashmir	Influenza A H1N1				2	214	0				2	214	0
	Influenza B				1	130	0				1	130	0
Jammu & Kashmir Total					3	344	0				3	344	0
Jharkhand	Anthrax				2	40	3	9	62	2	11	102	5
Jharkhand Total					2	40	3	9	62	2	11	102	5
Karnataka	Influenza A H1N1				1	0	0				1	0	0
	Influenza A H3N2							1	64	0	1	64	0
	Influenza B							1	16	0	1	16	0
	Kyasanur Forest Disease (KFD)	2	91	0	1	112	0	1	23	0	4	226	0
	Leptospirosis							3	14	0	3	14	0
Karnataka Total		2	91	0	2	112	0	6	136	0	10	342	0

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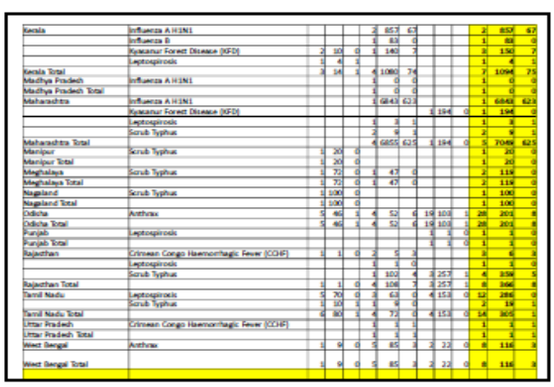

Kerala	Influenza A H1N1				2	857	67				2	857	67
	Influenza B				1	83	0				1	83	0
	Kyasanur Forest Disease (KFD)	2	10	0	1	140	7				3	150	7
	Leptospirosis	1	4	1							1	4	1
Kerala Total		3	14	1	4	1080	74				7	1094	75
Madhya Pradesh	Influenza A H1N1				1	0	0				1	0	0
Madhya Pradesh Total					1	0	0				1	0	0
Maharashtra	Influenza A H1N1				1	6843	623				1	6843	623
	Kyasanur Forest Disease (KFD)							1	194	0	1	194	0
	Leptospirosis				1	8	1				1	8	1
	Scrub Typhus				2	9	1				2	9	1
Maharashtra Total					4	6855	625	1	194	0	5	7049	625
Manipur	Scrub Typhus	1	20	0							1	20	0
Manipur Total		1	20	0							1	20	0
Meghalaya	Scrub Typhus	1	72	0	1	47	0				2	119	0
Meghalaya Total		1	72	0	1	47	0				2	119	0
Nagaland	Scrub Typhus	1	100	0							1	100	0
Nagaland Total		1	100	0							1	100	0
Odisha	Anthrax	5	46	1	4	52	6	19	103	1	28	201	8
Odisha Total		5	46	1	4	52	6	19	103	1	28	201	8
Punjab	Leptospirosis							1	1	0	1	1	0
Punjab Total								1	1	0	1	1	0
Rajasthan	Crimean Congo Haemorrhagic Fever (CCHF)	1	1	0	2	5	1				3	6	1
	Leptospirosis				1	1	0				1	1	0
	Scrub Typhus				1	102	4	1	257	1	4	359	5
Rajasthan Total		1	1	0	4	108	5	1	257	1	8	366	6
Tamil Nadu	Leptospirosis	5	70	0	1	63	0	4	153	0	12	286	0
	Scrub Typhus	1	10	1	1	9	0				2	19	1
Tamil Nadu Total		6	80	1	4	72	0	4	153	0	14	305	1
Uttar Pradesh	Crimean Congo Haemorrhagic Fever (CCHF)				1	1	0				1	1	0
Uttar Pradesh Total					1	1	0				1	1	0
West Bengal	Anthrax	1	9	0	5	85	3	2	22	0	8	116	3
West Bengal Total		1	9	0	5	85	3	2	22	0	8	116	3

4

Justification of proposal

- Leptospirosis is public health problem
- Frequent outbreaks of Leptospirosis are being reported
- Predominantly young adult males are affected
- Disease is easily treatable
- Mortality is preventable if detected and treated early
- The disease is preventable by judicious use of chemoprophylaxis

5

Pilot Project on Leptospirosis

Two Year Pilot project on Control of Leptospirosis : XIth Five Year Plan in March, 2008.

- **4 districts of Gujarat i.e. Surat, Navsari and Valsad;**
- **2 districts of Kerala i.e Kottayam and Allepey**
- **2 districts of Tamil Nadu i.e. Villupuram and Thiruchirapalli.**

Later expanded to two more states in2010 & 2011

- **2 districts of. Maharashtra (Ratnagiri& Thane)**
- **2 districts of Karnataka (Mangalore &Shimoga) in 2010-2011.**

Funds were allocated to the states –

- **Strengthening of diagnostic facilities,**
- **Trained manpower,**
- **Strengthening intersector3al coordination and**
- **IEC activities**

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Objective:

- Reduction of morbidity and mortality due to Leptospirosis
- Area of Implementation Endemic States:
  - Maharashtra
  - Gujarat
  - Karnataka
  - Tamil Nadu
  - Kerala
  - Andaman & Nicobar

7

Strategy:

a) Early detection of cases.

b) Strengthening of diagnostic facilities

c) Trained manpower – development

d) Strengthening of patient management facilities.

e) IEC activities

8

Activities at the Centre:

(1) Nodal agency NCDC, Delhi

- **Strengthening:**
  - Medical - Rs.60,000/-
  - DEO - Rs.10,406/-
  - Funds - professional Services
- **Development of Guidelines for prevention and Control of Leptospirosis.**
- **Expert group meetings:**
  - Experts : Medical, Veterinary, Animal husbandry, Agriculture
  - Duration : 1 day
  - Funds : OAE

9

Activities at the Centre: Contd....

(2) Training

- Training course for : Training of core trainers in early detection and patient management
- Duration : 1 day
- Funds : OAE

(3) Strengthening of Laboratory Diagnosis

- Identification of the Laboratories
- Training of core trainers in Laboratory techniques
- Duration : 2 day
- Funds : OAE

10

Activities at the Centre: Contd....

(4) IEC

Development of prototype IEC material

- Expert group meeting
- Expert : Medical, Veterinary, animal husbandry and CHEB
- Duration : 1day
- Funds : OAE and Advertisement & Publicity.

11

Activities of implementing State

- ❑ Signing of MOU
- ❑ Identification of Nodal Officer
- ❑ Strengthening for Early detection and management of patients
  - Training
    - Identification of core trainers for training at NCDC
    - Organize training of medical and paramedical staff by core trainers
  - Duration : 1 day each
  - Funds : OAE
- ❑ Ensuring proper infrastructure and logistics for patient management out of their own funds.

12

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Activities of implement State Contd....

- Strengthening of laboratory diagnosis
  - Identification of core trainers to be trained at NCDC in laboratories techniques
- Training courses for further training in laboratory diagnosis by core trainers
- Duration : 2 days each

  Funds : OAE

IEC:

Translation and Dissemination of the prototype IEC material in respective states.

- Funds : Advertisement & publicity.

13

Budget

Rs. In crore

Prevention and Control of Leptospirosis						
	1st year	2nd year	3rd year	4th year	5th year	Total
Professional services	–	0.108	0.12	0.12	0.12	0.468
Other Administrative Expenses	–	0.0066	0.0066	0.0066	0.0066	0.026
Material & Supply	–	0.2134	0.57	.057	0.57	1.924
Advertisement & Publicity	–	0.02	-	-	-	0.02
Travel Expenses	–	0.05	0.05	0.05	0.05	0.20
Other charges	–	0.102	0.1077	0.1077	0.1077	1.1151
Total	–	0.50	1.0843	1.0843	1.0843	3.753

14

Activities to be implemented as per operational guidelines

- Identification of District Focal Point.
- Identification of problem districts in the State and diseases mapping.
- Trainings on Diagnosis & Case Management of Leptospirosis.
- Strengthening Diagnostic facility.
- Strengthening case management facility.
- IEC Activities.
- Strengthening Surveillance for Leptospirosis
- Outbreak Reporting.
- Measures for Prevention and control of Leptospirosis-intersectoral coordination

15

Intersectoral co-ordination

- Sensitization of other sectors viz. veterinary and agriculture has resulted in establishment of intersectoral coordination for prevention and control of Leptospirosis

16

Present Status:

- Funds have been released to program state Gujarat, Tamil Nadu , Kerala, Karnataka, Maharashtra for the 2015-16.
- Meeting for sensitization of nodal officer and training of master trainers for implementation of the program has been carried out at NCDC.
- Guidelines for diagnosis case management prevention and control of leptospirosis have been published and uploaded on NCDC website
- Draft Operational Guidelines for the program circulated to the State Nodal Officers.
- Prototype IEC material has been developed for distribution to states.
- Mass media campaign through news paper advertisement carried out in 2015-16.

17

Issues

- GIA released in 2015-16 to all program states . None of the State could utilize the funds in 2015-16 therefore funds revalidated for utilization in current FY.
- State of Gujarat and Kerala and TN submitted activity reports Gujarat Submitted UC (Nil Utilization).
- Andaman and nikobar island –GIA could not be utilized as the funds provided under the single Budget head "Salary" and as per SFC , GIA is provided only for IEC and Trainings etc.

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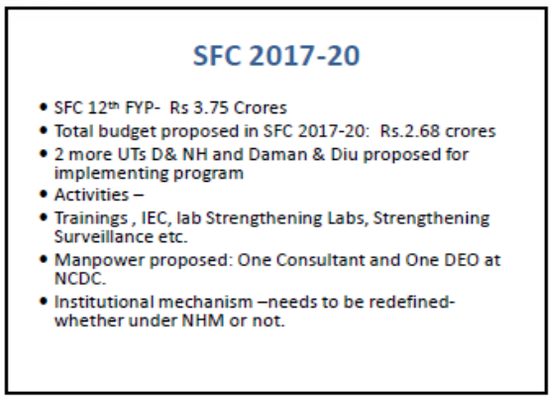

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3. Leptospirosis situation in Karnataka

Dr. Ravi Kumar, Senior Regional Director, Regional Office for Health & Family Welfare, Govt. of India. Bangalore.

It is an important public health problem in Karnataka. Prevalent in coastal and hilly areas. Often under diagnosed, under reported. IDSP attempts to conduct surveillance of leptospirosis through syndromic surveillance and laboratory surveillance. Many district level sentinel laboratories and medical college laboratories conduct tests. Found incidentally among fever cases in some outbreaks. There are 32 sentinel laboratories in the state. 5 District Public health laboratories namely Dakshina Kannada, Chitradurga, Chamarajnagara, Udupi, and Kolar conduct Leptospirosis IgM ELISA tests. Apart from them district surveillance unit at Uttara Kannada also conducts the tests. Referral laboratories at Bangalore Medical College, Shimoga Institute of Medical Sciences, Hassan Medical College, Vijaynagar Institute of Medical Sciences - Bellary, Bidar Medical College, Belgaum Institute of Medical Sciences, Karnataka institute of Medical Sciences, Mysore Medical College conduct tests for those samples sent during outbreak investigations. Leptospirosis project in Shimoga:19 lakh INR received by Shimoga district, 18.7 lakhs spent. Funds received for training, kits, lab supplies, administrative activities and IEC. Both RDT and IgM ELISA were conducted. 237 samples were tested by ELISA and RDT. 153 were negative by ELISA but out of which there were 3 positives by RDT. 84 were positive by ELISA but out of which only 1 positive by RDT.

The proposed activities for control of leptospirosis in Karnataka:

- **Early detection of cases:** Early detection of cases by dark field microscopy will be done by using dark ground microscope adopters to existing microscopes.
- **Strengthening of Diagnostic laboratories:** Enough number of kits will be ensured for detection of leptospirosis at all the 9 problematic districts.
- **Sensitization of Medical Officers and health workers:** Medical officers, health workers and ASHA workers will be sensitized on leptospirosis covering 15 taluks in 9 districts.

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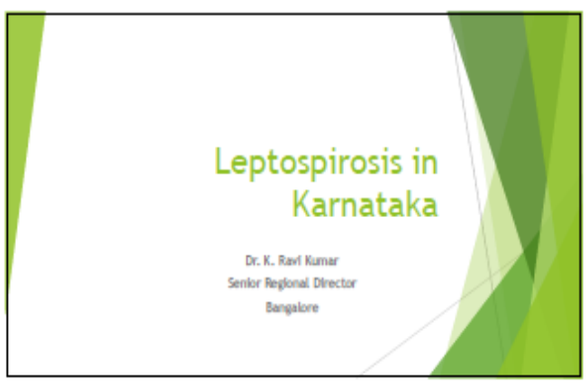
- **Serotyping and sequencing**: A collaboration work with Southern Regional Disease Diagnostic Laboratory (SRDDL) lab of Institute of Animal Health & Veterinary Biologicals (IAH&VB), Bengaluru and National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru.
- **Rodent control activities:** Collaboration with the agriculture department will be made to take up anti rodent activities in few affected areas.
- **IEC:** We have 15 affected taluks in 9 districts. IEC materials in the form of leaflets, Flex, banners, and hoardings will be prepared and distributed to all 15 taluks.
- **Animal leptospirosis surveillance:** Collaboration will be made with IAH & VB for the animal leptospirosis surveillance in the 15 human leptospirosis affected taluks.



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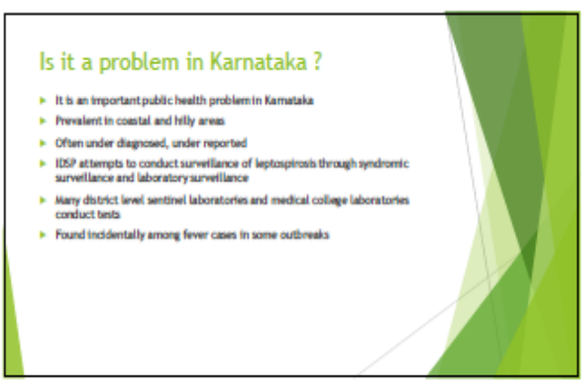

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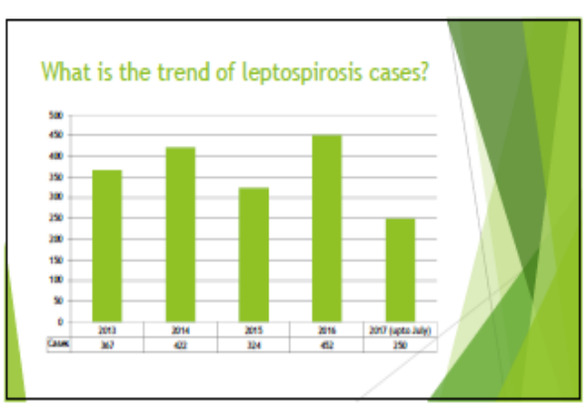

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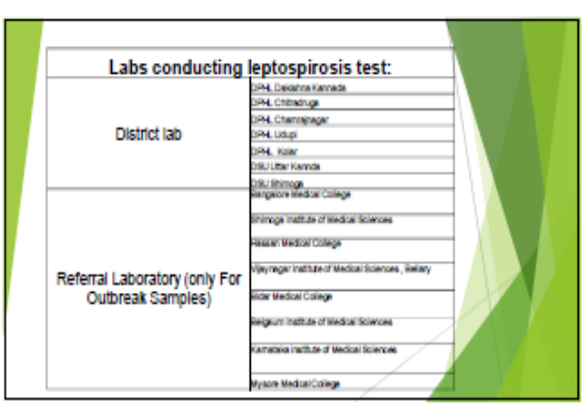

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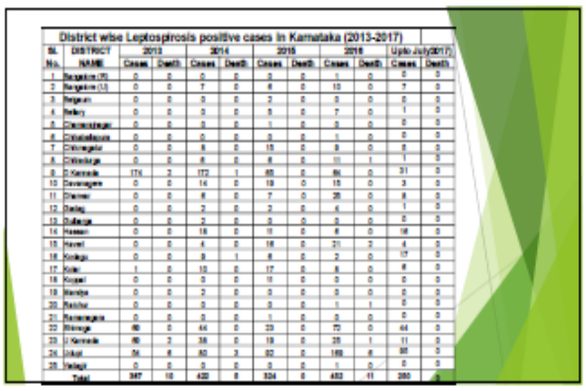

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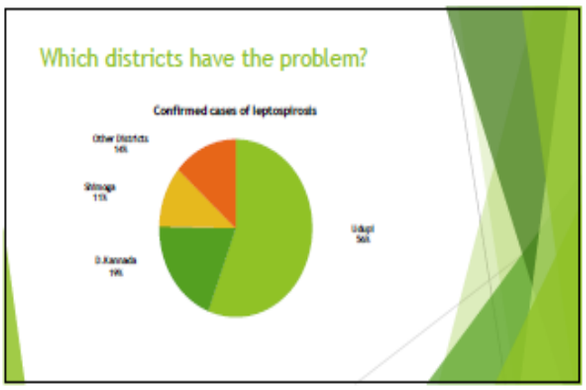

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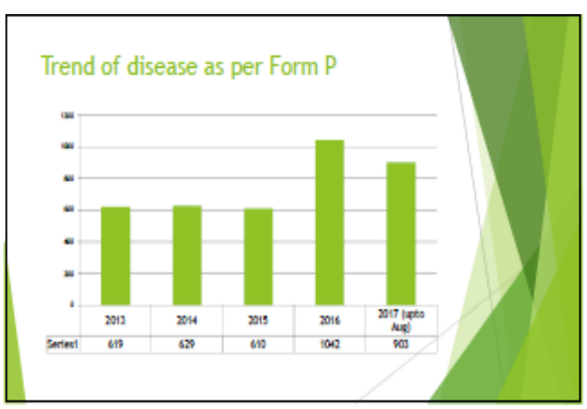

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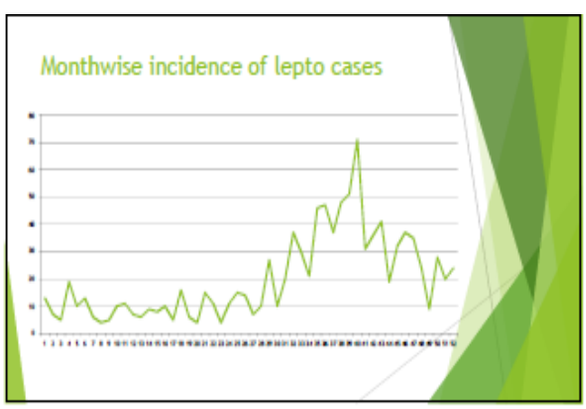

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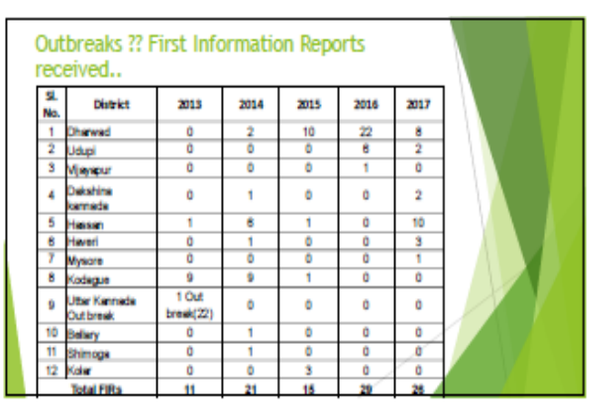

Sl. No.	District	2013	2014	2015	2016	2017
1	Dharwad	0	2	10	22	8
2	Udupi	0	0	0	6	2
3	Vijayapur	0	0	0	1	0
4	Dakshina kannada	0	1	0	0	2
5	Hassan	1	6	1	0	10
6	Haveri	0	1	0	0	3
7	Mysore	0	0	0	0	1
8	Kodagu	9	9	1	0	0
9	Uttar Kannada Out break	1 Out break(22)	0	0	0	0
10	Bellary	0	1	0	0	0
11	Shimoga	0	1	0	0	0
12	Kolar	0	0	3	0	0
	Total Fills	11	21	15	29	26

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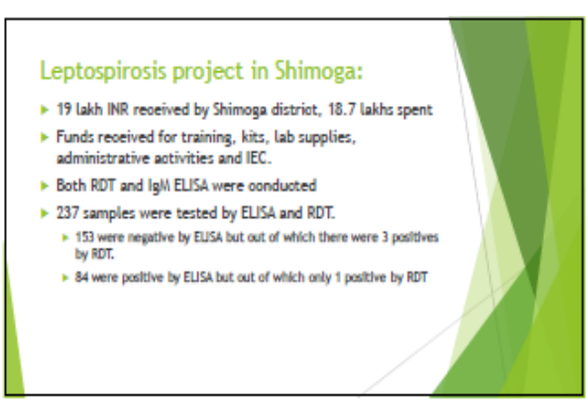

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Trend of lepto cases in Shimoga district

Year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Cases	0	7	23	51	20	14	69	85	47	69	194	95	42	29	46	38

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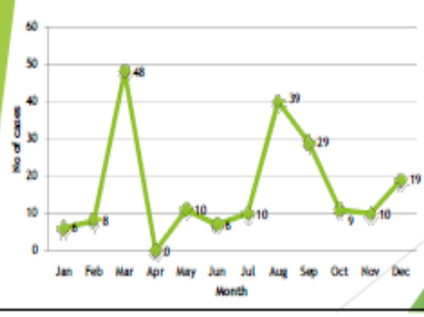
Month wise Distribution of Lepto cases-2012 -Shimoga



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Age and gender-wise distribution of cases

	< 5 yrs	5 to 10 yrs	10 to 15 yrs	> 15 yrs	Total
Male	0	3	4	93	100
Female	1	2	3	88	94
Total	1	5	7	181	194

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Dakshina Kannada project

	Samples tested	Positives	Deaths
2012	284	93	2
2013	318	76	2
2014	264	33	1
2015	343	54	0
2016	308	54	0
2017 (upto July)	152	17	0

- 19 lakh INR received by Dakshina Kannada district, 18.8 lakhs spent

14

The proposed activities for control of leptospirosis in Karnataka

- **Early detection of cases:** Early detection of cases by dark field microscopy will be done by using dark ground microscope adopters to existing microscopes.
- **Strengthening of Diagnostic laboratories:** Enough number of kits will be ensured for detection of leptospirosis at all the 9 problematic districts.
- **Sensitization of Medical Officers and health workers:** Medical officers, health workers and ASHA workers will be sensitized on leptospirosis covering 15 taluks in 9 districts.
- **Serotyping and sequencing:** A collaboration work with Southern Regional Disease Diagnostic Laboratory (SRDDL) lab of Institute of Animal Health & Veterinary Biologicals (IAH&VB), Bengaluru and National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru.
- **Rodent control activities:** Collaboration with the agriculture department will be made to take up anti rodent activities in few affected areas.
- **IEC:** We have 15 affected taluks in 9 districts. IEC materials in the form of leaflets, Flex, banners, and hoardings will be prepared and distributed to all 15 taluks.
- **Animal leptospirosis surveillance:** Collaboration will be made with IAH & VB for the animal leptospirosis surveillance in the 15 human leptospirosis affected taluks.

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4. Leptospirosis situation in Gujarat

Dr. DinkarRawal,Deputy Director (Epidemic),Commissionerate of Health, M.S. and M.E. Gandhinagar, Gujarat.

Leptospirosis is a zoonotic disease and accidently transmitted to human beings as an occupationalhazard. It prevailed throughout the world however their distribution is concentrated in tropical and sub-tropical countries where the soil pH and moisture are favorablefor their survival. It is closely associated with rice fields, rains and rodents. In additionto rats other animals like cow, goat, buffalos are also known to transmit this infection to man. Leptospirosis cases were seen for the first time in Gujarat in the Chikhli block of the old Valsad district in year 1994. Since then cases of Leptospirosis are continuously reported from Navsari, Valsad,Tapi& Surat districts of South Gujarat.In the year 2006 there were heavy floods in Surat city due to overflow in Tapi River. It resulted in occurrence of 379 cases of Suspected Leptospirosis in Surat city itself with 43 deaths. Surat Navsari, Valsad and Tapi are the 4 districts and Surat Municipal Corporation in the South Gujarat mainly affected due to Leptospirosis. It has found strong correlation with the community involved in farming and animal handling. Atmospherically it is associated with heavy rain fall, flood situations and salinity of soil. As per clinical symptoms deaths are due to renal complications earlier has shown shift over pulmonary complications since last three-four years. The majority of cases and deaths are prevalent from June to September months of every year. Mean of Case Fatality Rate (CFR) of last 20 years is 13.25%. Heavy clustering of cases is found around Gandevi District of Valsad and in the August months of every year and now in valod Taluka of Tapi.Massive anti-rodent measures and mass drug administration – chemoprophylaxis to high risk groups have been undertaken by District Administration in all districts of south Gujarat , still its effect in reducing cases & deaths due to leptospirosis is not as desired.Since last 3 years, numbers of cases of Leptospirosis have been tremendously decreased in Gujarat due to comprehensive efforts by various departments. Last year total 55 cases and 2 deaths have been noted. In current year till 7/9/2017, total 34 cases and 2 deaths have been occurred. This year maximum number of cases have been occurred in Surat district.

Special Control measures by State as mentioned below have paid rich dividends in reducing morbidity and mortality in past few years.

1) Special round the clock control rooms are made functional at the level of BHO, CDHO, NCH, SMC, RDD office, Surat & at Commissionerate(Health) office at State Level for easy flow of day to day information, feedback & corrective actions.
2) Day to day surveillance activities carried out at village level as per stipulated program so that every village gets surveyed at least once in a week's time.
3) Villages are stratified as per their endemicity in earlier years in High & Low risk groups so as to prioritize them in surveillance and providing Chemo prophylactic coverage with Cap. Doxycycline to high risk Group People staying in these villages at their doorsteps and under supervision on a weekly basis.
4) Special attention to track and follow up all fever cases in the endemic villages after onset of fever, during which effects of Presumptive & Radical Treatment for Malaria are also reviewed and follow up smears, are also collected.
5) Leptospirosis has been included as a State specific disease under IDSP program & primary training along with prescribed NICD modules has been given across the state as part of Integrated Disease Surveillance Programme Training Curriculum.

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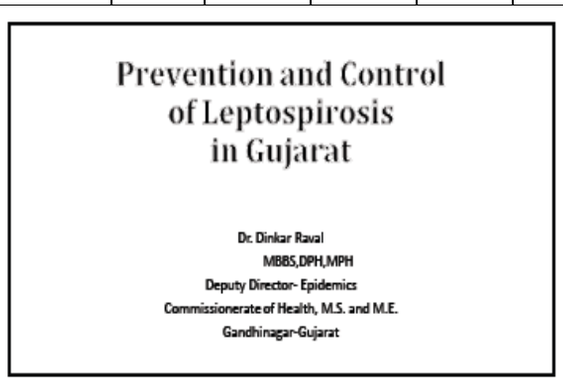
6) Lots of activities are directed towards strengthening of Inter Departmental Co-ordination with Dept. of Agriculture, Gujarat Agriculture University, Navsari, Dept. of Animal Husbandry, Dairy & Sugar Co Cooperatives, Irrigation as well as various Local Self Governmental institutions.
7) Interdepartmental meetings held since month of January regularly at state and regional level
8) Crisis Management Group meeting is held at state level before the start of outbreak and during outbreak
9) Drugs/ Logistics procured before the month of June and made available in the field
10) Module developed by experts from medical college
11) Modular Training to Block Health Officers, Medical officers and paramedical staffs
12) Sensitization of ASHA and other field staff by SATCOM
13) Experts like physician and Anesthetists deputed at CHC Bardoli& CHC Chikhli during outbreak
14) Intensive IEC done through Hoardings, Wall Paintings, Stickers, Banners, Pamphlets, LeptoRath, Bhavai, TV Scroll, Quickies, Radio Jingles, Radio Bytes, etc.
15) Case and Death audit is done by PSM Department of Medical Colleges

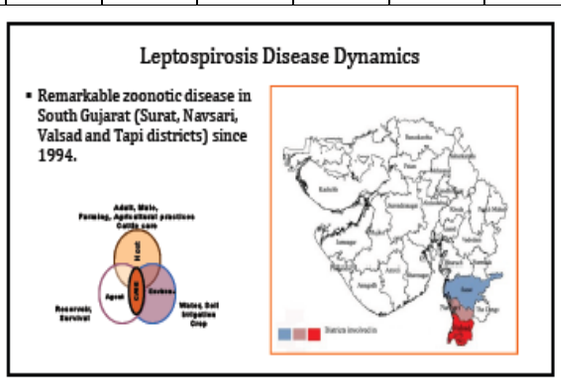
Actions required:

1. Systemic research in prevailing animal sera and its relevance in causing human disease.
2. Evaluation of role of chemoprophylaxis in prevention or reducing morbidity of leptospirosis.
3. Evaluation of role of anti-rodent measures in prevention or reducing morbidity of leptospirosis.
4. Further strengtheningof District Hospitals for Diagnosis and treatment in Leptospirosis prone area including ventilator facility up to CHC level in affected Taluka.
5. Identification of right mix of IEC so as to facilitate early reporting of cases.
6. Ways to strengthen interdepartmental coordination.

Status of Leptospirosis:-

Year →	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
No of Cases	621	575	225	611	919	157	308	90	41	55	34
No of Deaths	135	127	49	124	178	26	38	11	0	2	2
CFR	25.91	22.08	21.78	20.29	19.39	16.77	12.34	12.22	0	3.64	5.88





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Regional Profile (District wise) - 2017

Demography					Health Infrastructure			
District	Taluka	Population	Villages	Houses	PHC	CHC	Gen. Hosp.	Medical college
Surat	9	1652932	718	356932	58	14	1	2
Tapi	7	837079	564	175058	38	8	1	0
Navsari	6	1484492	462	319921	45	12	1	0
Valsad	6	1777670	470	357239	48	10	1	1
Total	28	5752173	2214	1209150	189	44	4	3

3

Mortality & Morbidity Status of Leptospirosis

District	2011		2012		2013		2014		2015		2016		2017	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D
SURAT	329	72	33	8	109	11	18	5	13	0	19	1	22	1
TAPI	293	50	73	12	69	11	34	4	8	0	10	0	5	1
NAVSARI	156	30	28	5	60	8	16	1	11	0	19	1	8	0
VALSAD	119	22	18	0	59	6	22	1	9	0	6	0	0	0
SMC	19	3	3	1	11	2	0	0	0	0	0	0	0	0
Total	916	177	155	26	308	38	90	11	41	0	55	2	35	2

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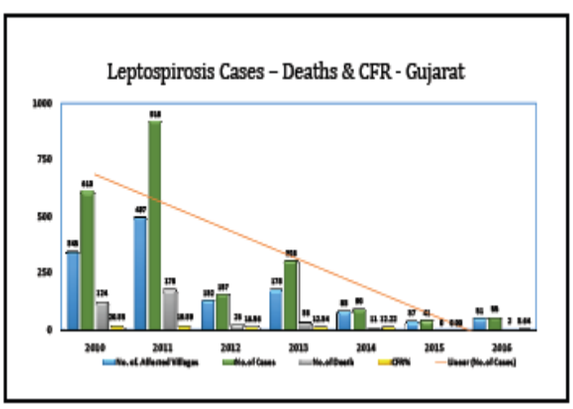

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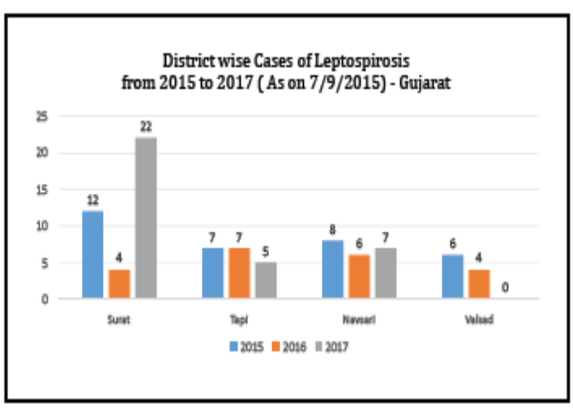

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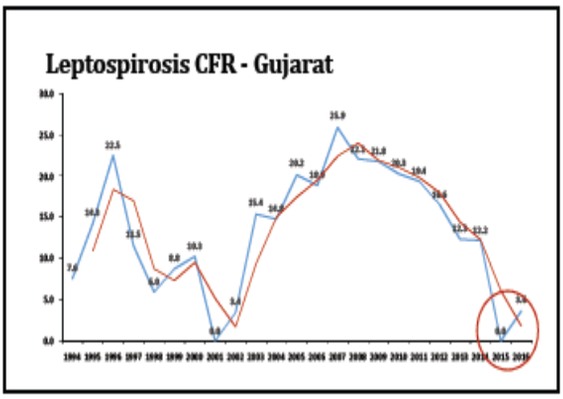

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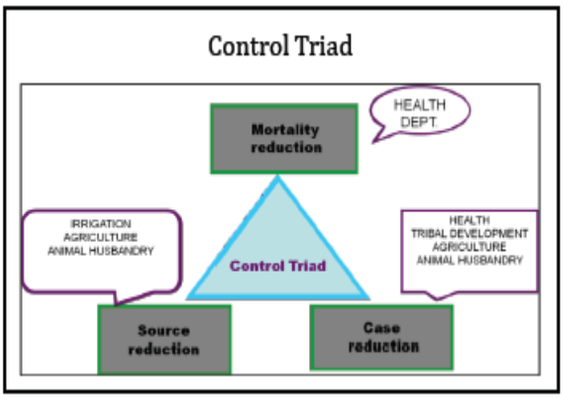

Activity plan for Leptospirosis

No	Activity	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
1.	State Task Force Meeting												
2	Identification of high risk villages												
3	Interdepartmental & review Meeting at region level												
4	Identification and strengthening of Treatment center												
5	Review IEC strategy												
6	Procurementof drug & RTK												
8	Fund Allocation												
9	Identification of Primary informer												

9

Continue....

No	Activity	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
10	Training of staff & ASHA												
11	Meeting with all field staff through SATCOM from BISAG												
12	Animal disease / Sero surveillance												
13	Anti-Rodent Activity (ARA)												
14	VHSC meeting												
15	Intensified IEC												
16	Media briefing												
17	Chemoprophylaxis												
18	Active Case Search						During Outbreak						
19	Case and Death investigation						Every 15th and 30th of month during outbreak						

10

Role of Different Departments

Department	Responsibility
Health & Family Welfare Department	• Preparing action plan, guidelines, protocols. • Training to medical, paramedical and other staff. • Chemoprophylaxis on Directly Observed Treatment(DOT)mode. • Strengthening of treatment centers • Active Search & Prompt treatment • IEC/BCC activities • Data analysis and feedback
Agriculture Department	• Preparation of Action plan for Anti Rodent Activity (ARA) • Fund for ARA and other activities • Procurement of drugs for ARA • Training of field workers • Intensive IEC for ARA specially in *'Krishi Mahotsav'* • Supervision & Monitoring
Animal Husbandry Department	• Disease Surveillance, particularly more emphasis to the high risk villages • Sero-surveillance to ascertain existing serovar & mapping of serovar . • Chemoprophylaxis to animals • Anti tick measures by spraying acaricide drug on all animals • Data sharing with health department

11

Role of Different Departments

Department	Responsibility
Panchayat Department Tribal Development Department	• Activating PRI members & the "Gram Sanjivani Samity" • Awareness regarding basic information of Leptospirosis • Local Group for immediate reporting and prompt responsive actions during monsoon season
Rural Development- Urban Development Department	• Proper waste disposal from residential area of village • Maintaining the cleanliness of entire village • Spraying of DTT and Gamaxine in cattle shade • Educate villagers regarding cleanliness of cattle shade and use of repellant • IEC regarding cleanliness and waste disposal among cattle handlers • Co-ordinate with staff of Health Department and Animal Husbandry Department.
Information Department	• Development of Media strategy • Role out appropriate communication materials in vernacular language • Facilitate availability of slots for media communication • Utilize the field publicity units for social mobilization. • Coordination of media for positive and scientific reporting to avert apprehension and fear in community

12

Chemoprophylaxis

- Timings decided at district level
- Weekly basis- for 6 weeks
- More than 29 lakh people to be covered.
- More than 5 crore Tab Doxycycline given:
- Field workers / ASHA ensures DOT. Are incentivized for chemoprophylaxis and suspected case search.

Sr. No.	District	High risk Population	No of Persons given Chemo	No of Cap Doxy used	Person Tab Azithro given	%
1	Surat	667078	652316	1230410	8828	99.1
2	Tapi	694132	655230	1219068	7277	95.4
3	Navsari	654756	622538	1177254	5025	95.8
4	Valsad	950770	947169	1721799	9297	100.6
		2966736	2877253	5348531	30427	98.0

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Anti Rodent Activity 2014 to 2017

Dist	2014		2015		2016		2017	
	Village covered for ARA	Case	Village covered for ARA	Case	Village covered for ARA	Case	Village covered for ARA	Case
Surat	435	18	607	12	673	19	625	22
Tapi	465	34	481	8	468	10	481	5
Navsari	386	16	386	12	388	19	388	7
Valsad	412	22	375	9	439	6	439	0
Total	1698	90	1849	41	1968	54	1933	34

14

Age & Sex wise distribution of Cases and Deaths of Leptospirosis 2016

Age group	Leptospirosis Cases				Total	
	Male		Female			
	Case	Death	Case	Death	Case	Death
0-8	0	0	0	0	0	0
9-15	1	0	0	0	1	0
16-45	32	1	11	0	43	1
46-60	7	0	2	1	9	1
>60	0	0	2	0	2	0
Total	40 (73%)	1	15 (27%)	1	55	2

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Leptospirosis cases Duration between onset and 1st Consultation -2016

Dist.	1-3 Days	4-5 Days	6-7 Days	>7 Days	Total
Surat	16	3	0	0	19
Tapi	7	2	1	0	10
Navsari	14	2	3	0	19
Valsad	6	0	0	0	6
Bharuch	1	0	0	0	1
Total	44	7	4	1	55

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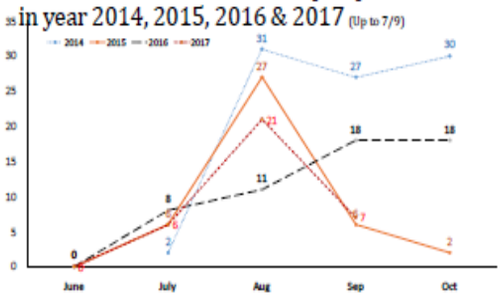
Month wise distribution of Leptospirosis cases in year 2014, 2015, 2016 & 2017 (Up to 7/9)



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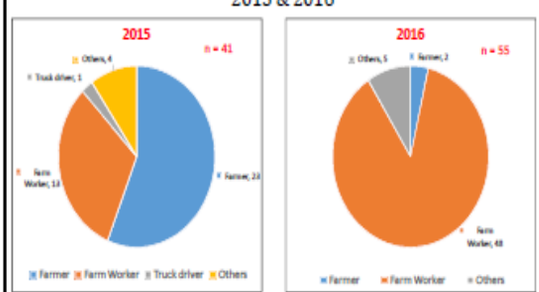
Occupation wise Distribution of Cases of Leptospirosis 2015 & 2016



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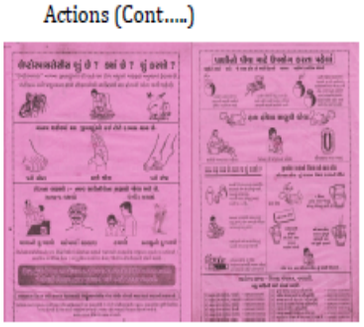
Actions.....

- Active surveillance from mid- June
- Survey of all fever cases (> 6 years of age)
- Cap Doxycycline along with antimalarial treatment.
- 24 /7 control room at District Panchayats and Regional Office Surat
- Modular Training to Taluka Health Officers, Medical officers and paramedical staffs- Module developed by experts from Medical colleges and Regional training center
- Sensitization of ASHA and other field staff by SATCOM
- Deputation of Experts at remote CHCs during outbreak
- Sero surveillance in animal

19

Actions (Cont.....)

- Hoardings,
- Wall Paintings,
- Stickers,
- Banners,
- Pamphlets,
- Lepto Rath,
- Bhavai,
- TV Scroll,
- Quickies,
- Radio Jingles,
- Radio Bytes,
- TV talk show.



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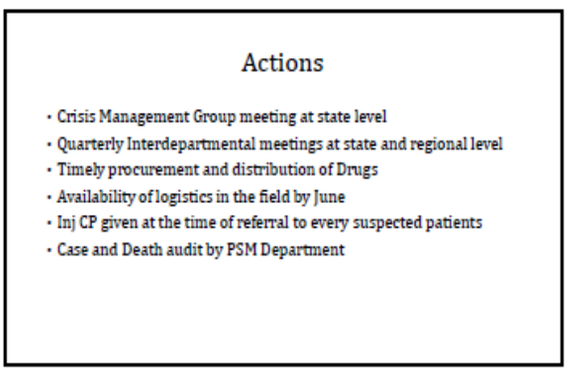


24

5.Leptospirosis situation in Andaman and Nicobar Island

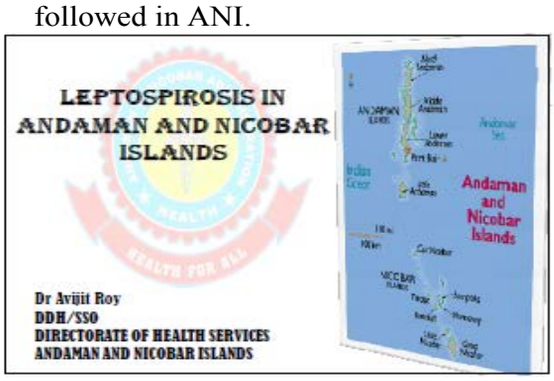
Dr. Avijit Roy, Joint Secretary, IDSP, Andaman & Nicobar Islands.

The first report of leptospirosis in India originated from Andaman Islands way back in 1930s.After this, apparently the disease disappeared or was neglected till it resurfaced in an explosive fashion as outbreaks of a haemorrhagic fever (called locally as Andaman Haemorrhagic Fever)in 1980s.Remained a mysterious disease till its leptospiral aetiology was revealed in 1993.The common circulating serogroups in the recent years are Icterohaemorrhagiae, Grippotyphosa and Australis. So far no confirmed cases reported from the Nicobar Island.Two separate clinical syndromes: one with hepato-renal involvement and the other with lung injury associated with pulmonary haemorrhages.The terminal events in most patients presenting haemorrhagic pneumonitis were massive haemorrhages into the tracheo-bronchial tree resulting in acute respiratory distress.Case fatality ratio upto 30 %.Spurt of cases is seen with the onset of the rain.Initial days cases were reported mostly from Diglipur of North Islands

.

however in recent day's cases are spurted in Middle Andaman also.South Andaman some sporadic cases are reported mostly in the peri-urban area.

During monsoon season advisory is issued to entire Andaman group of Islands to follow the protocol of treating all fever cases on par with Leptospirosis. The RMRC, Port Blair is WHO the collaborative centre for Leptospirosis and they keep updating the Health department about the various aspects of Leptospirosis. The RMRC collects blood sample from various health institutions directly on day to day basis. Further Medical officer I/Cs also send sample to RMRC directly from inter islands sectors. Even the RMRC is taking sample directly from patients,if the patients intend to get the sample tested at RMRC centres. State IDSP unit also keeps an active surveillance on Leptospirosis cases and regular reporting. Directorate of Health Services has put up ELISA reader in G.B.Pant Hospital, Port Blair and proposed to set up one in Dr R.P. Hospital, Mayabunder for N&M Andaman District. Rapid test kits provided to all the endemic areas of Andaman islandsDiagnocure for both IgG and IgM. Directorate of Health Services kept sufficient stock of Doxycycline medicine up to sub-centre level as a first line of management.No prophylaxis regimen is followed in ANI.



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HEALTH INSTITUTIONS ACROSS A & N ISLANDS

Item	S/Andaman District	N & M Andaman District	Nicobar District	Total
Referral Hospital	01	00	00	01
District Hospital	00	01	01	02
AYUSH Hospital	01	00	00	01
CHC	01	02	01	04
PHC	10	08	04	22
Urban Health Centres	05	00	00	05
Sub-centre	38	44	40	122

* PHC Garacharma under Up gradation to District Hospital South Andaman

3

SPECIALISTS / DOCTORS POSITION IN A & N ISLANDS ARE AS UNDER

	SANCTIONED	FILLED			VACANT
		Regular	Ad-hoc	Contract	
Specialists	49	11	1	1	36
Public Health Specialists	01	01			00
Medical Officer	107	61	02	14	30
Medical Officer (Homoeo)	09	-	-	07	02
Medical Officer (Ayur)	02	-	-	01	01
Medical Officer (Siddha)	01	-	-	-	01
Medical Officer (Unani)	01	-	-	-	01
Dental Surgeon	06	02	01	04 *	-

In the island the major problem facing by people regarding health is lack of super specialist doctor. 36 positions out of 49 are vacant.

4

Aerial View of Cellular Jail & G.B. Pant Hospital

ISLAND'S HELATH INDCATIORS

INDICATORS	NATIONAL	A & N ISLANDS
Total population	1236344631*	380581*
Literacy Rate	74.04%*	86.27%*
Birth Rate	21.4%#	14.6%#
Total Fertility Rate (TFR)	2.1	1.6
Sex Ratio	943/1000 males*	876/1000 males*
Child Sex Ratio	918/1000 males*	968/1000 males*
Infant Mortality Rate (IMR)	40#	15.16/1000###
Maternal Mortality Rate (MMR)	178	53.90/Lakh###
Full Antenatal Care	18.8##	48.5##
Institutional Delivery	41%**	96.5 %
Full Immunization	69##	83.5##

Sources: * census 2011, #SRS 2014, ** NFHS-3,##DLHS-3, ### DHS,DD(FW) + RBD

5 6

Background

- The first report of leptospirosis in India originated from Andaman Islands way back in 1930s.
- After this, apparently the disease disappeared or was neglected till it resurfaced in an explosive fashion as outbreaks of a haemorrhagic fever (called locally as Andaman Haemorrhagic Fever) in 1980s.
- Remained a mysterious disease till its leptospiral aetiology was revealed in 1993.
- The common circulating serogoups in the recent years are Icterohaemorrhagiae, Grippotyphosa and Australis.
- So far no confirmed cases reported from the Nicobar Island.

- Two separate clinical syndromes:
  - one with hepato-renal involvement and
  - The other with lung injury associated with pulmonary haemorrhages.
  - The terminal events in most patients presenting haemorrhagic pneumonitis were massive haemorrhages into the tracheo-bronchial tree resulting in acute respiratory distress.
- Case fatality ratio upto 30 %.
- Spurt of cases is seen with the onset of the rain.
- Initial days cases were reported mostly from Diglipur of North Islands however in recent days cases are spurted in Middle Andaman also.
- South Andaman some sporadic cases are reported mostly in the peri-urban area.

7 8

GUITAR ISLAND

YEAR WISE LEPTOSPIROSIS CASES

YEAR	NO OF CASES*	Deaths
2017(till August)	09	01
2016	255	03
2015	147	00
2014	142	06

* Sources : IDSP Portal and state report

9 10

PREVENTIVE STEP TAKEN

- During monsoon season advisory is issued to entire Andaman group of Islands to follow the protocol of treating all fever cases on par with Leptospirosis.
- The RMRC, Port Blair is WHO the collaborative centre for Leptospirosis and they keep updating the Health department about the various aspects of Leptospirosis.
- The RMRC collects blood sample from various health institutions directly on day to day basis. Further Medical officer I/Cs also send sample to RMRC directly from inter islands sectors.
- Even the RMRC is taking sample directly from patients ,if the patients intend to get the sample tested at RMRC centres

- State IDSP unit also keeps an active surveillance on Leptospirosis cases and regular reporting.
- Directorate of Health Services has put up ELISA reader in G.B.Pant Hospital, Port Blair and proposed to set up one in Dr R.P. Hospital , Mayabunder for N&M Andaman District.
- Rapid test kits provided to all the endemic areas of Andaman islands Diagnocure for both IgG and IgM.
- Directorate of Health Services kept sufficient stock of Doxycycline medicine up to sub-centre level as a first line of management.
- No prophylaxis regimen is followed in ANI.

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6. Leptospira Research activities at Government Medical College Surat, Gujarat

Dr.NeetaKhandelwal, Professor & Head, Department of Microbiology, Government Medical College, Surat, Gujarat.

Leptospirosis is endemic in South Gujarat, the laboratory receive sample from all over Gujarat and beside Gujarat from western India and Northern India. Leptospirosis laboratory is state of art in western zone, well equipped with basic as well molecular equipment.(RT-PCR machine).Laboratory is doing battery of test for leptospirosis, like Rapid,ELISA,MAT,PCR and Culture.The faculties are well trained from reference centre of India, (WHO ICMR) Andaman,Portblair.One of the training was awarded by Dr.Ramdas, Health minister of India in 2005. Awarded by Health & family welfare department of government of Gujarat for excellent work done in Leptospirosis control in post flood outbreak in Surat in 2007. On-going Research Activity: Immuno-proteomics in leptospirosis: towards laboratory diagnosis of pathogen and non-pathogenic leptospirosis and candidate vaccine.

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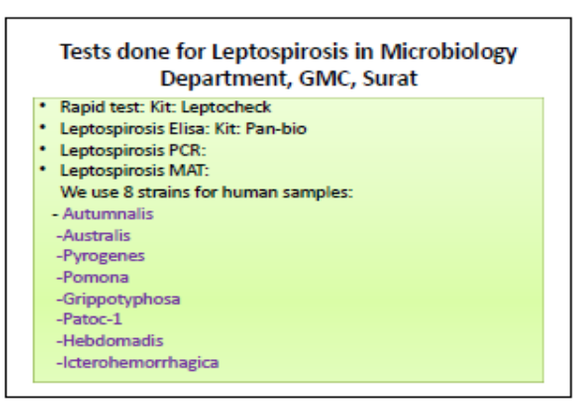

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Infrastructure:

- Leptospirosis laboratory is located at 3rd floor, Department of Microbiology having total 8 rooms & common lobby like
  - 1 DGI & MAT room
  - 1 Culture room
  - 1 Pre PCR room
  - 1 Autoclave and washing room
  - 1 PCR room
  - 1 reporting room
  - 1 In charge room
  - 1 ELISA room

3

Equipments of Laboratory

- Deep freeze: (-80°C): 1
- Deep freeze: (-20°C): 1
- Biosafety cabinets: 2
- Centrifuge Spin column: 1
- Centrifuge Digital: 1
- Refrigerated Centrifuge : 3
- PCR machine: 4
- DGI microscope: 1
- Autoclave: 1
- ELISA reader: 2
- ELISA washer: 1
- Bio safety cabinet: 4
- Water bath- 2

4

Leptospirosis annual human sample load 2011-2017

Test/ Year	2011		2012		2013		2014		2015		2016		2017 till 6-9-17	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P
Rapid	533	108	406	143	237	117	67	28	29	8	50	31	42	25
ELISA	983	474	564	250	561	354	115	58	63	27	100	56	83	50
PCR	118	46	381	57	411	81	92	11	46	3	67	2	68	8
MAT	984	424	564	218	544	216	98	30	56	22	77	41	78	46

T- Total, P- Positive

5

Predominant strains 2011-2016-Human

Districts/ Year	2011	2012	2013	2014	2015	2016
Surat	australis	australis	australis	australis, autumnalis	australis	australis, autumnalis
Navsari	australis, autumnalis	australis	autumnalis	autumnalis	autumnalis	australis
Valsad	australis, pyrogens	australis	australis	australis	-------	-------
Tapi	australis, autumnalis	australis, autumnalis	australis, autumnalis	australis	-------	australis, autumnalis

6

Leptospirosis data 2016-Human

7

(DIFFERENT LABORATORY DIAGNOSTIC TEST)

FROM 01-01-2016 to 28-12-2016

DISTRICTS	Rapid		IgM ELISA		MAT-1		PCR	
	Total	Positive	Total	Positive	Total	Positive	Total	Positive
SURAT	31	20	64	24	30	15	38	00
NAVSARI	08	06	10	06	08	04	11	00
VALSAD	02	02	02	01	02	00	02	01
TAPI	00	00	01	01	01	01	01	00
MISCELL.	09	03	19	04	13	02	14	01
TOTAL	50	31	100	36	76	22	66	02

8

Leptospirosis Analysis -2017

9

(DIFFERENT LABORATORY DIAGNOSTIC TEST)
FROM 01-01-2017 to 09-09-2017

DISTRICTS	Rapid		IgM ELISA		MAT-1		PCR	
	Total	Positive	Total	Positive	Total	Positive	Total	Positive
SURAT	29	15	39	21	34	19	41	04
NAVSARI	09	08	11	07	12	05	13	02
VALSAD	01	00	04	02	02	01	07	01
TAPI	02	01	02	01	02	01	02	01
MISCELL	01	01	07	02	09	03	05	00
TOTAL	42	25	63	33	59	29	68	08

10

Leptospirosis data from 2011-2017-Animal

YEAR	LEPTO ANIMAL	
	MAT	LEPTO PCR
2011	439	--
2012	2335	1507
2013	1407	1407
2014	1978	1615
2015	5	--
2016	1910	864
2017 till 09.09.17	1600	750
TOTAL	9674	6143

11

Leptospirosis research activity

12

Leptospirosis research papers by Department of Microbiology, GMC, Surat

- Tanvi P, Summaiya M, Parul P. Seroprevalence of leptospirosis in south gujarat region by evaluating the two rapid commercial diagnostic kits against the mat test for detection of antibodies to leptospira interrogans. *National journal of community medicine* 2011;2(1):64-70.
- Tanvi P, Sangita BR, Summaiya M. To evaluate the different rapid screening tests for diagnosis of leptospirosis. *J of Clin and Diag Res* 2015;9(2):21-24.
- Tanvi P, Summaiya M. Seroprevalence of the cattle leptospirosis in south Gujarat region of India. *Journal of Agriculture and Veterinary Science* 2015;8(2):8-11.

13

Research papers cont..

- Tanvi P, Summaiya M. To identify the prevalence leptospira serogroups in the cases from southern Gujarat region. *National Journal of Laboratory Medicine* 2016;5:34-38.
- Summaiya M, Tanvi P. Polymerase Chain Reaction: An Important Tool for Early Diagnosis of Leptospirosis Cases. *Journal of Clinical and Diagnostic Research*,2016 ;10(12): DC08-DC11.
- Summaiya M, Tanvi P. Epidemiological study on human, cattle and rodent leptospirosis in South Gujarat region of India. In publication-Accepted in Annals of pathology and laboratory medicine journal.

14

On going thesis on Leptospirosis

15

Comparative study for employing Microscopic Agglutination Test using patient *Leptospira isolates with the reference strains of Leptospira.*

16

Objectives of the study

By comparing the results of Microscopic Agglutination Test using the patient isolates with the reference strains we come to know if there is any new or different strain of *Leptospira circulating in South Gujarat because till now no such studies has been done over this aspects.*

17

Inclusion criteria

- For comparison of the Microscopic Agglutination Test using patient *Leptospira* isolates with the reference strain of *Leptospira,* samples whose Igm ELISA Serion-virion titre ≥100 units or IgM ELISA Panbio titre ≥25 Panbio units were only included.

18

Methodology:

- Samples from either New Civil Hospital or from peripheral health centres that came to the Department of Microbiology with proper requisition were included in the study and processed further.
- MAT was performed with both, the reference strains of Leptospira (according to the recommendation of WHO) as well as the isolates form the population of South Gujarat.

19

Contd...

- 8 reference strains and 32 isolated strains were taken as antigens for performing MAT.
- The results of MAT obtained, were recorded.
- Analysis and results are under process.

20

Leptospirosis data 2011-2017-Animal

21

Animal sample surveillance data 2016-2017 till now

DISTRICTS	MAT		PCR	
	Total sample tested 2016	Total sample received 2017 till now	Total sample tested 2016	Total sample received 2017 till now
NAVSARI	660	750	444	750
VALSAD	450	350	420	Not requested
TAPI	700	450	Not requested	Not requested
SURAT	100	50	Not requested	Not requested
TOTAL	1910	1600	864	750

22

Predominant strains in animal 2011-2016

Year	Predominant strain
2011	australis, petoc
2012	australis, icterohemorrhagica
2013	australis, petoc
2014	australis, petoc
2015	----------
2016	australis

23

Surveillance data
Leptospirosis

24

Total healthy human samples received

- Navsari: 64
- Valsad: 49
- Tapi: 56

25

IgM ELISA

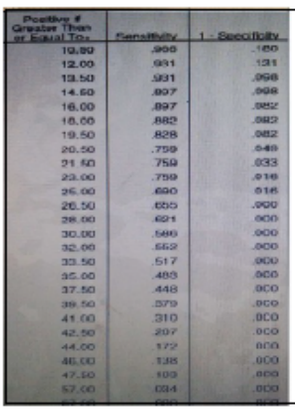
- Panbio ELISA kit: routine kit
- Cut off as per kit:
  <9 panbio unit: Negative
  9-11 panbio unit: Inderminate
  >11 panbio unit: Positive
- ROC analysis: To evaluate baseline cutoff according to geographic location where disease is endemic

26

Receiver Operating Characteristics ROC

- Compilation of total testing data of healthy human, confirm positive and other disease positives and decision on cutoff

27



Navsari
Best cut off value 14.5 panbio units

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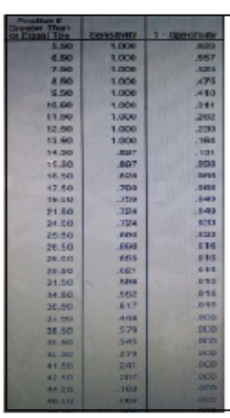
Valsad
Best cut off value 14.5 panbio units

29



Tapi
Best cut off value 15.5 panbio units

30

Microscopic agglutination test (MAT)

- Gold standard for diagnosis
- Panel of leptospira strains are pyrogen, australis, automonalis, Icterohemorrhagica, Grippotyphosa, Patoc, Pomona and Hebdo.
- Titre >400 suggestive of infection or
- Rising titre in paired sera is suggestive of infection
- Cross reactions can occur

31

Navsari

- Pyrogen: 0
- Australis: 11
- Automonalis: 1
- Gripho: 0
- Patoc: 1
- Pomona: 2
- Ictro: 0
- Hebdo: 1
- Canicola: 0
- Hardgio: 0
- Ballum: 0
- Batavia: 0

32

Valsad

- Pyrogen: 0
- Australis: 1
- Automonalis: 5
- Grippo: 2
- Patoc: 1
- Pomona: 2
- Ictero: 0
- Hebdo: 2
- Canicola: 0
- Hardgio: 3
- Ballum: 1
- Batavia: 2

33

Tapi

- Pyrogen: 0
- Australis: 14
- Automonalis: 17
- Grippo: 1
- Patoc: 0
- Pomona: 1
- Ictero: 1
- Hebdo: 4
- Canicola: 1
- Hardgio: 9
- Ballum: 2
- Batavia: 0

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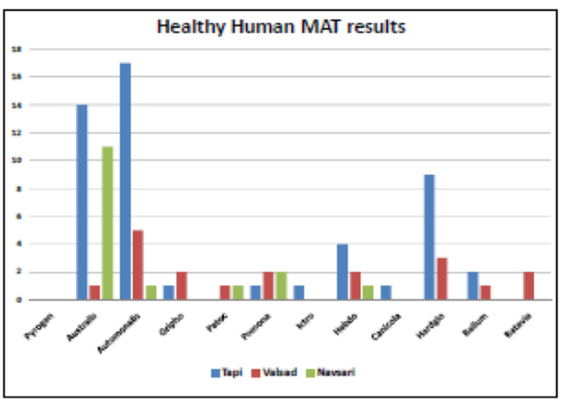

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Human healthy sample surveillance data 2016 for determination of cut off titre

DISTRICTS	MAT			ELISA	
	Total sample tested	Samples with significant titre(≥50)	Samples with titre not significant (<50)	Total sample tested	Percentage of Positive %
NAVSARI	64	05	59	30	57.00
VALSAD	49	04	45	31	26.00
TAPI	56	18	38	30	40.00
TOTAL	169	27	142	91	41.00

36

7. Leptospira Research activities at NIMHANS, Bengaluru

Dr. Nagarathna S,Professor, Department of Neuromicrobiology, NIMHANS, Bangalore

Leptospirosis is a neglected zoonotic disease that is often associated with animal carriers and contamination of the environment via infected urine by affecting animals and humans caused by infection with Leptospira.In developing countries such as India, leptospirosis is often underdiagnosed because ofprotean clinical manifestations, leading to significantmorbidity and mortality. The clinical spectrum canrange from an asymptomatic, subclinical infectionto a fatal hepatorenal syndrome (Weil's disease).Neurological manifestations seen in about 10-15 per cent of cases are protean and remain unrecognized and diverse. The clinical manifestations include aseptic meningitis, encephalitis, intracranial bleed, cerebellitis, movement disorders, myelitis, GBS, facial palsy etc.

The study conducted at NIMHANS consisted ofa total 31 patients treated during the five year period. We evaluated the pattern of nervous system involvement in leptospirosis, among patients presenting to the emergency services of a tertiary care neurological centre in

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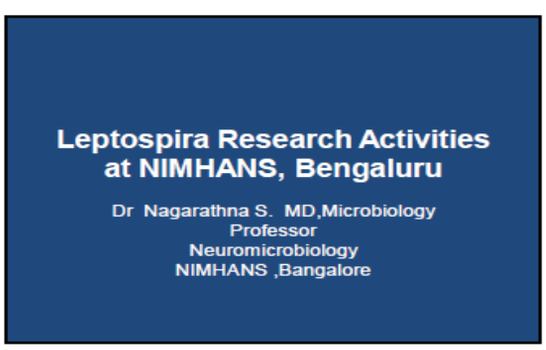
south India, and also analysed the outcome and prognostic indicatorsover a five year period which was published.The diagnosis of Neuroleptospirosis was based on clinical and laboratory evidence of hepato-renal syndrome, and serum or CSF positivity for antileptospiral antibody by a macroscopic agglutination test (MAT) and by IgM ELISA.A total of 31 patients (M:F 27:4, age range 6-68 yr, mean 36.4 ± 14.3 yr) were treated during the five year period.Acute fever with chills and rigors,headache and vomiting were the presentingmanifestations; 25 patients (81%) had altered sensorium for a period ranging from 1-8 days, four (12.9%) being deeply comatose. Eleven (35.5%) had acute symptomatic seizures at the time of presentation. Conjunctival congestion with or without haemorrhage was seen in 12 patients (38.7%), icterus in 14 (45%) and mild hepatosplenomegaly in 11 (35.5%). Early papilloedema was observed in three patients. Only three patients had localizing deficits.CT scan was normal in 18 of 27 (67%), while 7 (26%) had diffuse cerebral oedema. CSF pleocytosis with lymphocytic predominance (mean 50 cells/µl) and elevated protein levels (mean 115.5 ± 67.5 mg %) were noted. Leptospira antibody was detected in serum of all, and 5 of 22 in CSF samples. Eight patients (26%) succumbed.Deep altered sensorium at presentation and raised CSF protein were two poor prognostic indicators. Pathological study of brain in five cases revealed encephalitic features and in addition immune mediated acute disseminated encephalomyelitis (ADEM) like pathology in two cases.Neuroleptospirosis should be considered in the differential diagnosis of neuroinfections associated with hepatorenal dysfunction, in endemic areas.On-going project: ICMR funded prospective case control study on Neuroleptospirosis. This present study aims to analyse the clinical features, treatment response and the factors which lead to variable case fatality rate among cases of Neuroleptospirosis and also to know whether suspected cases of viral meningoencephalitis is in fact Neuroleptospirosis.



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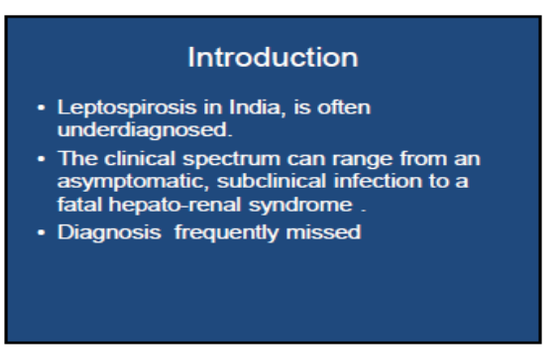

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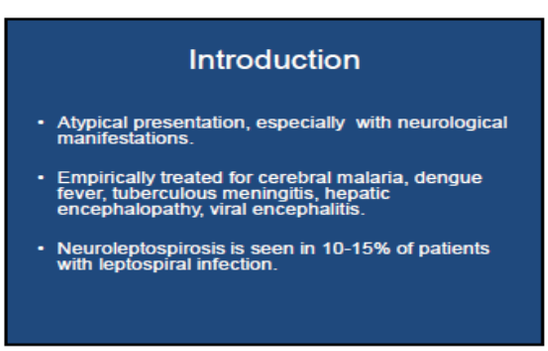

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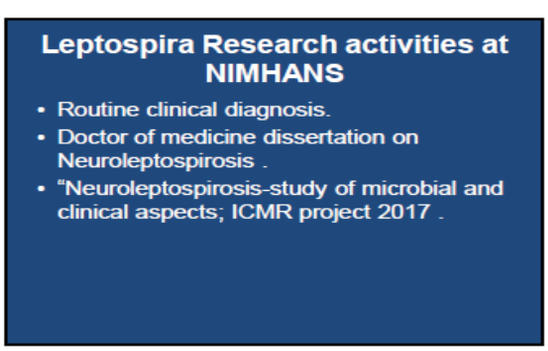

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Neuroleptospirosis - revisited: experience from a tertiary care neurological centre from south India

5

DM thesis

- The patients presenting with symptoms and signs referable to nervous system involvement, biochemical evidence of hepato-renal dysfunction, and serological evidence of leptospiral infection were analyzed.

6

DM Thesis

- Study period 1998-2003
- 31 cases fulfilling the inclusion criteria were evaluated.
- All the cases were positive for serum antileptospira antibody, 30 by MAT and one by ELISA (IgM antibody).
- In addition, 5 of 22 CSF samples tested by MAT were positive for antileptospira antibody.

7

Age & Gender

- The mean age of the cohort was 36.4 ± 14.3 yr (range 6-68 yr).
- Majority of patients were in the age group of 20-40 yr;
- 27 patients (87.1%) were males.

8

Occupation

- Majority of patients were farmers (51.6%) and manual labourers (22.6%), living in low socio-economic conditions.
- There was a definite seasonal association, majority of cases (84%) presenting during the months of October to January.

9

Neurological presentations

- The commonest neurological presentation, was altered sensorium, followed by seizures.
- Pure meningitic presentation was noted in four patients (13%),
- Pure encephalitic presentation in 8 (26%)
- Meningoencephalitic picture in 17 (55%) patients.
- Though the commonest neurological abnormality reported in literature was aseptic meningitis, majority of patients presented with altered sensorium (encephalitic picture)

10

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- This may be due to a referral bias, as only very sick and seriously ill patients are referred to the tertiary care neurological centre.
- In a general hospital setting the number of patients with aseptic meningitis could be higher.

11

- Clinically detectable jaundice was noted in 45%
- Conjunctival congestion/haemorrhage - 38.7%
- This indicates that icterus and conjunctival changes are not universal and their absence should not be taken as evidence against the diagnosis of leptospiral infection.

12

CSF parameters

- Mean CSF cell count was 50.2 ± 72 cells/µl (range 1 to 350 cells/µl);
- 28% had normal CSF cell count.
- Lymphocytic pleocytosis was noted in 72%
- Mean CSF protein was 115.5 ± 67.5 mg per cent with a range of 5-323mg per cent.
- CSF protein was elevated in 88%
- Six patients (24%) had CSF sugar less than 60 mg per cent and only one had sugar < 40 mg per cent.

13

Treatment

- Patients strongly suspected to have leptospirosis clinically, received crystalline penicillin even before the laboratory confirmation of the diagnosis.

14

Outcome

- Of the 31 patients, eight (26%) succumbed to the infection.
- Remaining patients were either discharged and/or referred to general hospital after the general condition improved.

15

Prognostic indicators

- The two statistically significant parameters for poor prognosis observed were elevated CSF protein and the degree of altered sensorium at the time of admission.

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Table. Prognostic factors in the patients with neurological manifestation

Parameter	Patients survived (group A) (n=23)	Patients expired (group B) (n=8)
Age (yr)	37.6 ± 16.2	33.0± 5.98
Seizures (%)	30.4	50
Icterus (%)	47.8	37.5
Total WBC count (cells/µl)	10693 ± 4459	12775 ± 5083
Neutrophils (%)	68.2 ± 14.9	75.9 ± 9.8
Platelet count (cells/µl)	142266 ± 106688	86400 ± 54454
Blood urea (mg %)	76.4 ± 61	107.8 ± 46.4
Serum creatinine (mg %)	1.69 ± 1.28	2.19 ± 1.17
SGOT (u/l)	146	236
SGPT (u/l)	116	171.5
CSF protein (mg %)	90.6 ± 45.7	183.3 ± 73.2*
Deep coma (%)	8.7	25**

*Values are given as median
SGOT, serum glutamate–oxaloacetate transaminase
SGPT, serum glutamate–pyruvate transaminase
*$P < 0.001$ compared to group A (t test)
**$P < 0.037$ compared to group B (Fisher's exact test)

17

Autopsy

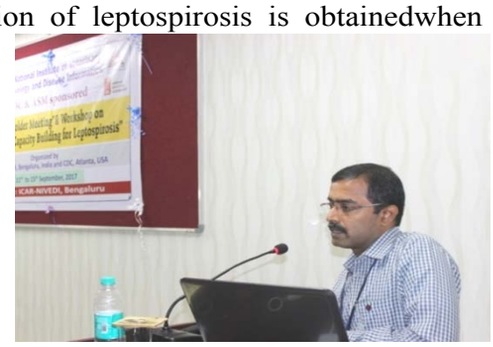
- Partial autopsy was carried out in four cases, while needle biopsy of the liver in 5 and transnasal brain biopsy through cribriform plate in one case.

18

8. Leptospira Research activities at TANUVAS, Chennai

Dr. T.M.A. Senthil Kumar, Professor, Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu.

Leptospirosis is an anthropozoonosis of ubiquitousdistribution, caused by spirochaetes of the pathogenicLeptospira species. Leptospirosis affects awide range ofhostsincluding humans, domestic and wild animal species.Laboratory confirmation of leptospirosis is obtainedwhen either the pathogen is isolated or a positive serologicalresult is obtained. The microscopic agglutination test (MAT)is considered as the reference test for leptospirosis.As all the existing tests have advantages as well aslimitations, the development of new diagnostics assumesgreater significance since leptospirosis has become animportant public health problem in most countries of theworld with many outbreaks reported in the recent past. Proteins located on the leptospiral outer membrane areof the greatest interest, as outer membrane proteins(OMPs) are potentially exposed to the host immunesystem. Recombinant antigens of these OMPs (LipL32, LipL41, LipL36, transmembraneprotein(OmpL1)havebeen produced and evaluated for sero-diagnosis of human and animal leptospirosis. Recombinant LipL32 antigen-based single serum dilution ELISA for detection of canine leptospirosis. Latex agglutination test and Flow-through immunoassay have been developed for direct screening of leptospiral antibodies in humans by simple visual identification.Further, development of rapid flow-through-based dot-immunoassay forsero-diagnosis ofleptospirosis in dogs.A simple dot-immunobindingassay was developed based on the flow-through principleutilizing the recombinant LipL41 (rLipL41) proteinexpressed in *E.coli* as capture antigen.Evaluation of the cocktail recombinant antigens, LipL32and LipL41 for sero-diagnosis ofcanine leptospirosis.Two immune-dominantrecombinant antigens LipL32 and LipL41 have beencombined and evaluated in IgG ELISA and Latex agglutinationtest for sero-diagnosis of canine leptospirosis.The rapidity, simplicity and economicsof the LAT were found to fulfill the requirements ofa rapid screening test for leptospiral antibodies. Theadvantages of using the recombinantcocktail antigens usedin the diagnosis of canine leptospirosis include the rapidityand the quantity of antigen production, safe since it eliminatesthe preparation of whole cell antigenic extracts ofleptospires and also the stability of antigens.



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Stakeholders Meeting on Leptospirosis

Development and evaluation of recombinant Outer membrane proteins-based serodiagnostics for leptospirosis

Dr. T.M.A. SENTHILKUMAR, Ph. D
Professor

Department of Animal Biotechnology,
Madras Veterinary College,
Tamil Nadu Veterinary and Animal Sciences University

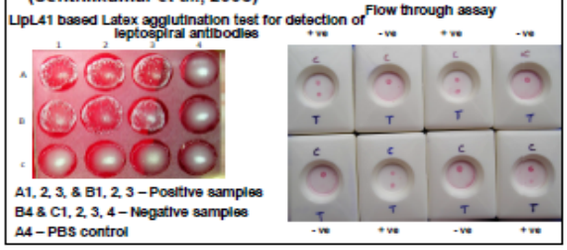
2017

1

- All the existing tests have advantages as well as Limitations.
- Development of new diagnostics assumes greater significance.
- Proteins located on the leptospiral outer membrane are of the greatest interest, as outer membrane proteins (OMPs) are potentially exposed to the host immune system.
- Antigenic conservation of leptospiral proteins was demonstrated in immunoblot studies.
- From the clinical and epidemiological stand point, rapid methods specific for pathogenic strains of leptospires are needed.

2

Latex agglutination test and Flow-through immunoassay have been developed for direct screening of leptospiral antibodies in humans by simple visual identification (Senthilkumar *et al.*, 2008)



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Comparison of Latex agglutination test and Microscopic agglutination test in humans

		MAT		Total
		Positive	Negative	
LAT	Positive	148	15	163
	Negative	17	142	159
		165	157	322

Sensitivity: 89.7%
Specificity: 90.45%
Concordance: 90.06%
$\chi^2 = 206.72^{**}$
K = 0. 80
** Highly significant P< 0. 01

4

Comparison of IgG-ELISA and Microscopic agglutination test in human beings

		MAT		Total
		Positive	Negative	
ELISA	Positive	132	6	138
	Negative	29	106	135
		161	112	273

Sensitivity: 82%
Specificity: 95%
Accuracy: 87%
$\chi^2 = 155.17^{**}$
K = 0. 74
** Highly significant P< 0. 01

5

Comparison of Flow through assay and MAT in human beings

		MAT		Total
		Positive	Negative	
Flow through	Positive	147	35	182
	Negative	18	122	140
		165	157	322

Sensitivity: 89.09%
Specificity: 77.7%
Concordance: 83.54%
$\chi^2 = 130.22^{**}$
K = 0. 63

6

.

Serodiagnosis of bovine leptospirosis by IgG-ELISA and Latex agglutination test using recombinant LipL41 (Senthilkumar *et al.*, 2010)

Comparison of IgG-ELISA and Microscopic agglutination test

			Total
	Pos	Neg	
Pos	165	11	176
Neg	25	229	254
	190	240	430

Sensitivity: 86.84%
Specificity: 95.42%
Concordance: 91.63%
$\chi^2 = 296.79^{**}$
K = 0. 83

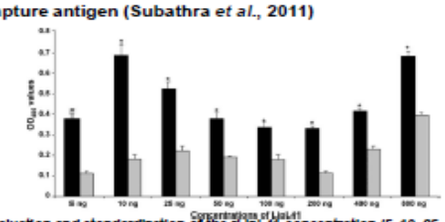
Comparison of LAT and Microscopic agglutination test

			Total
	Pos	Neg	
Pos	177	4	181
Neg	13	236	149
	190	240	430

Sensitivity: 93.16%
Specificity: 98.33%
Concordance: 96.05%
$\chi^2 = 346.18^{**}$
K = 0. 92

7

A simple dot-immunobinding assay was developed based on the flow-through principle utilizing the recombinant LipL41 (rLipL41) protein expressed in *Escherichia coli* as capture antigen (Subathra *et al.*, 2011)



Evaluation and standardization of the rLipL41 concentration (5, 10, 25, 50, 100, 200, 400 and 800 ng) at a serum dilution of 1:100. P 0.01.

8

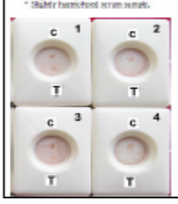
Comparison of IgG–ELISA and MAT for serodiagnosis of canine leptospirosis.

	MAT		
	+	–	Total
IgG-ELISA			
+	123 (a)	11 (b)	134
–	40 (c)	153 (d)	193
Total	163	164	327

$\chi^2 = 159.77^{**}$; $K = 0.68$. Sensitivity: 75.46%; specificity: 93.29%; concordance: 84.40%. **Highly significant $P \leq 0.01$.

9

Flow-through-based dot-immunobinding assay



Modules 1 and 3, positive reactions
Modules 2 and 4, negative reactions
C, control reactions
T, test reactions

10

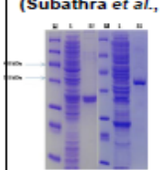
Comparison of flow-through-based dot-immunoassay and MAT for serodiagnosis of canine leptospirosis.

	MAT		
	+	–	Total
Flow-through			
+	143 (a)	17 (b)	160
–	20 (c)	147 (d)	167
Total	163	164	327

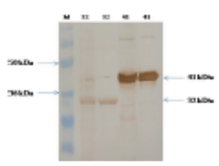
$\chi^2 = 195.80^{**}$; $K = 0.77$. Sensitivity: 87.73%; Specificity: 89.63%; Concordance: 88. 69%. **Highly significant $P \leq 0.01$.

11

Evaluation of the cocktail recombinant antigens, LipL32 and LipL41 for serodiagnosis of canine leptospirosis (Subathra *et al.*, 2011)



Purified recombinant proteins LipL32 & LipL41 in SDSPAGE.
Lane M: Medium range protein marker;
Lane 32: Purified rLipL32;
Lane 41: Purified rLipL41.
Lane L: Lysate



Western blotting of recombinant proteins rLipL32 and rLipL41
Lane M: Prestained protein marker
Lane 32: Purified rLipL32
Lane 41: Purified rLipL41

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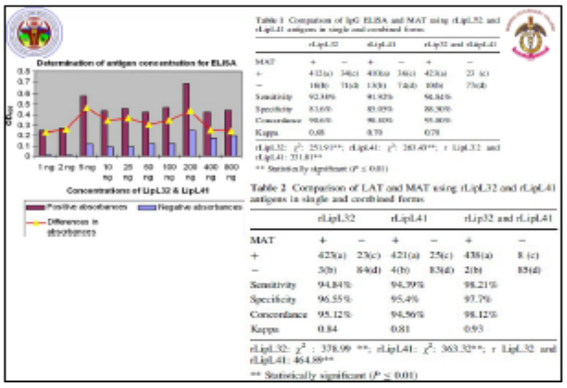

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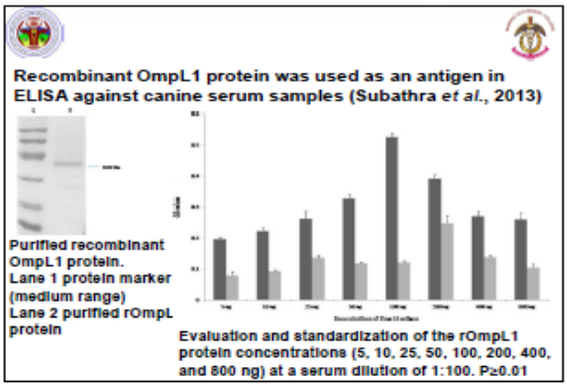

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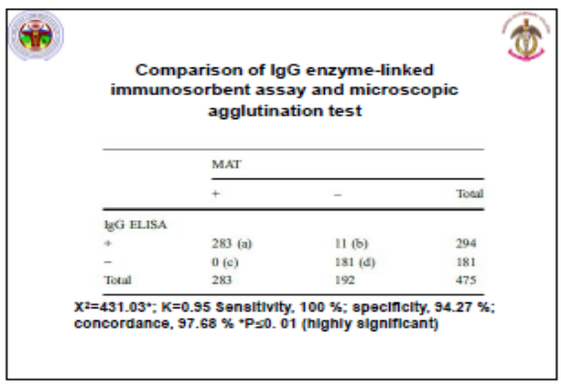

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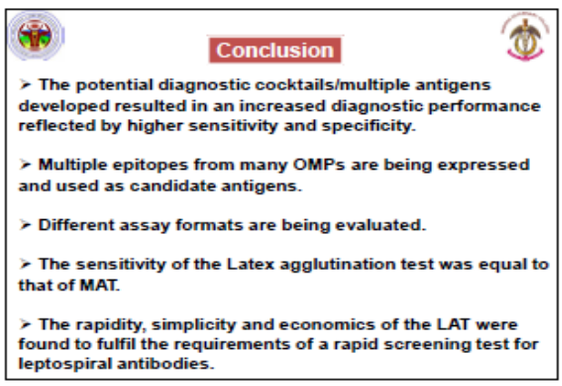


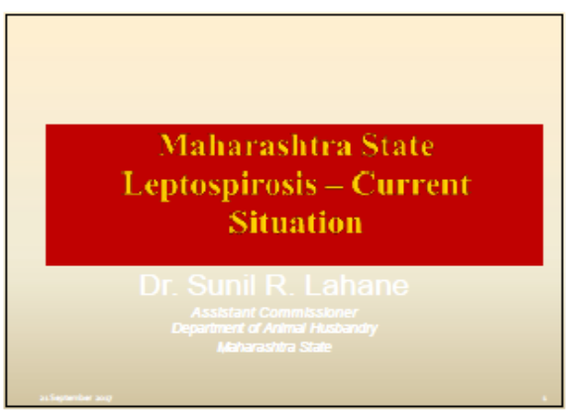
16

9. Leptospirosis situation in Animals-Maharashtra

Dr. Sunil Lahane, Asst. Commissioner of Animal Husbandry, Western Regional Disease Diagnostic Laboratory, Pune, Maharashtra.

Western Regional Disease Diagnostic Laboratory (WRDDL) is rendering disease diagnostic facility to 6 States under the Jurisdiction of this laboratory which includes Maharashtra, Gujarat, Madhya Pradesh, Chhattisgarh, Goa, Diu Daman & Dadra Nagar Haveli.Most importantly testing for Sexually Transmitted Diseases such as Tuberculosis, Johns Disease, Brucellosis,Infectious Bovine Rhinotrachitis, Trichomoniasis and Campylobacter is carried out regularly in the jurisdiction including Semen Stations .Objectives of Regional Disease Diagnostic Laboratory (Western Zone). Collection of biological material from outbreaks • The RDDL to be designated will serve as a Regional Referral Laboratory (RRL) for economically important livestock diseases (bacterial, viral, parasitic) by providing diagnostic services (both primary and confirmatory) to the states of the region. • The RDDL will maintain a data bank on the epidemiology of the economically important diseases of the region and it will be circulated to all the states on a periodic basis. • The RDDL will maintain a repository of virus; bacteria and parasitic agents isolated and make available a portion of the same for the national repository to .

be maintained at the National reference laboratory. The RDDL will follow the approved technique and standard reagents for generation of the diagnostic results. It will also maintain and monitor the standard of the test system followed by the state diagnostic laboratory. The RDDL will also train scientists / officers from the state diagnostic laboratories in the state of art technology and help in transfer of technology to the state diagnostic laboratories. In addition they will provide consultancy and expert services to the states of the region for speedy and accurate diagnosis of animal diseases. Testing of animals for screening of livestock and poultry diseases of National importance like Tuberculosis, Johns Disease, Brucellosis, Salmonellosis etc. for systemic control of these diseases. A special surveillance program is to be initiated for BSE / Scrapie as per the directions and terms of Department of Animal Husbandry and Dairying, GOI. and provide the necessary information to the department.Structure and working of Disease Investigation Section, Pune. The Institute is headed by Joint Commissioner of A H (Group A). The deputy Commissioners, Assistant Commissioners, and Livestock Development officers are engaged for the work of Disease Diagnosis and research. Following Laboratories are working for the objectives mentioned above.1) Bacteriology Laboratory 2) Mycology Laboratory 3) Parasitological Laboratory 4) Toxicology Laboratory 5) Pathology Laboratory 6) Virology & Cell culture Laboratory 7) Poultry Disease Diagnosis Laboratory 8) Foot & Mouth Disease Diagnostic Laboratory 9) Cattle Disease Surveillance Laboratory 10) Cattle Disease Laboratory. There are seven Regional Disease Investigation Laboratories, located at Chiplun, Kolhapur, Pune, Nasik, Aurangabad, Akola and Nagpur. These Laboratories are working at Regional Level for Diagnosis of Diseases, guidance of field officers etc. Veterinary Polyclinics are working at each district. Disease Diagnosis facilities are available with each polyclinic as District Laboratory.



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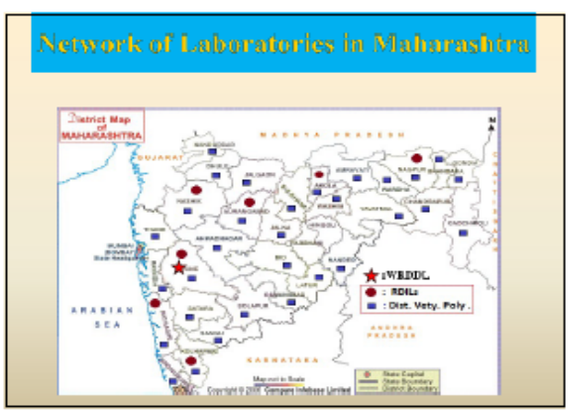

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Objectives of WRDDL

- Quick, accurate and precise diagnosis of various infectious and non infectious diseases of animals.
- To suggest measures to prevent the spread of disease to other animals.
- To advise the farmers about control measures to reduce further deaths and thus prevent economic losses.
- Epidemiological Investigation of the disease.
- To serve as a referral laboratory for Animal Diseases in Western Region of India.

3

Jurisdiction of WRDDL

4

Human Leptospirosis Situation

Sr No.	Dist/ Mun.Corp	2016				2017(up to 03/03/2017)			
		No.of Suspected	Sample Tested	Positive	Death	No.of Suspected	Sample Tested	Positive	Death
1	Thane Dist	1	1	1	0	0	0	0	0
2	Raigad	0	0	0	0	0	0	0	0
3	Palghar	0	0	0	0	0	0	0	0
4	Sindhudurg	4411	4411	25	2	1966	1966	8	1
5	Ratnagiri	331	331	66	0	45	45	5	0
6	Wardha	0	0	0	0	25	6	6	0
7	Kolhapur	0	0	0	0	1	1	1	1
8	BMC,Corp	122352	8541	267	9	64861	4856	147	3
9	Thane Corp	5	5	5	1	1	1	1	1
10	Bhivandi Corp	1	1	1	0	0	0	0	0
11	Vasai-Virar Corp	1	1	1	0	0	0	0	0
12	PCMC	0	0	0	0	1	1	1	0
13	PMC	0	0	0	0	10	10	10	1
14	Nashik Corp	1	1	1	1	0	0	0	0
Total District		4743	4743	91	2	2037	2018	20	2
Total Corp.		122360	8549	275	11	64873	4868	159	5

5

Map of Maharashtra with district wise Leptospirosis incidences

6

State Leptospirosis Situation - Corporation wise

sr	Corporation	2012		2013		2014		2015		2016		2017 (Up to 03.03.17)	
		Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death
1	Br. Mumbai Corp.	327	3	233	3	79	4	176	19	267	9	147	3
2	Navi Mumbai	5	0	0	0	1	1	0	0	0	0	0	0
3	Thane Corp	19	4	24	7	8	1	17	1	5	1	1	1
4	Kalyan Dombivali	3	0	0	0	0	0	0	0	0	0	0	0
5	Vasai-Virar	0	0	0	0	0	0	0	0	1	0	0	0
6	Bhivandi	0	0	0	0	0	0	0	0	1	0	0	0
7	Meera Bhyender	0	0	1	0	0	0	0	0	0	0	0	0
8	PCMC	0	0	0	0	0	0	0	0	0	0	1	0
9	PMC	0	0	0	0	0	0	0	0	0	0	10	1
10	Nashik	0	0	0	0	0	0	0	0	1	1	0	0
	Corp.Total	354	7	258	10	88	6	193	20	275	11	159	5

7

State Leptospirosis Situation – District wise

sr	Districts	2012		2013		2014		2015		2016		2017 (Up to 03.03.17)	
		Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death	Cases	Death
1	Thane	10	3	14	1	4	1	1	0	1	0	0	0
2	Raigad	12	0	16	3	0	0	0	0	0	0	0	0
3	Palghar	0	0	0	0	0	0	0	0	0	0	0	0
4	Pune	1	1	1	1	0	0	0	0	0	0	0	0
5	Nanded	0	0	0	0	0	0	3	1	0	0	0	0
6	Kolhapur	0	0	0	0	0	0	0	0	0	0	0	0
7	Ratnagiri	82	0	101	3	84	0	37	0	66	0	5	0
8	Sindhudurg	51	5	51	1	3	0	4	0	25	2	8	1
9	Kolhapur	0	0	0	0	0	0	0	0	0	0	1	1
10	Wardha	0	0	2	1	0	0	0	0	0	0	6	0
11	Gadchiroli	0	0	10	0	0	0	0	0	0	0	0	0
12	Bhandara	4	0	0	0	0	0	0	0	0	0	0	0
	Dist.Total	160	9	195	10	91	1	45	1	92	2	20	2
	Corp.Total	354	7	258	10	88	6	193	20	275	11	159	5
	State Total	514	16	453	20	179	7	238	21	367	13	179	7

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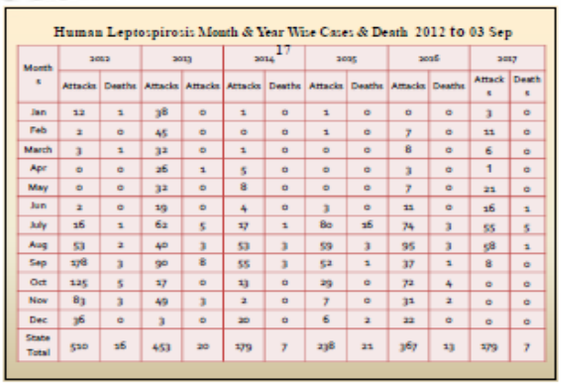

Human Leptospirosis Month & Year Wise Cases & Death 2012 to 03 Sep

Months	2012		2013		2014		2015		2016		2017	
	Attacks	Deaths	Attacks	Attacks	Attacks	Deaths	Attacks	Deaths	Attacks	Deaths	Attacks	Deaths
Jan	12	1	38	0	1	0	1	0	0	0	3	0
Feb	2	0	45	0	0	0	1	0	7	0	11	0
March	3	1	32	0	1	0	0	0	8	0	6	0
Apr	0	0	26	1	5	0	0	0	3	0	1	0
May	0	0	32	0	8	0	0	0	7	0	21	0
Jun	2	0	19	0	4	0	3	0	11	0	16	1
July	16	1	62	5	17	1	80	16	74	3	55	5
Aug	53	2	40	3	53	3	59	3	95	3	58	1
Sep	178	3	90	8	55	3	52	1	37	1	8	0
Oct	125	5	17	0	13	0	29	0	72	4	0	0
Nov	83	3	49	3	2	0	7	0	31	2	0	0
Dec	36	0	3	0	20	0	6	2	22	0	0	0
State Total	510	16	453	20	179	7	238	21	367	13	179	7

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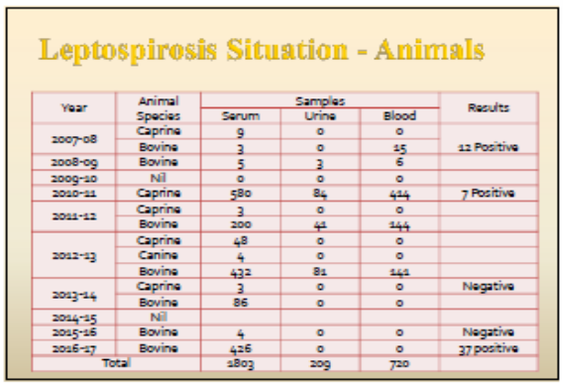

Leptospirosis Situation - Animals

Year	Animal Species	Samples			Results
		Serum	Urine	Blood	
2007-08	Caprine	9	0	0	12 Positive
	Bovine	3	0	15	
2008-09	Bovine	5	3	6	
2009-10	Nil	0	0	0	
2010-11	Caprine	580	84	414	7 Positive
2011-12	Caprine	3	0	0	
	Bovine	200	41	144	
2012-13	Caprine	48	0	0	
	Canine	4	0	0	
	Bovine	432	81	141	
2013-14	Caprine	3	0	0	Negative
	Bovine	86	0	0	
2014-15	Nil				
2015-16	Bovine	4	0	0	Negative
2016-17	Bovine	426	0	0	37 positive
Total		1803	209	720	

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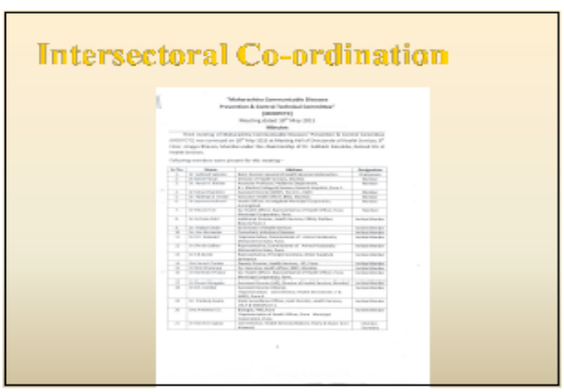

Intersectoral Co-ordination

11

Thankyou

12

10.Leptospira Research activities at SVVS, Tirupati, Andhra Pradesh

Dr. Raniprameela,Professor,State Disease Investigation Laboratory,Sri VenkateswaraVeterinary University, Tirupati, Andhra Pradesh.

Leptospirosis is considered as the most wide spread zoonotic disease in the world. It affects humans and a wide variety of animals. The disease is common in cattle, buffaloes, sheep, goat, dog & equines. It isconsidered to be economic significance disease and cause economic losses to the farmers due to abortions, decreased milk production, mastitis, death of young adult due to haemolytic anaemia and reproductive failures. In humans, the disease ranges from sub-clinical infection to severe syndromes of multi organ infection with high mortality. It is caused by a pathogenic spirochaete of the genus *Leptospira* belongs to the family *Leptospiraceae* of the order *Spirochaetales*. Worldwide in distribution and has been reported from USA, UK, Australia, New - Zealand, USSR, Europe, Asia, Germany, Spain, Portugal, including India. From India Uttar - Pradesh, Uttaranchal, West Bengal, Haryana, Andaman, Orissa, Tamil Nadu, Karnataka, Kerala, Including Andhra Pradesh &Telangana.The Seroepidemiological study was conducted using MAT on 2,705 serum samples collected from apparently healthy cattle, sheep, goat, dogs & pigs revealed 16.67%(451 Positives). Similarly, 34.15 % of sero positivity (207 Positives) recorded from clinically suspected cases of cattle,

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sheep, pigs, dogs & Humans (606 Serum samples). In wild animals a total of 64 serum samples collected from clinically suspected cases of Jackals, Hyenas, Deer, Leopard, Lion, Tiger & Elephants revealed sero positivity of 34.37 % (22 Positives).A total of 17 Leptospira isolates were recovered from animals, rats, rice field water and humans. *L.hardjo, L.pomona*commonly circulating serovars and *L.inadaii, L.naguchii*rarely occurring serovars and a new genome species Leptonema were isolated and characterized first time from Andhra Pradesh. A Trivalent Inactivated Vaccine against Leptospirosis using commonly circulating serovars namely *L.grippotyphosa, L.hardjo&L.autumnalis* was prepared, standardized and immune response was evaluated in rabbits. Further initiated the research work on "Development of Novel Vaccine against Pathogenic Leptospira" through pangenomic reverse vaccinology and it is under progress.

1

Leptospirosis from Andhra Prtadesh

- Leptospirosis is a major public health concerned.
- One of the re-emerging infectious diseases world wide.
- Economically important disease affecting domestic animals & wild life.

2

One of the important diseases in animals responsible for

- Abortions.
- Still births, infertility.
- Decreased milk production .
- Mastitis.
- Death of young adults .
- Reproductive failures.
- Zoonotic importance.

3

Efforts were made to study

- ✓Epidemiology of the disease
- ✓Isolation and characterization of Leptospira Circulating in the state
- ✓Attempts in vaccine development
  - Whole cell Inactivated Vaccine
  - Recombinant Vaccine – Reverse Vaccinology

4

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Financial Support received : **45.00 lakhs**

(SVVU, State plan)

- Established Leptospira Diagnostic laboratory to cater the needs of the farmers of the state

5

Reference Strains of Leptospires used in present study

S.No	Serogroup	Serovar	Strain
1	*L.autumnalis*	Rachmati	Rachmat
2	*L.ballum*	Ballum	Mus 127
3	*L.canicola*	Canicola	HV IV
4	*L.grippotyphosa*	Grippotyphosa	Moskova
5	*L.hardjo*	Hardjo	Hardjo prajinto
6	*L.hebdomedis*	Hebdomedis	Hebdomedis
7	*L. icterohaemorrhageae*	Copenhageni	M20
8	*L. icterohaemorrhageae*	Icterohaemorrhagiae	RGA
9	*L.javanica*	Poi	Poi
10	*L.Patoc*	Patoc	Patoc I
11	*L.Pomona*	Pomona	Pomona

6

Goats :

Apparently healthy

- 237 – 34 (14.35%)
- L.hardjo (38.23%)
- L.grippotyposa (29.41%)
- L.javanica (26.47%)
- L.autumnalis (6.45%)

7

PIGS :

Apparently healthy	Clinically suspected cases
213 – 52 (24.40%)	35 – 18 (51.42%)
L.grippotyposa (26.92%)	*L.pomona* (38.88%)
L. autumnalis (23.07%)	*L.hardjo* (27.77%)
L.canicola (21.15%)	*L. grippotyposa* (16.66%)
L.hardjo (30.76)	*L.canicola* (11.11%)
	L. autumnalis (5.55%)

8

Dogs:

Apparently healthy	Clinically suspected cases
149 – 21 (14.09 %)	61 – 30 (49.6%)
L.canicola and javanica (38.09%)	*L.canicola* (40.00%)
L. autumnalis (13.33%)	*L.hardjo* (20.00%)
L.hardjo (9.5%)	*L. autumnalis* (13.3%)
	L. ictero (20.00%)
	L.pomona (5.5%)

Humans :

Clinically suspected cases

- 70 – 46 (65.7 %)
- *L.hardjo* (26.08 %)
- *L. autumnalis* (17.4%)
- *L.hebdomedis* (13.04%)
- L. canicola and grippotyposa (10.86%)

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- The predominance of *L. hardjo* in human patients could be related to increase in dairy farming in particular the association with cattle, the maintenance host for *L. hardjo*.
- Increase in the seropositivity in humans could be related to
  - ✓increase in population
  - ✓overflow of sewages
  - ✓agricultural operations
  - ✓increase in association with pet dogs, domestic and wild life

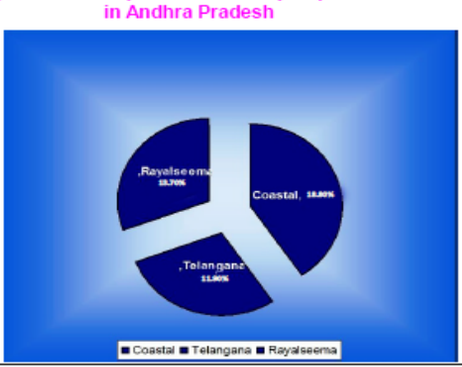
10

Region wise seroprevalence

- Coastal region – 1624 (18.80%)
- Rayalaseema region – 897 (13.70%)
- Telangana region – 184 (11.90%)
- High prevalence in coastal region is due to geographical and environmental factors with high humidity and water logged areas play an important role in perpetuation and spread of leptospirosis.

11

Region wise Seroprevalence of Leptospiral antibodies in Andhra Pradesh



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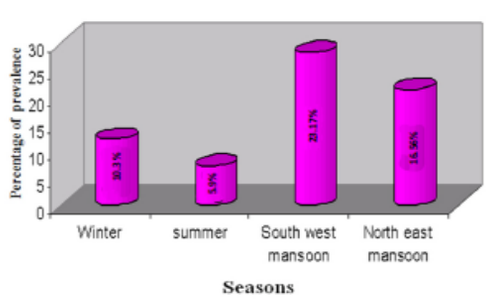
Season wise seroprevalence of leptospiral antibodies in Andhra Pradesh



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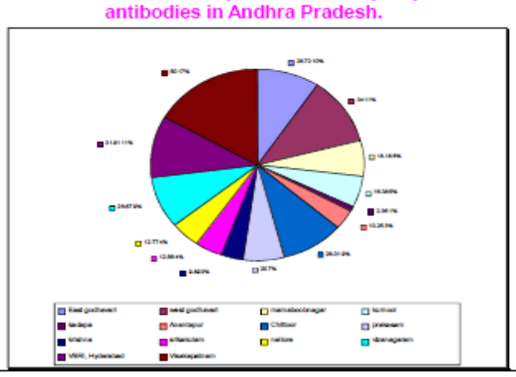
District wise seroprevalence of leptospiral antibodies in Andhra Pradesh.



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District wise seroprevalence

- Highest seropositivity in West Godavari (34.0%) followed by
- East Godavari (28.72%)
- Lowest in Anantapur District (4.83%)

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16

West Godavari :

Rich in natural vegetation with marshy lands.

- Small ponds with humidity and temperature
- Habit of bathing in water bodies contaminated with infected urine as one of the main source of transmission of leptospira.

17

- Second highest prevalent district is East Godavari (28.72%).
- Presence of more no of rice fields infested with rats that act as carrier for *Leptospira.*
- Warm wet climatic conditions with a PH close to the neutral slightly alkaline provides optimum for growth of *Leptospira.*

18

- Lowest prevalence in Anantapur (4.83%) and Kadapa (5.63%) Districts
- Low prevalence was attributed to low rainfall with high temperature prevailing in the Districts.
- The maintenance of animals in households separately for milking purpose under clean hygienic conditions could also be one of the factors for low prevalence in the area.

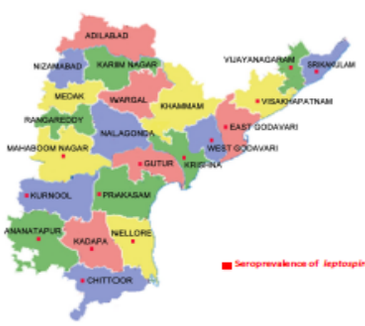
19

Seroepidemiology : Wild animals

S.No	Wild Animal Species	Total No.Of samples screened	No of Positives	% Positivity	Leptospira Serovar			
					L.Javanica	*L.Icterohae morrigiae*	*L.hardjo*	*L.Pomona*
1	Jackal	16	8	50	4	-	2	2
2	Deer	12	4	33.33	1	2	1	-
3	Elephants	5	2	40	-	-	2	-
4	Hyenas	10	1	10	-	1	-	-
5	Lions	6	2	33.33	-	1	-	1
6	Tigers	15	5	33.33	2	-	1	2
Grand Total		64	22	34.37%	7	4	6	5
Seropositivity					31.8 %	18.18 %	27.30 %	22.70 %

20

Fig. 3: Seroprevalence of leptospirosis in different districts of Andhra Pradesh



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Isolation of Leptospira

- From natural infected animals
- Reservoir hosts (rodents)
- To find out epidemiological link between animals, humans and rats

22

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Table 5: Details of clinical samples collected and leptospiral isolates recovered

S.No	Source of isolation	No. of samples subjected for isolation	No. of samples found positive	Percent positivity
1	Rats	299	5	1.67
2	Sheep	42	5	11.91
3	Pigs	15	4	26.6
4	Humans	53	2	3.77
5	Rice field	10	1	10
6	Cattle	26	-	-
7	Dogs	13	-	-

23

Table 4: Details of samples collected from rats for isolation of leptospira

S.No	Place of collection	No.of samples collected	No.of samples tested for isolation	No. of samples positive
1	Govindarajaswamy Temple area	22	22	2 samples positive
2	Railway station area	38	38	3 positive
3	R.S.J unction area	20	20	Negative
4	Bhavani Nagar area	15	15	Negative
5	Bus station area	19	19	Negative
6	Medical college area	185	185	Negative

24

Table 27: Results of 16S rRNA of Leptospiral isolates

Leptospiral isolate	Samples		Molecular diagnostic test
	Source	Type of material	
S1	Sheep	Blood	+
S2	Sheep	Blood	+
S3	Sheep	Blood	+
S4	Sheep	Blood	+
S5	Sheep	Blood	+
RR1	Rat	Kidney	+
RG1	Rat	Kidney	+
RR2	Rat	Kidney	+
RG2	Rat	Kidney	+
RG3	Rat	Kidney	+
P1	Pigs	Aborted material	+
P2	Pigs	Aborted material	+
P3	Pigs	Aborted material	+
P4	Pigs	Aborted material	+
H1	Humans	Blood	+
W1	Rice field	water sample	-
H2	Human Blood	Blood	*

25

Molecular Epidemiology

sheep – Leptonema, *L.hardjo* and *L. inadii*

Rats - *L.naguchii* and Leptonema

Pigs – *L.pomona*

26

Vaccine Trails :

- ✓ Based on seroepedimiological studies L. grripotyphosa, L. hardjo, & L. autumnalis were selected as vaccine candidates for the preparation of trivalent vaccine.
- ✓ Preparation

  Trivalent inactivated (formalin) and adjuvanted (Al OH; Mountanide) vaccine

- ✓ Immune response

  six month - satisfactory protective immunity in rabbits

27

Recombinant Vaccine :

- To identify the vaccine candidates in designing vaccine against pathogenic Leptospira.
- Complete Proteomes of *L.borgopetersenii hardjo bovis* JB 197 & L550 were screened to identify common surface exposed proteins.
- Insilico analysis of L.borgpetersenii JB197 and L550 retrieved.
  a. Ton B dependent receptor containing single epitope,
  b. ABC permease protein with three epitopes
  c. Uvr ABC protein B with single epitope.

28

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- The primers were designed PCR was standardized for the amplification of ABC permease gene of *L.ballum*.
- The purified PCR product was cloned in PR SET vector using E-coli DH5α cells and expressed in E-Coli BL21 (DE3) cells.
- The recombinant protein was characterized using SDS PAGE yielding 20KD of expected recombinant Protein.

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BLAST analysis of sequencing results

S.No	Strain	Identity(%)
1	*Leptospira borgpetersenii* str. 4E chromosome	99%
2	*Leptospira borgpetersenii* serovar Ballum strain 356604	99%
3	*Leptospira borgpetersenii* serovar Hardjo strain NVSL S 818	98%
4	*Leptospira borgpetersenii* serovar Hardjo strain NVSL S 1343	98%
5	*Leptospira borgpetersenii* serovar Hardjo strain BK-30	98%
6	*Leptospira borgpetersenii* serovar Hardjo strain BK-9	98%
7	*Leptospira borgpetersenii* serovar Hardjo strain BK-6	98%
8	*Leptospira borgpetersenii* serovar Hardjo-bovis JB197	98%
9	*Leptospira borgpetersenii* serovar Hardjo-bovis L550	98%
10	*Leptospira santarosai* serovar Shermani str. LT 821	89%

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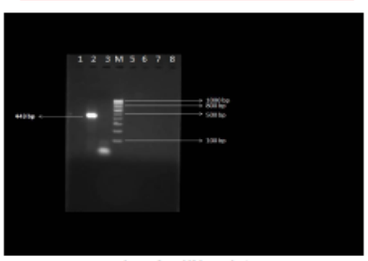
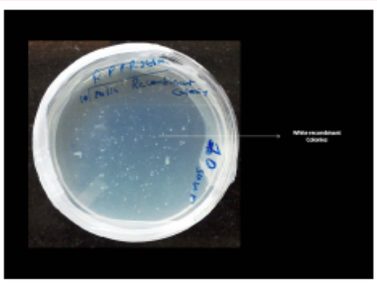
Amplification of ABC Permease gene



Lane – 2 : 443 bp product
M : 100bp ladder

31

LB Agar Plate with White Recombinant Colonies



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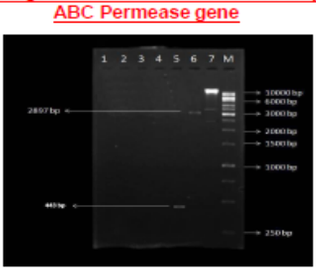
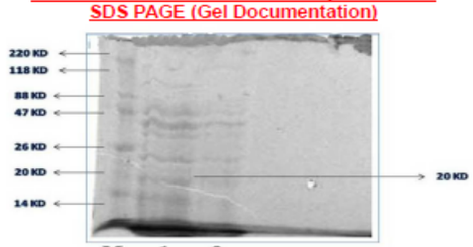
Restriction digestion PRSET A vector and amplified in ABC Permease gene



Restriction digestion of PRSETA Vector and amplified ABC permease gene
Lane – 5 : Restriction digested amplified ABC Permease Gene
Lane – 6 : Restriction digested PRSETA Vector
Lane – 7 : Undigested PRSETA Vector
M : 1kb ladder

33

Characterization of recombinant protein with SDS PAGE (Gel Documentation)



M - Marker
Lane - 1 - Induced
Lane - 2 - Un Induced

34

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Leptospiral Transmission

- **Water (Water bodies)**
  - Ponds, Rivers and sewage water
  - Heavy rains, floods and cyclones
- **Soil**
- **Reservoir hosts**
  - Rodents
  - Cattle
  - Dogs

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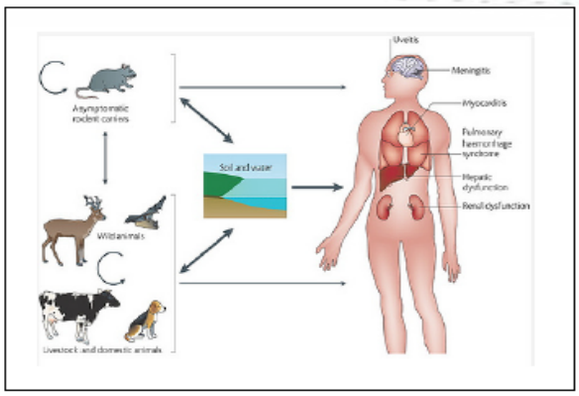

36

- **Occupational Activities**
  - Vets
  - Para Vets
  - Dairy farmers
  - Agricultural Workers

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Conclusion

- ✓ A total of 2705 serum samples collected randomly from apparently healthy animals of Bovines, sheep, goats, pigs and dogs on MAT revealed seropositivity of 16.28%, 16.57%, 14.35%, 24.40% & 14.09% respectively.
- ✓ Of 606 serum samples from clinically suspected cattle, sheep, pigs, dogs and humans the seropositivity on MAT was found to be 22.1%, 32.5%, 51.42%, 49.6% and 65.7% respectively.

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- The percentage of seroprevalence of leptospirosis in clinically suspected cases was high compared to the apparently healthy ones indicating active infection
- *L.pomona* & *L.grippotyposa* followed by *autumanlis* and hardjo in cattle
- *L. hardjo* & *L.pomona* followed by autumnalis & hebdomadis in sheep
- L. hardjo, L.autumnalis followed by grippotyposa and javanica in goats
- *L. pomona, L. hardjo, L.grippotyposa, L.canicola* and *L. autumanlis* in pigs
- *L. canicola, L.hardjo, L. autumanlis, L.ictero and l. pomona* in dogs
- *L. hardjo, L. autumnalis, L. grippotyposa, L.hebdomedis and L. canicola* n humans were found to be commonly circulating serovars.

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- The seroprevalence of leptopsirosis was noticed high during south west & north east.
- The seroprevalence of leptopsirosis in coastal regions was high (18.80%) followed by Rayalaseema (13.70%) and Telangana (11.90%)
- District wise seroprevalence of leptopsirosis was found to be high in West Godavari (34.0%) followed by East Godavari (28.72%), and lowest in Anantapur (4.83%) and Kadapa districts (5.63%)
- On isolation and characterization revealed L.hardjo, L.pomona, Leptonema, L.Inadii and L.noguchii.
- Trivalent Inactivated whole cell adjuvanted vaccine
- Recombinant vaccine through PANGENOME Reverse vaccinology

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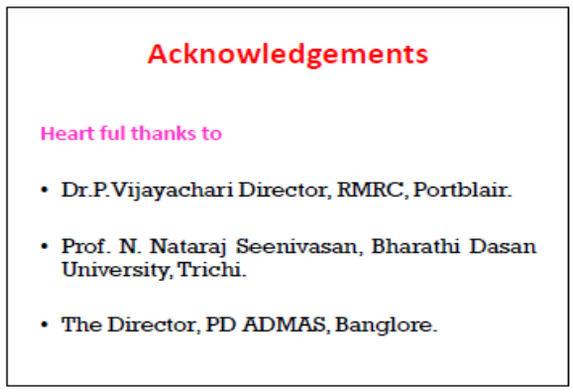

41 42

11.Leptospira Research activities at ICAR-Indian Veterinary Research Institute, UP

Dr. Sabarinath,Scientist,IVRI Deemed University,Izatnagar, Bareilly, Uttar Pradesh.

In IVRI, recombinant Leptospira Immunoglobulin like protein B (rLigB) based diagnostic tests such as ELISA, Latex Agglutination Test (LAT) and Dipstick assay have been developed. A total of 1135 serum samples (Dog n=423, Pig n=372, Human n=340) were collected. All the serum samples were tested using MAT as well as by rLigB based ELISA, LAT and Dipstick assay. The overall seroprevalence of leptospirosis in dogs was 28.6%, 31.44%, 24.58% and 22.69% as detected by MAT, rLigB based ELISA, LAT and Dipstick test, respectively whereas in pigs it was found to be 26.07%, 28.22%, 24.45% and 22.31% respectively by foresaid methods. In human beings, 22.94%, 27.64%, 19.41% and 18.82% human sera showed seropositivity by foresaid methods. The predominant serovar reported in the study was Icterohaemmorhagiae and its prevalence in seropositive cases was found to be 35.41%, 36.54%, 53.40% in dogs, pigs and human, respectively. In this study it was observed that almost identical serovars were reported from livestock, pet animals and high risk humans which were ample proof to confirm the anthropozoonotic potential of leptospirosis. In case of dogs, breed, dog's access to rodent infested garbage den and vaccination status were regarded as risk factors ($P<0.001$) for contracting leptospirosis. Non-descript feral pigs belonging to rural areas are more prone to contracting leptospirosis ($P<0.001$). In humans, it was found by calculating odds ratio that humans belonging to rural, monsoon affected areas who utilize public bathing places and having contact with animals are nearly two times more prone to contracting leptospirosis than general population.The potential ofrLigB protein based LAT as a DIVA strategy tool was evaluated using 54 MAT +ve vaccinated sera received from Polyclinic, IVRI. The fact that 46 out of 54 canine sera did not show any agglutination for rLigB protein based LAT is ample proof that rLigB protein based LAT holds promise as a DIVA strategy tool. The 8 sera which tested positive by rLigB protein based LAT might have occurred due to natural infection following vaccination. IVRI has developed a Loop-mediated isothermal amplification (LAMP) assay utilizing a novel set of primers targeting LigB gene for rapid and visual detection of pathogenic *Leptospira* in urine samples. Pre-addition of dyes such as Hydroxyl napthol blue (HNB), SYBR GREEN I and calcein were done to record test results. Analytical sensitivity of LAMP was as few as 1 X 10^1leptospiral organisms in spiked urine samples from cattle and dog. The diagnostic

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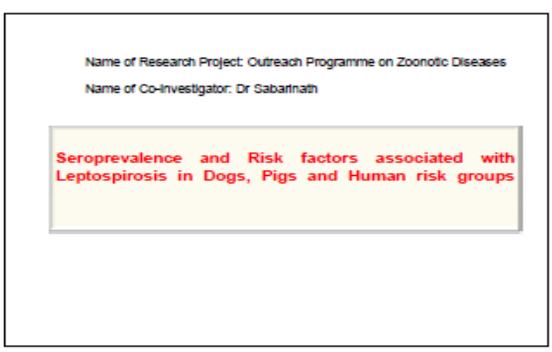
specificity of LAMP was 100% when compared to SYBR green qPCR for detection of *Leptospira* in urine samples. *Leptospirabiflexa*serovarPatoc, a non-pathogenic Leptospira species, and eight non-Leptospira species included in the study showed a negative reaction on LigB-LAMP.IVRI has played a pivotal role in providing diagnostic services pertaining to leptospirosis to zoological parks located in various parts of India. A total of 76 sloth bear sera samples (56 sera from Wild life rescue park, Agra and 20 sera from Bannerghatta, Karnataka) were screened for leptospirosis by MAT. 32 sera samples tested positive for various serovars of leptospira. Pyrogenes and Icterohaemorrhagiae were the predominant serovar reported from Wild life Rescue Park, Agra and Bannerghatta respectively. Leptospirosis has also been reported from M.C. Zoological Park, Chhatbir, Punjab and Jodhpur Zoo, Rajasthan were wild feline sera tested positive by both MAT and rLigB based LAT. Icterohaemorrhagiae was present in all the positive sera samples in M.C. Zoological Park while Icterohaemorrhagiae as well as Pomona, Grippotyphosa, Javanica and Australis were implicated in Jodhpur Zoo. The feeding of buffalo carcass without removing offals such as Kidneys, reproductive organs seem to be responsible for the disease outbreak in Jodhpur Zoo while rodent infestation seem to be the prime reason for the disease occurrence in M.C. Zoological Park.

1



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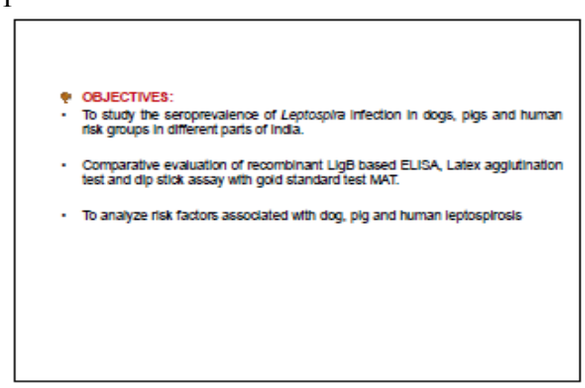

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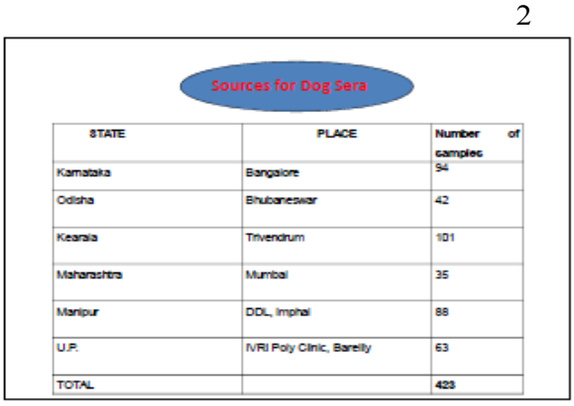


STATE	PLACE	Number of samples
Karnataka	Bangalore	94
Odisha	Bhubaneswar	42
Kearala	Trivendrum	101
Maharashtra	Mumbai	35
Manipur	DDL, Imphal	88
U.P.	IVRI Poly Clinic, Bareilly	63
TOTAL		423

4

.

Sources for Pig Sera

STATE	PLACE	Numberof samples
UP	Aligarh	51
	Nekpur Bareilly	147
	Barbanki	36
	IVRI Piggery Farm	50
Maharshtra	Deonar slaughter house, Mumbai	42
Odisha	Local Slaughter unit	36
TOTAL		372

5

Sources for Human Sera

STATE	PLACE	Number of samples
UP	GSVM Medical college, Kanpur	100
ODISHA	S.C.B Medical College Hospital, Cuttack	170
KARNATAKA	SRI DEVRAJ URS Medical College, Kolar	70
TOTAL		340

N.B. Animal attenders, Agriculture workers, Abattoir workers

6

- MAT was performed following standard protocol by OIE (OIE, 2011)
- Recombinant clone of LigB available from GEB laboratory of B&M was expressed, purified and its immunogenicity was tested by western blot analysis.
- Recombinant LigB based ELISA , latex agglutination test , DIPSTICK Assay were standardized for sero diagnosis of leptospirosis in dogs, pigs and human
- Analysis of risk factors were done using SPSS software (SPSS, 16.0)

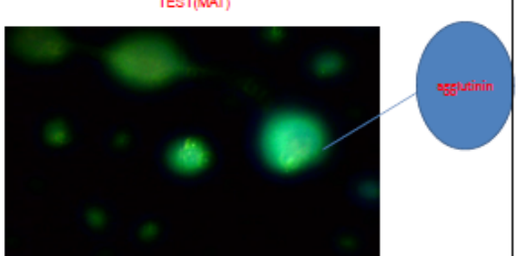
7

ANTIGEN PANELS FOR CONDUCTING MAT

Sl No.	Serovars	Strain
1	Australis	Ballico
2	Autumnalis	Rachmati
3	Ballum	Mus 127
4	Bataviae	Bataviae
5	Canicola	Hond Utrecht IV
6	Cyanopteri	Cyanopteri
7	Dejasiman	Dejasiman
8	Grippotyphosa	Moskva V
9	Hardjoprajitno	Hardjoprajitno
10	Hedomadis	Hedomadis
11	Icterohaemorrhagiae	RGA
12	Javnica	Poi
13	Louisiana	Louisiana
14	Patoc	Patoc
15	Pomona	Pomona
16	Pyrogenes	Salinem
17	Tarassovi	Perepelicin

8

MICROSCOPIC AGGLUTINATION TEST(MAT)



≥1:100 titre considered as positive

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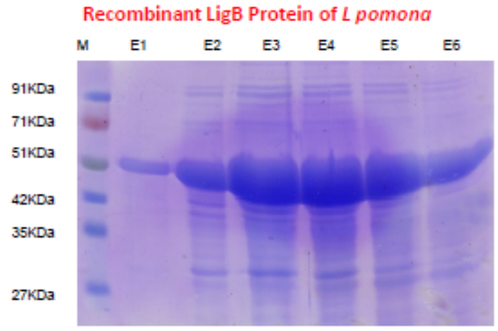
Recombinant LigB Protein of *L pomona*



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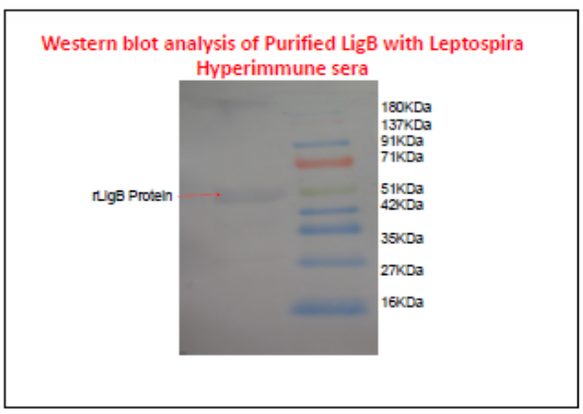

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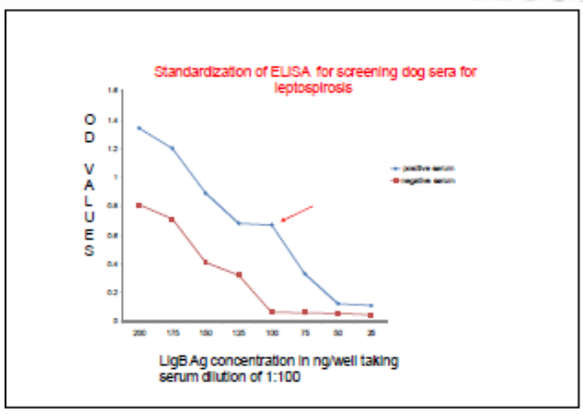

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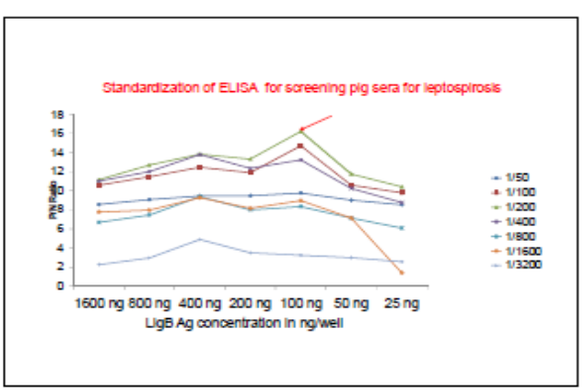

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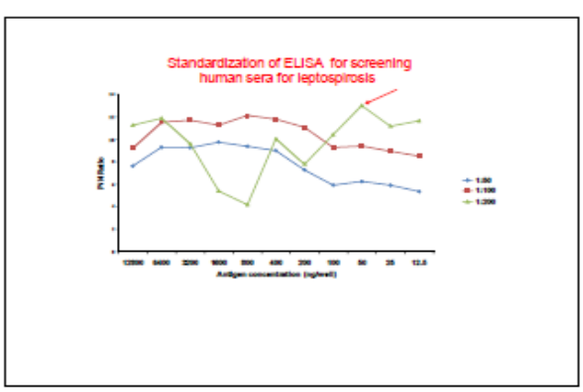

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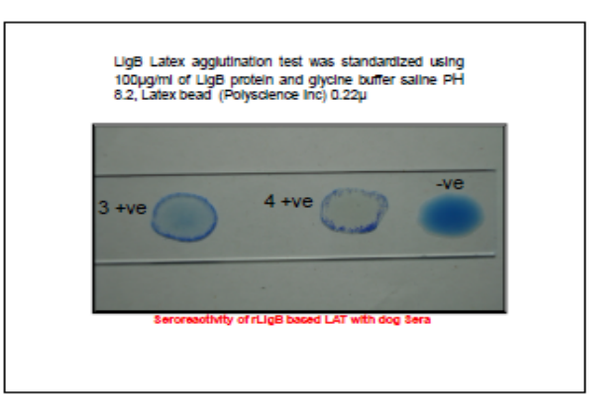

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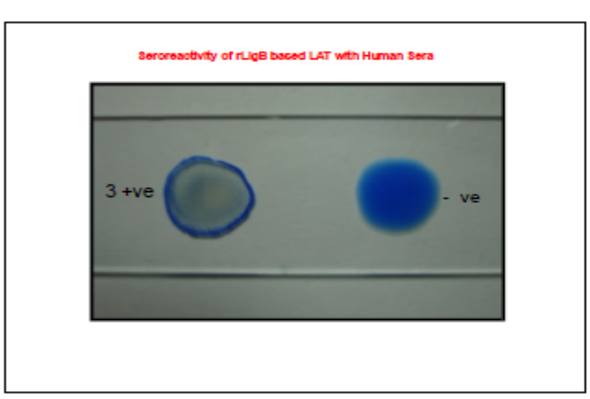

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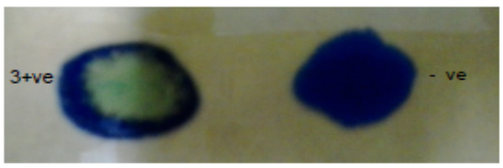
Seroreactivity of rLigB based LAT with pig Sera



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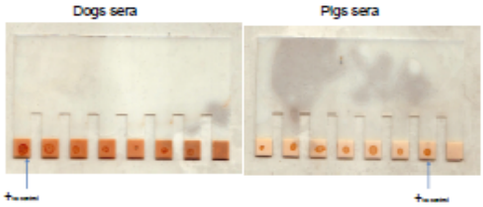
DIPSTICK ASSAYS

1 µg protein/tip of dipstick. 1:2000 conjugate dilution. 1:50 dilution serum



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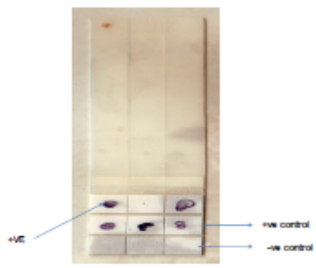
Human sera



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Species specific seroprevalence of leptospirosis by different tests

Species and No. of samples	Recombinant LigB IgGELISA(%)	Recombinant LigB LAT(%)	Recombinant LigB Dipstick(%)	Microscopic agglutination test (%)
DOGS (423)	133 (31.44)	104(24.58)	96(22.69)	121(28.6)
PIGS (372)	105(28.22)	91(24.46)	83 (22.31%)	97(26.07)
HUMAN (340)	74(27.64)	61(19.41)	64(18.82%)	66(22.94)

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Distribution of serovars in different species

Species No. and total serovars	Serovars of *Leptospira Interrogaans*
DOGS(N=288/423)	Icterohaemmorhagiae102 (36.41%), Grippotyphosa 71 (23.95%), Pyrogens 35 (13.19%), Javanica 27 (9.37%), Canicola 12 (4.16%), Pomona 11(3.81%), Australis 08 (2.77%), Autumnalis 06 (2.08%) %), Dejasiman 07(2.43%), Cyanopteri 06 (2.08%), Tarassovi 02 (0.69%)
PIGS (N=197/372)	Icterohaemmorhagiae 72 (36.64%), Grippotyphosa 49 (24.87%), Pomona 34(17.25%), Tarassovi 31 (15.73%) Australis 06 (3.04%), Javanica 05 (2.53%)
HUMAN (N=88/340)	Icterohaemmorhagiae 47 (53.40%), Grippotyphosa 26 (29.55%), Australis 10 (11.36%), Hebdomadis 03 (0.34%), Hardjoprajitno 01 (0.11%), Autumnalis 01 (0.11%)

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States	species	
Odisha	Dogs	Icterohaemmorhagiae11(61.11%), Grippotyphosa 05 (27.77%), Javanica 2 (11.11%),
	Pigs	Icterohaemmorhagiae06(40%), Grippotyphosa 04 (26.66%), Javanica05(33.3%)
	Human	Icterohaemmorhagiae 31(%), Grippotyphosa 10 (%), Australis 07(%), Hebdomadis 03 (%), Hardjoprajitno 01 (%)
UP	Dogs	Icterohaemmorhagiae22(73.3%), Grippotyphosa 06 (20%), Australis02 (6.66%)
	Pigs	Icterohaemmorhagiae49(33.56%), Grippotyphosa 32 (21.91%), Pomona 31(21.31%), Tarassovi 28(19.17%), Australis 06(4.16%)
	Human	Icterohaemmorhagiae11(52.38%), Grippotyphosa 09(42.85%), Australis 1(4.76%)
Karnataka	Dogs	Icterohaemmorhagiae18(31.57%), Grippotyphosa 15 (26.31%), Pyrogens10 (17.54%), Javanica 4 (7.01%), Canicola 2(3.5%), Pomona 05(8.7%), Australis 03 (5.2%)
	Human	Grippotyphosa07(46.66%) Icterohaemmorhagiae 05(30%) Australis02(13.33%), Autumnalis 01(6.66%)
Maharashtra	Dogs	Icterohaemmorhagiae 4(50%), Grippotyphosa 02 (25%%), Canicola 02(25%)
	Pigs	Icterohaemmorhagiae17(44.73%), Grippotyphosa 13 (34.21%), Pyrogens 02 (5.2%), Pomona 03(7.9%) Tarassovi 03(7.9%)
Kerala	Dogs	Icterohaemmorhagiae30(26.31%), Grippotyphosa 24 (21.05%), Pyrogens14 (12.3%), Javanica 15(13.16%), Canicola 5(4.3%), Pomona 02(1.8%), Australis 04(3.5%), Autumnalis 05 (4.4%), Dejasiman 07(6.14%), Cyanopteri 06 (5.25%), Tarassovi 02 (1.75%)
Manipur	Dogs	Grippotyphosa 19 (31.14%), Icterohaemmorhagiae17(27.8%), Pyrogens11(18.03%), Javanica 6 (9.83%), Canicola 3 (4.91%), Pomona 04 (6.6%), Autumnalis 01(1.6%)

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Risk factors for swine leptospirosis

Risk factors	Leptopositive	Leptonegative	Crude OR	95%CI	P-value
Age					
0-2 yrs	46 (20.81%)	175 (79.18%)	0.591		
2-4yrs	47 (34.05%)	91 (65.94%)	1.162		
>4yrs	4 (30.76%)	9 (69.23%)	1		
Sex				0.509, 1.412	
Male	68 (25.19%)	202 (74.81%)	0.847		
Female	29(28.43)	73 (71.56%)	1		
Breed			1.961	1.184, 3.247	<0.001
ND	70 (30.83%)	157 (69.16%)	1		
CB	27 (18.62%)	118 (81.37%)			
Feral country pigs	62 (36.12%)	145 (63.87%)	1.203	0.111, 0.369	<0.001
Small holder farm	15 (10.34%)	130(89.65%)	1		
Rural	62 (31.41%)	179(68.58%)	2.932	1.603, 5.362	<0.001
Perturban	15 (13.51%)	96(86.48%)	1		
Access to muddy wallowing area			2.932	1.603, 5.362	<0.001
Yes	62 (31.41%)	179(68.58%)	1		
No	15(13.51%)	96(86.48%)			
Contact with other animals					
Yes	37(32.46%)	77(67.54%)	0.974	0.753, 1.261	
No	0	50(100%)	1		
Not Known	60 (28.85%)	148(71.15%)			

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Risk factors for dog leptospirosis

Risk factors	Leptopositive	Leptonegative	Crude OR	95%CI	P- value
Age					
0-5 yrs	78(25.06%)	227(74.91%)	0.578		
5-10yrs	34 (37.78%)	56 (62.22%)	1.049		
>10yrs	11 (36.66%)	19 (63.33%)	1		
Sex			1.368	0.891, 2.10	
Male	73(31.46%)	159(68.53%)	1		
Female	46(25.13%)	140(74.86%)			
Breed			2.491	1.562,3.996	
ND	48(42.47%)	65(57.52%)	1.243	0.631, 2.448	
CB	14(26.92%)	38(73.07%)	1		
Pure-Breed	59(22.96%)	198(77.13%)			
Valley land	27(30.68%)	61(69.31%)	1.121	0.682, 2.153	
Low lying water logged area	52 (29.21%)	126 (70.78%)	1.130	0.700, 1.824	
Dry land	42 (26.75%)	115 (73.24%)	1		
Urban	70(25.73%)	202 (74.26%)	0.679	0.441, 1.046	
Perturban	51(33.77%)	100(66.23%)	1		
Access to garbage den with rodents			2.253	1.438, 3.561	P<0.001
Yes	48(41.37%)	68(58.62%)	1		
No	73(23.77%)	234(76.22%)			
Vaccination			0.538	0.350, 0.826	P<0.005
Yes	63(23.77%)	202(76.23%)	1		
No	58 (36.70%)	100(63.29%)			
Heptopathy, Jaundice, Renal failure	54(36%) 67(24.54%)	96(64%) 206(75.46%)	1.729 1	1.122, 2.666	

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Risk factors for human leptospirosis

Risk factors	Leptopositive	Leptonegative	Crude OR	95%CI	P-value
Age					P<0.005
<20yrs	4 (10.26%)	35(89.74%)	0.313	0.113, 0.986	
20-30	12 (11.42%)	93 (88.57%)	0.334	0.191, 0.745	
30-40	8 (12.90%)	54(87.09%)	0.377	0.114, 0.967	
>40	42 (31.34%)	92 (68.66%)	1		
Sex			0.982	0.547, 1.764	
Male	46(19.32%)	192 (80.67%)	1		
Female	20 (19.60%)	82 (80.39%)			
Rural	48(24.74%)	146(75.25%)	2.075	0.958, 4.504	
Perturban	09 (11.25%)	71 (88.75%)	0.817	0.304,2.192	
Urban	09 (13.63%)	57 (90.47%)	1		
Wet surroundings with history of flooding			1.974	1.141, 3.417	
Yes	40(25%)	120(75%)	1		
No	26 (14.44%)	154(85.56%)			
Contact with animals	55(21.57%)	200(78.43%)	1.850	0.919,3.726	
Yes	11(12.94%)	74(87.05%0	1		
No					
Use of public bathing place			1.664	0.777, 3.561	
Yes	57(20.80%)	217(79.19%)	1		
No	9(13.63%)	57(86.36%)			

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Comparison of rLigB based ELISA, Dipetick ELISA & LAT with MAT

Test parameters	Dogs			Pigs			Human		
	rLigB ELISA	rLigB LAT	rLigB Dipetick	rLigB ELISA	rLigB LAT	rLigB Dipetick	rLigB ELISA	rLigB LAT	rLigB Dipetick
Diagnostic Sensitivity	100%	83.47%	96.86%	98.95%	91.75%	97.46%	94.87%	82.05%	95.46%
Diagnostic Specificity	92.94%	99.05%	88.57%	96.72%	99.28%	93.77%	92.36%	99.25%	99.36%
Accuracy	97.16%	94.56%	90.80%	97.31%	97.31%	94.70%	92.94%	95.29%	98.82%
Kappa value (Perfect agreement)	0.95	0.93	0.93	0.95	0.96	0.92	0.92	0.94	0.94

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Conclusion

- According to our Study, there is similarity in distribution of *Leptospira* serovar in dogs, pigs and human beings which infers that there is a vicious cycle of transmission among animals and man.
- Seroprevalence was found to be 28.6%, 26.07%, and 22.94% in dogs, pigs and human risk groups respectively by adopting gold standard test MAT
- All the sera which tested positive against Icterohaemmorhagiae were 35.41%, 36.54%, 53.40% in dogs, pigs, and human respectively
- Sensitivity , Specificity of all the tests rLigB ELISA, LAT, DIPSTICK were found to be in the range of 80% to 90%, in dogs, pigs and humans , signifies utilities of these tests in serodiagnosis of leptospirosis
- *Leptospira* seroprevalence in pigs is significantly associated with rural, wallowing muddy area, feral nondescript pigs where as in dogs it is associated with garbage den with rodent infestation
- In human risk groups, *Leptospira* seroprevalence is significantly higher in >40 years age group of people

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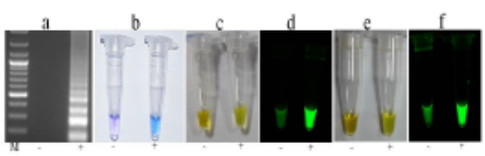
Detection of Leptospira DNA by LigB-LAMP



Detection of the LigB LAMP products by different visual methods. (a) Visualization of LAMP products on 2% Agarose gel electrophoresis, (b) by HNB dye, (c) by adding calcein dye in white light and (d) UV , (e) using SYBR GREEN I in white light and (f) under UV.

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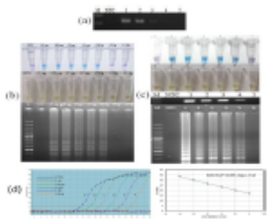
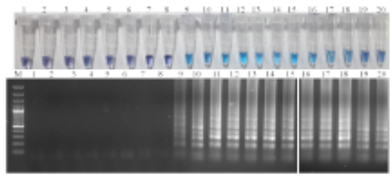
Sensitivity of LigB-LAMP for detection of Leptospira DNA



(a) Sensitivity of conventional PCR with purified DNA (M: 100 bp DNA ladder; NTC: negative template control; 1: 1.5 ng; 2: 150 pg; 3: 15 pg; 4:1.5 pg; 5: 150 fg)
(b) sensitivity of LigB-LAMP assay with purified DNA (from 1.5 ng to 1.5 fg) by visual detection using pre-addition of HNB dye and calcein dye and detection of LAMP product by agarose gel electrophoresis
(c) sensitivity of LigB-LAMP in dog urine spiked with Leptospira organisms - visual detection by pre-addition of HNB dye and calcein dye (tube no.1: NTC, 2: 1 X10^6 leptospiral cells; 3: 1 X10^4; 4: 1 X10^3; 5: 1 X10^2; 6: 10^1; 7: 1 leptospiral cell)

29

Specificity of LigB-LAMP for detection of Leptospira DNA



Specificity of LigB- LAMP analyzed by visual detection and on agarose gel.
Lane M-100bp DNA ladder; 1: *E.coli*; 2: *S. Aureus*; 3: *P. Multocida*; 4: *M. Bovis*; 5: *M. Leachii*; 6:*B. abortus*;
7: *Streptococcus bovis*; 8: *Leptospira biflexa* Patoc; 9: *L. interrogans* serovar Pomona;
10: *L. interrogans* serovar Icterohaemorrhagiae; 11: *L. kirschneri* serovar Grippotyphosa; 12: *L. interrogans* serovar Hardjoprajitno;
13: *L. kirschneri* serovar Cynopteri ; 14: *L. interrogans* serovar Australis; 15: *L. interrogans* serovar Autumnalis;
16: *L. interrogans* serovar Canicola; 17: *L. interrogans* serovar Copenhageni; 18: *L. interrogans* serovar Bataviae;
19: *L. interrogans* serovar Hebdomadis; 20: *L. interrogans* serovar Pyrogenes).

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Screening of Wild life sera samples for Leptospirosis using rLigB based LAT:

M.C. Zoological Park, Chhatbir, Punjab:

- 27 wild feline sera (18 Tiger, 8 Lion and 1 Jaguar) tested
- all the Lion sera and 15 tiger sera tested +ve for Leptospirosis by both rLigB based LAT and MAT.
- Icterohaemorrhagiae was present in all the positive sera samples (Lion 1:800) & Tiger (1:400) titre
- Only one Lion sera tested positive for serovar Pomona.

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Screening of Wild life sera samples for Leptospirosis using rLigB based LAT:

- **Jodhpur Zoo, Rajasthan:**
  - 42 sera (8 Tiger, 4 Lion, 6 Leopard, 2 cheethal, 1 Black Buck, 12 Buffalo and 9 Human sera) and 3 live rodents tested
  - 7 tiger sera, all the 4 lion sera, 2 leopard and 2 cheethal sera tested positive for Leptospirosis by LAT and MAT.
  - Icterohaemorrhagiae was the main serovar involved in all the animals while in Panther, the serovars Pomona (titre 1:800), Grippotyphosa and Australis were implicated.

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Jodhpur Zoo, Rajasthan:

- All the buffalo sera whose carcass were fed to the wild felines tested positive
- kidney and urine collected aseptically from all the 3 rodents tested negative on cultural examination
- feeding of buffalo carcass without removing offals was responsible for the disease outbreak in the Zoo.
- Zoo staff showed a basal level sera titre (1:100) indicating exposure to the pathogen without any premonitory symptoms

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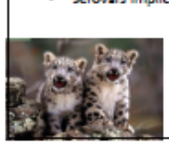
Screening of Wild life sera samples for Leptospirosis using rLigB based LAT:

Van Vihar National Park, Bhopal, Madhya Pradesh:

- 8 (4 Tiger, 3 Leopard and 1Lion sera) received.
- All the animals tested +ve by LAT and MAT (Icterohaemorrhagiae)

Bhiwani Mini Zoo, Haryana:

- 3 sera samples (2 lion and 1 Tiger) received
- 1Lion and 1 tiger tested +ve by both MAT and LAT.
- serovars implicated were Icterohaemorrhagiae and Grippotyphosa



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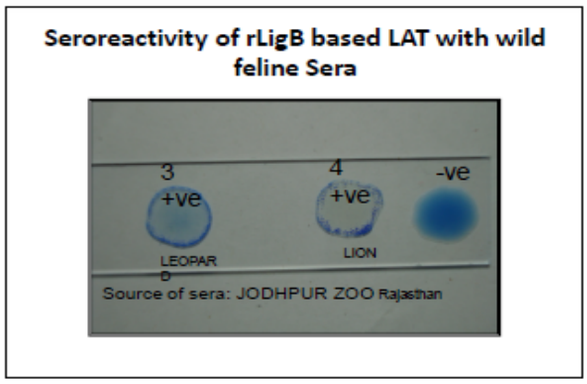

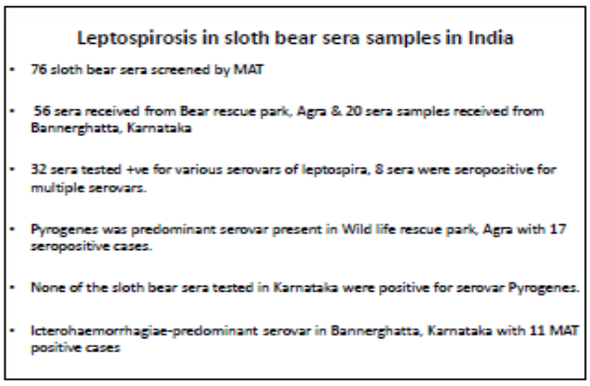

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12.Leptospira Research activities at IIT, Guwahati, Assam

Dr.Manish Kumar,Associate Professor,Dept. of Biosciences and Bioengineering, Indian Institute of Technology(IIT), Guwahati, Assam.

Our laboratory group is involved in studying novel outer membrane proteins of pathogenic *Leptospira* to extend list of new diagnostics and vaccine candidate for Leptospirosis. One of the approaches of finding new candidates is to employ diverse host factors like catecholamine hormone, osmotic pressure or temperature and evaluate selective membrane transcripts of *Leptospira* under *in vitro* condition by real-time reverse transcription-PCR(qRT-PCR) technique. In this regard, one of the projects aims at understanding modulation of gene transcription in *Leptospira interrogans*on exposure to catecholamines under *in vitro* condition. We analyze selective transcripts of outer membrane proteins (OMPs) of *L.interrogans* Copenhageni in response to Epinephrine/Norepinephrine and its antagonist propranolol (PO). We anticipate that this approach will facilitate the identification of OMPs responding to host chemical signals with the potential to serve as virulence factors, new serodiagnostic antigens, and vaccine candidates. Currently, using this approach we have developed two different diagnostic antigen that has 90-100 % specificity and sensitivity to detect Leptospirosis.In another approach we are investigating role of CRISPR-Cas (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats–CRISPR associated sequence proteins), a genetically encoded RNA-mediated adaptive defence system in *Leptospira interrogans*. It is a known fact that genetic manipulation of *L. interrogans* to study its pathogenesis is still under infancy despite the availability of advanced techniques for other spirochetes. With the recent advancement of genetic manipulation using CRISPR-Cas system, one can device this as a tool to study virulent gene using reverse genetics approach in *Leptospira* too. Therefore, study of biochemical activities on diverse CRISPR-Cas proteins may prove vital in molecular biology similar to DNA restriction enzymes that have revolutionized cloning and DNA manipulation. Based on the CRISPR finder program, *L. interrogans* Copenhageni have a *cas* operon in close proximity to CRISPR locus. These *cas* operon has an arrangement typical to type I-B that has been pre-defined.We have recently shown in *Leptospira* using bioinformatics and transcription analysis to possess CRISPR-Cas subtype I-B system where *cas4, cas1, cas2* and *cas6*, *cas3*, *cas8, cas7, cas5* are clustered together in two independent operons. We are in process to understand why genetic manipulation of pathogenic form of *Leptospira* is difficult in comparison to saprophytic forms.

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Research activities at IIT Guwahati

Dr. Manish Kumar
Associate Professor

Department of Biosciences and Bioengineering,
Indian Institute of Technology Guwahati,
Guwahati -781039, Assam, India

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Highlights of the Lab Activities at IIT Guwahati

- **Pathogenesis**: Modulation of gene expression in *Leptospira* due to host stress factor : catecholamines (Epi/NE)
- **Diagnostics**: Characterizing proteins in search of novel diagnostic antigen and vaccine candidate
- **Virulence**: Understanding CRISPR-Cas system of pathogenic *L. interrogans*
- **Treatment**: Targeting caseinolytic protease of *L. interrogans* as an alternatives to traditional antibiotics

2

Analysis of Differential Expression of Outer Membrane Proteins of pathogenic *Leptospira* in response to catecholamine: a host stress factor

3

Figure 1

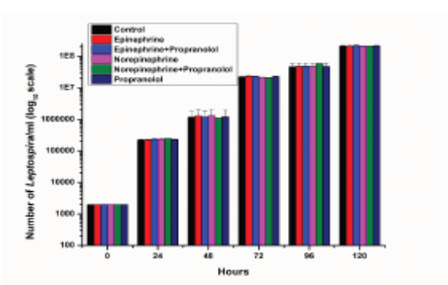

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Figure 2

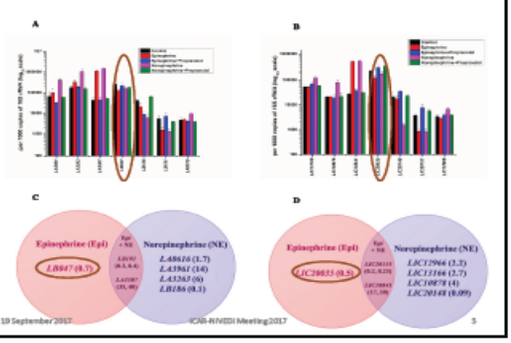

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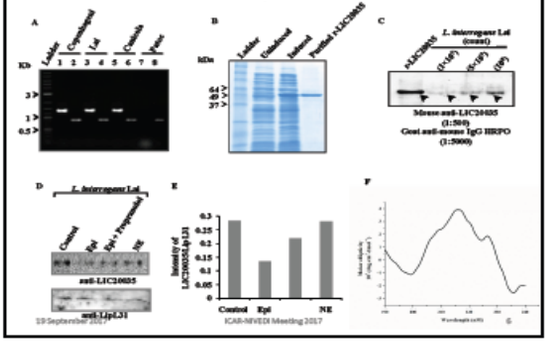
Figure 3



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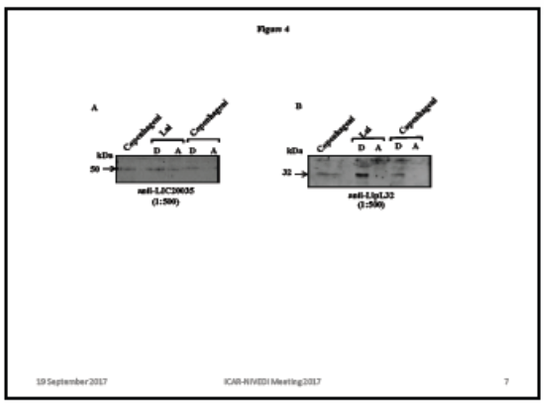

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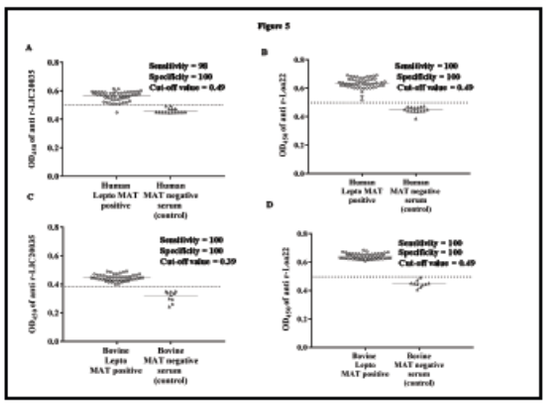

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What is CRISPR-Cas system?

The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR associated proteins)

- Adaptive defense system in bacteria and archaea
- Mediates immunity in 3 step process:

1. Adaptation:
   - ✓ A small fragments of foreign nucleic acids (protospacers) are first recognized and inserted into CRISPR array of host by help of Cas1 and Cas2 proteins.
2. Expression:
   - ✓ The transcripts of CRISPR-array is processed by Cas proteins into short CRISPR-RNAs (crRNA)
3. Interference:
   - ✓ The transcript in the form of Cascade complex interfere with the cognate foreign target nucleic acid

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Why to study CRISPR-Cas in *Leptospira* ?

- ❑ Genetic manipulation of *Leptospira interrogans* to study its pathogenesis is still under infancy
- ▪ Question: Does *Leptospira interrogans* possess strong immunity by virtue of its CRISPR-Cas defense array against foreign invading genomes?
- ❑ Bioinformatics analysis shows CRISPR-Cas exists only in pathogenic strains
- ▪ Logical explanation: Relatively gene manipulation is easier in *L. biflexa* than that of other pathogenic *Leptospira* strains

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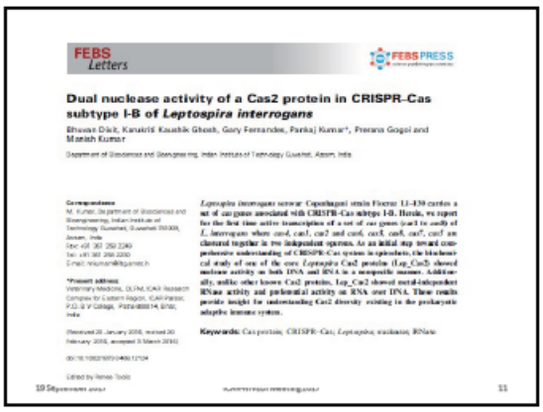

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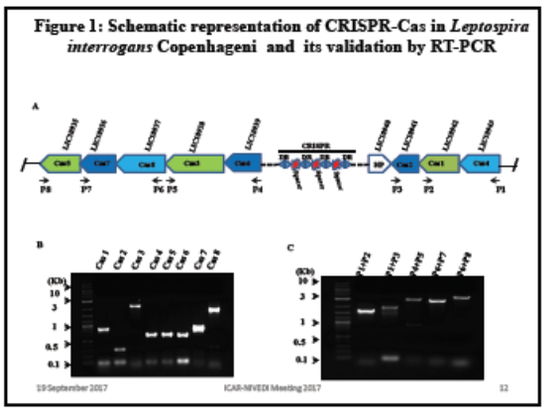

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13.Leptospira Research activities at ICAR-NIVEDI, Bengaluru

Dr. V. Balamurugan, Principal Scientist, ICAR-NIVEDI, Bengaluru. Karnataka

ICAR-NIVEDI is a pioneer research institute in the Leptospirosisofanimalssince inception 1987.The laboratory has made a mark for its technical expertise in the field in veterinary fraternities in the country. The main focus of the research area include epidemiological study, prevalence of leptospirosis in livestock, development of effective need based diagnostic methods for surveillance; risk factors identification, assessment and impact of leptospirosis in livestock and humans,Imparting laboratory capacity building program or "hands-on" training to the research scholars, or research / medical officers /personnel in the leptospira research area. Institute has all the facilities required for conducting basic, applied and molecular research work on Leptospira. These include, dark field microscopic examination, microscopic agglutination test, isolation and maintenance of reference leptospira serovars,molecular diagnostic PCR techniques,typing of leptospiral isolates to species level by molecular based approaches, providing leptospirosis diagnostic services to the livestock farmers, Veterinarians as well as Medical doctors. Surveillance /prevalence of leptospirosis study in livestock in endemic states of India using Serum repository facility of institute. Institute is having a National livestock serum repository (NLSR) with sera of all the livestock species from different parts of the country, which have been screened for economically important livestock diseases in the country.The research activities in leptospirosis since inception has led to development or formulation of a new user friendly, sensitive simple leptospira staining technique for diagnosis of leptospirosis (Leptospria Staining kit), commercially available kit and are being used widely in the country. Development of transport medium for sending the field materials to laboratory. Recording of the Leptospira abortions in bovines and other animal species. Isolation of Leptospira spp. from hosts. First isolation of *L. inadai*from rodent reservoir hosts and a rabbit and also from two fatal human cases.Base line information about the distribution and prevalence of serogroup specific antibodies in endemic states of India. Scientists of the Institute are delivering lectures on epidemiology and diagnosis of leptospirosis in the Veterinary College, National and International seminars and Institute training courses,

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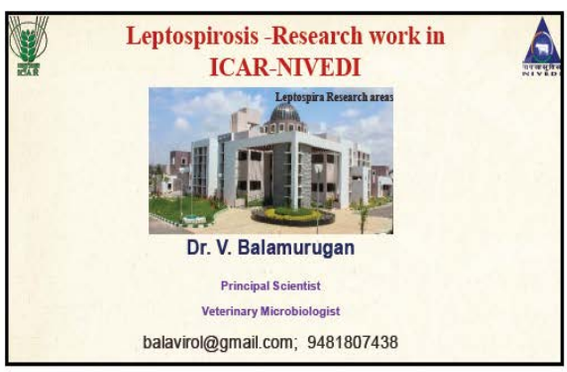
etc.,Institute scientists are guiding M.V.Sc and Ph.D scholars on the topics related to Leptospira research with major emphasis on the diagnosis.Future area of the research include,Identification of risk factors for occurrence of leptospirosis in bovine, analysis of economic burden of leptospirosis on human health- DALY'S, production, welfare loss, averting behaviour and control cost, impact on production parameters in animals, Knowledge, Attitude and Behaviour of at-risk human groups (KAP studies),Spatial analysis and landscape epidemiology of leptospirosis ie., Mapping of human leptospira outbreaks and seroprevalence analysis of leptospirosis in livestock using SoftwareQGIS, Epi info, etc., At present NIVEDI, has two externally funded project on leptospirosis research; ICMR project on development of recombinant antigen based diagnostics for bovine and human diagnosis and another one on sero-prevalence and risk factor analysis study of bovine leptospirosis by ICAR. Details of the research work done at ICAR-NIVEDI will be discussed during deliberation.

Please find the detailed information as given below:



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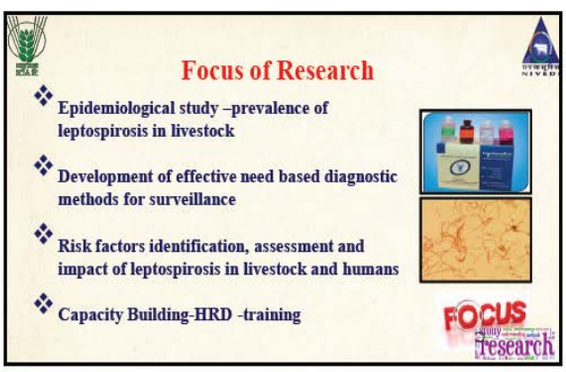

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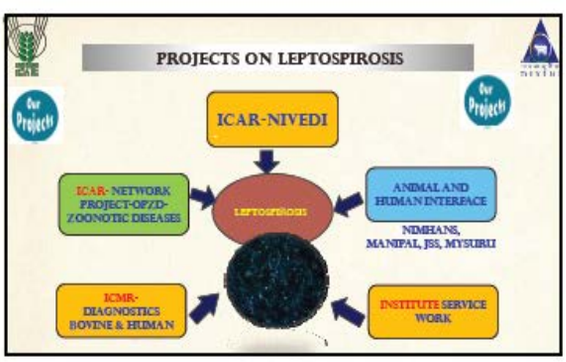

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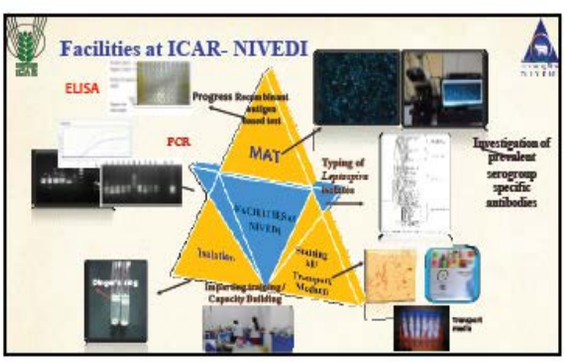

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Maintaining The Panel of *Leptospira* Reference Serovars

Species	Serovar	Strain	Serogroup
L. interrogans	Australis	Ballico	Australis
L. interrogans	Bunkiseng	Bunkiseng I	Australis
L. interrogans	Canicola	HondUtrech IV	Canicola
L. interrogans	Hardjo	Hardjoprajitno	Sejroe
L. interrogans	Hebdomadis	Hebdomadis	Hebdomadis
L. interrogans	Pyrogenes	Salinem	Pyrogenes?
L. borgpetersenii	Tarassovi	Perepelkin	Tarassovi
L. interrogans	Icterohaemorrhagiae	RGA(ATCC#43642)	Icterohaemorrhagiae
L. interrogans	Pomona	Pomona	Pomona
L. Santarosai	Shermani	1342 K	Shermani
L. kmetyi	Kmety	LT 64 - 60	Tarassovi
L. kirschneri	Grippotyphosa	MoskvaV	Grippotyphosa
L. fainei	Hurstbridge	BUT 6	Hurstbridge
L. borgpetersenii	Javanica	Poi	Javanica
L. noguchii	Panama	CZ 214 K	Panama
L. interrogans	Djasiman	Djasiman	Djasiman
L. interrogans	Copenhageni	M 20	Icterohaemorrhagiae
L. interrogans	Bataviae	Swart	Bataviae

(ORIGINAL SOURCE:- WHO REFERENCE LABORATORY - ICMR-RMRC, PORTBLAIR, ANDAMAN ISLANDS)

5

SAMPLE SCREENING- Leptospirosis

DFM - Dark Field Microscopy

Silver Staining technique

MAT–Microscopic Agglutination Test

Multiplex PCR /Real time PCR

LAT (Latex Agglutination Test)

Bovine Hardjo ELISA kit (Linnodee kit) for Bovine

are used in screening of samples for diagnosis of leptospirosis

6

Sero-epidemiology Work at ICAR-NIVEDI

Sero prevalence study and
Distribution of group specific Leptospira antibodies against speicifc Serovars in endemic region by MAT

7

Seroprevalence of leptospirosis by MAT

Odisha

Samples collected During : 2011–2014

Species : Livestock

Overall seroprevalence : 36.69% (197/537)

Overall seroprevalence (36.69%)

36.13%

54.28%

44.44%

28.33%

Prevalence of serogroup specific antibodies

Sejroe (30.4%), Tarassovi (20.8%), Australis (19.19%), Autumnalis (18.18%), Pomona (18.88%), Hebdomadis (11.11%), Pyrogenes (10.1%), Bataviae (9.68%), Icterohaemorrhagiae (9.09%),

(Balamurugan et al., 2017)

8

Seroprevalence of leptospirosis by MAT

Konkan Region of Maharashtra

Samples collected During : 2011–2013

Species : Livestock-Bovine Purposive sampling

Overall seroprevalence : 41.04% (236/575)

Cattle – 34.5%

Buffaloes – 52.2 %

Bullocks – 32.1% and

Bulls - 29.4 %

Prevalence of serogroup specific antibodies

Australis (23.61%), Sejroe (19.44%), Hebdomadis (16.67%), Autumnalis (15.28%), Icterohaemorrhagiae (14.58%), Tarassovi (9.03%)

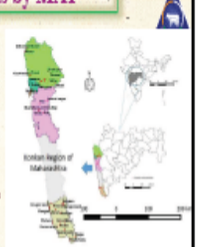

Figure 1: Map showing Study Area

(Balamurugan et al., 2017)

9

Seroprevalence of leptospirosis by MAT

Gujarat

Samples collected During : 2011–2012

Species : Livestock-Purposive sampling

Overall seroprevalence : 22.64% (48/212)

2015

34 % sero-positivity in Bovine (82/241) Purposive Random sample- Navarari and Suart District- Gujarat

55.41% sero-positivity in Livestock (Cattle, Buffaloes, sheep and goats) (179/323) Purposive Random sample- Surat District- Gujarat

Prevalence of serogroup specific antibodies

Sejroe, Hebdomadis, Icterohaemorrhagiae, Pyrogene, Pomona, Tarassovi Australis, Autumnalis, Grippotyposa Shermani, Hurstbridge, Javanica, etc.,

(Balamurugan, et al., in process)

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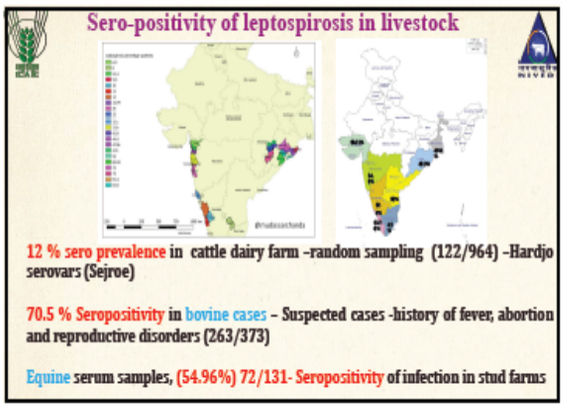

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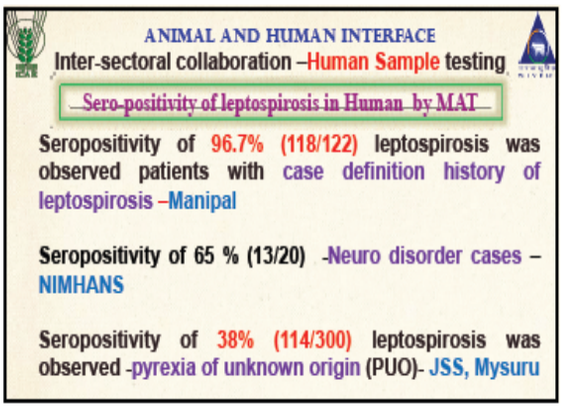

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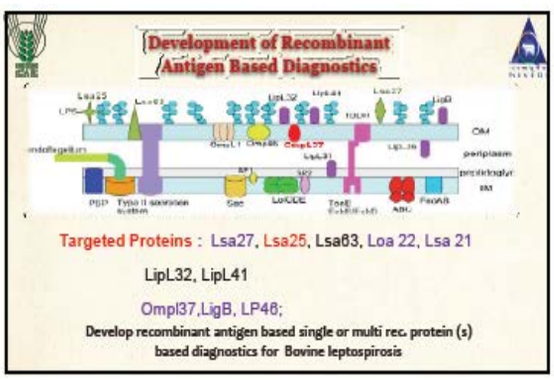

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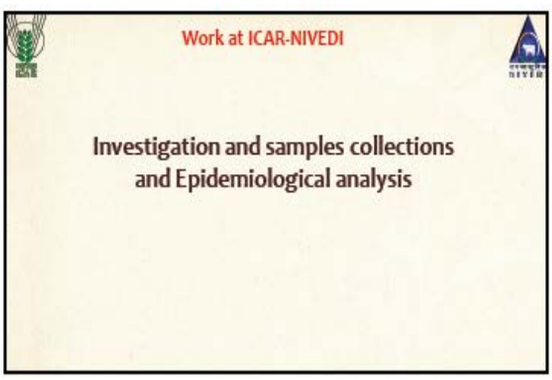

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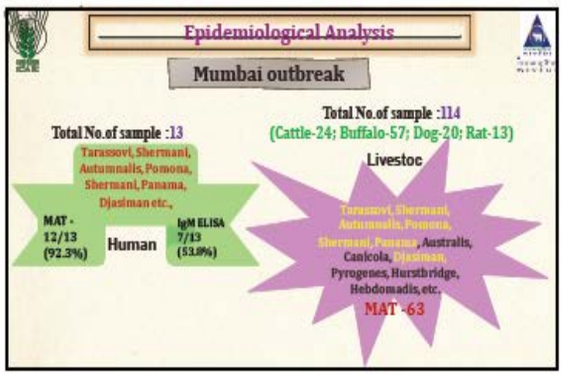

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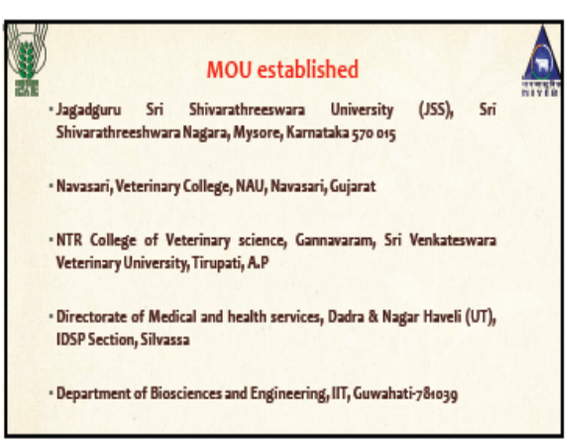

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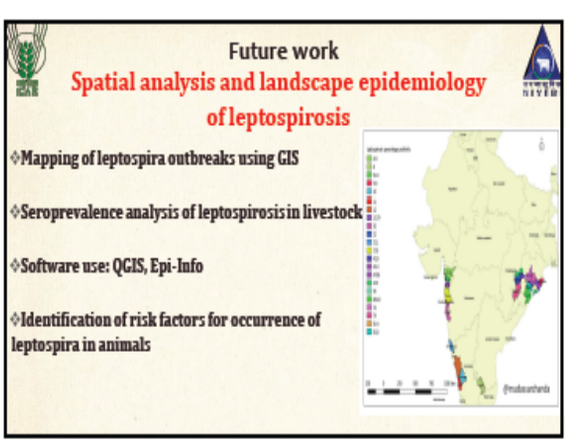

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Future work

Economic impact of Leptospirosis

if funding is provided from external agency like ICMR, DST, etc.,

- Economic burden of leptospirosis on human health- DALY'S, production, welfare loss, averting behaviour and control cost
- Impact on production parameters in animals
- Knowledge, Attitude and Behaviour of at-risk human groups- KAP studies
- Work done: Foot and Mouth Disease (FMD), HS, PPR, Avian Influenza, Sheep and Goat pox, etc.

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Summary/Publications

❖Prevalence of *Leptospira intermediate* species *(L.wolffi)* identified during the disease monitoring in the livestock (Balamurugan et al., 2013)


Characterization of leptospira isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India

24

Maharashtra State

❖Prevalence of serogroup specific antibodies identified in Konkon region of Maharashtra state- 41 % - 2011-2016

(Balamurugan et al., 2016)


Research Article

25

Odisha State

❖Prevalence of serogroup specific antibodies identified in Odisha 2012 – 42 % (Balamurugan et al., 2013)

❖Distribution of Leptospira serogroup specific antibodies in Odisha –in different climates 2011-2014 (Balamurugan et al., 2017)-36.69 % (197/537)

50.76% prevalence of multiple serovars

26

Recombinant antigen based Latex agglutination test for Bovine leptospirosis using Lig B protein of Hardjo serovar

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❖Prevalence of *Leptospira intermediate* species serovars –group specific antibodies identified during the disease monitoring in the livestock

(Balamurugan et al., 2016)

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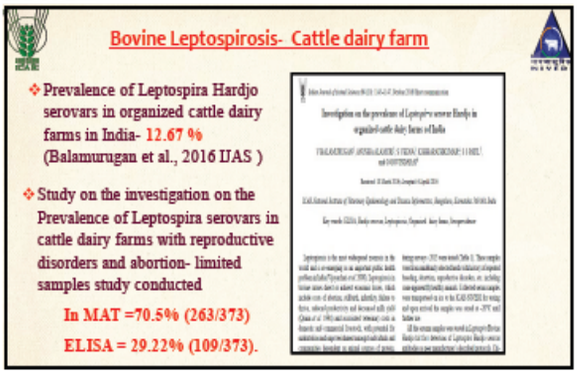

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14.Leptospira Research activities at MCVR, Manipal University, Karnataka

Dr. G. Arun Kumar,Professor& Head,MCVR, Manipal University, Manipal.

The AFI Surveillance project being conducted by the Manipal Centre for Virus Research (MCVR), Manipal University under the Global Health Security Agenda (GHSA) has been implemented in 33 Sentinel hospitals distributed across 10 states of India including Karnataka, Kerala, Assam, Goa, Gujarat, Maharashtra, Jharkhand, Tripura, Tamil Nadu and Odisha in close coordination and collaboration with the respective state health services.A total of 29952 cases have been recruited from the different sentinel sites during the period of June 2014- September 2017; out of which we were able to provide a definitive diagnosis in 13179 (44%) cases. Six pathogens account for 79% of the diagnosis including Influenza (38%), Dengue (17%), Malaria (10%), Kyasanur Forest Disease (5%), scrub typhus (5%), leptospirosis (4%) and others (21%). The tests and assays used by MCVR in leptospirosis diagnostics include Leptospira IgM ELISA (Panbio), Leptospira Uniplex Real Time PCR (CDC protocol) and Microscopic Agglutination Test (MAT). Out of 510 lab confirmed leptospirosis cases, 495 (97%) were diagnosed by Leptospira IgM alone, while 13(3%) were diagnosed by Leptospira PCR. The Prevalent Leptospira serovars identified at MCVR based on MAT include *L. georgia, L. bratislava, L.canicola and L. wolffi.* Poor sensitivity for PCR after 3 days from onset of illness is a challenge in leptospirosis diagnostics. While performing MAT the availability of acute and convalescent samplesis a major constraint.An integrated one health approach would be the ideal way forward for leptospirosis research and diagnostics in India.

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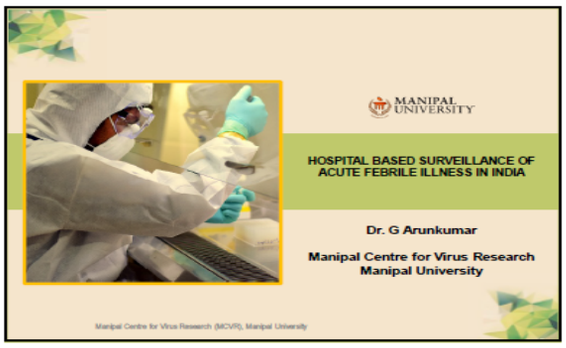
MANIPAL UNIVERSITY
HOSPITAL BASED SURVEILLANCE OF ACUTE FEBRILE ILLNESS IN INDIA
Dr. G Arunkumar
Manipal Centre for Virus Research
Manipal University

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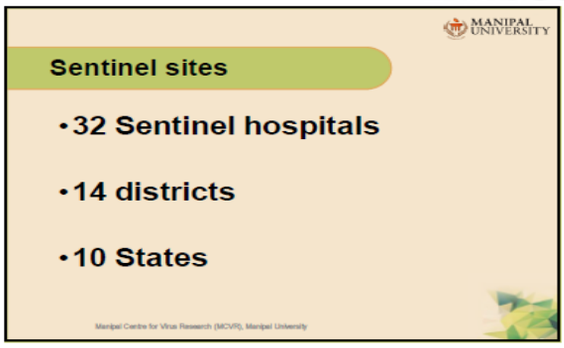
MANIPAL UNIVERSITY
Sentinel sites
•32 Sentinel hospitals
•14 districts
•10 States

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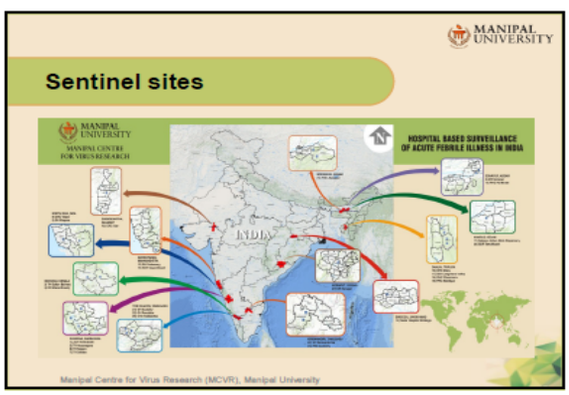
MANIPAL UNIVERSITY
Sentinel sites
HOSPITAL BASED SURVEILLANCE OF ACUTE FEBRILE ILLNESS IN INDIA
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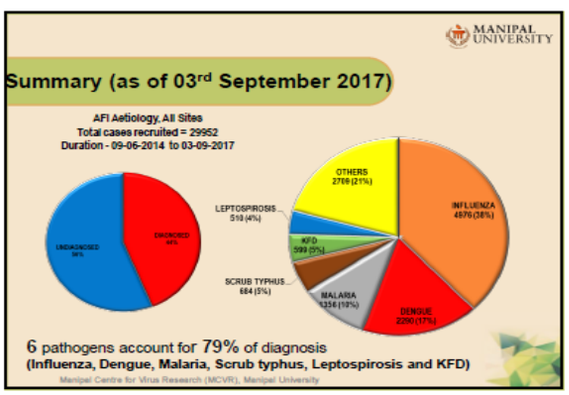
MANIPAL UNIVERSITY
Summary (as of 03rd September 2017)
AFI Aetiology, All Sites
Total cases recruited = 29952
Duration - 09-06-2014 to 03-09-2017
OTHERS 2709 (21%)
INFLUENZA 4870 (38%)
LEPTOSPIROSIS 510 (4%)
KFD 599 (5%)
SCRUB TYPHUS 684 (5%)
MALARIA 1356 (10%)
DENGUE 2290 (17%)
6 pathogens account for 79% of diagnosis
(Influenza, Dengue, Malaria, Scrub typhus, Leptospirosis and KFD)

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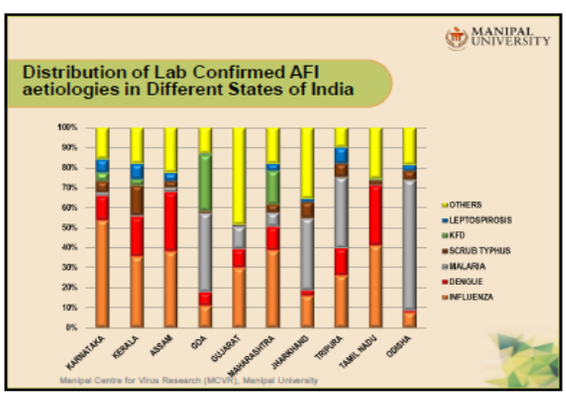
MANIPAL UNIVERSITY
Distribution of Lab Confirmed AFI aetiologies in Different States of India
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
KARNATAKA
KERALA
ASSAM
GOA
GUJARAT
MAHARASHTRA
JHARKHAND
TRIPURA
TAMIL NADU
ODISHA
OTHERS
LEPTOSPIROSIS
KFD
SCRUB TYPHUS
MALARIA
DENGUE
INFLUENZA

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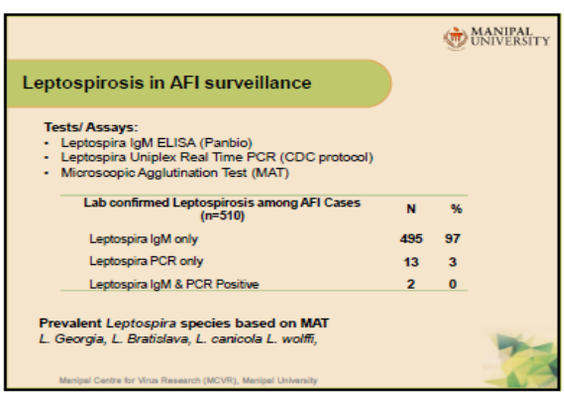
MANIPAL UNIVERSITY
Leptospirosis in AFI surveillance
Tests/ Assays:
• Leptospira IgM ELISA (Panbio)
• Leptospira Uniplex Real Time PCR (CDC protocol)
• Microscopic Agglutination Test (MAT)
Lab confirmed Leptospirosis among AFI Cases (n=510)
N
%
Leptospira IgM only
495
97
Leptospira PCR only
13
3
Leptospira IgM & PCR Positive
2
0
Prevalent Leptospira species based on MAT
L. Georgia, L. Bratislava, L. canicola L. wolffi,

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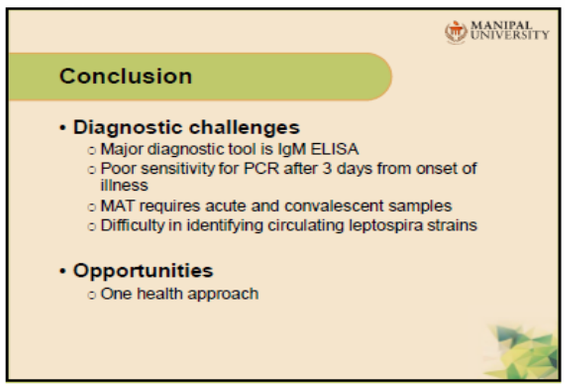

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BrainstormingSession

Session was chaired by Dr. Parimal Roy, Director, ICAR-NIVEDI, Bengaluru, and Dr. Daniel L. Garcia, Senior Laboratory Advisor, Division of Global Health Protection,CDC-India, New Delhi.

Deliberations and Brainstorming by different experts and resource persons on Identifying collaborative research issues and preparing roadmap for control of leptospirosis under one health approach has been taken place.

Recommendations

- Leptospirosis is endemic throughout the country and to understand entire status and plan for a road map, inter sectoral participation for surveillance is of paramount importance. The importance of capacity building among various stake holders was also stressed.
- The need for working together in leptospirosis to understand and control the disease in the country was discussed in the meeting. Further geographic genomics, pathogenomics and pharmacogenomics studies for understanding the leptospirosis epidemiology and control were stressed.
- The meeting highlighted the importance of surveillance and capacity building and ICAR-NIVEDI was identified to collaborate in all the aspects as a lead centre for animal surveillance and RMRC, Port Blair for human surveillance.
- During the meeting, the need for uniform and quality diagnosis and availability of diagnostics at various centres was felt by various stake holders.
- Handling of human samples at veterinary institutes and their ethical modality were discussed and it was recommended to write to heads of ICAR and ICMR to seek permission and approval for the same.

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Workshop on Laboratory Capacity building for leptospirosis (12th-15th Sep, 2017)

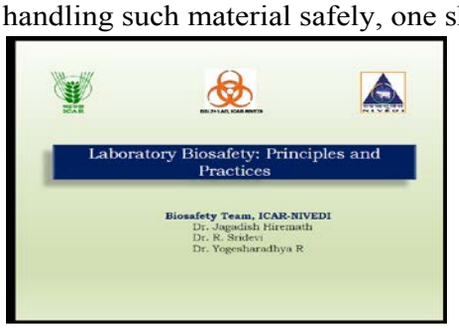
Hands-on training on different diagnostic techniques for diagnosis of leptospirosis jointly conducted by experts from ICAR-NIVEDI, Bengaluru, India and CDC, Atlanta, USA.

Workshop Training Presentations

Laboratory Biosafety: Principles and Practices

Dr. Jagadish Hiremath, Scientist cum Biosafety Officer, ICAR-NIVEDI, Bengaluru.

Biosafety refers to all the measures to protect self, people around and environment against the biological risk posed by handling of various biohazards in laboratory conditions. Laboratory biosafety involves the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release. Depend upon the nature of agent handled and risk group categorization, the containment requirements in the form of laboratory practices, safety equipment, facility design and laboratory biosafety levels for safe handling of the agent is developed. There are different levels of controls put in place to regulate the movement of materials and personnel in to and outside of the biosafety laboratories. The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and unless proficient in the practices and techniques required for handling such material safely, one should not indulge in to such works.



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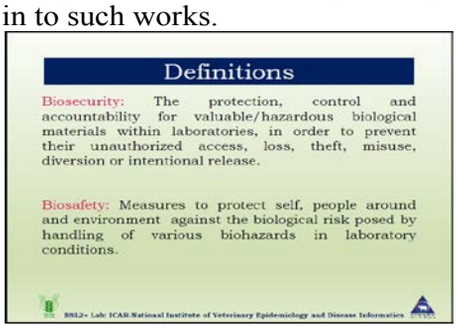

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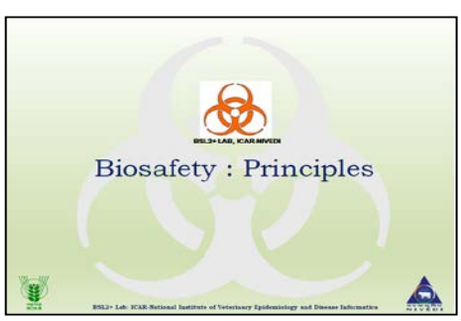

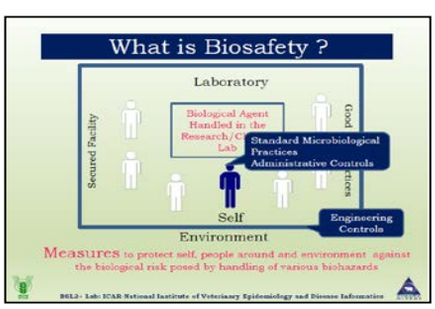

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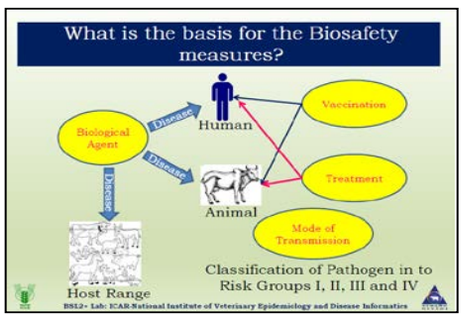
What is the basis for the Biosafety measures?
Biological Agent
Disease
Human
Vaccination
Disease
Disease
Treatment
Animal
Mode of Transmission
Host Range
Classification of Pathogen in to Risk Groups I, II, III and IV
BSL2+ Lab: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

4


RISK GROUP CATOGORIZATION
Agent
Risk Group
Individual Risk
Community Risk
Pure Culture handled (Yes/No) if yes, purpose: diagnostics, vaccine etc.
Biohazard Material Handled :Blood, Body fluids, organ specimen, Fecal, Urine, any other
Survival in Environment Limited, moderate, long term survival
Mode of Disease Transmission Aerial /Close Contact/ Vector/IH
Inter-species Transmission Limited, readily
Geographical Spread if released, Limited, moderate, wide spread
Direct Animal to Animal Transmission Limited, easily occur
Confinement of animal to prevent disease transmission Minimal, nanecsary, obligatory Control of animal movement Minimal, necessary, obligatory
Economic significance of the disease Low, moderate, high
BSL2+ Lab: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

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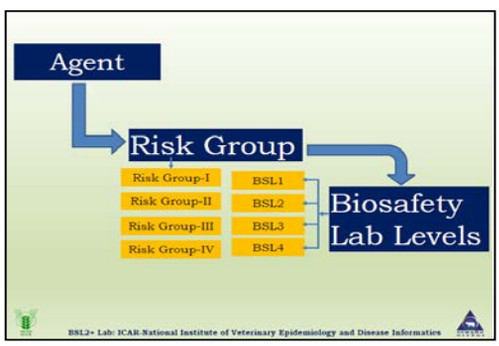
Agent
Risk Group
Risk Group-I
Risk Group-II
Risk Group-III
Risk Group-IV
BSL1
BSL2
BSL3
BSL4
Biosafety Lab Levels
BSL2+ Lab: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

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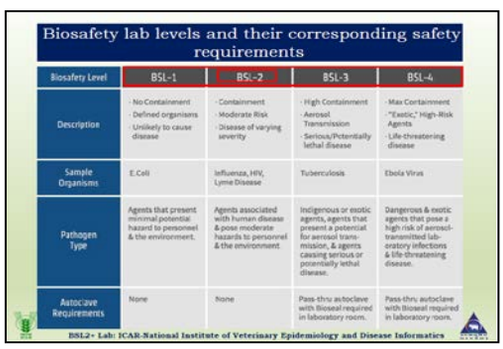
Biosafety lab levels and their corresponding safety requirements
Biosafety Level
BSL-1
BSL-2
BSL-3
BSL-4
Description
No Containment
Defined organisms
Unlikely to cause disease
Containment
Moderate Risk
Disease of varying severity
High Containment
Aerosol Transmission
Serious/Potentially lethal disease
Max Containment
"Exotic," High-Risk Agents
Life-threatening disease
Sample Organisms
E.Coli
Influenza, HIV, Lyme Disease
Tuberculosis
Ebola Virus
Pathogen Type
Agents that present minimal potential hazard to personnel & the environment.
Agents associated with human disease & pose moderate hazards to personnel & the environment
Indigenous or exotic agents, agents that present a potential for aerosol transmission, & agents causing serious or potentially lethal disease.
Dangerous & exotic agents that pose a high risk of aerosol-transmitted laboratory infections & life-threatening disease.
Autoclave Requirements
None
None
Pass-thru autoclave with Bioseal required in laboratory room.
Pass-thru autoclave with Bioseal required in laboratory room.
BSL2+ Lab: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

7


Measures to Achieve the Safety Levels
Hierarchy of Controls
Most effective
Least effective
Elimination
Physically remove the hazard
Substitution
Replace the hazard
Engineering Controls
Isolate people from the hazard
Administrative Controls
Change the way people work
PPE
Protect the worker with Personal Protective Equipment
http://www.biosafety-cabinets.com/2015_08_01_archive.html

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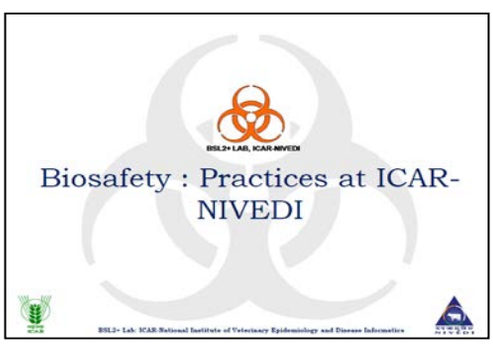
BSL2+ LAB, ICAR-NIVEDI
Biosafety : Practices at ICAR-NIVEDI
BSL2+ Lab: ICAR-National Institute of Veterinary Epidemiology and Disease Informatics

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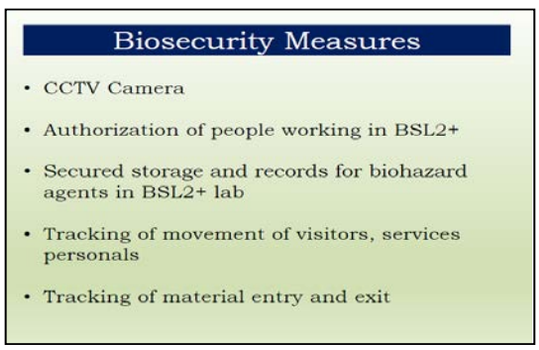

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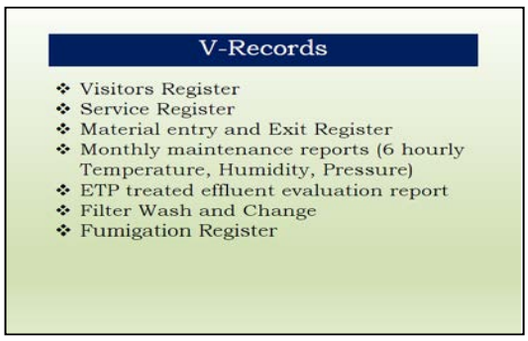

16

Application of Real time PCR for diagnosis of Diseases

Dr.V.Balamurugan, Principal Scientist, ICAR-NIVEDI, Bengaluru

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Application of real time PCR for diagnosis of diseases
Dr. V. Balamurugan
Principal Scientist
ICAR-NIVEDI, Bangalore
balavirol@gmail.com; 9481807438

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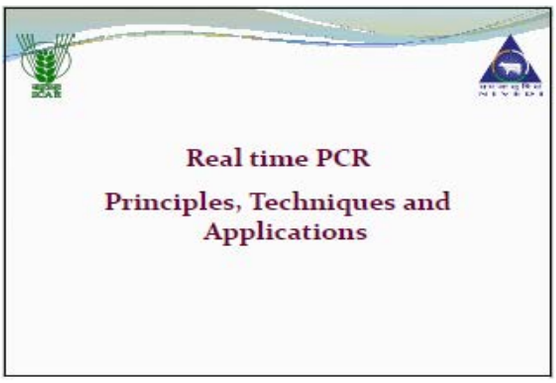
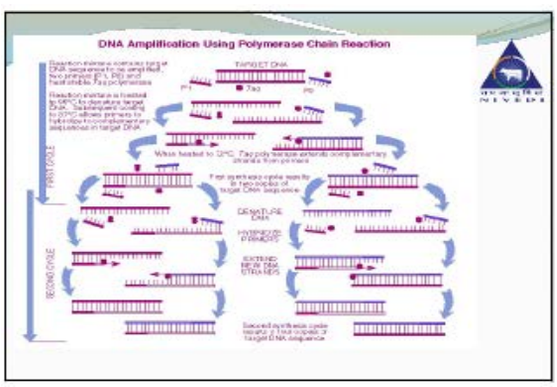
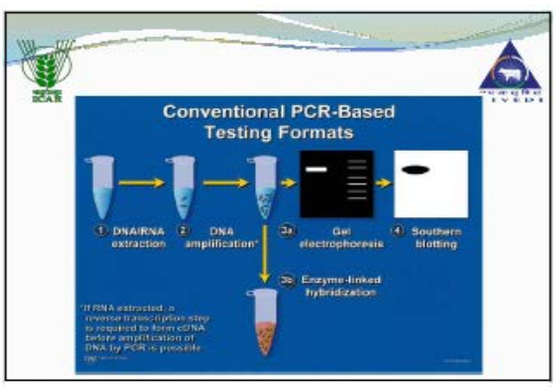

Real time PCR
Principles, Techniques and Applications

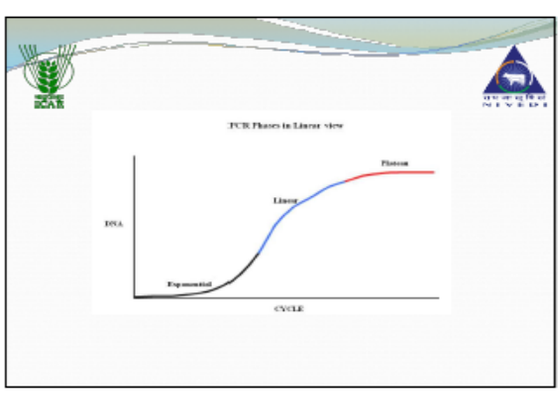
2


DNA Amplification Using Polymerase Chain Reaction

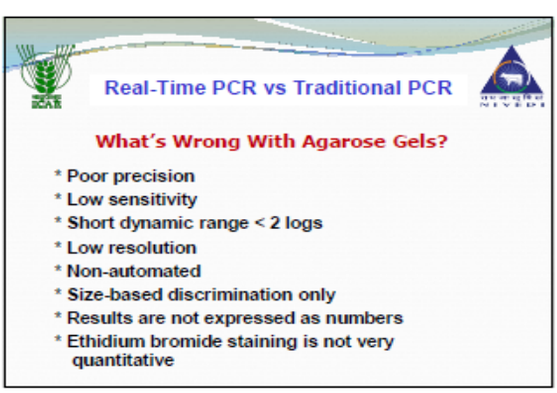
3


Conventional PCR-Based Testing Formats
DNA/RNA extraction
DNA amplification*
Gel electrophoresis
Southern blotting
Enzyme-linked hybridization

4


PCR Phases in Linear view
Plateau
Linear
DNA
Exponential
CYCLE

5


Real-Time PCR vs Traditional PCR
What's Wrong With Agarose Gels?
* Poor precision
* Low sensitivity
* Short dynamic range < 2 logs
* Low resolution
* Non-automated
* Size-based discrimination only
* Results are not expressed as numbers
* Ethidium bromide staining is not very quantitative

6

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Real-Time PCR

Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production at each PCR cycle (in real time) as opposed to the endpoint detection

7

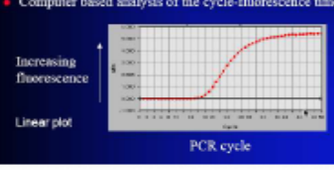
Real-time Principle

- Based on the detection and quantitation of a fluorescent reporter
- The first significant increase in the amount of PCR product (C_T - threshold cycle) correlates to the initial amount of target template

8

Real-time PCR is kinetic

- Detection of "amplification-associated fluorescence" at each cycle during PCR
- No gel-based analysis at the end of the PCR reaction
- Computer based analysis of the cycle-fluorescence time course



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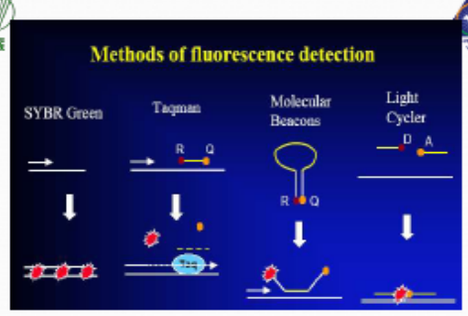
Methods of fluorescence detection



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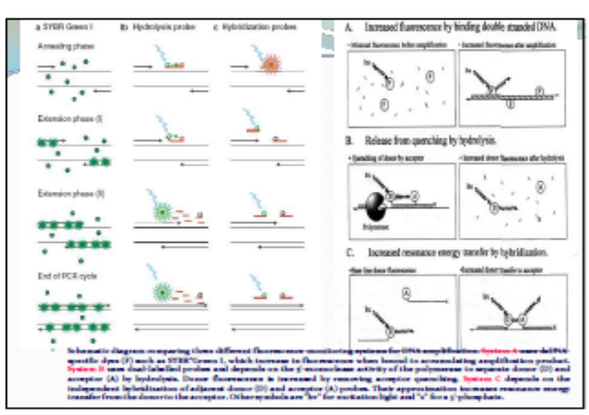

11

Three general methods for the quantitative detection

1. Hydrolysis probes
(TaqMan, Beacons, Scorpions)
2. Hybridisation probes
(Light Cycler)
3. DNA-binding agents
(SYBR Green)

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Non-specific detection using DNA binding dyes

SYBR Green I technique:

- SYBR Green I fluorescence is enormously increased upon binding to double-stranded DNA.
- During the extension phase, more and more SYBR Green I will bind to the PCR product, resulting in an increased fluorescence.
- Consequently, during each subsequent PCR cycle more fluorescence signal will be detected.

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The SYBR Green:

- First to be used in real-time PCR.
- It binds to double-stranded DNA and emits light when excited.
- Unfortunately, it binds to any double-stranded DNA which could result in inaccurate data, especially compared with the specificity found in the other two methods

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SYBR Green(double-stranded DNA binding dye):

- Emits a strong fluorescent signal upon binding to double-stranded DNA
- Non specific binding is a disadvantage
- Requires extensive optimisation
- Requires melting point curve determination
- Longer amplicons create a stronger signal
- May be multiplexed when coupled with melting point analysis

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SYBR® Green Assay

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Advantages and Disadvantages

Advantage:

- The biggest advantage of SYBR it binds to any dsDNA
- The advantage of dsDNA-binding include simple assay design, ability to test multiple genes quickly without designing multiple probes.
- Probes cost more
- SYBR Green –cost effective

Disadvantage:

- Both specific and non-specific products generate signal, DNA binding DNA bind to any dsDNA.
- Standardization should be perfect

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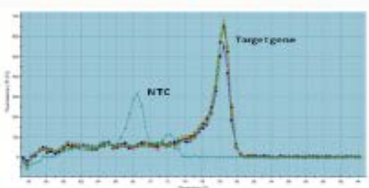
Interpretation (Melting curve analysis):



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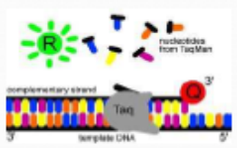
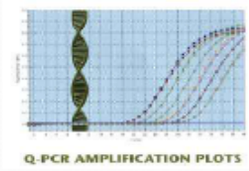
Specific detection using DNA Probes

TaqMan probe real time





Q-PCR AMPLIFICATION PLOTS

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Hydrolysis probe technique

- The hydrolysis probe is conjugated with a quencher fluorochrome, which absorbs the fluorescence of the reporter fluorochrome as long as the probe is intact.
- However, upon amplification of the target sequence, the hydrolysis probe is displaced and subsequently hydrolyzed by the Taq polymerase.
- This results in the separation of the reporter and quencher fluorochrome and consequently the fluorescence of the reporter fluorochrome becomes detectable.
- During each consecutive PCR cycle this fluorescence will further increase because of the progressive and exponential accumulation of free reporter fluorochromes.

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TaqMan Probes

- FRET = Förster/fluorescence resonance energy transfer & DNA Polymerase 5' exonuclease activity
- * Tm value 10° C higher than primers
- * Runs of identical nucleotides (no consecutive Gs)
- * G+C content 30-80%
- * More Cs than Gs
- * No G at the 5' end

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Molecular Beacons:

- The molecular beacon method utilizes a reporter probe that is wrapped around into a hairpin. It also has a quencher dye that must be in close contact to the reporter to work.
- An important difference of the molecular beacon method in comparison to the TaqMan® method is that the probe remains intact throughout the PCR product, and is rebound to the target at every cycle.
- Utilize molecular beacons that are complementary to a sequence in the middle of the expected amplicon.
- The length of the loop sequence should be chosen so that the probe-target hybrid is stable at the annealing temperature. Whether a molecular beacon actually exhibits these designed features is determined by obtaining thermal denaturation profiles.
- The molecular beacons with appropriate thermal denaturation characteristics are included in each reaction at a concentration similar to the concentration of the primers.

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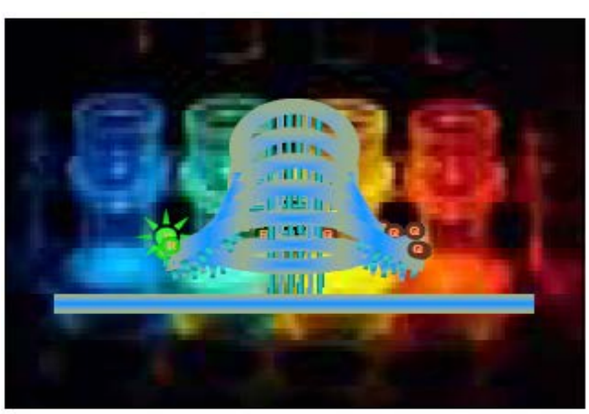

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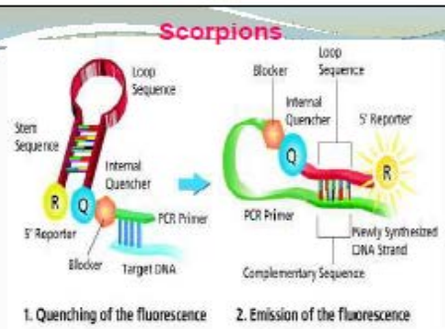
Scorpions



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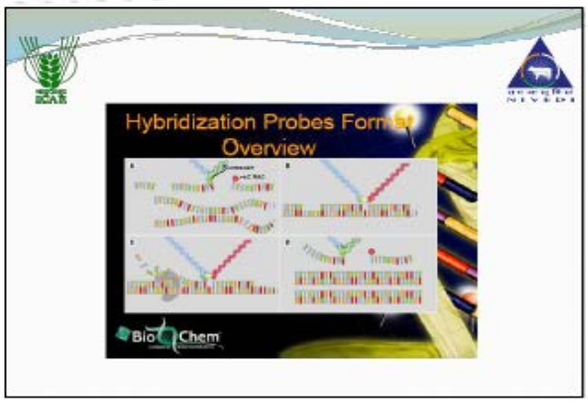

25

Reporter, Quencher and Internal Reference Dyes

The classical reporter dye:

- 6-FAM (6 carboxy fluorescein),
- HEX (5-hexa chloro fluorescein) and
- Cy5 (carbocyanin-cyanine dyes).

Other reporters used for multiplexing are

- JOE (4-5 Dichloro carboxy fluorescein) and
- VIC (4,7, 2 trichloro carboxy fluorescein)
- red dyes also used as a reporters.

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Quencher

TAMRA (6-carboxy tetramethyl rhodamine).

- Dark dyes, DABYCL (Fluorophore 4-dimethylamino phenyl azobenzoic acid)
- Black hole quenchers-BHQ I and II (Biosearch Technologies).
- TAMRA-quenched probes do not require a reference dye; they can use the TAMRA itself.
- Single probe reactions quenched by dark dyes should use an internal reference dye, classically ROX (5 or 6-carboxy-x-rhodamine-dark red).
- Multiplex reactions usually use dark quenchers and ROX.

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Threshold Cycle

- Threshold cycle or the C_T value is the cycle at which a significant increase in DRn is first detected
- It is the parameter used for quantitation
- C_T value of 40 or more means no amplification and cannot be included in the calculations

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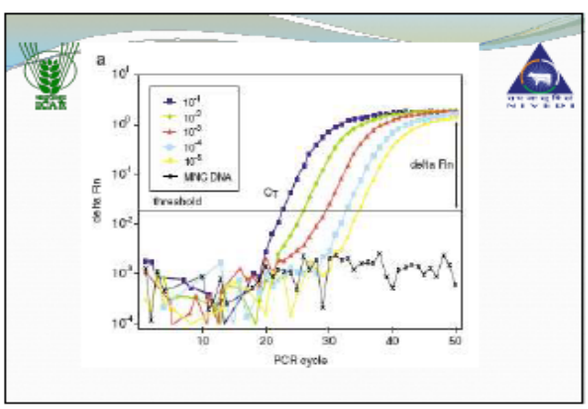

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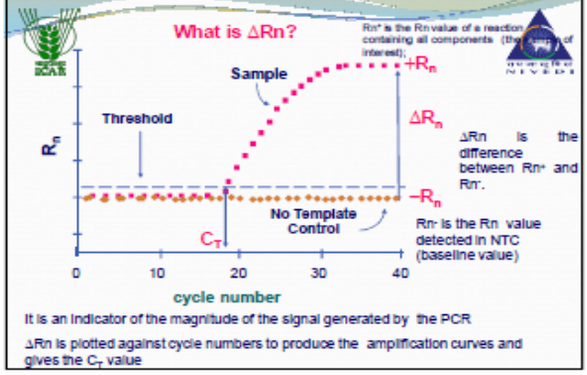
What is ΔRn?



It is an indicator of the magnitude of the signal generated by the PCR

ΔRn is plotted against cycle numbers to produce the amplification curves and gives the C_T value

30

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Performance and Evaluation

31

Efficiency-Optimization-SC

- The slope of the log-linear phase is a reflection of the amplification efficiency
- The efficiency of the reaction can be calculated by the following equation:

$$Eff = 10^{(-1/slope)} - 1$$

The efficiency of the PCR should be 90-100% (ideal slope = -3.3)

- A number of variables can affect the efficiency of the PCR.
  - length of the amplicon,
  - secondary structure,
  - primer design,
- Approximation vs Pfaffl method (Efficiency Determination)

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Assay Validation

Test primer pairs in all combinations with the probe with a known template (plasmid clone, cDNA, RNA)

- Use standard assay conditions: 300-400 nM primers; 100 nM probe, 3 mM $MgCl_2$
- Choose the primer pair that gives the highest DRn and the lowest C_T
- Make a dilution of a template, either cDNA, cRNA or total RNA for a standard curve
- Correlation coefficient of the standard curve > 0.99
- If the slope of the standard curve of the best primer pair is around -3.6 increase the $MgCl_2$ to 6 mM
- If the slope is higher than -3.6, change primers
- An ideal assay will have a slope of -3.3

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Calculation

The slope of the standard curve can be used to determine the exponential amplification and efficiency of the PCR reaction by the following equations:

$$\text{Exponential Amplification} = 10^{(-1/slope)}$$
$$\text{Efficiency} = [10^{(-1/slope)}] - 1$$

The following table shows the amplification and efficiency for various values of the slope:

Slope	Amplification	Efficiency
-3.60	1.8957	0.8957
-3.55	1.9129	0.9129
-3.50	1.9307	0.9307
-3.45	1.9492	0.9492
-3.40	1.9684	0.9684
-3.35	1.9884	0.9884
-3.30	2.0092	1.0092
-3.25	2.0309	1.0309
-3.20	2.0535	1.0535
-3.15	2.0771	1.0771
-3.10	2.1017	1.1017

As the table illustrates, optimal PCR efficiency is indicated by a slope of –3.3.

Stratagene Application Notes #10

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Using the PCR Equation

$$X_n = X_0(1 + E)^n$$

X_n = PCR product after cycle n
X_0 = initial copy number
E = amplification efficiency
n = cycle number

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Effect of Amplification Efficiency

$X_n = X_0(1+E)^n$

Case 1: E = 0.9
$X_n = 100\ (1+0.9)^{30}$
$X_n = 2.3 \times 10^{10}$

Case 2: E = 0.8
$X_n = 100\ (1+0.8)^{30}$
$X_n = 4.6 \times 10^{9}$

A difference of 0.1 in amplification efficiencies created a five-fold difference in the final ratio of PCR products after 30 cycles

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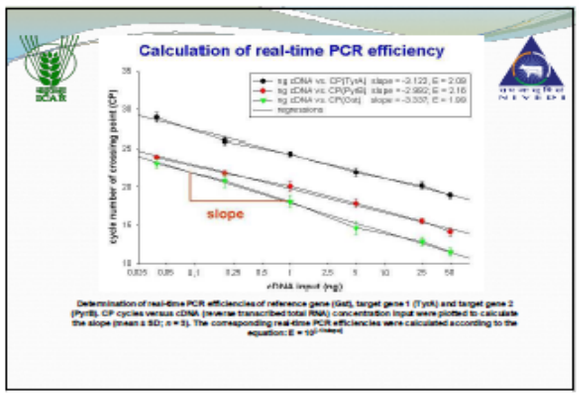

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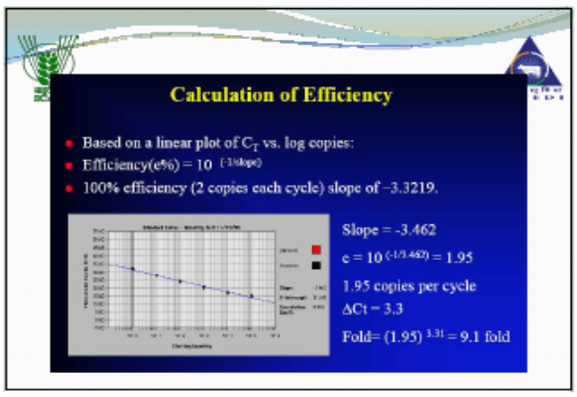

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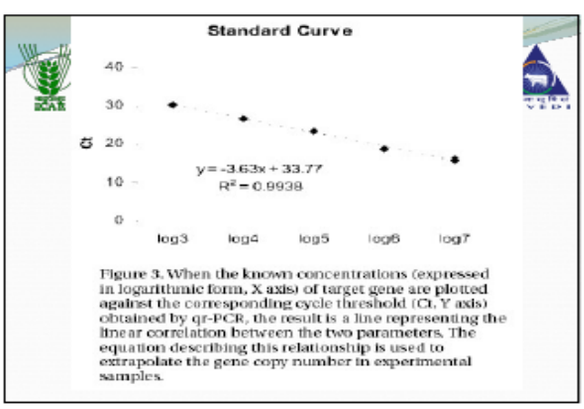

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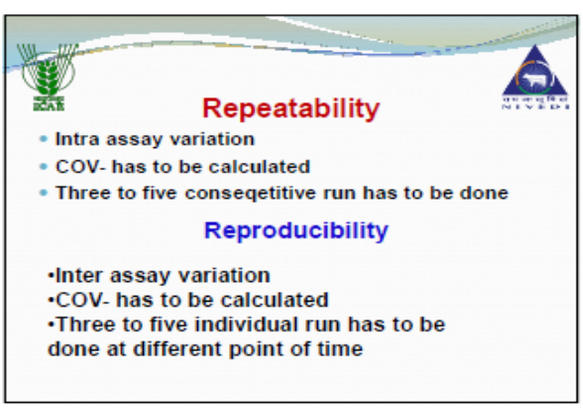

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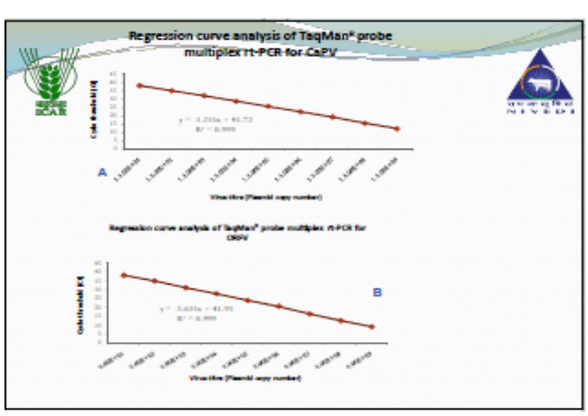

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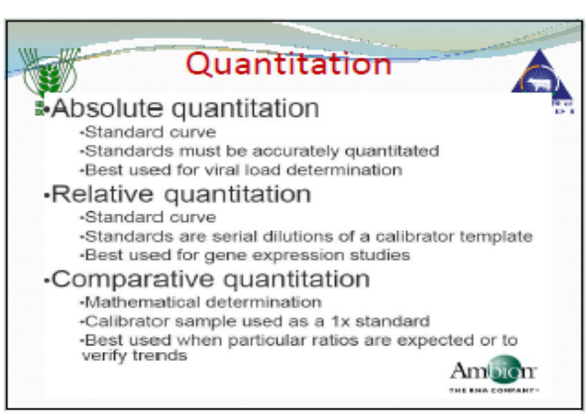

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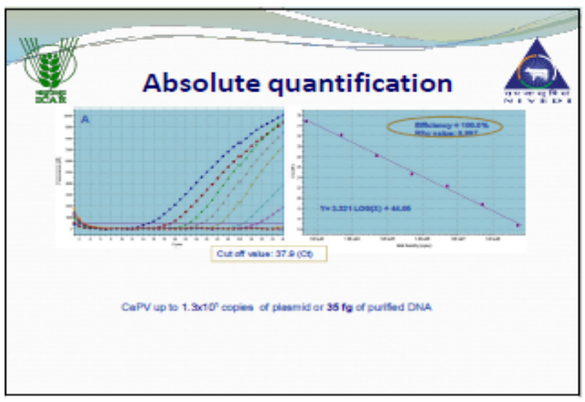

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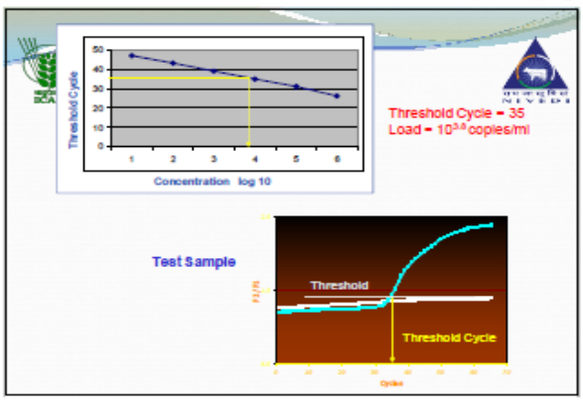

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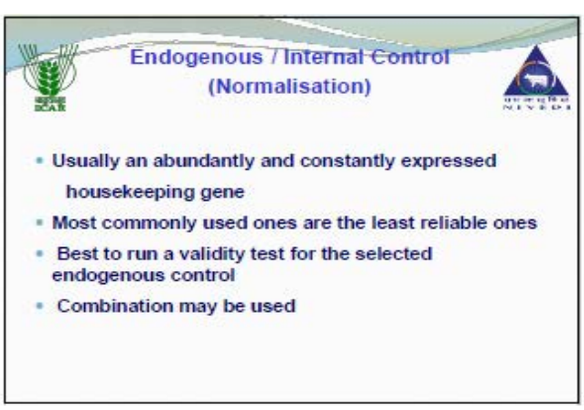

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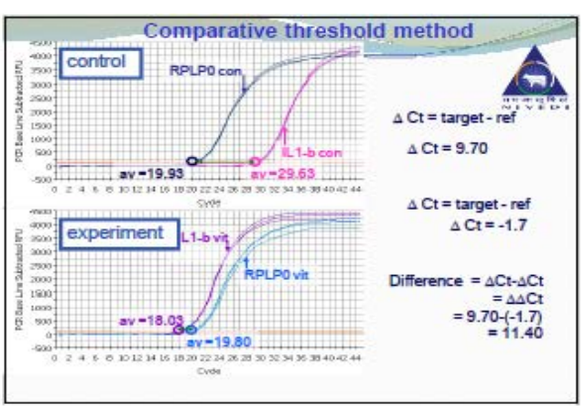

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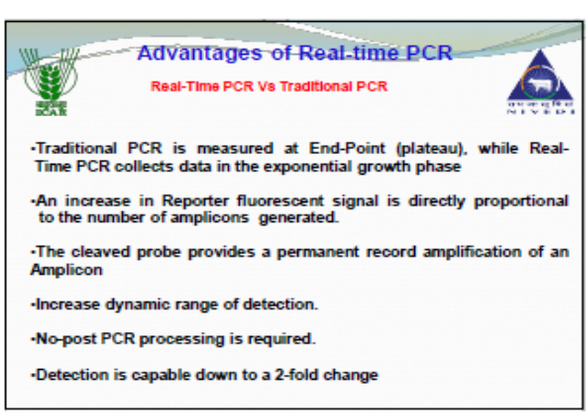

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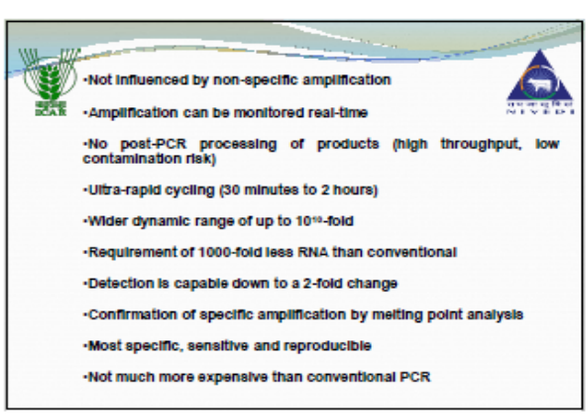

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Disadvantages of Traditional /conventional PCR:

- Poor precision
- Low sensitivity
- Short dynamic range < 2 logs
- Low resolution
- Non-automated
- Size-based discrimination only
- Results are not expressed as numbers
- Ethidium bromide staining is not very quantitative

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Disadvantages of Real-time PCR:

- Not ideal for multiplexing in general
- Setting up requires high technical skill and support
- High equipment cost
- Intra- and inter-assay variation
- RNA lability
- DNA contamination (in mRNA analysis)

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Practicals

Detection of Leptospira nucleic acid by Real time PCR

DNA Extraction:

- DNA extracted from clinical samples/isolates, by firstly heat killing .
- Commonly used samples are tissues, whole blood, serum, body fluids etc.,
- Oftenly used samples for PCR diagnosis of human samples are Blood/plasma.

Materials required:

- Centrifuge, vortexer, water bath, microcentrifuge tubes, ethanol (96-100 %),
- Qiagen QIAamp DNA Mini Kit includes:

  QIAamp spin column, collection tubes, Buffer ATL; Buffer AL; Proteinase K; Buffer AW1; Buffer AW2 and Buffer AE

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1. Transfer one ml of bacterial culture into a 1.5 ml microcentrifuge tube and centrifuge for 5 min at 13,000 rpm.
2. Discard the supernatant without disturbing the pellet or concentrate.
3. To the pellet add 180µl of Buffer ATL and 20 µl of Proteinase K, mix by vortexing and incubate at 56°C for 20 minutes (until the pellet is completely lysed).
4. Briefly centrifuge the tube to remove the drops from the inside of the lid.
5. Add 200 µl of Buffer AL to the sample, mix by pulse-vortexing for 15 seconds and incubate further at 70°C for 10 min.
6. Add 200 µl ethanol (96-100 %) to the sample and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tube to remove the drops from inside the lid.
7. Carefully apply the mixture (including the precipitate if any) to the QIAamp spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate from the collection tube.

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8. Add 500 µl of Buffer AW1 without wetting the rim and centrifuge at 13,000 rpm for 1min. Discard the filtrate from the collection tube.
9. Add 500 µl of Buffer AW2 without wetting the rim and centrifuge at 14,000 rpm for 3 min. Discard the collection tube containing filtrate.
10. Place the QIAamp spin column in a new collection tube (provided) and centrifuge at 14,000 rpm for 1 min.
11. Place the QIAamp spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate.
12. Add 50 µl of Buffer AE and incubate at room temperature for 5 min and centrifuge at 13,000 rpm for 2 min.
13. store the DNA at -20° C for further use.

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Primer and Amplicon Design

Oligonucleotide primers to be designed against a conserved region for diagnosis. The primers can be designed with software http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). A commercially available program such as Beacon Designer software performs both primer design and amplicon selection.

Guidelines to be followed for amplicon design:

- Amplicon designed should be 75–200 bp. shorter amplicons are typically amplified with higher efficiency. An amplicon should be at least 75 bp to easily distinguish it from any primer-dimers that might form.
- Secondary structure is to be avoided if possible. Use programs such as m fold (http://www.bioinfo.rpi.edu/applications/mfold/) to predict whether an amplicon will form any secondary structure at annealing temperature.
- Templates with long (>4) repeats of single bases is to be avoided.
- GC content of 50–60% to be maintained.

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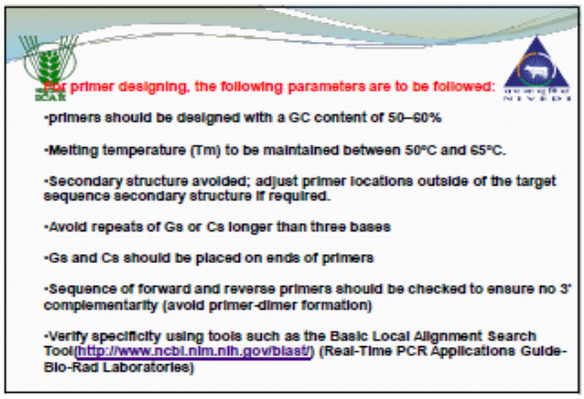

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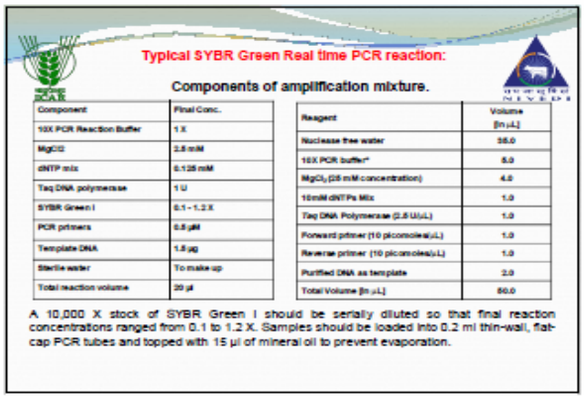

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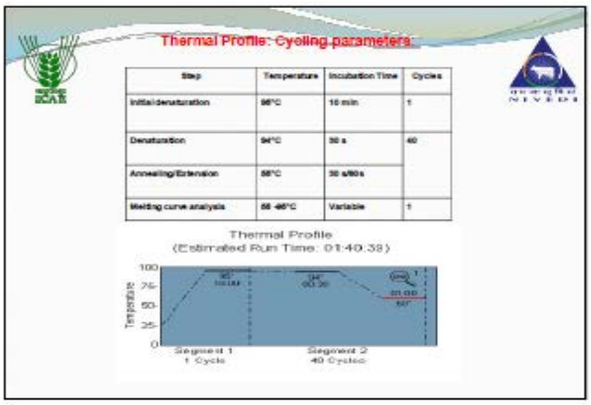

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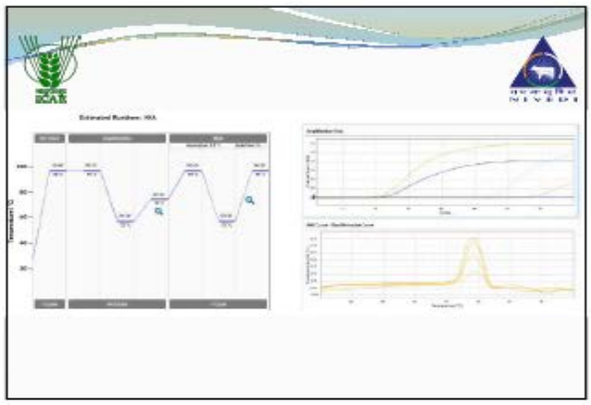

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Economic impact of leptospirosis in animals and human and KAP studies

Dr. G. Govindaraj, Senior Scientist, ICAR-NIVEDI, Bengaluru.

Leptospirosis is the most widespread zoonosis in the world and an important public health problem. Though the disease prevails throughout the world, high incidence has been recorded in tropical and sub-tropical regions where the climatic conditions highly favour the existence of leptospires. The *Leptospira* affect human and animals. Till date, research world over was focused mainly on the study of human leptospirosis because of the explicit disease manifestations in man. In animals, the disease escapes early attention of the veterinary clinician as the symptoms are invariably masked. In animals, the production losses are abortion, still berth, milk loss etc. It also acts as an indirect carrier for human transmission. In humans, the disease reduces household production and also incurs cost on public health regulation. The household cost includes, medical cost, productivity loss, pain and suffering etc., The public health sector cost includes disease surveillance cost, cost of investigating the outbreak and investment on control measures. Some cost are monetizable and some are non-monetizable in nature. Before implementing any public health programme for prevention or control or eradication, the Knowledge, Attitude and Practice (KAP) evaluation study is essential for knowing the base level of KAP across individual/groups. It will help the policy makers for designing better public health programme. KAP studies is also an important tool for ex-post assessment of any intervention programmes.

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Economic Impact of Leptospirosis in Animals and Humans and KAP studies

Dr.G.Govindaraj
ICAR-NIVEDI

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What is an impact?

2

IMPACT




3

Definition

- Impact Assessment is defined as the process of identifying the future consequences of a current or proposed action.
- Impact assessment (IA) is a structured process for considering the implications, for people and their environment, of proposed actions. It is applied at all levels of decision-making, from policies to specific projects.
- The process involves the identification and characterisation of the most likely impacts of proposed actions (impact prediction/forecasting), and an assessment of the social significance of those impacts (impact evaluation).

4

Data Collection Designs and Their Characteristics

Characteristics evaluation design	Cost	Reliability	Technical expertise	Types of evaluation	Ability to measure what is happening
Case study: one measurement (actual vs. planned)	low	very low	low	reporting	very low
Case study: two measurements(before and after)	medium	low	low	process evaluation	good
Time series design (prior trend vs. actual)	relatively low, if feasible	medium	medium	impact evaluation	very good
Case study with one measurement and a control group (with and without)	medium	low	low	formative evaluation	low
Quasi-experimental design	relatively high (variable)	relatively high (variable)	relatively high	impact evaluation	very good
Experimental design	expensive			evaluation research	very good

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Why do we need Impact analysis for livestock and human diseases?

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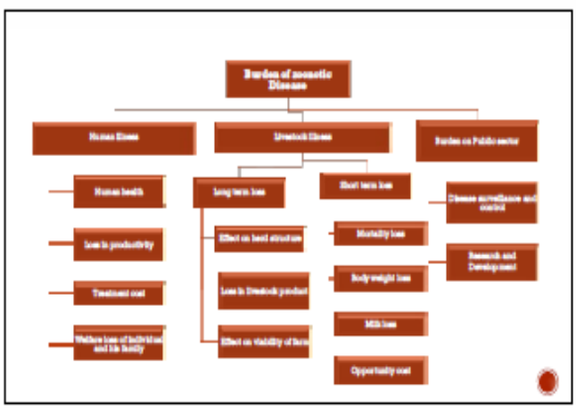
Burden of zoonotic Disease
Human Illness
Livestock Illness
Burden on Public sector
Human health
Loss in productivity
Treatment cost
Welfare loss of individual and his family
Long term loss
Short term loss
Effect on herd structure
Loss in livestock product
Effect on viability of farm
Mortality loss
Body weight loss
Milk loss
Opportunity cost
Disease surveillance and control
Research and Development

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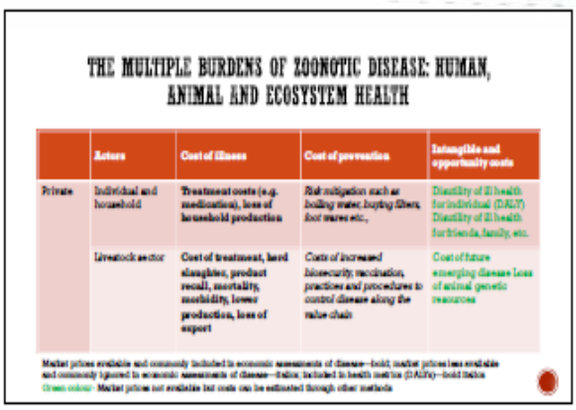
THE MULTIPLE BURDENS OF ZOONOTIC DISEASE: HUMAN, ANIMAL AND ECOSYSTEM HEALTH
Actors
Cost of illness
Cost of prevention
Intangible and opportunity costs
Private
Individual and household
Treatment costs (e.g. medication), loss of household production
Risk mitigation such as boiling water, buying filters, footwares etc.,
Disutility of Ill health for individual (DALY) Disutility of Ill health for friends, family, etc.
Livestock sector
Cost of treatment, herd slaughter, product recall, mortality, morbidity, lower production, loss of export
Costs of increased biosecurity, vaccination, practices and procedures to control disease along the value chain
Cost of future emerging disease Loss of animal genetic resources

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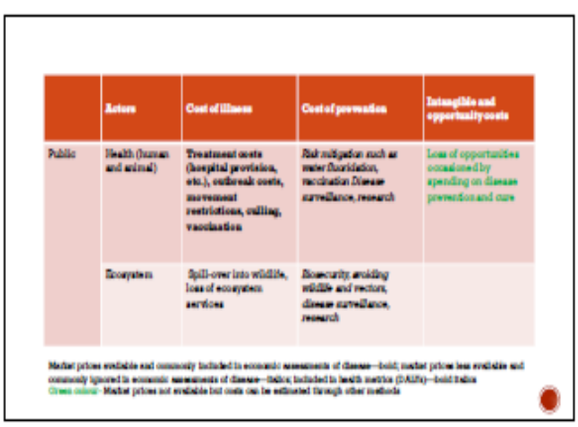
Actors
Cost of illness
Cost of prevention
Intangible and opportunity costs
Public
Health (human and animal)
Treatment costs (hospital provision, etc.), outbreak costs, movement restrictions, culling, vaccination
Risk mitigation such as water fluoridation, vaccination Disease surveillance, research
Loss of opportunities consumed by spending on disease prevention and cure
Ecosystem
Spill-over into wildlife, loss of ecosystem services
Biosecurity avoiding wildlife and vectors, disease surveillance, research

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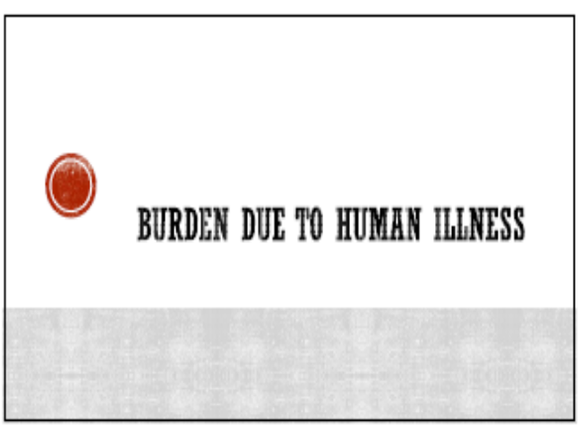
BURDEN DUE TO HUMAN ILLNESS

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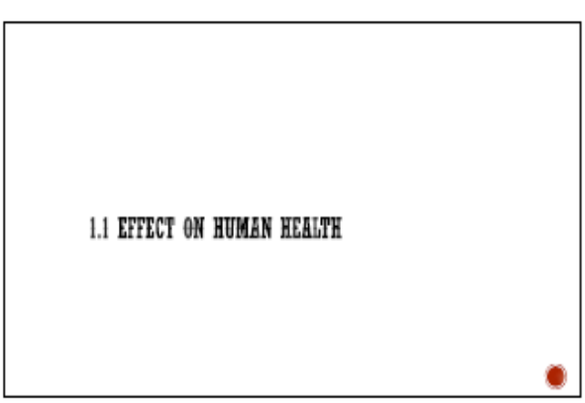
1.1 EFFECT ON HUMAN HEALTH

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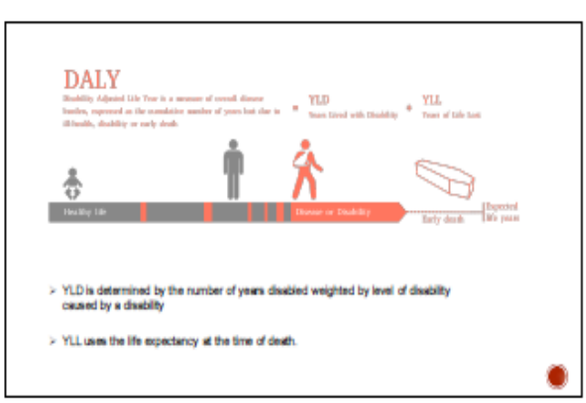
DALY
YLD
Years Lived with Disability
YLL
Years of Life Lost
Healthy life
Disease or Disability
Early death
Expected life years
YLD is determined by the number of years disabled weighted by level of disability caused by a disability
YLL uses the life expectancy at the time of death.

12

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1.2 LOSS IN PRODUCTIVITY

Loss of productivity of labour(P)

P = (Number of working days per year * Average daily earning)

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(Number of day actually worked due to disease persistence* Average daily earning)

13

1.3 Welfare loss of individual and his family

It is difficult to quantify the welfare loss in terms of pain experienced by the patient and happiness foregone by him and his family

WTP may be used as proxies

1.4 Cost of averting behaviour

Such as boiling milk, Using shoes, etc., in order to reduce the risk of disease

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1.3 TREATMENT COST

- Coping cost which is contributed out of individual's or household's pocket, suffering from the disease is calculated as follows

Cost incurred in treating human

= number of times visited to hospital * (average fees of doctors + Average expenditure on drugs)

15

BURDEN DUE TO LIVESTOCK ILLNESS

16

2.1 MORTALITY LOSS (A)
2.2 BODY WEIGHT LOSS
2.3 MILK LOSS
2.4 MILK LOSS DUE TO ABORTION
2.5 MILK LOSS DUE TO INCREASED INTER-CALVING PERIOD
2.6 TREATMENT COST
2.7 OPPORTUNITY COST OF LABOUR

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BURDEN ON PUBLIC SECTOR

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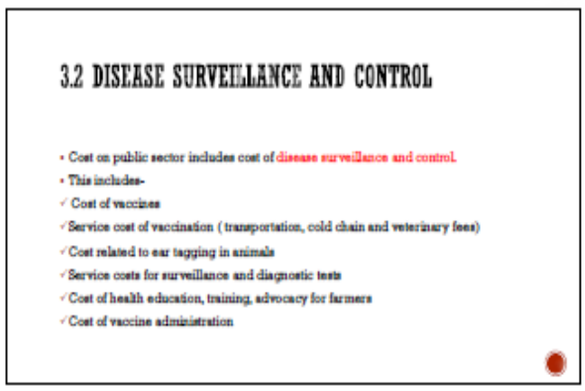

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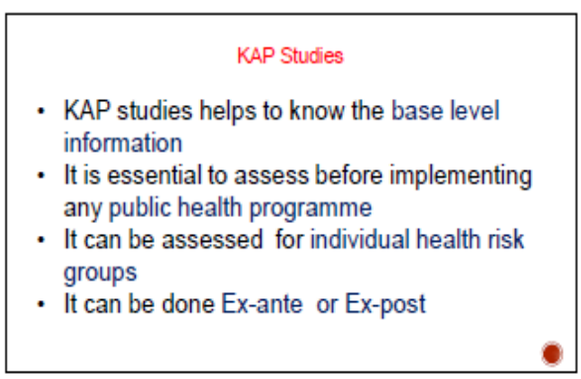

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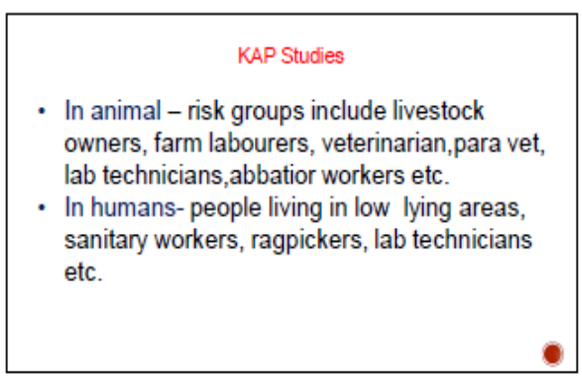

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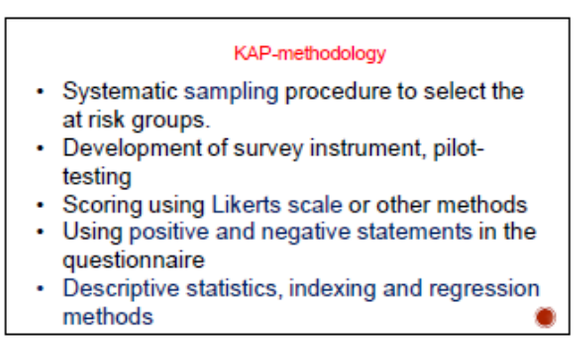

Application of GIS in understanding the Spatial Epidemiology of Leptospirosis in India

Dr. Md.Mudassar Chanda, Scientist, ICAR-NIVEDI.

A geographic information system (GIS) is a system designed to capture, store, manipulate,analyse, manage, and present all types of spatial or geographical data in a computer. GIS is a system of computer software, hardware and data, and the personnel to enter, manipulate, analyse the data.The hardware component of computer is on which a GIS operates. The software components of GIS rely on the underlying Database Management System (DBMS). The data is the most important component of GIS comprising of geographic features

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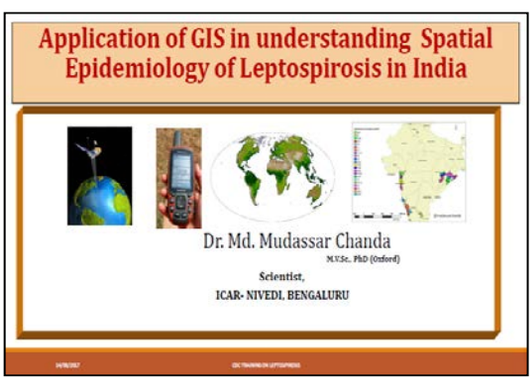
and attribute data. There are two types of datastored in the database of GIS. The attribute data gives information about the data like for example the livestock population. The spatial feature gives the information about where the feature is located in spatial domain, for example Leptospirosis cases/outbreaks in different districts of India. Spatial data can be either stored in raster or a vector format. A raster data is a continuous surface and the attribute data has rows and columns of number with a Digital Value/Number (DN) for each cell. Units are usually represented as square grid cells that are uniform in size. The satellite images, aerial photography or scanned images can all be stored in raster format. The vector data are discrete features of spatial data and they can be of three different forms- points, lines and polygons. Vector data are stored as x and y coordinates or a series of x & y coordinates. The GIS can be very helpful in mapping spatial distribution of Leptospirosis in India. The GIS can help us to understand the spatial features of Leptospirosis cases/outbreaks revealing hidden patterns, trends etc., which may not be apparent in spreadsheets. The application of GIS is not only restricted to better visualization but can also be used for many other purposes -***Field surveys, Mapping the point data and interpolation, Choropleth mapping, Overlaying disease data with other layers, Analysis of disease data.***



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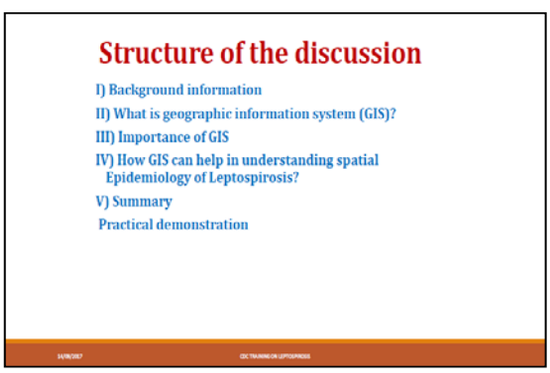

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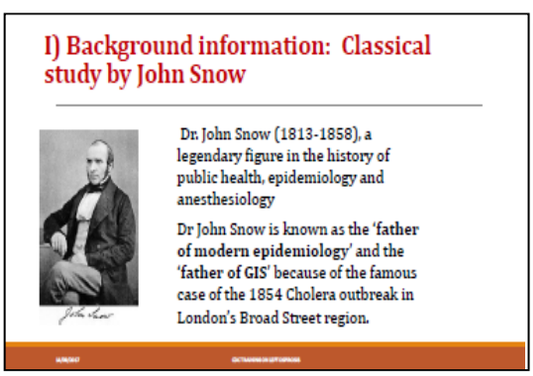

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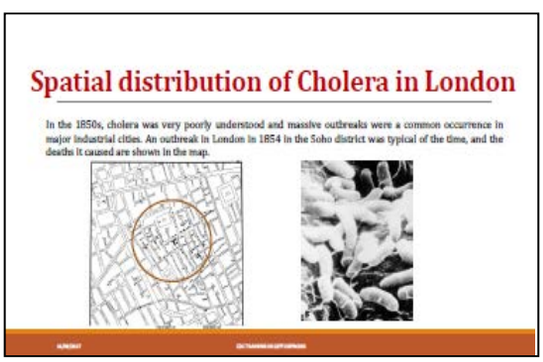

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Cholera mapping

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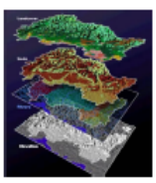
II) What is Geographical Information system(GIS)?

GIS: A type of software
- A computer system that allows us to handle information about the location of features or phenomena on the Earth's surface
- Has all the functionality of a conventional DBMS plus much of the functionality of a computer mapping system
- GIS as a DBMS that allows us to explicitly handle the spatial
- Common examples:
  - ArcView
  - ArcGIS
  - MapInfo



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Types of data in GIS

Two types of data are stored for each item in the database

1. Attribute data:
   - Says *what* a feature is
     - Eg. statistics, text, images, sound, etc.
2. Spatial data:
   - Says *where* the feature is
   - Co-ordinate based
   - Vector data – discrete features:
     - Points
     - Lines
     - Polygons (zones or areas)
   - Raster data:
     - A continuous surface

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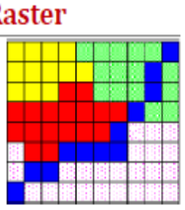
Types of data in GIS- Raster

Raster data

Stores images as rows and columns of numbers with a Digital Value/Number (DN) for each cell.

Units are usually represented as square grid cells that are uniform in size.



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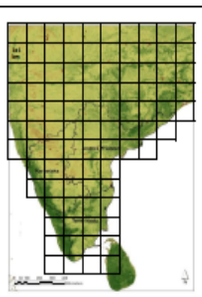

Satellite image in the raster format showing vegetation in South India.

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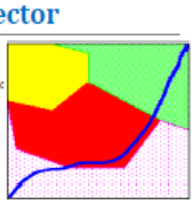
Types of data in GIS- Vector

Vector data

Allows user to specify specific spatial locations and assumes that geo broken up into discrete grid squares

We store features as sets of X,Y coordinate pairs



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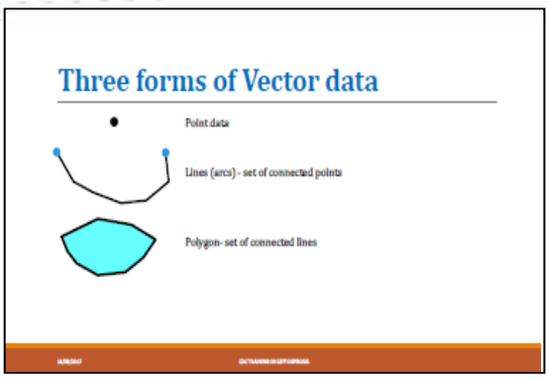

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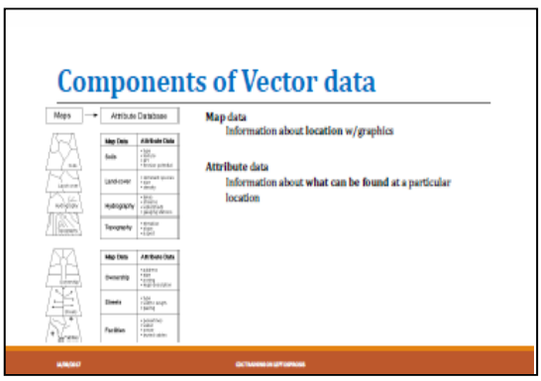

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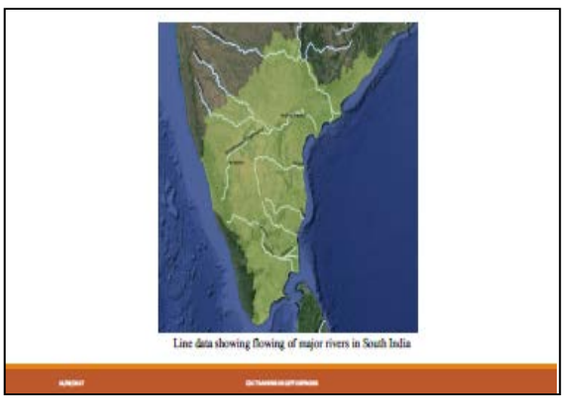

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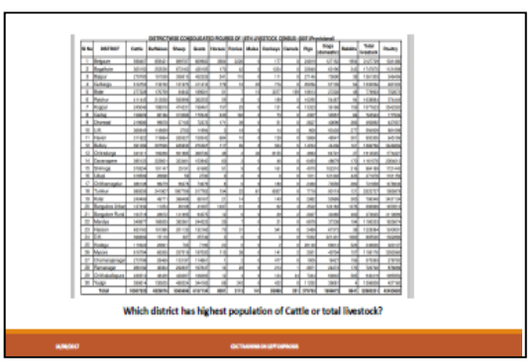

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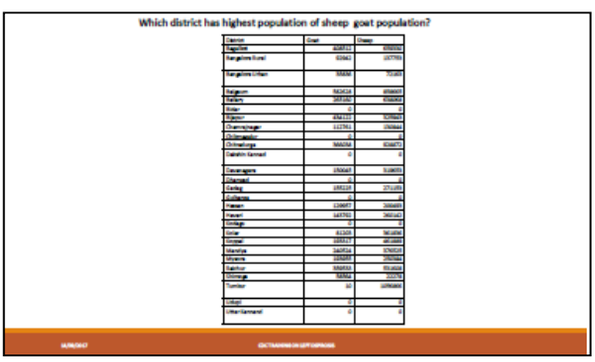

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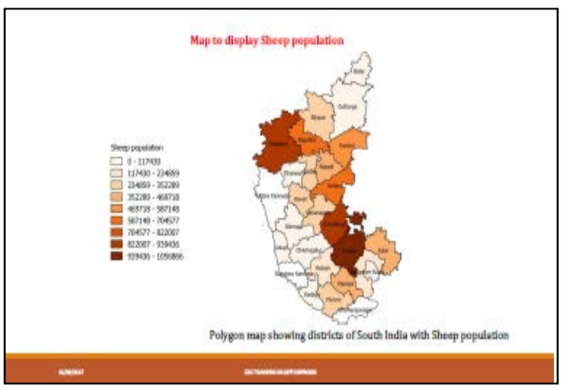

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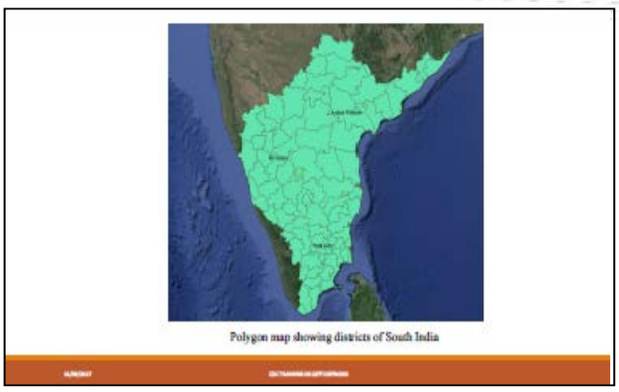

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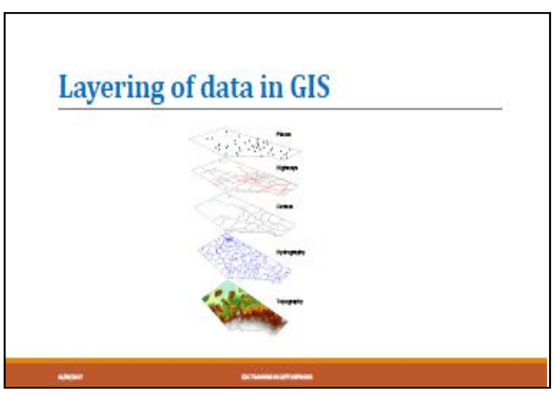

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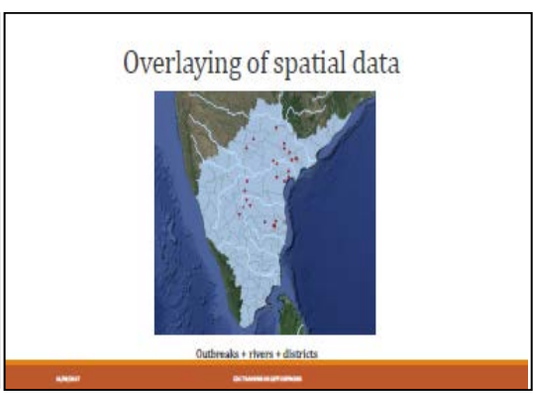

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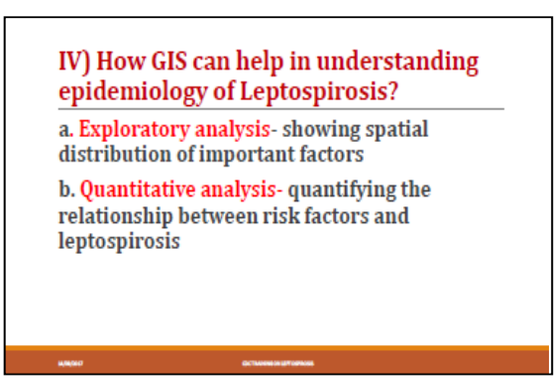

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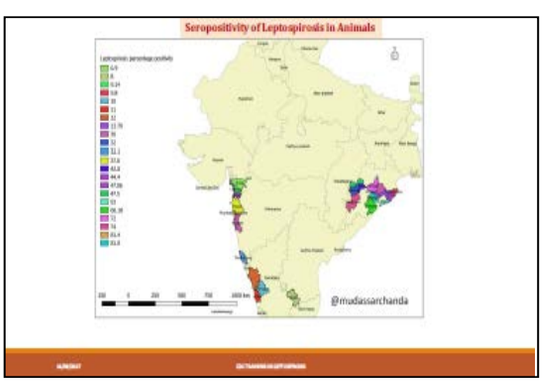

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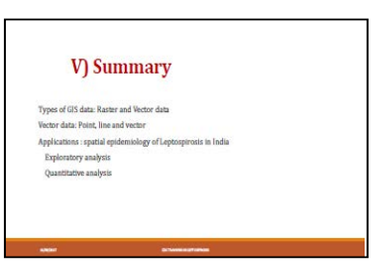

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Annexure 1

List of Participants

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List of Participants-Trainees

Workshop on Laboratory Capacity Building for Leptospirosis

1. **Mrs. AnilaRajendran**, Laboratory Technician, NCDC, New Delhi.
2. **Mr. Chandan Singh**, Laboratory Technician, NCDC, New Delhi.
3. **Mr. Aman Prakash**, Research Scholar, Department of Biosciences and Bioengineering, IIT, Guwahati.
4. **Mrs. SayaniMaji,** Senior Research Fellow, Department of Neuromicrobiology, NIMHANS, Bengaluru, Karnataka.
5. **Ms. Anjana K**, Research Assistant, MCVR, Manipal University, Manipal, Karnataka.
6. **Dr. Karikalan**, Scientist, ICAR-IVRI, Izatnagar, Bareilly,Uttar Pradesh.
7. **Ms. SowjanyaKumari**, Senior Research Fellow, ICAR-NIVEDI, Bengaluru.
8. **Mrs. LinshaLakshmanan**, Lab Assistant, ICAR-NIVEDI, Bengaluru.
9. **Mrs. ParulbenrameshchandraNaik**, Senior Laboratory Technician, Department of Microbiology,Govt. Medical College, Surat, Gujarat.
10. **Mrs Prameela** Microbiologist, State Surveillance Unit, Bengaluru,Karnataka.
11. **Dr. Neeta Khandelwal**, Professor& Head, Department of Microbiology, Government Medical College, Surat, Gujarat.
12. **Dr. Sunil Lahane**, Asst. Commissioner of Animal Husbandry, Western Regional Disease Diagnostic Laboratory, Pune, Maharashtra.
13. **Dr. Nagarathna S**, Professor, Dept. of Neuromicrobiology, NIMHANS, Bengaluru, Karnataka.
14. **Mr. Dominic Xavier Mani.A**,Senior Laboratory Technician,NIMHANS,Karnataka.
15. **Dr.Manga Devi.N**,Teaching Assistant,SLDL,SVVU,Tirupathi, Andhra Pradesh.
16. **Mrs.Anusha Alamuri,** Senior Research Fellow, ICAR-NIVEDI, Bengaluru.
17. **Mrs.ShilpaShiju,** Microbiologist, State Surveillance Unit, Bengaluru,Karnataka.









ICAR- National Institute of Veterinary Epidemiology and Disease Informatics
CDC & ASM sponsored
"Stakeholder Meeting" & Workshop on
"Laboratory Capacity Building for Leptospirosis"
Venue: ICAR-NIVEDI, Bengaluru


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ICAR-NIVEDI, Bengaluru, India and CDC, Atlanta, USA
11th to 15th September, 2017
Venue: ICAR-NIVEDI, Bengaluru









Organized by
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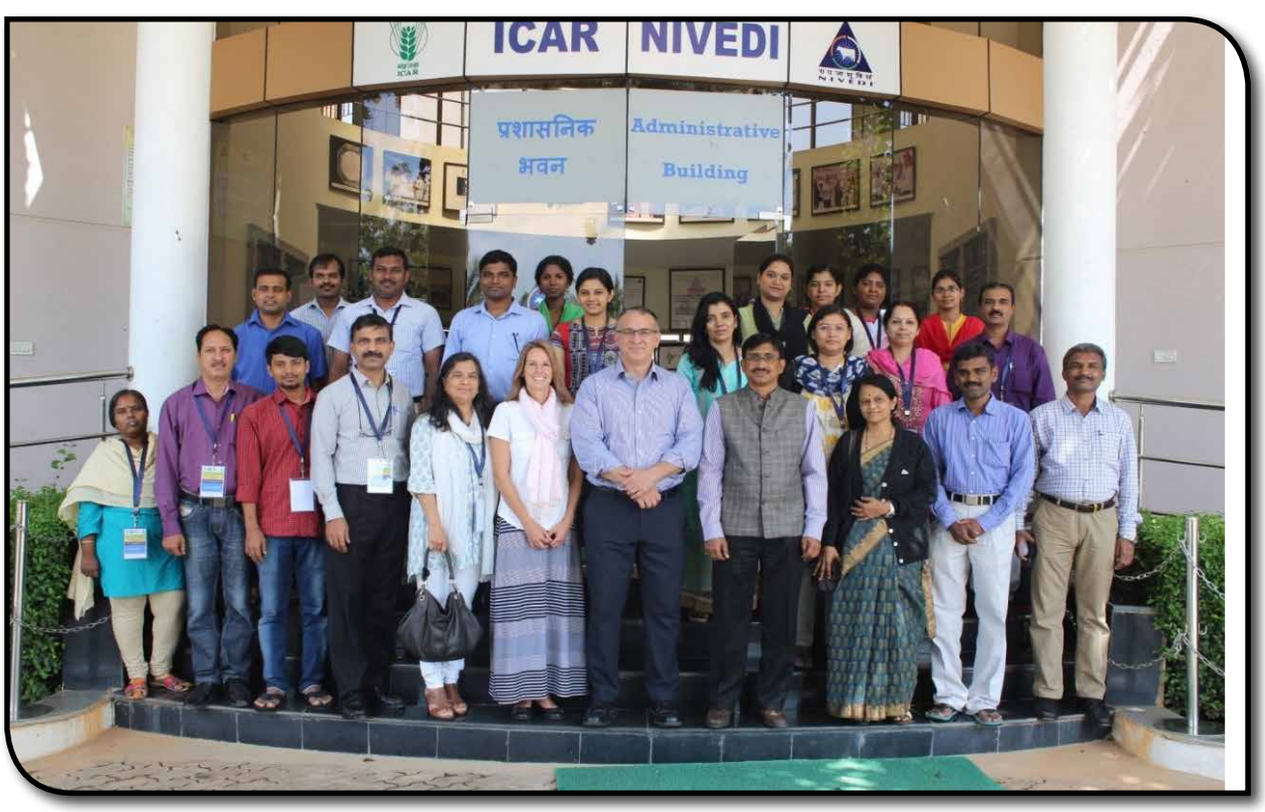
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