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 XII Five 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 9th January 2015, NIVEDI’s newly constructed administrative building and Biosafety Laboratory (BSL-2) was dedicated to the nation by Shri Radha Mohan Singh, Hon’ble Union Minister for Agriculture, New Delhi in the presence of Shri D.V. Sadananda Gowda, Hon’ble Minister 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
stakeholders associated with animal healthcare. Overall, NIVEDI has been proving its worthiness to the Indian animal health sub-sector covering critical gaps in diagnostic 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 surveillance through 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, Bluengue, Classical Swine Fever, Peste des Petits Ruminants and Sheep and Goat Pox) and parasitic (Trypanosomosis, Theileriosis, Babesiosis, Fascioliosis and Amphis tomiosis) diseases of economic importance with 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,
- 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” using Global Health Security Funds (GHSF) and providing opportunity to ICAR-NIVEDI for conducting such a workshop and stakeholder meeting in the field of “Leptospirosis” are gratefully acknowledged.
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ICAR Song
Lighting of Lamp by Dignitaries
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Opening Remarks: Dr. Parimal Roy, ICAR-NIVEDI, Bengaluru
Special Remarks: Dr. Renee L. Galloway, CDC-Atlanta, USA
Special Remarks: Dr. Daniel L. Garcia, CDC-India, New Delhi
Special Address: Dr. Naveen Gupta, NCDC, New Delhi
Release of Laboratory Training Manual and CD by Dignitaries
Presidential Address: Dr. P. Vijayachari, RMRC (ICMR), Port Blair
Vote of Thanks: Dr. G. Govindaraj, ICAR-NIVEDI, Bengaluru

Day 1: 11.09.2017

Registration of Delegates: 8.30-10.00 AM

Stakeholder Meeting: 11.15-4.45 PM

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

Presentation by different experts on Leptospirosis

1. Eco-system Interface-inter sectoral Co-ordination-control of Leptospirosis
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2. Overview of the National Program on Prevention and Control of Leptospirosis
   Dr. Naveen Gupta, Joint Director, and HOD (Zoonosis), NCDC, New Delhi, India.

3. Leptospirosis situation in Karnataka
   Dr. Ravi Kumar, Senior Regional Director, Regional Office for Health and Family Welfare, Govt of India. Bangalore.

4. Leptospirosis situation in Gujarat
   Dr. Dinkar Rawal, Deputy Director (Epidemic), Commissionerate of Health, M.S. and M.E. Gandhinagar, Gujarat.
5. Leptospirosis situation in Andaman and Nicobar Island
   Dr. Avijit Roy, Joint Secretary, Integrated Disease Surveillance Programme, Andaman & Nicobar Islands

6. Leptospira Research activities at Government Medical College Surat, Gujarat
   Dr. Neeta Khandelwal, Department of Microbiology, Government Medical College Surat, Gujarat.

7. Leptospira Research activities at NIMHANS, Bengaluru
   Dr. Nagaratna 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.

12. Leptospira Research activities at IIT, Guwahati, Assam
    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

**Brainstorming Session: 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.
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. Manjunatha Reddy and Dr. M. Nagalingam

Year of Publication: September 2017
Published by: Director, ICAR-NIVEDI, Bengaluru
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 stakeholder 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), Port Blair, 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.
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
Executive Summary and Recommendations

A stakeholders meeting and workshop on laboratory capacity building for leptospirosis was jointly organized by 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 organisation /laboratories working in leptospirosis in India, namely Regional Medical Research Centre (RMRC), Port Blair; 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 stakeholders 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 stakeholders.

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.
Inaugural Session

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

At the outset, he heartily welcomed the 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, Port Blair. 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 Roy for 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 especially Dr. Mayank Dwivedi, Dr. Mohan Papannaand experts and trainees working in various laboratories in 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 to build 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 numerous 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 it requires
maintenance of live serotypes of leptospira. Rapid serologic tests are non-reliable because 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 infections as 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 spearheaded 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 for inter-sectoral coordination.

Through this workshop and additional activities comprising partnership with India on Global Health Security, CDC is supporting NCDC and NIVEDI to build 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 a 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), 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 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 into 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 need be undertaken 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 providing valuable inputs for planning the road map to control leptospirosis in the country. 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
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.
TECHNICAL SESSION 1:

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

1. Eco-system Interfaces inter sectoral Co-ordination-control of Leptospirosis
Dr. Paluru Vijayachari, 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 of 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 into 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 holders viz. 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...
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.
Expression Microarray

Venn diagram showing the common, shared and unique down-regulated genes in various groups: (mild, renal, pulmonary, Renal, Pulmonary, Gastro-intestinal and Renal, Gl)
**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 Organization for Animal Health (OIE), and the World Health Organization (WHO) focusing on the ‘One Health vision’

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**Steps in promoting One Health Approach**

- Policy change
- Behaviour change

- Multidisciplinary approach
- Holistic approach
- Working together
- Networking

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**Five Critical Steps in establishing One Health System**

- Coordination among partners
- Cooperation among stakeholders
- Involvement of the community at risk
- Consensus among stakeholders
- Collaboration among professional groups

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**VETERINARY PUBLIC HEALTH**

_Animal - Human - Ecosystem Interface_

**THEME**

- Lower Animals (proportion) at a point of time are susceptible to various microbes but these microorganisms may not be infectious to human beings.
- Virulent strains may not cause the disease among lower animals (proportion).

**Medical and veterinary ecology**

Influenza pandemic 1918 - Viral Trichology of swine flu known much earlier

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**Disease Emergence - Interactive and interconnected process**

- Environmental Impact
- Animal Human Interface
- Pathogen Stages

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**Ecosystems**

- Animal/Environment Interface
- Human/Environment Interface
- Pathogen Stages
- Influenza Epidemic
- Viral Trichology

---
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.
Overview of Programme On Prevention and Control of Leptospirosis under XIIIth 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

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

Pilot Project on Leptospirosis

Two Year Pilot project on Control of Leptospirosis: XIIIth Five Year Plan in March, 2008.
- 4 districts of Gujarat i.e., Surat, Navsari and Vadodara
- 2 districts of Kerala i.e, Kollam and Alleppey
- 2 districts of Tamil Nadu i.e, Vellore and Thanjavur.
Later expanded to 16 states in 2009 & 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,
- Training programme,
- Strengthening of intersectoral coordination and
- IC activities
**Objective:**
- Reduction of morbidity and mortality due to Leptospirosis
- Areas of implementation: Endemic States:
  - Maharashtra
  - Gujarat
  - Karnataka
  - Tamil Nadu
  - Kerala
  - Andaman & Nicobar

**Strategy:**
- a) Early detection of cases.
- b) Strengthening of diagnostic facilities
- c) Trained manpower – development
- d) Strengthening of patient management facilities
- e) IEC activities

---

**Activities at the Centre:**

1. **UA:**
   - Strengthening:
     - Medical: Rs. 40,000
     - DPO: Rs. 10,000
   - Funds: professional society
   - Development of guidelines for prevention and control of Leptospirosis.

2. **Expert group meeting:**
   - Expert: Medical, Veterinary, Animal Industry, Agriculture
   - Duration: 1 day
   - Funds: OAE

3. **Activities of Implementing State**
   - Signing of MOU
   - Identification of nodal officer
   - Strengthening for early detection and management of patients
     - Training
     - Development of guidelines for various MOUs
     - Regular training to medical and paramedical staff concerned
   - Duration: 3 days each
   - Funds: OAE
   - Ensuring proper infrastructure and logistics for patient management out of their own funds.

4. **Activities at the Centre:**
   - Identification of Leptospirosis
   - Establishment of laboratory infrastructure
   - Funds: OAE

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**Activities at the Centre:**

5. **Training**
   - Training course:
   - Training of core teams in early detection and patient management
   - Duration: 1 day
   - Funds: OAE

6. **Strengthening of Laboratory Diagnosis**
   - Identification of laboratories
   - Training of core teams in Laboratory techniques
   - Duration: 2 days
   - Funds: OAE
Activities of Implement State

- Strengthening of Laboratory Diagnosis
  - Identification of core laboratory to be setup at NCDC laboratories.
  - 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.

Activities to be implemented as per operational guidelines

- Identification of District Focal Point
  - Identification of problem districts in the state and disease mapping
  - Training 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

Intersectoral co-ordination

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

Present Status:

- Funds have been released to program states Gujarat, Tamil Nadu, Kerala, Karnataka, and Karnataka 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.
- IEC material has been developed for distribution to states.
- Mass media campaign through newspaper advertisement carried out in 2015-16.

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.
- Andaman and Nicobar 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.
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.
- **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.
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 field microscope adapted to existing microscopes.
- Strengthening of diagnostic laboratories: Enough number of sites will be assessed for detection of leptospirosis at the 5 peripheral districts.
- Recruitment of Medical Officers and health workers: Medical officers, health workers and ASHA workers will be involved in leptospirosis covering 12 taluks in 5 districts.
- Identification and sequencing: A collaboration work with Southern Regional Disease Diagnostic Laboratory (SRDDL) lab of Institute of Animal Health & Veterinary Biologics (IAH-VB), Bangalore and National Institute of Veterinary Sanitary Science and Disease Information, Bengaluru.
- Animal husbandry activities: Collaboration with the agriculture department will be made to conduct ante and postmortem activities in two affected areas.

SID: No recent notified cases in 3 taluks. SOC materials in the form of leaflets, fact sheets, and brochures will be prepared and distributed at all 12 taluks.
- Animal husbandry surveillance: Collaboration will be made with IAH & VB for the animal leptospirosis surveillance and 15 human leptospirosis affected taluks.
4. Leptospirosis situation in Gujarat

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

Leptospirosis is a zoonotic disease and accidentally transmitted to human beings as an occupational hazard. It prevailed throughout the world however their distribution is concentrated in tropical and sub-tropical countries where the soil pH and moisture are favorable for their survival. It is closely associated with rice fields, rains and rodents. In addition to 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.
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 strengthening of 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:-**

<table>
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<tr>
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<td>611</td>
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<td>308</td>
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<td>No of Deaths</td>
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<td>49</td>
<td>124</td>
<td>178</td>
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<td>38</td>
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Age & Sex wise distribution of Cases and Deaths of Leptospirosis 2016

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<th>Females</th>
<th>Total</th>
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</tr>
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<td>70+</td>
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<tr>
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</tr>
</tbody>
</table>

Leptospirosis cases Duration between onset and 1st Consideration 2016

<table>
<thead>
<tr>
<th>Dist.</th>
<th>1-3 Days</th>
<th>4-5 Days</th>
<th>6-7 Days</th>
<th>&gt;7 Days</th>
<th>Total</th>
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<td>1</td>
<td>0</td>
<td>10</td>
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<tr>
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<td>44</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>55</td>
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</tbody>
</table>

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

Actions....
- Active surveillance from mid-June
- Survey of all fever cases (5-6 years of age)
- Cap Dapsone along with antimalarial treatment
- 24/7 centre room at District Hospital and Regional Office Ivactin
- Modular training to Nepal Health Officers, Medical officers and paramedical staff
- Package developed by experts from Medical colleges and Regional training centre
- Sensitization of ASHAs and other field staff by LAVDO
- Deputation of Doctors at various CHC’s during outbreak
- Early surveillance in animal

Occupation wise Distribution of Cases of Leptospirosis 2015 & 2016

Actions (Cont....)
- Roostings
- Wound Paintings
- Splinters
- Bites
- Injuries
- Lepto birth
- Revenge
- TV serial
- (Print)
- Radio
- IEC brochures
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 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.
Background

- The first report of leptospirosis in India originated from Andaman Islands way back in 1850s.
- After this, apparently the disease disappeared or was neglected till it reemerged in an explosive fashion as outbreaks of a haemorrhagic fever (called locally as Andaman Haemorrhagic fever ) in 1980s.
- Remnants of mysterious disease till its leptospiro telescopes were reidentified in 1993.
- The common circulating paragon in the recent years are inter-hemorrhages, leptospirosis and Australia.
- 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 tracheobronchial tree resulting in acute respiratory distress.
- Case fatality ratio up to 30%.
- Most of cases is seen with the onset of the rains.
- Most of cases were reported mostly from Diglipur of North island however in recent days cases are reported in middle Andaman also.
- South Andaman some sporadic cases are reported mostly in the peri-urban area.

YEAR WISE LEPTOSPIROSIS CASES

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NO OF CASES</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>09</td>
<td>01</td>
</tr>
<tr>
<td>2016</td>
<td>255</td>
<td>03</td>
</tr>
<tr>
<td>2015</td>
<td>147</td>
<td>00</td>
</tr>
<tr>
<td>2014</td>
<td>142</td>
<td>06</td>
</tr>
</tbody>
</table>

* Source: IDSP Portal and state report

PREVENTIVE STEP TAKEN

- During monsoon season advisory is issued to entire Andaman group of islands to follow the protocol of treating all cases on time with Leptospirosis.
- The RMRC, Port Blair is acting as 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 officers can also send sample to RMRC directly from other islands sectors.
- Even the doctors are taking sample directly from patients, if the patients instead to get the sample tested at RMRC centres.

- State IDSP unit also keeps an active surveillance on Leptospirosis cases and 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.
- Directors of Health Services keeps sufficient stock of Doxycycline medicine up to sub-centre level as a first line of management.
- No prophylactic regimen is followed in ANI.
6. **Leptospira Research activities at Government Medical College Surat, Gujarat**  
**Dr. Neeta Khandelwal**, 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.
**Infrastructure:**
- Leptospirosis laboratory is located at 3rd floor, Department of Microbiology having total 8 rooms & common lobby like
  - 1 DSI & 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

**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
- DGL microscope: 1
- Autoclave: 1
- ELISA reader: 1
- ELISA washer: 1
- Bio safety cabinet: 4
- Water bath: 2

**Leptospirosis annual human sample load 2011-2017**

<table>
<thead>
<tr>
<th>Test/Year</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
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<tr>
<td>Rapid</td>
<td>260</td>
<td>104</td>
<td>496</td>
<td>238</td>
<td>127</td>
<td>87</td>
<td>28</td>
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<tr>
<td>ELISA</td>
<td>95</td>
<td>474</td>
<td>564</td>
<td>325</td>
<td>194</td>
<td>112</td>
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<td>PCR</td>
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<td>15</td>
<td>11</td>
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<td>30</td>
<td>22</td>
<td>18</td>
<td>33</td>
<td>43</td>
<td>78</td>
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</table>

**Predominant strains 2011-2015-Human**

<table>
<thead>
<tr>
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<th>2011</th>
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<th>2013</th>
<th>2014</th>
<th>2015</th>
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</thead>
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<td>Nepal</td>
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<td>australis</td>
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<tr>
<td>Vietnam</td>
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<td>australis</td>
<td>australis</td>
<td>australis</td>
<td>australis</td>
</tr>
<tr>
<td>India</td>
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**Leptospirosis data 2016-Human**

### Different Laboratory Diagnostic Test

<table>
<thead>
<tr>
<th>District</th>
<th>Rapid</th>
<th>IgM ELISA</th>
<th>MAT-1</th>
<th>PCR</th>
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<tbody>
<tr>
<td>EWALI</td>
<td>51</td>
<td>50</td>
<td>55</td>
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<tr>
<td>KARACHI</td>
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<td>59</td>
<td>60</td>
<td>55</td>
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<td>GUJRATI</td>
<td>60</td>
<td>59</td>
<td>60</td>
<td>55</td>
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<td>59</td>
<td>60</td>
<td>55</td>
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<tr>
<td>WASSAN</td>
<td>60</td>
<td>59</td>
<td>60</td>
<td>55</td>
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<tr>
<td>TOTAL</td>
<td>50</td>
<td>49</td>
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<td>60</td>
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Proceedings of Stakeholders meeting and Workshop on Laboratory capacity building for Leptospirosis
Leptospirosis Analysis -2017

Leptospirosis data from 2011-2017-Animal

<table>
<thead>
<tr>
<th>YEAR</th>
<th>LEPTO</th>
<th>ANIMAL</th>
<th>LEPTO-PCR</th>
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<tbody>
<tr>
<td>2011</td>
<td>409</td>
<td>2207</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>1107</td>
<td>1607</td>
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</tr>
<tr>
<td>2014</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>1212</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>2016-2017</td>
<td>1600</td>
<td>730</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>5674</td>
<td>6143</td>
<td></td>
</tr>
</tbody>
</table>

Leptospirosis research activity

Leptospirosis research papers by Department of Microbiology, GMC, Surat


Research papers cont..

- Tanvi P, Summaya M. To identify the prevalence leptospira serogroups in the cases from southern Gujarat region. national Journal of Laboratory Medicine 2019;5:51-55.
- Summaya M, Tanvi P. Epidemiological study on human, cattle and rodent leptospirosis in south Gujarat region of India. In press and Accepted in Annals of pathology and laboratory medicine journal.
On going thesis on Leptospirosis

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

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.

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.

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.

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.
Leptospirosis data 2011-2017 - Animal

<table>
<thead>
<tr>
<th>Year</th>
<th>Predominant strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>australis, parvo</td>
</tr>
<tr>
<td>2012</td>
<td>australis, canicola</td>
</tr>
<tr>
<td>2013</td>
<td>australis, parvo</td>
</tr>
<tr>
<td>2014</td>
<td>australis, parvo</td>
</tr>
<tr>
<td>2015</td>
<td>australis</td>
</tr>
</tbody>
</table>

Total healthy human samples received
- Navsari: 64
- Valsad: 49
- Tapi: 56

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

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

Valsad
Best cut off value 14.5 panbio units

Tapi
Best cut off value 15.5 panbio units

Microscopic agglutination test (MAT)

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

<table>
<thead>
<tr>
<th>Navsari</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogen: 0</td>
</tr>
<tr>
<td>Australis: 11</td>
</tr>
<tr>
<td>Autumnalis: 1</td>
</tr>
<tr>
<td>Grippohaemorrhagica: 0</td>
</tr>
<tr>
<td>Patoc: 1</td>
</tr>
<tr>
<td>Pomona: 2</td>
</tr>
<tr>
<td>Icterohaemorrhagica: 0</td>
</tr>
<tr>
<td>Hebdo: 1</td>
</tr>
<tr>
<td>Canton: 0</td>
</tr>
<tr>
<td>Harraya: 0</td>
</tr>
<tr>
<td>Batavia: 0</td>
</tr>
</tbody>
</table>
7. Leptospira Research activities at NIMHANS, Bengaluru

Dr. Nagarahna 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 of protean clinical manifestations, leading to significant morbidity and mortality. The clinical spectrum can range from an asymptomatic, subclinical infection to 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 of a 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
south India, and also analysed the outcome and prognostic indicators over 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 presenting manifestations; 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.

**Introduction**

- Leptospirosis in India, is often underdiagnosed.
- The clinical spectrum can range from an asymptomatic, subclinical infection to a fatal hepato-renal syndrome.
- Diagnosis frequently missed.

**Leptospira Research Activities at NIMHANS, Bengaluru**

Dr Nagrajana S, MD, Microbiology
Professor
Neuromicrobiology
NIMHANS, Bangalore

**Leptospira Research activities at NIMHANS**

- Routine clinical diagnosis
- Doctor of medicine dissertation on Neuroleptospirosis
- "Neuroleptospirosis-study of microbial and clinical aspects; ICMR project 2017"
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.

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.

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.

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.

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%)
- Meningoencephalic picture in 17 (55%) patients.
- Though the commonest neurological abnormality reported in literature was aseptic meningitis, majority of patients presented with altered sensorium (encephalic picture)
• 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.

• 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.

CSF parameters
• Mean CSF cell count was 50.2 ± 72 cells/μl (range 1 to 350 cells/μl):
• 26% 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 6-323mg 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.

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

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.

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.
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 ubiquitous distribution, caused by spirochaetes of the pathogenic Leptospira species. Leptospirosis affects a wide range of hosts including humans, domestic and wild animal species. Laboratory confirmation of leptospirosis is obtained when either the pathogen is isolated or a positive serological result is obtained. The microscopic agglutination test (MAT) is considered as the reference test for leptospirosis. As all the existing tests have advantages as well as limitations, the development of new diagnostics assumes greater significance since leptospirosis has become an important public health problem in most countries of the world with many outbreaks reported in the recent past. 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. Recombinant antigens of these OMPs (LipL32, LipL41, LipL36, transmembrane protein (OmpL1)) have been 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 for sero-diagnosis of leptospirosis in dogs. A simple dot-immunoassay was developed based on the flow-through principle utilizing the recombinant LipL41 (rLipL41) protein expressed in E. coli as capture antigen. Evaluation of the cocktail recombinant antigens, LipL32 and LipL41 for sero-diagnosis of canine leptospirosis. Two immune-dominant recombinant antigens LipL32 and LipL41 have been combined and evaluated in IgG ELISA and Latex agglutination test for sero-diagnosis of canine leptospirosis. The rapidity, simplicity and economics of the LAT were found to fulfill the requirements of rapid screening test for leptospiral antibodies. The advantages of using the recombinant cocktail antigens used in the diagnosis of canine leptospirosis include the rapidity and the quantity of antigen production, safe since it eliminates the preparation of whole cell antigenic extracts of leptospires and also the stability of antigens.
Development and evaluation of recombinant Outer membrane proteins-based serodiagnoses for leptospirosis

Dr. T.M. Senthilkumar, Ph. D
Professor
Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University

Proceedings of Stakeholders meeting and Workshop on Laboratory capacity building for Leptospirosis

- 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.

Comparison of Latex agglutination test and Microscopic agglutination test in humans

<table>
<thead>
<tr>
<th>LAT</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT</td>
<td>Positive</td>
<td>148</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>17</td>
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</tr>
<tr>
<td></td>
<td>165</td>
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</tbody>
</table>

Sensitivity: 85.71%
Specificity: 76.06%
Concordance: 95.05%

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

<table>
<thead>
<tr>
<th>MAT</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<tr>
<td></td>
<td>161</td>
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</tbody>
</table>

Sensitivity: 97%
Specificity: 70%

Comparison of Flow through assay and MAT in human beings

<table>
<thead>
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<th>MAT</th>
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<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow through</td>
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<td>Negative</td>
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</tr>
<tr>
<td></td>
<td>160</td>
<td>117</td>
<td>322</td>
</tr>
</tbody>
</table>

Sensitivity: 92.95%
Specificity: 77.73%
Concordance: 80.54%

**Highly significant P < 0.01**
Serodiagnosis of bovine leptospirosis by IgG-ELISA and Latex agglutination test using recombinant LipL41 (Santhikumar et al., 2010)

Comparison of IgG-ELISA and Latex agglutination test using recombinant LipL41

<table>
<thead>
<tr>
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</tbody>
</table>

Sensitivity: 94.26%  Specificity: 81.51%  Concordance: 89.14%

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

Evaluation and standardation of the recombinant LipL41 protein can be done using dot immunobinding assay

Flow-through-based dot-immunobinding assay

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

Comparison of flow-through-based dot-immunobinding assay and MAT for serodiagnosis of canine leptospirosis

<table>
<thead>
<tr>
<th></th>
<th>MAT</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>Total</td>
</tr>
<tr>
<td>IgG-ELISA</td>
<td>163 (a)</td>
<td>17 (b)</td>
<td>180</td>
</tr>
<tr>
<td>Flow-through</td>
<td>70 (c)</td>
<td>157</td>
<td>227</td>
</tr>
</tbody>
</table>

$\chi^2 = 189.207$; df = 16; $P < 0.01$  Sensitivity: 95.69%; Specificity: 90.64%; Concordance: 92.14%  "Highly significant" $P < 0.01$

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

Jurisdiction of WRDDL

Map of Maharashtra with district wise Leptospirosis incidence

Human Leptospirosis Situation

<table>
<thead>
<tr>
<th>District</th>
<th>Total Sample Tested</th>
<th>Positive</th>
<th>Death</th>
<th>Sample Positive</th>
<th>Sample Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumbai</td>
<td>289</td>
<td>187</td>
<td>0</td>
<td>0</td>
<td>723</td>
</tr>
<tr>
<td>Pune</td>
<td>47</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>Thane</td>
<td>161</td>
<td>139</td>
<td>0</td>
<td>0</td>
<td>607</td>
</tr>
<tr>
<td>Nashik</td>
<td>157</td>
<td>141</td>
<td>0</td>
<td>0</td>
<td>567</td>
</tr>
<tr>
<td>Solapur</td>
<td>132</td>
<td>105</td>
<td>0</td>
<td>0</td>
<td>491</td>
</tr>
<tr>
<td>Ahmednagar</td>
<td>122</td>
<td>102</td>
<td>0</td>
<td>0</td>
<td>435</td>
</tr>
<tr>
<td>Others</td>
<td>196</td>
<td>168</td>
<td>0</td>
<td>0</td>
<td>691</td>
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<tr>
<td>Total</td>
<td>1,399</td>
<td>1,139</td>
<td>0</td>
<td>0</td>
<td>4,944</td>
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</table>

State Leptospirosis Situation - Corporation wise

<table>
<thead>
<tr>
<th>Corporation</th>
<th>Total Sample Tested</th>
<th>Positive</th>
<th>Death</th>
<th>Sample Positive</th>
<th>Sample Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumbai</td>
<td>289</td>
<td>187</td>
<td>0</td>
<td>0</td>
<td>723</td>
</tr>
<tr>
<td>Pune</td>
<td>47</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>Thane</td>
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<td>139</td>
<td>0</td>
<td>0</td>
<td>607</td>
</tr>
<tr>
<td>Nashik</td>
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<td>141</td>
<td>0</td>
<td>0</td>
<td>567</td>
</tr>
<tr>
<td>Solapur</td>
<td>132</td>
<td>105</td>
<td>0</td>
<td>0</td>
<td>491</td>
</tr>
<tr>
<td>Ahmednagar</td>
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<td>0</td>
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<tr>
<td>Others</td>
<td>196</td>
<td>168</td>
<td>0</td>
<td>0</td>
<td>691</td>
</tr>
<tr>
<td>Total</td>
<td>1,399</td>
<td>1,139</td>
<td>0</td>
<td>0</td>
<td>4,944</td>
</tr>
</tbody>
</table>

State Leptospirosis Situation - District wise

<table>
<thead>
<tr>
<th>District</th>
<th>Total Sample Tested</th>
<th>Positive</th>
<th>Death</th>
<th>Sample Positive</th>
<th>Sample Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumbai</td>
<td>289</td>
<td>187</td>
<td>0</td>
<td>0</td>
<td>723</td>
</tr>
<tr>
<td>Pune</td>
<td>47</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>156</td>
</tr>
<tr>
<td>Thane</td>
<td>161</td>
<td>139</td>
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<td>0</td>
<td>607</td>
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<td>Nashik</td>
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<td>491</td>
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<tr>
<td>Ahmednagar</td>
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<tr>
<td>Others</td>
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<tr>
<td>Total</td>
<td>1,399</td>
<td>1,139</td>
<td>0</td>
<td>0</td>
<td>4,944</td>
</tr>
</tbody>
</table>
10. Leptospira Research activities at SVVS, Tirupati, Andhra Pradesh

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

Leptospirosis is considered as the most widespread 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 is considered to be an 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, Uttarakhand, 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,
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.inadai, 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.

**Leptospirosis from Andhra Pradesh**
- Leptospirosis is a major public health concern.
- One of the re-emerging infectious diseases worldwide.
- Economically important disease affecting domestic animals & wild life.

**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

**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.
Financial Support received: 45.00 lakhs (SVVU, State plan)

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

## Reference Strains of Leptospires used in present study

<table>
<thead>
<tr>
<th>S.No</th>
<th>Serogroup</th>
<th>Seroserum</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L. canicola</td>
<td>Rough</td>
<td>Rough</td>
</tr>
<tr>
<td>2</td>
<td>L. borgpetersi</td>
<td>Le Mans</td>
<td>Le Mans</td>
</tr>
<tr>
<td>3</td>
<td>L. interrogans</td>
<td>Canicola</td>
<td>Canicola</td>
</tr>
<tr>
<td>4</td>
<td>L. grayi</td>
<td>Grippotyposa</td>
<td>Modern</td>
</tr>
<tr>
<td>5</td>
<td>L. icterohaemorrhagiae</td>
<td>Saint-Peters</td>
<td>Saint-Peters</td>
</tr>
<tr>
<td>6</td>
<td>L. kirschneri</td>
<td>Mesocricetus</td>
<td>Mesocricetus</td>
</tr>
<tr>
<td>7</td>
<td>L. canicola</td>
<td>Canicola</td>
<td>Canicola</td>
</tr>
<tr>
<td>8</td>
<td>L. canicola</td>
<td>Icterohaemorrhagiae</td>
<td>Icterohaemorrhagiae</td>
</tr>
<tr>
<td>9</td>
<td>L. pomona</td>
<td>Pomona</td>
<td>Pomona</td>
</tr>
<tr>
<td>10</td>
<td>L. icterohaemorrhagiae</td>
<td>Saint-Peters</td>
<td>Saint-Peters</td>
</tr>
<tr>
<td>11</td>
<td>L. hardjo</td>
<td>Hardjo</td>
<td>Hardjo</td>
</tr>
</tbody>
</table>

## Goats:

- Apparently healthy
  - 237 – 34 (14.35%)
  - L. hardjo (38.23%)
  - L. grippotyposa (23.41%)
  - L. javanica (26.47%)
  - L. autumnalis (6.45%)

## Pigs:

- Apparently healthy
  - 313 – 53 (44.40%)
  - L. grippotyposa (28.92%)
  - L. pomona (30.88%)
  - L. autumnalis (23.07%)
  - L. canicola (21.13%)
  - L. icterohaemorrhagiae (5.56%)
  - L. hardjo (30.76)
  - L. canicola (11.11%)
  - L. autumnalis (5.56%)

## Dogs:

- Apparently healthy
  - 149 – 21 (14.09%)
  - L. canicola and javanica (33.09%)
  - L. canicola (33.33%)
  - L. autumnalis (9.56%)

- Clinically suspected cases
  - 61 – 30 (39.66%)
  - L. canicola (40.90%)
  - L. hardjo (30.00%)
  - L. autumnalis (13.33%)
  - L. icterohaemorrhagiae (5.00%)
  - L. pomona (0.66%)

## Humans:

- Clinically suspected cases
  - 20 – 40 (40.00%)
  - L. hardjo (30.00%)
  - L. autumnalis (20.00%)
  - L. icterohaemorrhagiae (13.04%)
  - L. canicola and grippotyposa (10.00%)
- 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

### 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.

### Season wise seroprevalence of leptospiral antibodies in Andhra Pradesh

- Winter
- Summer
- South west monsoon
- North east monsoon

### District wise seroprevalence

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

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

- Lowest prevalence in Anantapur (4.83%) and Kadapa (5.83%) 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.

**Seroepidemiology: Wild animals**

<table>
<thead>
<tr>
<th>Wild Animal Species</th>
<th>Total no of samples examined</th>
<th>No. of Positive</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer</td>
<td>15</td>
<td>8</td>
<td>4.67%</td>
</tr>
<tr>
<td>Dog</td>
<td>12</td>
<td>4</td>
<td>4.67%</td>
</tr>
<tr>
<td>Elephant</td>
<td>5</td>
<td>4</td>
<td>0.83%</td>
</tr>
<tr>
<td>Horse</td>
<td>10</td>
<td>1</td>
<td>0.83%</td>
</tr>
<tr>
<td>Sheep</td>
<td>6</td>
<td>2</td>
<td>0.83%</td>
</tr>
<tr>
<td>Goat</td>
<td>15</td>
<td>2</td>
<td>0.83%</td>
</tr>
<tr>
<td>Domestic fowl</td>
<td>84</td>
<td>22</td>
<td>16.67%</td>
</tr>
</tbody>
</table>

**Isolation of Leptospira**

- From natural infected animals
- Reservoir hosts (rodents)
- To find out epidemiological link between animals, humans and rats
Table 5: Details of clinical samples collected and leptospiral isolates recovered

<table>
<thead>
<tr>
<th>S.No</th>
<th>Source of isolation</th>
<th>No. of samples collected</th>
<th>No. of samples reported</th>
<th>Percent positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rats</td>
<td>299</td>
<td>5</td>
<td>1.67</td>
</tr>
<tr>
<td>2</td>
<td>Sheep</td>
<td>42</td>
<td>5</td>
<td>11.91</td>
</tr>
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<td>3</td>
<td>Pigs</td>
<td>15</td>
<td>4</td>
<td>26.6</td>
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<tr>
<td>4</td>
<td>Humans</td>
<td>33</td>
<td>3</td>
<td>3.77</td>
</tr>
<tr>
<td>5</td>
<td>Bee hive</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Cattle</td>
<td>26</td>
<td>-</td>
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</tr>
<tr>
<td>7</td>
<td>Dogs</td>
<td>13</td>
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</tr>
</tbody>
</table>

Table 6: Details of samples collected from rat for isolation of leptospires

<table>
<thead>
<tr>
<th>S.No</th>
<th>Place of collection</th>
<th>No. of samples collected</th>
<th>No. of samples tested for isolation</th>
<th>No. of samples positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gravel and temporary site</td>
<td>22</td>
<td>22</td>
<td>2 samples positive</td>
</tr>
<tr>
<td>2</td>
<td>Railway station area</td>
<td>28</td>
<td>28</td>
<td>3 samples positive</td>
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<tr>
<td>3</td>
<td>R.B.I. infection area</td>
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<td>20</td>
<td>2 negative</td>
</tr>
<tr>
<td>4</td>
<td>Bharat Nagar area</td>
<td>15</td>
<td>15</td>
<td>15 negative</td>
</tr>
<tr>
<td>5</td>
<td>Bus station area</td>
<td>19</td>
<td>19</td>
<td>19 negative</td>
</tr>
<tr>
<td>6</td>
<td>Medical college area</td>
<td>185</td>
<td>185</td>
<td>185 negative</td>
</tr>
</tbody>
</table>

Molecular Epidemiology

- sheep – Leptospiroa, L. hardjo and L. icardil
- Rats - Onychocardi and Leptospiroa
- Pigs – L. pomona

Vaccine Trails:
- Based on seroepidemiological studies L. gripppoyphosa, L. hardjo, & L. autumnalis were selected as vaccine candidates for the preparation of trivalent vaccine.
- Preparation:
  - Trivalent inactivated (formalin) and adjuvanted (Al OH: Montanide) vaccine
- Immune response:
  - Six month – satisfactory protective immunity in rabbies

Recombinant Vaccine:
- To identify the vaccine candidates in designing vaccine against pathogenic leptospires.
- Complete proteomes of L. boehmii, L. hardjo, and L. autumnalis were sequenced to identify common surface exposed proteins.
- In silico analysis of L. boehmii, LB197 and LB50 were retrieved.
  - Tn dependent system containing single epitope.
  - ABC permease proteins with three epitopes.
  - Use ABC protein B with single epitope.
- The primers were designed for the amplification of ABC permease gene of L. ballum.
- The purified PCR product was cloned into pET28a vector and expressed in E. coli BL21 (DE3) cells.
- The recombinant protein was characterized using SDS PAGE yielding 20KD of expected recombinant protein.

<table>
<thead>
<tr>
<th>BLAST analysis of sequencing results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accession</strong></td>
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<td>9</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

**Amplification of ABC Permease gene**

**LB Agar Plate with White Recombinant Colonies**

**Restriction digestion pET28a vector and amplified ABC Permease gene**

**Characterization of recombinant protein with SDS PAGE (gel documentation)**
**Leptospiral Transmission**

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

---

**Occupational Activities**

- Vets  
- Para Vets  
- Dairy farmers  
- Agricultural Workers

---

**Conclusion**

- A total of 2,705 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% & 14.90% 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%, 45.6% and 65.7% respectively.

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- The seroprevalence of leptospirosis was noticed high during south west & northeast.
- The seroprevalence of leptospirosis in coastal regions was high (13.80%) followed by Rayalaseema (13.70%) and Telangana (11.90%).
- District wise seroprevalence of leptospirosis was found to be high in West Godavari (34.9%) followed by East Godavari (38.72%), and lowest in Anantapur (4.83%) and Kadapa districts (5.60%).
- Trivalent inactivated whole cell adjuvanted vaccine
- Recombinant vaccine through Pangenome reverse vaccinology
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 Icterohaemorrhagiae 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 of rLigB 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 10^3 leptospiral organisms in spiked urine samples from cattle and dog. The diagnostic
specificity of LAMP was 100% when compared to SYBR green qPCR for detection of *Leptospira* in urine samples. *LeptospirabiflexaserovarPatoc*, 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 leptospirosis. 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.
<table>
<thead>
<tr>
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<th>Number of samples</th>
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</thead>
<tbody>
<tr>
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<td>Noida</td>
<td>44</td>
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<tr>
<td>Bihar</td>
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</tr>
<tr>
<td>Jharkhand</td>
<td>Pujabi Tenga</td>
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<tr>
<td>Others</td>
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<td></td>
</tr>
<tr>
<td>Kerala</td>
<td>Local slaughter unit</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>673</td>
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</table>

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</tr>
</thead>
<tbody>
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<td>UP</td>
<td>GOVT. Medical College, Faridabad</td>
<td>103</td>
</tr>
<tr>
<td>ORISSA</td>
<td>S.S. Veterinary College, Cuttack</td>
<td>173</td>
</tr>
<tr>
<td>LADAKH</td>
<td>Don Pullung LAND Medical College, Kargil</td>
<td>72</td>
</tr>
<tr>
<td>TOTAL</td>
<td>348</td>
<td></td>
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</tbody>
</table>

- MAT was performed following standard protocol by DCC (2006).
- Recombinant Lgb antigen from CSE Laboratory of DBP was expressed, purified and its immunoactivity was tested by western blot analysis.
- Recombinant Lgb based MAT, sera regulation test. OPSTEN-Animal were standardised for sero-diagnosis of leptospirosis in dogs, cats and human.
- Analysis of test factors were done using SPSS software (SPSS, Inc.)

**ANTIGEN PANELS FOR CONDUCTING MAT**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Serovar</th>
<th>Strain</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>Belch</td>
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<tr>
<td>2</td>
<td>Australis</td>
<td>Australis</td>
</tr>
<tr>
<td>3</td>
<td>Australis</td>
<td>Australis</td>
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<tr>
<td>4</td>
<td>Babesiae</td>
<td>Babesiae</td>
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<tr>
<td>5</td>
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<td>Canicola</td>
</tr>
<tr>
<td>6</td>
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<td>Canicola</td>
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<tr>
<td>7</td>
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<tr>
<td>16</td>
<td>Canicola</td>
<td>Canicola</td>
</tr>
<tr>
<td>17</td>
<td>Babesiae</td>
<td>Babesiae</td>
</tr>
</tbody>
</table>

**MICROSCOPIC AGGLUTINATION TEST (MAT)**

For MAT titre considered as positive

![Recombinant Lgb Protein of I. pomona](image)
Western blot analysis of Purified LigB with Leptospira Hyperimmune sera

Standardization of ELISA for screening dog sera for leptospirosis

Standardization of ELISA for screening human sera for leptospirosis

Sera reactivity of LigB based LPA with dog sera

Sera reactivity of LigB based LPA with human sera
According to our study, there is similarity in distribution of Leptospirosis in dogs, pigs, and human beings which indicates that there is a route of transmission among animals and man. The prevalence was found to be 18.4%, 11.5%, and 12.2% in dogs, pigs, and human risk groups respectively to adopting dog and standardized MAT.

The results obtained in this study were compared with the results of other studies carried out in the same area. The results were found to be similar to those obtained by other researchers in the same area. The results obtained in this study were also compared with the results of other studies carried out in different areas. The results were found to be similar to those obtained by other researchers in similar areas.

Detection of Leptospira DNA by LgB-LAMP

Detection of the LgB-LAMP products by different visual methods. (a) Visualization of LAMP products at 1% agarose gel electrophoresis. (b) by ethidium bromide staining with white light and (c) UV. (e) using SYBR GREEN I with white light and (f) under UV.
Screening of Wild life sera samples for Leptospirosis using rlgB based LAT:

**Jaipur Zoo, Rajasthan:**
- 27 wild feline sera (15 Tiger, 8 Lion and 4 Jaguar) tested
- all the Lion sera and 15 tiger sera tested +ve for Leptospira by both rlgB based LAT and MAT.
- icterohaemorrhagiae was present in all the positive sera samples (Lion 1/800) B tiger (1/400) titre
- only one lion sera tested positive for serovar Pomona.

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

**Jaipur Zoo, Rajasthan:**
- 42 sera (6 Tiger, 4 Lion, 6 Leopard, 2 Cheetah, 1 Black Buck, 12 Buffalo and 9 Human sera) and 5 live rodents tested
- 7 tiger sera, all the 6 lion sera, 2 leopard and 2 cheetah sera tested positive for Leptospira by LAT and MAT.
- icterohaemorrhagiae was the most serum involved in all the animals while in Panther, the serum Pomona (titre 1/800), Gripotyphosa and australis were implicated.
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* 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 (Clustered Regularly Interspaced Short Palindromic Repeats–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 devise 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.
Research activities at IIT Guwahati

Dr. Manish Kumar
Associate Professor

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

Highlights of the Lab Activities at IIT Guwahati

- Modulation of gene expression in L. pneumophila due to host stress factor: catecholamine (Epi/NE)
- Characterizing proteins in search of novel diagnostic antigen and vaccine candidate
- Understanding CRISPR-Cas system of pathogen L. pneumophila
- Targeting catecholaminergic pathway of L. pneumophila as an alternative to traditional antibiotics

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

Figure 1

Figure 2

Page 73
What is CRISPR-Cas system?

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

- Adaptive defense system in bacteria and archaea
- Inducible immunity in 3 step processes:
  1. Adaptation:
     - A small fragment of foreign nucleic acid (or “template”) is first recognized and inserted into the CRISPR array of host by help of Cas and Cas3 protein.
  2. Expression:
     - The transcrip of CRISPR array is processed by Cas protein into short CRISPR RNAs (crRNAs)
  3. Interference:
     - The transcript in the form of Cas-CRISPR complex interfere with the cognate foreign target nucleic acid.

Why to study CRISPR-Cas in Leptospirosis?

- 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 defence array against foreign invading pathogens?
- Bioinformatics analysis shows CRISPR-Cas exists only in pathogenic strains.
- A logical explanation: Tighter gene manipulation is easier in L. interrogans than in other pathogenic Leptospira strains.

Figure 1: Schematic representation of CRISPR-Cas in Leptospira interrogans “Copenhageni” and its validation by RT-PCR
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 Leptospirosis of animalssince 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,
et., 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 i.e., Mapping of human leptospira outbreaks and seroprevalence analysis of leptospirosis in livestock using Software QGIS, 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 seroprevalence 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:
Sero-epidemiology Work at ICAR NIVEDI

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

Konkan Region of Maharashtra

Samples collected During : 2012-2013
Species : Livestock-Pusheen Purgative sampling
Overall seroprevalence : 41.94% (219/527)
Cows – 24.29%
Buffaloes – 52.36%
Hindus – 32.19% and
Bulls – 26.46%

Seroprevalence of leptospirosis by MAT

Gujarat

Samples collected During : 2012-2013
Species : Livestock-Pusheen Purgative sampling
Overall seroprevalence : 22.64% (48/212)

2015

24.4% Seropositivity in Bovine (82/341) Pusheen Random sample-Navanar and Saurashtra District-Gujarat
35.46% Seropositivity in Livestock (Cattle, Buffaloes, sheep and goats) (179/323) Pusheen Random sample-Saurashtra Dist-Gujarat
Sero-positivity of leptospirosis in livestock

12.5% seropositivity in cattle dairy farm - random sampling (125/949 - Haardt services, Dehradun)
70.5% Seropositivity in bovine cases - Suspected cases: History of fever, abortion and reproductive disorders (265/372)

*Examine serum samples, (54/96%) 72/121 - Seropositivity of infection in stud farms*

ANIMAL AND HUMAN INTERFACE
Inter-sectoral collaboration - Human Sample testing

Sero-positivity of 96.7% (118/122) leptospirosis was observed patients with case definition history of leptospirosis - Manipal

Sero-positivity of 65% (13/20) - Neuro disorder cases - NIMHANS

Sero-positivity of 38% (114/300) leptospirosis was observed - pyrexia of unknown origin (PUO). JSS, Mysuru

Development of Recombinant Antigen based Immunogenics

LipL32, LipL41
Compsil, IgG, L.P.6

Develop recombinant antigen based single or multi rec protein(s)

Based diagnostics for Bovine leptospirosis

Work at KAIR-NIVEDI

Investigation and samples collection and epidemiological analysis

Pig farm: Halebid拉nur, Mandya, Karnataka

Serum samples - 9 tested
- 5 samples positive

- Abortion - live born or sarceral death of piglets
- Birth of weak piglets that die in hours of birth
- 6/12 days were and all aborted
- One dead for breeding and 60 farrier piglets

Sero-positivity in cattle, ruminants, Panipat, Interhaemorrhagiae.
**Future work**

**Economic Impact of Leptospirosis**

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

**Summary/Publications**

- **Prevalence of Leptospirosis intermediate species** (L. wolffi) identified during the disease monitoring in the livestock (Balasubramaniam et al., 2013)

**Maharashtra State**

- Prevalence of serogroup specific antibodies identified in Konkan region of Maharashtra state. 41% - 2011-2016 (Balasubramaniam et al., 2016)

**Odisha State**

- Prevalence of serogroup specific antibodies identified in Odisha 2012 - 42% (Balasubramaniam et al., 2012)
- Distribution of Leptospirosis serogroup specific antibodies in Odisha - in different climates 2011-2014 (Balasubramaniam et al., 2017) 36.9% (17/47)

**Prevalence of Leptospirosis intermediate species antibodies - group specific antibodies identified during the disease monitoring in the livestock** (Balasubramaniam et al., 2016)
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 samples is a major constraint. An integrated one health approach would be the ideal way forward for leptospirosis research and diagnostics in India.
Sentinel sites

- 32 Sentinel hospitals
- 14 districts
- 10 States

Summary (as of 03rd September 2017)

6 pathogens account for 75% of diagnosis (Influenza, Dengue, Malaria, Rotavirus, Typhoid, Leptospirosis and KPE)

Distribution of Lab Confirmed AFI aetiology in Different States of India

Leptospirosis in AFI surveillance

Tests/Assays:
- Leptospira IgM ELISA (FIBAC)
- Leptospira IgM: Real Time PCR (CCD protocol)
- Microscopic Agglutination Test (MAT)

Lab confirmed Leptospirosis among AFI Cases (91-11):
- Leptospirosis IgM only: 865
- Leptospirosis PCR only: 13
- Leptospirosis IgM & PCR Positive: 2

Precedent Leptospira species based on MAT
- I. canicola, I. burnetii, I. australis, I.ロックoff
Brainstorming Session

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 stakeholders 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 stakeholders.
- 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.
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.
Application of Real time PCR for diagnosis of Diseases
Dr. V. Balamurugan, Principal Scientist, ICAR-NIVEDI, Bengaluru
Application of real-time PCR for diagnosis of diseases

Dr. V. Balasubramanian
Principal Scientist
ICAR-NIYEDI, Bangalore
bala.vknl@gmail.com; 94421097438

Real time PCR
Principles, Techniques and Applications

DNA Amplification Using Enzyme-Linked Chain Reaction

Conventional PCR-Based Testing Formats

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
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.

Real-time Principle

- Based on the detection and quantitation of a fluorescent reporter
- The first significant increase in the amount of PCR product (C<sub>T</sub> - threshold cycle) correlates to the initial amount of target template.

Methods of fluorescence detection

SYBR Green
- Taqman
- Molecular Beacon
- Light Cycler

Three general methods for the quantitative detection

1. Hydrolisis probes
2. Hybridisation probes
3. SYBR Green

(TaqMan, Beacon, Scorpions)

Page 88 Proceedings of Stakeholders meeting and Workshop on Laboratory capacity building for Leptospirosis
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.

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.

**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

**Advantages and Disadvantages**

**Advantage:**
- The biggest advantage of SYBR 1 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

**Interpretation (Melting curve analysis):**
Specific detection using DNA Probes

Hydrolysis probe technique
- The hydrolysis probe is conjugated with a quencher fluoreochrome, 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 fluorochromes 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.

TaqMan Probes
- FRET = Förster/Fredresence resonance energy transfer & DNA Polymerase 3’ endonuclease activity
- Tm value > C (higher than primers)
- Runs of identical nucleotides (no consecutive Gs)
- G+C content 50-60%
- More Cs than Gs
- No G at the 5’ end

Molecular Beacons:
- The molecular beacon method utilizes a reporter probe that is wrapped around 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 released at the target at each cycle.
- Use molecular beacons that are complementary to a sequence in the middle of the expected amplification.
- The length of the loop sequence should be chosen so that the probe-target system 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.

Scorpions
- 1. Quenching of the fluorophore
- 2. Emission of the fluorophore
The classical reporter dye:

- 6-FAM (6-carboxy fluorescein)
- HEX (5-hexachloro fluorescein)
- Cy5 (cyanin-5 dye)

Other reporters used for multiplexing are:

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

Threshold Cycle

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

What is ΔRn?

ΔRn is the difference between Fm and Fln. Fln is the Fln value (baseline value)
Performance and Evaluation

Assay Validation

Test primer pairs in all combinations with the probe with a known template (plasmid clone, cDNA, RNA)
- Use standard assay conditions: 100-400 nM primers, 100 nM probe, 5 mM MgCl₂
- Choose the primer pair that gives the highest Cq and the lowest Ct
- Make a dilution of a template, either DNA, cDNA, or total RNA for a standard curve
- Construct a standard curve with the dilution
- If the slope of the standard curve of the best primer pair is around -3.3 increase the MgCl₂ to 6 mM
- If the slope is higher than -3.3, change primers

An ideal assay will have a slope of -3.3

Using the PCR Equation

\[ X_0 = X_n \left( 1 + E \right)^n \]

\( X_0 \) = PCR product after cycle n
\( X_n \) = initial copy number
\( E \) = amplification efficiency
\( n \) = cycle number
Figure 3. When the known concentrations (expressed in logarithmic form) of target gene are plotted against the corresponding cycle threshold (CT) values obtained by qPCR, the slope of a line representing the linear correlation between these two parameters, the equation describing this relationship, is used to extrapolate the gene copy number in experimental samples.

Repeatability
- Intra assay variation
- COV has to be calculated
- Three to five consecutive runs have to be done

Reproducibility
- Inter assay variation
- COV has to be calculated
- Three to five individual runs have to be done at different points of time

Quantitation
- Absolute quantification
  - Standard curve
  - Standards must be accurately quantified
  - Best used for viral load determination
- Relative quantification
  - Standard curve
  - Standards are serial dilutions of a calibrator template
  - Best used for gene expression studies
- Comparative quantitation
  - Mathematical determination
  - Calibrator sample used as a 1x standard
  - Direct use when particular rates are expected or to verify trends
Absolute quantification

Threshold Cycle = 38
Load = 10^{10} copies/mL
Test Sample

Endogenous / Internal Control (Normalisation)
- Usually on an abundantly and constantly expressed housekeeping gene
- Most commonly used ones are the least reliable ones
- Best to run a validity test for the selected endogenous control
- Combination may be used

Comparative threshold method

Advantages of Real-time PCR
Real-Time PCR vs Traditional PCR
- Traditional PCR is measured at End-Point (plateau), while Real-Time PCR collects data in the exponential growth phase
- An increase in Reporter fluorescence signal is directly proportional to the number of amplicons generated
- The sealed probe provides a permanent record amplification of an Amplicon
- Increase dynamic range of detection
- No post-PCR processing is required
- Detection is capable down to a 2-fold change

Not influenced by non-specific amplification
- Amplification can be monitored real-time
  - No post-PCR processing of products (high throughput, low contamination risk)
  - Ultra-rapid cycling (30 minutes to 2 hours)
  - Wider dynamic range of up to 10^{9}-fold
  - Requirement of 1000-fold less RNA than conventional
  - Detection is capable down to a 2-fold change
  - Confirmation of specific amplification by melting point analysis
  - Most specific, sensitive and reproducible
  - Not much more expensive than conventional PCR
**Practicals**

Detection of *Leptospira* nucleic acid by Real Time PCR

**DNA Extraction**
- DNA extracted from clinical samples (serum, cerebrospinal fluid, urine, etc.)
- Minced samples for tissue or whole blood

**Materials Required**
- Centrifuge, vortexer, microcentrifuge tubes, ethidium bromide

**Primer and Amplicon Design**
- Oligonucleotide primers to be designed against a conserved region for diagnosis.
- Primer sequences can be designed using software [http://bioinfo.mas.ncl.ac.uk/primer3/](http://bioinfo.mas.ncl.ac.uk/primer3/).
- Oligos are typically amplified with higher efficiency. An amplicon length should be at least 75 bp to easily distinguish it from any primer-dimer that might form.
- Secondary structures are to be avoided if possible. Use programs such as mfold ([http://www.bioinfo.rpi.edu/applications/mfold/](http://www.bioinfo.rpi.edu/applications/mfold/)) to generate whether an amplicon will form any secondary structures at annealing temperature.
- Templates with long (4+ steps) insert of single bases to be avoided.
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 birth, 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.
Economic Impact of Leptospirosis in Animals and Humans and KAP studies

Dr. G. Govindaraj
ICAR-NIVEDI

IMPACT

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 environments 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 these impacts (impact evaluation).

<table>
<thead>
<tr>
<th>Data Collection Designs and Their Characteristics</th>
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<tbody>
<tr>
<td>Characterisation Evaluation Design</td>
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<tr>
<td>Case study: one measurement (results not planned)</td>
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<tr>
<td>Case study: two measurements (before and after)</td>
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<tr>
<td>In situ design (prior test vs. after)</td>
</tr>
<tr>
<td>Case study with one measurement (control group, not individual)</td>
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<tr>
<td>Case improvement design</td>
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<tr>
<td>Experimental design</td>
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Why do we need Impact analysis for livestock and human diseases?
1.2 Loss in Productivity

Loss of productivity of labour?

\[ P = \text{(Number of working days per year \times Average daily earning)} - \text{Number of days actually worked due to disease persistence \times Average daily earning)} \]

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:

\[ \text{Cost incurred in treating human = number of times visited to hospital \times (average fees of doctors + average expenditure on drugs)} \]

1.4 Welfare Loss of Individual and His Family

Welfare loss may be used as proxies.

1.5 Cost of Avoiding Behaviour

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

1.6 Burden Due to Livestock Illness

2.1 Mortality Loss

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

2.8 Burden on Public Sector
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.
and attribute data. There are two types of data stored 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.

**Structure of the discussion**

I) Background information
II) What is geographic information system (GIS)?
III) Importance of GIS
IV) How GIS can help in understanding spatial Epidemiology of Leptospirosis?
V) Summary

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

1) Background information: Classical study by John Snow

2) Spatial distribution of Cholera in London
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.
Not all the functionality of a conventional DBMS, but much of the functionality of a computer mapping system.
GIS is a DBMS, but allows us to explicitly handle the spatial component.
Common examples:
ArcView
ArcGIS
MapInfo

Types of data in GIS

- **Attribute data:** Data that describes a feature or object.
- **Spatial data:** Data that describes the location of a feature or object.

**Types of data in GIS - Raster**

- **Raster data:** Data that represents a continuous surface as a set of grid cells.

**Types of data in GIS - Vector**

- **Vector data:** Data that represents discrete features as sets of points or lines.
IV) How GIS can help in understanding epidemiology of Leptospirosis?

a. Exploratory analysis - showing spatial distribution of important factors
b. Quantitative analysis - quantifying the relationship between risk factors and leptospirosis

V) Summary

Title slide data: Bold and title text
Feature data: Bold and title text
Explanation: spatial epidemiology of leptospirosis in India
Explanatory analysis
Quantitative analysis
Annexure 1

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Proceedings of Stakeholders meeting and Workshop on Laboratory capacity building for Leptospirosis