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JOINT EVENTS



THE 13TH CONFERENCE OF THE INTERNATIONAL LEPTOSPIROSIS SOCIETY

THE 4TH MEETING OF THE EUROPEAN LEPTOSPIROSIS AND OTHER RODENT-BORNE HAEMORRHAGIC FEVERS SOCIETY

ABSTRACT BOOK

2-4 SEPTEMBER, 2024

ART & HISTORY MUSEUM, BRUSSELS

THE 4TH MEETING
EUROPEAN LEPTOSPIROSIS
AND OTHER RODENT-BORNE HAEMORRHAGIC FEVERS
SOCIETY

THE 13TH CONFERENCE
INTERNATIONAL LEPTOSPIROSIS
SOCIETY

2-4 SEPTEMBER, 2024
ART & HISTORY MUSEUM, BRUSSELS

DESKTOP PUBLISHING

Goldgraphic

Via Gen. F. Pignatelli, 33C - 80141 Naples, Italy
Tel +39 347 6765367 - info@goldgraphic.com - www.goldgraphic.com

CONTENTS

5 WELCOME

7 SCIENTIFIC COMMITTEE

9 VENUE WITH FLOOR PLAN

11 PROGRAM

15 SPONSORS

17 INVITED SPEAKERS

27 OPENING CEREMONY

31 **SESSION 1** EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

93 **SESSION 2** NEW CHALLENGES FOR CLINICS AND TREATMENT OF HUMAN LEPTOSPIROSIS

111 **SESSION 3** ANIMAL LEPTOSPIROSIS IN DOMESTIC AND WILDLIFE SPECIES

157 **SESSION 4** IMMUNOLOGY AND PROTECTIVE IMMUNITY

189 **SESSION 5** RODENT-BORNE HAEMORRHAGIC FEVERS

201 **SESSION 6** GENETICS, MOLECULAR EPIDEMIOLOGY AND GENOMICS

237 **SESSION 7** RECENT ADVANCES ON DIAGNOSTIC APPROACHES

265 AUTHOR INDEX

WELCOME



Dr. Marcella Mori
Joint ILS-ELS Meeting2024 Chairwoman



Dr. Renee L Galloway
ILS President



Dr. Mathieu Picardeau
ELS Vice President

Dear Colleagues and Friends,

It is with great pleasure that we welcome you to the first joint event combining the 13th Conference of the International Leptospirosis Society (ILS) and the 4th Meeting of the European Leptospirosis and other Rodent-Borne Haemorrhagic Fevers Society (ELS). This joint venture will be held in one of the cities in the heart of Europe and co-hosted by Sciensano, the Belgian One-Health Scientific Institute.

Started in 1970 for ILS and in 2012 for ELS, these international gatherings aim to promote exchanges and advancements in understanding and managing leptospirosis, while encouraging scientific networking valuable for researchers, clinicians, pharmaceutical companies, and stakeholders involved in risk assessment and policy. We are thrilled to host this event from September 2 to 4, 2024, at the Art & History Museum of Brussels, which houses antiquities, ethnographic, and decorative arts from around the world.

Leptospirosis remains a significant public health challenge, particularly in tropical and subtropical regions. Climate change, characterized by rising of global temperatures and increasingly

unpredictable weather patterns, has contributed to the spread of leptospirosis beyond its traditional areas. This shift has broadened the endemicity of the disease, highlighting the risk of both human and animal infections worldwide and making leptospirosis a pressing planetary health issue.

The Scientific Committee has prepared an engaging program comprising 8 keynote lectures, including Prof. Mahillon, Prof. Dreyfus, Prof. Agampodi, Dr. Nally, Prof. Haake, Prof. Van Gorp, Dr. Rajeev and Prof. Srisawat, who will share their experiences in session involving One-Health epidemiology, Human and Animal leptospirosis, Immunity, Genetics and '-OMICS', and diagnostics of leptospirosis as well insights on rodentborne haemmorragic fevers. Additionally, 27 oral communications and numerous poster presentations will spark exchanges, collaborations, and cutting-edge knowledge among participants. We count on your contributions to make this congress a comprehensive platform for sharing research findings, innovative methodologies, and effective strategies for the prevention, diagnosis, and treatment of leptospirosis, fostering interdisciplinary approaches to combat this disease.

In addition to the scientific sessions, we have planned social events to encourage networking and the building of lasting professional relationships. We hope you take this opportunity to explore Brussels, with its rich cultural heritage and vibrant community.

We extend our grateful thanks to the organizing-scientific committee, sponsors, keynote speakers and volunteers whose work has made this congress possible. We also express our gratitude to all participants for contributing their knowledge and expertise to this important event.

We look forward to meeting you in Brussels and welcome you to the Joint ILS-ELS Meeting!

Dr. Marcella Mori
Joint ILS-ELS Meeting2024 Chairwoman

Dr. Renee L Galloway
ILS President

Dr. Mathieu Picardeau
ELS Vice President

SCIENTIFIC COMMITTEE

Ben Adler (Australia)

Suneth Agampodi (South Korea)

Josipa Habus (Croatia)

David Haake (USA)

Tinne Lernout (Belgium)

Walter Lilenbaum (Brasil)

Anneleen Matthijs (Belgium)

Marcella Mori (Belgium)

Jarlath Nally (USA)

Alda Natale (Italy)

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Sree Rajeev (USA)

Simone Schuller (Switzerland)

Nattachai Srisawat (Thailand)

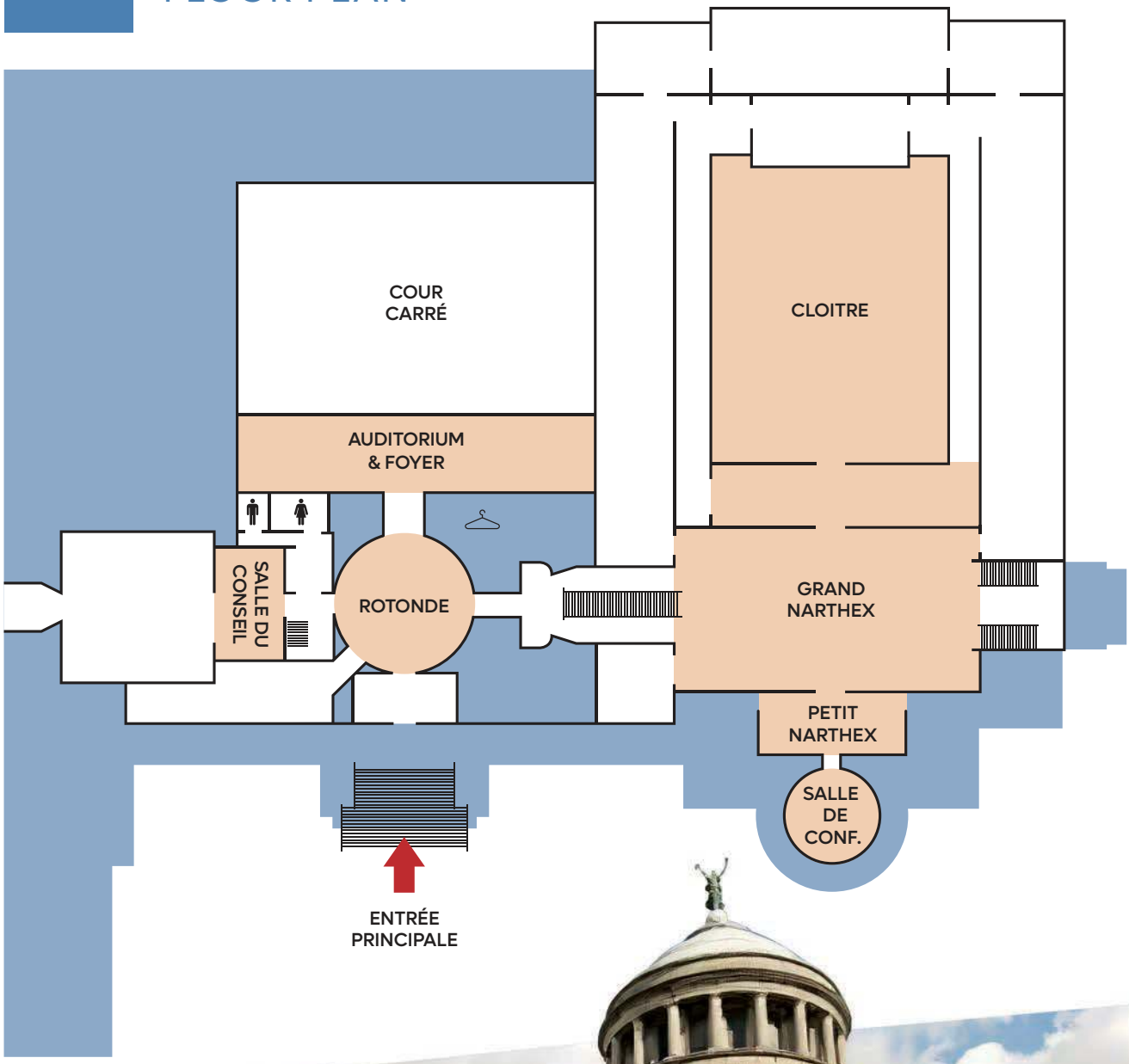
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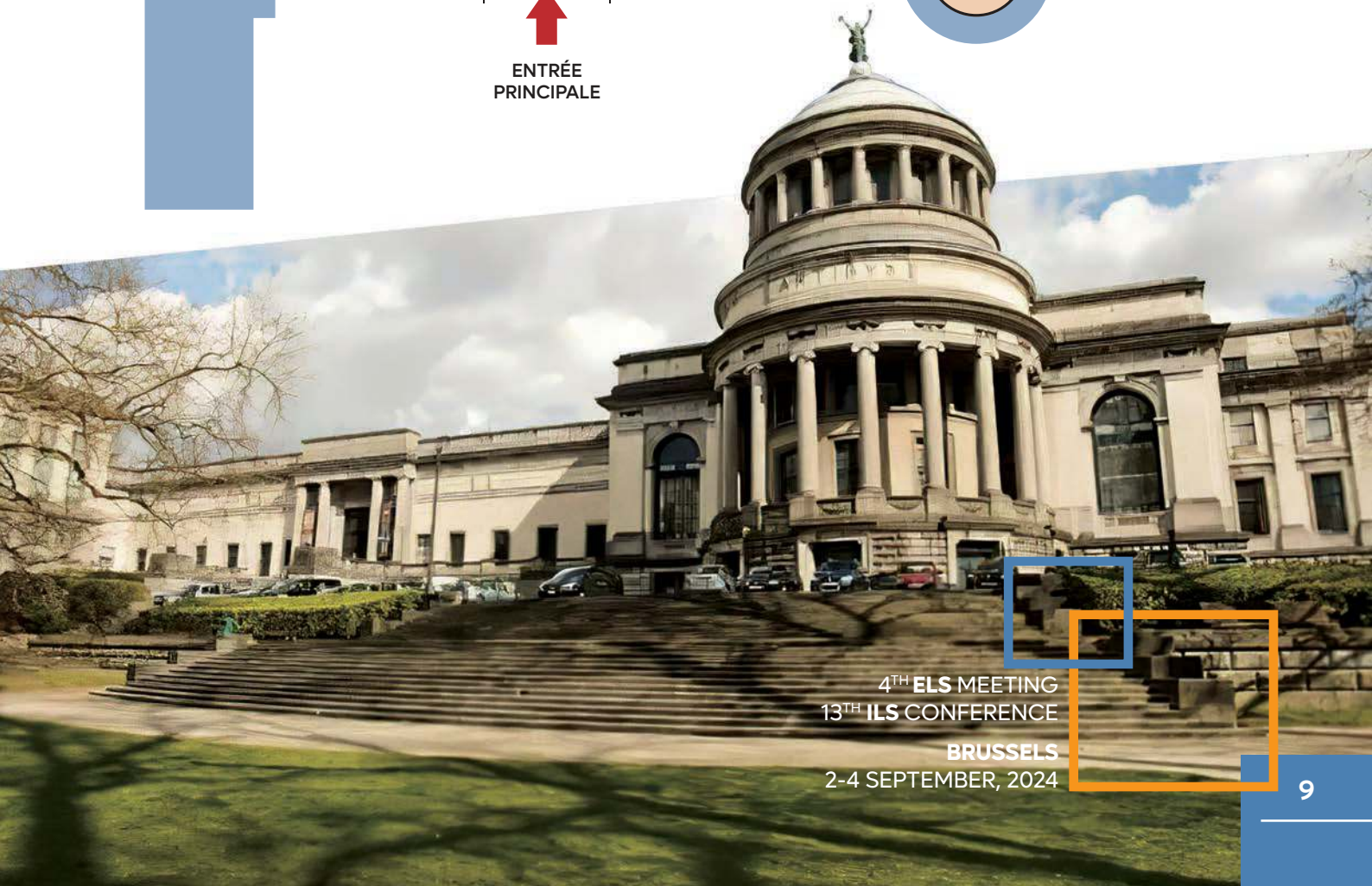
Pablo Tortosa (Réunion, France)

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Eric van Gorp (The Netherlands)

ART & HISTORY MUSEUM, BRUSSELS
FLOOR PLAN





4TH **ELS** MEETING
13TH **ILS** CONFERENCE

BRUSSELS
2-4 SEPTEMBER, 2024

PROGRAM

DAY 1 SEPTEMBER 2 - 2024

08:30 Registration and Welcome Coffee

09:00 Opening Ceremony

09:30 Living on a microbial planet: A One-Health - One-World perspective
Keynote Speaker J. Mahillon

SESSION EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

Moderators: Marcella Mori & Ben Adler

10:00 Leptospirosis research in East Africa using a One Health Approach
Keynote Speaker A. Dreyfus

10:40 Between Soil Grains and Raindrops: Understanding *Leptospira's* Environmental Journey
T. Roman

11:00 Coffee break

11:20 Estimation of Environmental Transmission of *Leptospira* spp.
G. Monti

11:40 Unveiling the genetic diversity of *Leptospira* strains circulating in mainland France
M. Picardeau

12:00 Livestock Vaccine Launched Against Emerging Genotype to Protect Humans Against Leptospirosis in New Zealand
C. Heuer

12:20 One Health approach on serosurvey of *Leptospira* spp. in former black slave (quilombola) communities and their dogs in southern Brazil
L. B. Kmetiuk

12:40 Poster session

13:20 Lunch

SESSION NEW CHALLENGES FOR CLINICS AND TREATMENT OF HUMAN LEPTOSPIROSIS

Moderators: Yupin Suputtamongkol & Robyn A. Stoddard

14:20 Obstacles in Diagnosing Leptospirosis Clinically and Evidence-Based Treatment Approaches
Keynote Speaker S. Agampodi

15:00 Epidemiological Trends and Cases of Leptospirosis in Pediatric Patients
A. Grindstaff

15:20 Updates for Cochrane systematic reviews of treatments for Leptospirosis
N. Lee

15:40 Coffee break

16:00 Inflammatory Protein Profiling in Human Leptospirosis: Unveiling Biomarkers for Severity
U. Limothai

16:20 Disruption of epithelial cell mechanosensing during pathogenic *Leptospira* infection
C. Toma

16:40 Poster session

17:10 Poster session + ELS business meeting

18:10 End of the day

DAY 2 SEPTEMBER 3 - 2024

08:30 Special Tribute Session: Homage to Marga Goris by AMC colleagues

SESSION ANIMAL LEPTOSPIROSIS IN DOMESTIC AND WILDLIFE SPECIES

Moderators: Josipa Habus & Cord Heuer

09:00 **Animal Leptospirosis in Domestic and Wildlife Species**
Keynote Speaker J. Nally

09:40 **Potential leptospiral shedding in vaccinated and asymptomatic pack hounds: a prospective study in 56 dogs**
F. Ayral

10:00 **Seroprevalence of Leptospirosis in Selected Livestock Species and the First Report of *Leptospira borgpetersenii* Serovar Tarassovi Isolated in The Philippines**
M. Villanueva

10:20 **Ophthalmic Manifestation of Acute Canine Leptospirosis**
I. Zečević

10:40 **Monitoring Leptospirosis in Israel's Wild Fauna: A Ten-Year Study Highlighting the Spread of Serogroup Pomona**
S. Blum

11:00 **Coffee break**

11:20 **Poster session**

12:40 **Lunch**

SESSION IMMUNOLOGY AND PROTECTIVE IMMUNITY

Moderators: Lourdes Isaac & Catherine Werts

13:40 **Host-Pathogen Interactions in Leptospirosis - A Collaborative Program Project**
Keynote Speaker D. Haake

14:20 **Molecular and Cellular Mechanisms of Leptospirosis Pathogenesis: Central Role of Virulence Modifying Proteins Encoded by the PF07598 DUF1561 Gene Family**
J. Vinetz

15:00 ***In-vivo* Host-*Leptospira interrogans* Transcriptomes Reveal Key Virulence Factors for Pathogen Adaptation and Dissemination**
A. Giraud-Gatineau

15:20 **Coffee break**

15:40 **No Evidence of Cytokine Storm but Signs of Organ Dysfunction Associated with the Death of Mice Upon Acute Leptospirosis**
S. Papadopoulos

16:00 ***Leptospira* infection triggers renal senescence and leads to sustained senescence-associated inflammatory responses and kidney fibrosis in the hamster**
S. H. Hsu

SESSION RODENT-BORNE HAEMORRHAGIC FEVERS

Moderators: Arent Zbigniew

16:20 **Emerging infections in the global village: the rodent and mosquito borne hemorrhagic infections**
Keynote Speaker E.C.M. van Gorp

17:00 **Urban Rats in Norway as a Source for Rodent-Borne Haemorrhagic Fever**
S. F. Hansen

17:20 **Poster session + Subcommittee on the taxonomy of Leptospiraceae**

18:10 **End of the day**

DAY 3 SEPTEMBER 4 - 2024

08:30 ILS business meeting

SESSION GENETICS, MOLECULAR EPIDEMIOLOGY AND GENOMICS

Moderators: Mathieu Picardeau & Renee Galloway

09:30 **Using "OMICS" Approach to Study *Leptospira***
Keynote Speaker S. Rajeev

10:10 **Adaptation of *Leptospira* to Oxidative Stress and Its Implication for the Emergence of Pathogenicity**
N. Benaroudj

10:30 **Transcriptomic Analysis of *Leptospira* forming Biofilm reveals Adaptations to Starvation and General Stress while maintaining Virulence**
G. Davignon

10:50 **A Novel CRISPR-Prime Editing Strategy for Single-Nucleotide Resolution Mutations in *Leptospira* spp.**
L. Fernandes

11:10 **Rfb cluster barcoding to assign serovar of *Leptospira* P1+ species**
F. Veyrier

11:30 **Coffee break**

11:50 **Poster session**

13:00 **Lunch**

SESSION RECENT ADVANCES ON DIAGNOSTIC APPROACHES

Moderators: Nattachai Srisawat & Paula Ristow

14:00 **Clinical Challenges in early diagnose human leptospirosis and prediction disease severity: an unmet need**
Keynote Speaker N. Srisawat

14:20 **Objectification of Evaluation Criteria in MAT Using Deep Learning**
R. Ozuru

14:40 **Immunogenicity study of the outer membrane protein LipI41 from Iranian isolates of *Leptospira interrogans* applicable for a promising diagnostic ELISA test**
N. Golab

15:00 **Development and Validation of a Competitive Enzyme-Linked Immuno Sorbent Assay (cELISA) for the Detecion of *Leptospira* Serovar Grippotyphosa Antibodies in Cattle Sera**
M. Van Nimmen

15:20 **Proteomic Signatures Identify Acute Leptospirosis and Elucidate Immune and Inflammatory Response Pathways in Human Hosts**
M. McClain

15:40 **Final discussion, end of the Meeting and Closing Ceremony**

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INVITED SPEAKERS



Jacques Mahillon

Pr Jacques Mahillon headed the Laboratory of Food and Environmental Microbiology, where he conducted research on the genetic and genomic plasticity of members of the *Bacillus cereus* group, with a particular focus on i) the contribution of mobile genetic elements and conjugative plasmids to the genomic flexibility and virulence of *B. cereus* and ii) the functional characterisation of bacterial phages and plasmids. He has also worked on the development of strategies for the biocontrol of foodborne and plant pathogens, using bacteria, antimicrobial molecules and bacteriophages, in the context of One-Health. He is member of different microbiology societies, including the *Belgian Society for Food Microbiology* (Founder and Chairman 2015-2022) and the *National Committee for Microbiology*. Pr Mahillon was also the Dean (2009-2013) and Vice-Dean (2003-2007) of the Faculty of Bioscience Engineering at UCLouvain. Expert in different national and international research councils and institutions (*e.g.* ANR, AFSCA, FNRS, ESA, EFSA, ISF, IFS). He has also been actively involved in cooperative projects with Benin, Bolivia, China, Cuba, Ecuador, Russia and Vietnam. He has co-authored more than 220 publications and (co-)supervised more than 40 Ph.D. theses.



Anou Dreyfus

For the last 15 years, I have researched leptospirosis at the human-animal interface in various countries, including New Zealand, Uganda, Madagascar, Bhutan, and Switzerland. My interests focus on zoonotic pathogens in non-malarial fever and the research and control of neglected tropical diseases through a One Health approach. Currently, I am a senior researcher at the University of Zürich, collaborating with Swiss and Ugandan partners from Makerere University to study leptospirosis and rickettsiosis in acute undifferentiated fever patients in Uganda, where we have established a research group and diagnostic capacity (MAT and qPCR).
I have served as a veterinary and human epidemiologist, public health expert and senior lecturer at various institutions, including the Food and Agriculture Organization in Rome, the Swiss Federal Office of Public Health, the University of Zürich, the Swiss Tropical and Public Health Institute, and as deputy head of the Epidemiology and Clinical Research Unit at the Institut Pasteur of Madagascar.
As a steering committee member of GLEAN (Global Leptospirosis Environmental Action Network), I aim to enhance the visibility, research, and control of leptospirosis, a significantly neglected tropical disease despite its high burden.



Suneth Agampodi

Dr. Suneth Agampodi is a Research Scientist at the International Vaccine Institute in Seoul, South Korea. With a robust foundation as a public health physician, infectious disease epidemiologist, and Professor in public health, he has dedicated himself to the study of leptospirosis since 2008. Dr. Agampodi's primary research on leptospirosis stems from Sri Lanka, a nation with one of the highest incidences of the disease globally. His work spans a spectrum of key areas, including clinical and epidemiological research, molecular studies, diagnostics, and disease control strategies. Since 2018, Dr. Agampodi has served as an active executive member of the International Leptospirosis Society, and currently working on leptospirosis disease burden assessment and vaccine development.



Jarlath Nally

Jarlath Nally completed his Ph.D. in Veterinary Microbiology in 2001 at the University of Kentucky. He completed his postdoctoral research in the Division of Infectious Diseases, Department of Medicine at the University of California, Los Angeles. His postdoctoral research focused on understanding pathogenic mechanisms of the zoonotic disease leptospirosis (Weil's disease) and in particular, the severe pulmonary forms of this disease, and for which he received a National Research Service Award from NIH. In 2005, Dr. Nally joined the School of Veterinary Medicine, University College Dublin, where he was awarded The President of Ireland Young Researcher Award from Science Foundation Ireland to further elucidate pathogenic mechanisms of leptospirosis. As a Veterinary Microbiologist, his research interests include zoonotic pathogens, proteomics and animal models of infection. In 2014, he returned to the United States to join the Infectious Bacterial Diseases of Livestock Research Unit, at the National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture where he is lead scientist on Spirochete research and continues to work on animal leptospirosis.



David A. Haake

I have been an infectious diseases specialist with an interest in research on leptospirosis and *Leptospira* species since 1989. My laboratory has been focused on leptospiral outer membrane proteins and their role(s) in leptospiral pathogenesis and protective immunity, regulation of leptospiral virulence factors, and the host response to leptospirosis. My laboratory has also worked on innovative approaches for rapid identification and antimicrobial susceptibility testing of bacterial pathogens directly from clinical specimens. I serve as Chief Medical Officer at Qvella Corporation, which is developing rapid diagnostics for bloodstream pathogens. It has been my great honor to serve as Chair of the Gordon Research Conference on the Biology of Spirochetes in 2006, as a standing member of the National Institutes of Health Host Interactions with Bacterial Pathogens Study Section from 2009-2013, and as President of the International Leptospirosis Society from 2013-2017. I am currently serving as Principal Investigator on a five-year, multisite NIH/NIAID Program Project investigating host-pathogen interactions in leptospirosis.



Eric C.M. van Gorp

Prof. dr. Eric C.M. van Gorp is an internist/infectiologist/virologist at the Erasmus MC, heading the clinical working group of Emerging Viral infections [EVI] in the Department of Viroscience.
The EVI group is involved in research in the field of Emerging [outbreak] viruses like Dengue, Ebola, Zika and COVID-19. Vaccine effectiveness studies [multicenter] in risk groups and optimizing vaccination implementation strategies is a second field of expertise. His field of interest is the social and scientific impact of infectious diseases. He is founder of the Cirion foundation [www.cirion.net], initiating and facilitating initiatives and projects to promote prevention of infectious diseases. One of the initiatives is the EU supported international education and prevention project Viruskenner for scholars [www.viruskenner.nl]. The research line, with several PhD's, he is supervising is focussing on the emerging viral infections, with an extensive (inter)national network in the Netherlands, the Caribbean, Surinam, Brazil , South Africa, and Indonesia. He is supervisor of the Vaccination outpatient clinic/ Travel clinic and the Clinical trial unit from the Viroscience department on which fase I and II vaccination studies are conducted [e.g. Horizon 2020, CEPI, ZonMW].



Sree Rajeev

Dr. Sreekumari (Sree) Rajeev is a professor of infectious diseases and the director of the Veterinary Bacteriology and Mycology Diagnostic Laboratory at the University of Tennessee College of Veterinary Medicine. She is board certified by the American College of Veterinary Microbiologists in the areas of Bacteriology and Mycology, Virology, and Immunology as well as the American College of Veterinary Pathologists (Anatomic Pathology). Dr. Rajeev's research focuses on advancing the field of infectious diseases, specifically leptospirosis, a widespread zoonotic disease impacting humans and animals. Dr. Rajeev maintains an integrated system approach focusing on microbiology, molecular biology, pathology, host-pathogen interaction, and epidemiology to develop improved vaccines and diagnostics by applying traditional and advanced genome-based techniques.



Nattachai Srisawat

My main research focused in critical care nephrology field, focusing on and acute kidney injury (AKI) and tropical infection associated AKI such as dengue infection, leptospirosis. For AKI, we conducted one of the largest registry of AKI epidemiology to study prevalence, risk factors, and outcome of AKI in Southeast Asia. Currently, we also explored the role of post AKI care in AKI survivors to improve long term outcome. In the field of leptospirosis, our group are studying on clinical scoring (Thai Lepto score), biomarkers to predict severe leptospirosis by using high throughput technique, and organ support therapy.

4TH **ELS** MEETING
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OPENING CEREMONY

4TH ELS MEETING
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Living on a microbial planet: A *One-Health - One-World* perspective

Mahillon J
Full Professor Emeritus and Invited Professor, Université catholique de Louvain (UCLouvain), Faculty of Bioscience Engineering, Earth and Life Institute, Louvain-la-Neuve, Belgium

Although the concept of *One-Health* is rather recent, sharing earth's space and resources with a plethora of other living organisms is an old reality that started with the early days of life. And this is particularly true when it comes to living with the smallest, invisible and most numerous planet's inhabitants, the microbes. Plants and animals indeed heavily depend on their activities, whether they are friends or foes. After briefly reminding the bright side of microbes, the darker side of their adverse interactions with plants and animals will be illustrated through a few examples in recent history. The lecture will then focus on the arms race engaged on the antimicrobial resistance battle, that started some sixty years ago. Facing these challenges, new and alternative routes and strategies, designed to better anticipate and control microbial diseases and pandemics, will be presented, in the context of *One-Health - One-World* vistas.

SESSION 1

EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

Moderators:
Marcella Mori & Ben Adler

4TH ELS MEETING
13TH ILS CONFERENCE
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Leptospirosis research in East Africa using a One Health Approach

Dreyfus A
PhD, MSc, Dr. med. vet., DVM, Veterinary Epidemiologist, Vetsuisse Faculty, Winterthurerstrasse 270 8057 Zurich

Growing evidence from East Africa indicates that bacterial zoonotic diseases are significantly underdiagnosed and underreported. The causes of acute undifferentiated fever are often incorrectly attributed to malaria. The associated morbidity, mortality, and healthcare costs of febrile illnesses are exacerbated by the limitations of current diagnostic tools and the inappropriate use of antimicrobials. Zoonotic diseases like leptospirosis are frequently misdiagnosed in healthcare settings, and many countries lack the evidence base to inform national treatment guidelines.
Following a brief introduction to leptospirosis in East Africa, I will discuss the One Health approach within the context of leptospirosis research. I will present examples from past and current research on leptospirosis epidemiology in East Africa, highlighting both the challenges and the added value of the One Health approach.

LIVESTOCK VACCINE LAUNCHED AGAINST EMERGING GENOTYPE TO PROTECT HUMANS AGAINST LEPTOSPIROSIS IN NEW ZEALAND

Heuer C, Yupiana Y, Wilkinson DA, Sokolova M, Collins-Emerson JM, Vallee E, Benschop J, Nisa S, Wilson PR, Subharat S

School of Veterinary Science, Massey University, Palmerston North, New Zealand

Hc.heuer@gmail.com

Introduction: Almost all dairy herds in New Zealand use two or 3-way vaccines annually for all cattle. A nationwide epidemiological survey in 2016 identified a hitherto unknown genotype (strain Pacifica) of the *Leptospira borgpetersenii* species in 27% of dairy herds and 3% of cows (n=4000). Data from human cases (1999-2017) showed an increasing trend of leptospirosis patients among dairy farmers MAT-seropositive for serogroup Tarassovi, suggesting a link between an emerging new strain in dairy cows and humans. This study examines this link.

Materials and Methods: We reviewed currently available evidence about the distribution and production effects of serovar Tarassovi in ruminant livestock and wildlife, and in human leptospirosis patients over the past 20 years. We also report about the development of a vaccine by one commercial supplier.

Results: Serologically (MAT), there is strong association between Pacifica PCR sequences and Tarassovi titres in dairy cattle. No PCR positive cattle had Tarassovi sequences. No associations were observed between Pacifica and production loss in dairy herds. Tarassovi was found serologically about seven times more often in human patients, especially dairy farmers, than serovars currently included in 3-way cattle (Pomona, Hardjo, Copenhageni) vaccines, potentially indicating Pacifica infection. Tarassovi was recently added to one of the commercially available 3-way vaccines, a cornerstone for preventing leptospirosis in humans in New Zealand.

Conclusions: Pacifica is now regarded as being highly adapted to and readily transmitted between dairy cattle. Due to seemingly serologically synonymous, or possibly identical, surface proteins shared between the known Tarassovi and the new Pacifica, it is expected that Tarassovi will protect cattle against shedding Pacifica, and hence prevent humans from being exposed to Pacifica by way of vaccinating cattle.

ONE HEALTH APPROACH ON SEROSURVEY OF *LEPTOSPIRA* SPP. IN FORMER BLACK SLAVE (*QUILOMBOLA*) COMMUNITIES AND THEIR DOGS IN SOUTHERN BRAZIL

Kmetiuk LB[1*], Lisboa R[2*], Cuba Gaspar R[3], Kalempa Panazzolo G[2], Domingues OJ[2], Langoni H[3], Monti G[4], Fávero GM[2], Biondo AW[5]

[1]Zoonosis Surveillance Unit, City Secretary of Health, Curitiba, PR, Brazil; [2]State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil; [3]São Paulo State University, Botucatu, São Paulo, Brazil; [4]Wageningen University and Research, Wageningen, Netherlands; [5]Federal University of Paraná, Curitiba, Paraná, Brazil; *Both authors have equally contributed to the study

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Introduction: Brazilian quilombos are enduring rural and semi-isolated communities of former black slaves and their descendants. They have traditionally relied on subsistence livestock and agriculture, and historically lacked specific public health policies. However, this study aims to fill the gap by conducting a concomitant serosurvey of former black slaves and their dogs, to shed light on the prevalence of leptospirosis in this vulnerable population.

Material and methods: We assessed populations in four Brazilian quilombos situated in southern Brazil. Serum samples from both humans and dogs were tested for anti-Leptospira spp. antibodies against 30 different serovars using MAT.

Results: None of the 208 humans and 18.7% (15/80; 95%CI 10.9-29.0) dogs were seropositive. An overall 80% (12/15) of seropositive dogs reacted to L. species serovar Butembo, 26.6% (4/15) to L. serovar Pomona, 6.6% (1/15) to L. serovar Grippotyphosa, and 3 dogs reacted to more than one serovar.

Conclusion: These results may indicate multiple sources of *Leptospira* spp. infection in Brazilian *quilombos*, such as bovine, pigs, wild and synanthropic rodents. Although no human seropositivity was observed, dogs were exposed, and they could be used as environmental sentinels for *Leptospira* spp. circulation in *quilombola* communities.

References: Sohn-Hausner N, et al. One Health Approach to Leptospirosis: Dogs as Environmental Sentinels for Identification and Monitoring of Human Risk Areas in Southern Brazil. Trop Med Infect Dis., 2023, Sep 6;8(9):435. do Couto AC, et al. One health approach on serosurvey of anti-Leptospira spp. in homeless persons and their dogs in South Brazil. One Health, 2022 Jul 25;15:100421.

ESTIMATION OF ENVIRONMENTAL TRANSMISSION OF *LEPTOSPIRA* SPP.

Monti G

Wageningen University and Research, Quantitative Veterinary Epidemiology Group, Wageningen, The Netherlands

gustavo.monti@wur.nl

Introduction: Leptospirosis is an emergent zoonotic disease in Europe, a 2023 ECDC reported for 2007-2021, a high hospitalisation rate (> 90%) with an average mortality rate of 3% and an increasing incidence trend since 2015. However, accurate figures on the burden of the disease in both humans and animals is limited and largely underestimated. Leptospires are an attractive as a model for studying pathogen-host relationships and infections, both in animals and humans, that are commonly transmitted through the environment (E), which in general has been less studied compared to direct transmission. Although dynamic modelling for environmental transmission presents several challenges, environmental contamination mainly observed in laboratory settings can differ significantly from the natural transmission. Accurate and comprehensive data on environmental contamination is essential to identify the parameters for shedding rate, decay rate, and transmission rate.
This type of transmission requires suitable estimation methods as the parameters are not jointly identifiable. To solve this, is presented a stochastic environmental transmission model with a novel scaling method for shedding rate parameter and estimation method that distinguishes transmission rate and decay rate parameter.

Material and methods: For quantifying transmission, environmental contamination is modelled using the basic equation (Chang and de Jong, 2023).

$$\frac{d\,E\,(t)}{dt} = \varphi\, I(t) - \mu\, E\,(t)$$

It was adopted a stochastic SIS compartmental model with an extra environment compartment and infections are assumed to occur only through E (Figure 1). The transmission rate is obtained using a binomial GLM model with cumulated E(t) for each recipient, which is cumulated over time and over the area covered by the individual in that time, as offset.
With this exposure-based estimation it can be estimated the decay rate parameter m and the transmission rate parameter.
The method and results, with examples from dairy farms in Chile, will be presented more extensively during the conference.

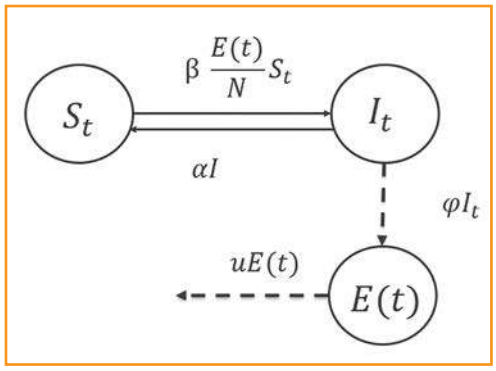


Figure 1

Reference

Chang, Y. and de Jong, M.C.M., 2023. A novel method to jointly estimate transmission rate and decay rate parameters in environmental transmission models. Epidemics 42, 100672.

UNVEILING THE GENETIC DIVERSITY OF *LEPTOSPIRA* STRAINS CIRCULATING IN MAINLAND FRANCE

Bourhy P[1], Lorioux C[1], Mariet J-F[1], Zinini F[1], Soares A[2], Trombert-Paolantoni S[3], Boucraut C[4], Delattre D[5], Museux K[6], Picardeau M[1]

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Introduction: France has a long history of passive surveillance of leptospirosis and reports the highest incidence of leptospirosis in Europe. We observe an increase in disease incidence since 2014 with more than 600 cases of human leptospirosis every year, mostly autochtonous cases. However, there is a considerable deficit in the understanding of basic aspects of the epidemiology of the disease. Better understanding of the diversity of *Leptospira* circulating strains is important to (i) identify strains or genotypes responsible for severe infections, (ii) evaluate the accuracy of current diagnostic tools and whole *Leptospira*-vaccines and (iii) develop targeted prevention strategies for *Leptospira* serovars associated with particular animal reservoirs.

Material and methods: Culture isolation is challenging and serological data are not always a reliable indicator of infecting serogroups, We have therefore used *lfb1* gene sequencing to gain a better understanding of the strains circulating in mainland France, both in patients and in dogs, as well as other animal samples when available.

Results: We sequenced the lfb1 gene amplification products from >300 and>200 biological samples collected from humans and dogs, respectively, from 2019 to 2024. *L. interrogans* serogroup icterohaemorrhagiae is the predominant serogroup isolated in both patients and dogs and this serogroup is responsible for the most severe forms. However, there are many other *lfb1* genotypes circulating, including some not associated with any known serovar or serogroup.

Conclusion: Our study reveals a high diversity of *Leptospira* genotypes, thus extending our current knowledge of strains circulating in mainland France. Leptospirosis is now added in the list of notifiable diseases since August 2023. The mandatory reporting of leptospirosis cases should significantly improve our knowledge of the epidemiology in mainland and french oversea-territories.

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BETWEEN SOIL GRAINS AND RAINDROPS: UNDERSTANDING *LEPTOSPIRA'S* ENVIRONMENTAL JOURNEY

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Introduction: Leptospires transmission, strongly influenced by pathogenic *Leptospira's* environmental survival, has been understudied in its ecosystem context. While previous research focused mainly on direct animal-to-human routes, the significant role of environmental factors and ecosystem interactions has been neglected. Our present study aims at filling this gap by examining the ecological dynamics of *Leptospira* survival across contrasted hydro-telluric habitats. Its objective is to uncover how soil abiotic factors and rainfall-induced resuspension dynamics contribute to *Leptospira's* survival and dispersion, a key approach to understand human exposure risk.

Material and methods: We explored pathogenic *Leptospira's* natural habitats characteristics by examining 90 physico-chemical properties across 102 soil samples and assessed *Leptospira* presence by qPCR. These properties were reproduced into soil and liquid microcosms to evaluate which chemical and physical parameters affect *Leptospira* survival. We developed innovative transparent soil microcosms and investigated by confocal microscopy the role of soil's texture in bacterial colonization. We also tracked *Leptospira* dispersion in rivers during intense rainfall using automated water sampling, linking bacterial presence to environmental and hydrological factors.

Results: Our soil analysis revealed that 35% of the samples were positive for the presence of pathogenic *Leptospira*. Microcosm experiments outlined soil clusters with distinct *Leptospira* survival patterns linked to specific physicochemical characteristics. We discovered that soluble complexes like $MgSO_4$ and K_2SO_4 promoted *Leptospira* survival, whereas others like $NaNO_2$ and Na_2CO_3 were detrimental. The study also highlighted the significant role of soil texture, by evidencing the influence of soil textural heterogeneity on bacterial proliferation and spatial dispersion. Field studies during rainfall events revealed that *Leptospira* resuspension peaked at rain onset and exhibited secondary spikes in slightly turbid post-flood waters.

Conclusion: This study on abiotic factors influencing pathogenic *Leptospira's* persistence in soils and waterways deepens our understanding of their hydro-telluric habitats and the conditions favorable to their survival and spread. Identifying soils and

sediments as primary reservoirs and uncovering key hydrological and environmental triggers for bacterial survival and resuspension are crucial for efficient risk mapping. In this regard, the results of this study will allow to devise effective interventions and policies to mitigate leptospirosis risk, particularly in areas already exhibiting or susceptible to develop comparable ecological characteristics upon climate change.

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List of poster abstracts

EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

D1-1 Alinaitwe L

LIVESTOCK *CARRIERS OF PATHOGENIC LEPTOSPIRES IN UGANDA AND* IMPLICATIONS FOR SLAUGHTERHOUSE WORKERS

D1-2 Alla L

EPIDEMIOLOGICAL CHARACTERISTICS OF LEPTOSPIROSIS IN ALBANIA

D1-3 Atherstone C

EPIDEMIOLOGICAL TRENDS OF LEPTOSPIROSIS, UNITED STATES, 2014-2020

D1-4 Ayral F

TRACKING POTENTIAL LEPTOSPIRA SOURCES FOLLOWING HUMAN CASES OF LEPTOSPIROSIS: A ONE HEALTH APPROACH APPLIED TO AN ECOSYSTEM IN BRITTANY, FRANCE

D1-5 Biondo AW

SEROSURVEY OF *LEPTOSPIRA SPP*. IN INDIGENOUS COMMUNITIES AND THEIR DOGS OF SOUTHERN BRAZIL

D1-6 Biondo AW

OCCUPATION AS ASSOCIATED RISK FACTOR FOR LEPTOSPIROSIS IN BRAZIL

D1-7 Brauneisen P

BETWEEN A PUBLIC HEALTH PROBLEM AND A HEALTH "PUBLIC PROBLEM": CLUSTER CASES OF LEPTOSPIROSIS IN BRITTANY, FRANCE

D1-8 Campo RDC

DETECTION OF *LEPTOSPIRA* SPP. IN BIOFILMS COLLECTED FROM SELECTED AREAS WITHIN THE UNIVERSITY OF THE PHILIPPINES MANILA CAMPUS

D1-9 Cao Y

SEROPREVALENCE OF LEPTOSPIRA IN STRAY DOG FROM CHONGQING, SOUTHWESTERN CHINA

D1-10 Collins-Emerson J

UNIFYING AND ENHANCING LEPTOSPIROSIS DIAGNOSTICS TO HARMONISE HUMAN/ANIMAL/ENVIRONMENTAL HEALTH AND IMPROVE HEALTH INEQUITIES IN NEW ZEALAND

D1-11 Cruz Romero A

EXPOSURE TO *LEPTOSPIRA* SP. OF STUDENTS AND PROFESSORS OF VETERINARY MEDICINE AND ZOOTECHNICS OF VERACRUZ, MEXICO

List of poster abstracts

EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

D1-12 Cruz Romero A

SEROLOGICAL FREQUENCY OF LEPTOSPIRA IN DESMODUS ROTUNDUS IN VERACRUZ, MEXICO

D1-13 Cruz Romero A

SEROLOGICAL FREQUENCY OF *LEPTOSPIRA* SP. IN CATTLE FROM CENTRAL ZONE OF VERACRUZ, MÉXICO

D1-14 dos Santos Ribeiro P

CORRELATION BETWEEN PATHOGENIC *LEPTOSPIRA* BURDEN AND SOIL PHYSICOCHEMICAL PARAMETERS IN POORLY SANITIZED COMMUNITIES

D1-15 Douchet L

CLIMATE-DRIVEN EARLY WARNING SYSTEM FOR LEPTOSPIROSIS DYNAMICS IN PACIFIC ISLANDS

D1-16 Fornazari F

ASSESSMENT OF *LEPTOSPIRA* SHEDDING IN DOGS AND CATS FROM TWO ANIMAL SHELTERS, SOUTHEASTERN BRAZIL

D1-17 Hamond C

TEMPORAL AND SPATIAL ANALYSIS OF LEPTOSPIROSIS CASES IN RIO DE JANEIRO, BRAZIL IN BETWEEN 2005-2022

D1-18 Kirabo AV

ONE HEALTH ASPECTS IN RESEARCH ON THE ROLE OF LEPTOSPIROSIS IN ACUTE UNDIFFERENTIATED FEVER PATIENTS IN UGANDA

D1-19 Kmetiuk LB

RISK FACTORS ASSOCIATED WITH ANTI-LEPTOSPIRA SPP. ANTIBODIES IN HOMELESS PERSONS OF BRAZIL

D1-20 Koffi KS

THE ONE HEALTH APPROACH APPLYING TO THE CIRCULATION OF PATHOGENIC LEPTOSPIRES IN A PERI-URBAN REGION IN CÔTE D'IVOIRE, WEST AFRICA

D1-21 Lagares A

SOCIO-ENVIRONMENTAL DETERMINANTS OF LEPTOSPIROSIS INCIDENCE IN CARIBBEAN AREA, COLOMBIA

D1-22 Limsiaco MLN

DETECTION AND CHARACTERIZATION OF *LEPTOSPIRA* IN SELECTED FARMING COMMUNITIES IN TUGUEGARAO CITY, MUNICIPALITY OF TOLOSA, AND DIPOLOG CITY

EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

D1-23 Majid K

GENETIC DIVERSITY AND ZOONOTIC IMPORTANCE OF UNCULTURED PATHOGENIC LEPTOSPIRA SPECIES IN AFRICA: INSIGHTS FROM TANZANIA AND SOUTH AFRICA

D1-24 Nascimento Silva AM

MAPPING ENVIRONMENTAL ENGINEERING INFRASTRUCTURE IN URBAN SLUMS: A TOOL FOR UNDERSTANDING AND TACKLING LEPTOSPIROSIS TRANSMISSION

D1-25 Neela VK

SPATIAL STUDY ON POTENTIAL DRIVERS OF LEPTOSPIROSIS IN SELANGOR STATE OF MALAYSIA

D1-26 Nilsson V

A ONE HEALTH PERSPECTIVE ON LEPTOSPIROSIS IN NORWAY: CASE STUDY AND DIAGNOSTIC VALIDATION

D1-28 Rahelinirina S

RISK OF LEPTOSPIROSIS TRANSMISSION IN A PERI URBAN AREA IN MADAGASCAR

D1-29 Ramos T

HUMAN LEPTOSPIROSIS IN PORTUGAL: A RETROSPECTIVE STUDY FROM 2019 TO 2023

D1-30 Ramos Vazquez JR

COINFECTION OF LEPTOSPIRA INTERROGANS AND DENGUE IN A FEMALE PATIENT IN VERACRUZ, MÉXICO

D1-31 Ramos Vazquez JR

ISOLATION AND IDENTICATION OF *LEPTOSPIRA* SP. IN RODENTS FROM A LIVESTOCK UNIT OF VERACRUZ, MEXICO

D1-32 Renjifo-Ibañez MC

EFFORTS AIMED AT LONG-TERM CONSERVATION OF "FASTIDIOUS" MICROORGANISMS: CASE *LEPTOSPIRA* SP.

D1-33 Richter MH

OUTBREAK OF LEPTOSPIROSIS IN SEVERAL GERMAN FEDERAL STATES LINKED TO PET RATS

D1-34 Rodríguez-Rodríguez V

HUMAN LEPTOSPIROSIS IN A REGION OF THE COLOMBIAN CARIBBEAN, 2017-2021 FROM THE "ONE HEALTH" APPROACH

List of poster abstracts

EPIDEMIOLOGY OF LEPTOSPIROSIS INCLUDING THE ONE HEALTH PERSPECTIVE

D1-35 Sohail ML

Seroprevalence and risk factor analysis of human leptospirosis in distinct climatic regions of Pakistan

D1-36 Vasconcelos L

ACIDIC pH ALTERS BIOFILM FORMATION AND CELL VIABILITY IN SAPROPHYTIC *LEPTOSPIRA* STRAINS

D1-37 B Waitt

LEPTOSPIROSIS IN A CANADIAN LANDSCAPE

D1-38 Yasir A

EPIDEMIOLOGICAL INVESTIGATION OF LEPTOSPIROSIS IN PUERTO RICO THROUGH HOSPITAL ACTIVE SURVEILLANCE AND COMMUNITY-BASED ANIMAL SAMPLING

LIVESTOCK CARRIERS OF PATHOGENIC LEPTOSPIRES IN UGANDA AND IMPLICATIONS FOR SLAUGHTERHOUSE WORKERS

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Introduction: *Leptospira* is a group of bacteria that cause leptospirosis in humans and animals. In Uganda, *Leptospira* exposure has been reported in febrile patients from geographically distinct areas, with domestic animals being speculated as the source. However, corresponding infection in animals has only been shown in cattle, dogs, and pigs, leaving the status of other potential animal carriers unknown. Moreover, it is not clear how humans get infected. We determined the status of *Leptospira* infection, circulating *Leptospira* species and sequence types among livestock, and corresponding exposure in slaughterhouse workers in Uganda.

Methods: From a nationwide cross-sectional survey in slaughter facilities, we tested 2,030 livestock kidney samples by real-time PCR, SecY gene typing, and MLST. Corresponding sera were tested by the microscopic agglutination test, except for cattle since previous reports existed. We also tested sera from 513 workers in the same slaughter facilities where we sampled the livestock.

Results: *Leptospira* infection was detected in 45 of the 2,030 livestock samples. The prevalence was 6.12% (6/114, 95% CI = 2.69–12.89) in sheep, 4.25% (30/820,95% CI = 2.91–5.98) in cattle, 2.08% (6/335, 95% CI = 0.91–4.38) in goats, and 0.46% (3/761, 95% CI = 0.12–1.31) in pigs. Sequence typing of the PCR-positive samples revealed the species *L. borgpetersenii*, *L. kirschneri* and *L. interrogans* as widespread in livestock from all regions of Uganda, with 13 novel sequence types identified. *Leptospira* seroprevalence was 26.67% (247/926, 95%CI 23.92–29.61) among pigs, and 21.81% (101/463, 95%CI 18.29–25.80) in goats and sheep (small ruminants), with *L. interrogans* Australis (Sg Australis) and *L. borgpetersenii* Tarassovi (Sg Tarassovi) being highly prevalent. Up to 14.42% (74/513, 95% CI = 11.65-17.73) of the workers were found to be seropositive, and mostly to serogroup Australis (70.73%). Seropositive individuals were found in all job positions except those in administration and supportive non-livestock trade. Up to 66.7% (22/33) of the latter worker group also reported participation in other equally risky activities like rearing livestock at home, crop farming, forest work, swimming, and fishing.

Conclusion: Livestock in Uganda are carriers of genetically diverse *Leptospira* types and could act as a source of human exposure. Finding similar *Leptospira* serogroups in both livestock and workers in the current study supports such speculations and

highlights the public health relevance of leptospirosis in Uganda. Appropriate risk control measures need to be implemented. The identified *Leptospira* sequence types contribute to the MLST database for East Africa and provide the basis for further research on serovars associated with these novel sequence types.

EPIDEMIOLOGICAL CHARACTERISTICS OF LEPTOSPIROSIS IN ALBANIA

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Keywords: Leptospirosis, incidence, disease, occupation

Introduction: Leptospirosis is an endemic zoonotic disease in Albania and is part of the Mandatory Disease Reporting System. The disease is still present in the country and poses a threat in areas where livestock, animals and rodents are present and influenced by other factors. Our aim is to make an overview of leptospirosis in Albania from 2014 to 2023 included and identify the main disease characteristics and its risk factors.

Material and Method: We collected the epidemiological data on human cases according to person-place-and time, using the data base of our department from 2014 -2020 and since 2020 using web based integrated surveillance system of infectious diseases data provided within both systems by any clinic or public health unit. The conventional ELISA test was performed for the estimation of IgM and IgG antibodies in all cases. For a descriptive analysis we used appropriate statistical methods within SPSS.

Results: In total, for the period 2014-2023, were reported 217 cases. The cumulative incidence is in the values of 7.8 cases/100,000 inhabitants, CI (95%) 6.8 to 8.8 cases 100.000 inhabitants for the whole period. Annual incidence in 2014 was (1.5 cases/100,000 inhabitants) and in 2023 has decreased respectively 0.2 cases/100,000 inhabitants. There was a significant decrease for 2020 - 2023 indicating the reduced activities of surveillance for other infectious diseases during COVID-19 pandemic. The most affected age groups were 45-59 years and 60+ years. Males show an incidence 5 times higher than women. The age and gender characteristics in human population are mainly related to their occupations such recreational activities, exposure to contaminated water surfaces contact with the urine of rodents. We did not include here the analyses of climate change and flooding as risk factors.

Conclusion: Despite its existence, the animal vaccination program is sporadic in Albania. Leptospirosis is an important infectious disease in Albania affecting especially males and rural and peri urban areas of north and the central part of Albania engaged in certain occupations. It is important that during pandemics or other emergencies to have a surveillance sustainability plan for so called neglected diseases.

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EPIDEMIOLOGICAL TRENDS OF LEPTOSPIROSIS, UNITED STATES, 2014-2020

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Introduction: In 2013, leptospirosis was reinstated as a nationally notifiable disease in the United States (U.S.). We aimed to describe the epidemiological trends of leptospirosis case data submitted between 2014-2020.

Material and methods: Analysis included confirmed and probable leptospirosis cases from jurisdictions reporting ≥ 1 case between 2014-2020. Analyzed data included reportable case surveillance (geographic and demographic) and voluntarily submitted supplemental data (exposures, travel history, clinical presentation, laboratory testing, treatment, and outcome). Logistic regression was used to determine associations between exposure type (animal, environmental, both) and demographic characteristics, occupation, activities, and location.

Results: Between 2014-2020, CDC received 1,053 case reports from 34 jurisdictions. The national incidence rate was 0.48 cases per 100,000 population. Since 2014, leptospirosis cases have been increasing, with an average gain of 13 cases per year (R^2 = 0.69). Cases increased in summer, peaking in early fall, corresponding with warmer weather and hurricane season. Among cases with data, 85% (n=606) of cases were hospitalized; 10% (n=74) died. More cases reported animal (n=236, [46%]) than environmental (fresh water or mud) (n=148, [29%]) or both exposures ((n=126, [25%]). 58% (n=414) of cases reported animal or environmental exposures at home from avocational activities like pet or livestock ownership, gardening, or yard work. We identified at least five pathogenic species and twenty serovars circulating in the U.S. There were regional differences in reported exposures, serovar reactivity on MAT, and leptospiral species identified by PCR.

Conclusions: An increasing number of individuals in the U.S. are being diagnosed with leptospirosis, almost entirely from domestic sources of infection. Changing epidemiological trends to exposure at home or from recreational activities highlights the need for interventions that mitigate transmission in these environments. A high percentage of cases were hospitalized and died. Educating healthcare providers about early identification and treatment for leptospirosis can improve patient outcomes.

TRACKING POTENTIAL *LEPTOSPIRA* SOURCES FOLLOWING HUMAN CASES OF LEPTOSPIROSIS: A ONE HEALTH APPROACH APPLIED TO AN ECOSYSTEM IN BRITTANY, FRANCE

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Keywords: Maintenance community, Cattle, Rodents, Reservoirs, Zoonosis, Rivers

Pathogenic *Leptospira* can cause leptospirosis: a widespread, potentially fatal bacterial zoonosis whose risk is strongly mediated by ecohydrological patterns and animal host distributions. When human cases of leptospirosis occur, it is challenging to track down their source because ecosystem-level epidemiological knowledge on Leptospira is needed.

As a first step in this process, we investigated leptospiral carriage in the main animal hosts found in a focal ecosystem in Brittany, France. Between 2016 and 2019, the region's human population experienced an outbreak and successive cases of leptospirosis attributable to *L. kirschneri* and *L. interrogans*. We sampled 143 nutria, 17 muskrats, and 10 Norway rats. DNA was extracted from their kidneys, lungs, and urine and subject to real-time PCR (RT-PCR) targeting the *Leptospira 16S rRNA* and *lfb1* genes. We also sampled serum from 439 cattle and used a microscopic agglutination test to detect the presence of antibodies against *Leptospira*. Urine samples were concomitantly obtained from 145 cattle and were used in two analyses: RT-PCR targeting the *Leptospira 16S rRNA* gene and *Leptospira* culturing.

We found that among the animal species tested, nutria and muskrats were the most likely source of the *L. interrogans* behind the human cases. The cattle tested negative for *Leptospira* DNA but positive for antibodies against the serogroups implicated in the human cases. We failed to identify the potential source of the *L. kirschneri* responsible for several human cases of leptospirosis. Our results call for further clarification of the *Leptospira* maintenance community, which may comprise known maintenance hosts, such as rodents, as well as taxa not commonly considered to be maintenance hosts but that can still spread *Leptospira*. Tracking potential *Leptospira* sources requires a consolidated monitoring system and the investigation of additional rodent species that may rise ethical concerns. Lastly, our results underline the fact that these investigations must be supported by typing tools and consider each genetic profile and their related epidemiology, independently.

This study was carried out using the One Health approach, as described in international health guidelines. Barriers and levers for the implementation of such a One Health workforce are also addressed to help future collaborative *Leptospira* source tracking.

SEROSURVEY OF *LEPTOSPIRA SPP.* IN INDIGENOUS COMMUNITIES AND THEIR DOGS OF SOUTHERN BRAZIL

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Introduction: Leptospirosis has historically caused human and animal epidemics and outbreaks in vulnerable populations, mostly due to inadequate infrastructure and communal living conditions. However, *Leptospira spp.* infection and associated risk factors in indigenous populations and their dogs remain to be fully established.

Material and Methods: Accordingly, the study herein serologically assessed responses in indigenous individuals and their dogs in 10 indigenous communities of southern Brazil by the Microscopic Agglutination Test (MAT) against 24 serovars to human samples, including Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Djasiman, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorraghiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc and Guaricura, and against 12 serovars to canine samples, including Australis, Bratislava, Autumnalis, Canicola, Djasiman, Grippotyphosa, Copenhageni, Icterohaemorraghiae, Pomona, Pyrogenes, Hardjo and Nupezo.

Results: Overall, 36/800 (4.5%) indigenous individuals and 7/169 (4.14%) dogs exhibited titers higher than 1:100. The Kopenoty community exhibited mostly seropositivity, with 11/36 (30.6%) individuals and 3/7 (42.8%) dogs. A seropositive owner and dog were identified, indicating common exposure. Seropositive individuals reacted to serovars Canicola, Whitcombi, and Hardjo, while all dogs reacted to Hardjo. Working-aged men were more serologically positive.

Conclusion: Although the seropositivity herein may be associated with isolation, inadequate sanitation, and water supply, the Kopenoty was among the communities with the best infrastructure. As indigenous individuals and their dogs commonly shared household and peri-domestic environments, potentially interacting with wildlife hosts, such contact may have explained the unusual *Leptospira* serovars.

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OCCUPATION AS ASSOCIATED RISK FACTOR FOR LEPTOSPIROSIS IN BRAZIL

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Introduction: This study provides a comprehensive assessment of leptospirosis cases and occupational risk from database of the Brazilian National Surveillance System from 2007 to 2022.

Material and methods: A total of approximately 55,000 confirmed cases were gathered and analyzed by associated risk factors. Occupational risk was assessed based on confirmed cases of 14-year or older, from the 100 largest cities to insure attendance and notification, along with censitary data on case occupation per Brazilian region. The occupational groups were divided into low, medium, and high risk to leptospirosis.

Results: The majority of the cases were males (80.1%) and aged20-59 (73.6%). The cases were predominantly white (44.9%) and brown (37.5%) colors, located in urban areas (54.7%) and household environment (40.3%). Despite 46.4% of cases had no report on occupation, workers (19.1%) were mostly males (91.6%), average age of 31.5 years, with similar infection place (urban 44.0%). Farmers (12.0%), construction workers (6.0%), and recycling material collectors (5.0%) were among the most exposed occupations. The work environment was identified as the most common infection place (75.5%). A higher occurrence of the disease was reported in southern Brazil, with high-risk occupations significantly more likely exposed.

Conclusion: The present study has provided important information on occupational leptospirosis, enabling better diagnosis, control, and prevention. Furthermore, the Brazilian national surveillance system serves as a clear indicator for effectiveness of public health actions and policies, as well as the leptospirosis dynamics on occupational exposure over time. References: Atil A., et al. Occupational determinants of leptospirosis among urban service workers. Int. J. Envir. Res. Publ. Health., v. 17, n. 2, p. 427, 2020. Galan, D. I., et al. Leptospirosis Risk among Occupational Groups in Brazil, 2010–2015. Am. J. Trop. Med. and Hyg., v. 109, n. 2, p. 376, 2023.

BETWEEN A PUBLIC HEALTH PROBLEM AND A HEALTH "PUBLIC PROBLEM": CLUSTER CASES OF LEPTOSPIROSIS IN BRITTANY, FRANCE

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As a zoonosis at the heart of the One Health concept, leptospirosis illustrates how difficult it is to implement not only risk analysis, but also environmental public action. In fact, grouped cases that occurred in 2006 in Brittany (France) raise the question of how the responsibilities of the various health sectors (human, animal and environmental) should be articulated. To explore this topic with a human sciences lens, we chose to use semi-structured interviews, focusing on three main areas: the characterization of leptospirosis as a public problem, the governance of environmental risk, and the management strategy implemented by local stakeholders. We have shown that publicization for this problem remains limited: concern about health risks, on the part of institutional players, municipalities and the general public, varies widely. As a result, it encounters several obstacles and masks that prevent coherent public health action. In addition, between collaboration and competition, it is sometimes difficult to bring together people who make up the leptospirosis control network, and yet the effectiveness of health monitoring depends on weaving this web. The uncontrollability of environmental risks calls into question the quest for mastery of a health problem that may never be mastered; consequently, the subject is not so much the management of the danger as the governance of the associated uncertainty. Finally, it is clear from the comments made by the persons interviewed that, in concrete terms, leptospirosis management requires more concerted action on the part of the various scientists involved, in addition to human and financial constraints. We have summarized a few prospects for improvement that were suggested by the various stakeholders involved in leptospirosis management in Brittany. Although it may seem a secondary issue, perhaps now is the time for health and scientific authorities to address a problem that is not yet a major one?

DETECTION OF *LEPTOSPIRA* SPP. IN BIOFILMS COLLECTED FROM SELECTED AREAS WITHIN THE UNIVERSITY OF THE PHILIPPINES MANILA CAMPUS

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Keywords: biofilm, *Leptospira*, coexisting bacteria, 23S rRNA (*rrl*) PCR, *fla*B PCR, VITEK®2

Introduction: Leptospirosis often spreads through contaminated environments rather than direct contact with infected animal urine.[1] *Leptospira* spp. has been reported to form biofilms, which could be a protective mechanism of pathogenic leptospires to thrive in the environment and infect humans.[2-4] However, *Leptospira* biofilms remain unexplored, especially in the Philippines despite its endemicity. Thus, this study aimed to detect the presence of *Leptospira* spp. in environmental biofilms collected from selected areas within the University of the Philippines Manila Campus.

Materials and Methods: Fifteen environmental biofilms were collected around the UP Manila Campus. The biofilms' physical characteristics and collection sites were described. Darkfield microscopy and 23S (*rrl*) rRNA PCR were employed to detect spirochetes and *Leptospira* spp. presence in the collected biofilms.[5,6] Nested *fla*B PCR was performed to determine its pathogenicity.[7] The single dominant co-existing bacteria with *Leptospira* were identified through VITEK®2.

Results: One biofilm was found to contain non-pathogenic *Leptospira* spp. Notably, *Sphingomonas paucimobilis* was the predominant coexisting bacterium in this biofilm. While the study did not detect pathogenic leptospires, it does not definitively negate their potential presence in the environmental biofilms collected. In addition, *S. paucimobilis* has been reported to favor growth of non-pathogenic leptospires over pathogenic strains.[8]

Conclusions and References: *Leptospira* spp. was detected from an environmental biofilm in one site around UP Manila campus. The isolated *Leptospira* spp. co-existed with *S. paucimobilis*, which was found to be resistant against four antimicrobials. Increased sampling in endemic regions may improve detection. Addressing leptospirosis requires coordinated efforts, including better sanitation, urban infrastructure, drainage systems, and policy reinforcement.

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SEROPREVALENCE OF *LEPTOSPIRA* IN STRAY DOG FROM CHONGQING, SOUTHWESTERN CHINA

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Leptospirosis is a worldwide re-emerging zoonosis caused by pathogenic Leptospira. All vertebrate species can be infected. Leptospires-carried stray dogs may act as a source of human infection. This study was designed to determine the prevalence of Leptospira in stray dogs in Chongqing, Southwestern China. A total of 108 samples were randomly collected from over 2000 stray kennels in the area. Information about the breed and sex of the stray dogs was gathered, and serum samples were analyzed using microscopic agglutination testing (MAT) to ascertain the antibody status of the dogs and evaluate the prevalence of leptospirosis based on antibody levels. The MAT (Titer ≥ 100) results indicated that 25 dogs (23.15 %) of the 108 dogs were tested positive for leptospirosis. The main serogroups of Leptospira prevalent in the stray dogs were L. interrogans Icterohaemorrhagiae (7.14 %), L. interrogans Javanica (9.25 %), L. interrogans Canicola (5.56 %), L. interrogans Australis (9.25 %), and L. interrogans Tarassovi (1.85 %). Among the positive cases, 9 dogs (8.33 %) were found to be infected with two or more serogroups. In summary, this study revealed a high infection rate of leptospirosis and complex seroprevalence in stray dogs, and more regional epidemiological investigations are urgently needed, and increase the awareness among public health institutions or practitioners.

UNIFYING AND ENHANCING LEPTOSPIROSIS DIAGNOSTICS TO HARMONISE HUMAN/ANIMAL/ENVIRONMENTAL HEALTH AND IMPROVE HEALTH INEQUITIES IN NEW ZEALAND

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Introduction: Traditionally MAT has been used to identify leptospirosis infections in New Zealand. Patients often present in the acute phase. The requirement for two blood tests, 2 weeks apart to demonstrate rising titres, can be inhibitory and is implicated in health inequities for rural dwellers and those in lower socioeconomic and particular ethnic groupings. A single, sensitive, one-visit diagnostic PCR in the acute phase, that could also be used for subsequent strain identification, would circumvent some of these issues. PCR is now commonly used but is currently restricted to identifying pathogenic *Leptospira* only. We developed a *glmU* PCR[1] that identifies pathogenic leptospiral DNA and can be used for strain/serovar identification by sequence analysed for endemic New Zealand strains. In collaboration with a hospital laboratory, we are testing a *glmU* PCR against their currently used 16s PCR, with the ultimate aim of introducing an improved, accredited diagnostic test, and standardising its use nationally.

Materials and Methods: The conventional *glmU* PCR has been optimised in our laboratory. We are currently developing this as a multiplex qPCR to identify the different endemic species. This multiplex will subsequently be tested in a hospital laboratory on sera that have been submitted for standard leptospirosis testing.

Conclusion: If the *glmU* PCR performs as well or better than the existing 16s PCR, the hospital will develop the test for accreditation and further interlaboratory testing with a view to national adoption of this PCR for human diagnostics. Ultimately, it would be desirable to work towards introduction of a standardised, unifying PCR in human/animal/environmental health, plus linked reporting/data sharing systems, that would improve health inequities, inform health policy, and enhance management of leptospirosis in New Zealand through a One Health approach.

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EXPOSURE TO *LEPTOSPIRA* SP. OF STUDENTS AND PROFESSORS OF VETERINARY MEDICINE AND ZOOTECHNICS OF VERACRUZ, MEXICO

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Introduction: Leptospirosis is the most prevalent zoonotic disease worldwide, which is caused by different serovars of *Leptospira* sp. In the urban environment, the exposed population corresponds to occupational groups, among which are students and professors of veterinary sciences [(1)]. Therefore, veterinary students and doctors may be exposed to different infectious pathogens such as *Leptospira* sp, due to the fact that in their undergraduate studies they handle different animal species in diverse subjects.

Methods: To determine the frequency of seroreactors in veterinary professors and students, blood samples were taken from 154 students and 7 professors and analyzed by microscopic agglutination test (MAT).

Results: The overall frequency of seropositivity to *Leptospira* sp. was 3.72%, all cases being identified among students. Participants with equine and canine contact had the highest rates of seropositivity, with 12.5% for those with equine contact and 14.3% for those with canine contact. In comparison, participants with ovine and bovine contact showed lower rates, with 8.3% and 7.3%, respectively. Finally, those with goat and swine contact had no cases.

Conclusion: It was demonstrated that students and physicians are exposed to *Leptospira* sp, due to the manipulation of different animal species in various educational experiences throughout the degree. Therefore, it is essential that health professionals and students receive adequate training on prevention measures and safe handling of situations that may expose them to this bacterium. In addition, research and epidemiological surveillance are crucial to better understand the prevalence and risks associated with exposure to this bacterium.

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SEROLOGICAL FREQUENCY OF *LEPTOSPIRA* IN *DESMODUS ROTUNDUS* IN VERACRUZ, MEXICO

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Introduction: Bats play a crucial role in the transmission of zoonotic diseases. This study aims to determine the frequency and serovars of pathogenic *L. interrogans* in *Desmodus rotundus* across different localities in Veracruz, Mexico. The hypothesis proposes that given the widespread distribution of *D. rotundus* in Veracruz, the frequency of *Leptospira* serovars in these bats will be similar in the northern and central regions of the state.

Methods: The sites were selected according to the presence of bat bites in cattle; two sites were chosen from two municipalities of Veracruz, Mexico (Figure 1). The livestock herds were left in the animals pens during three days prior to capture; this was to bait the bats with their prey (cattle). This made it possible to identify the possible entry routes for the bats to the pen and decide on the placement of nets (6 m long x 2 m high, with 2 mm mesh). Blood samples were taken with microtainers without anticoagulants to separate the serum and subsequently perform the microagglutination test (MAT) in the Infectious Diseases Laboratory of the University of Veracruz. The MAT was performed using five serovars of *Leptospira interrogans*. Absolute and relative frequencies were calculated by geographical area and sex of the bats. A χ^2 test was conducted to determine the association between collection region, sex, and the frequency of seropositive bats.

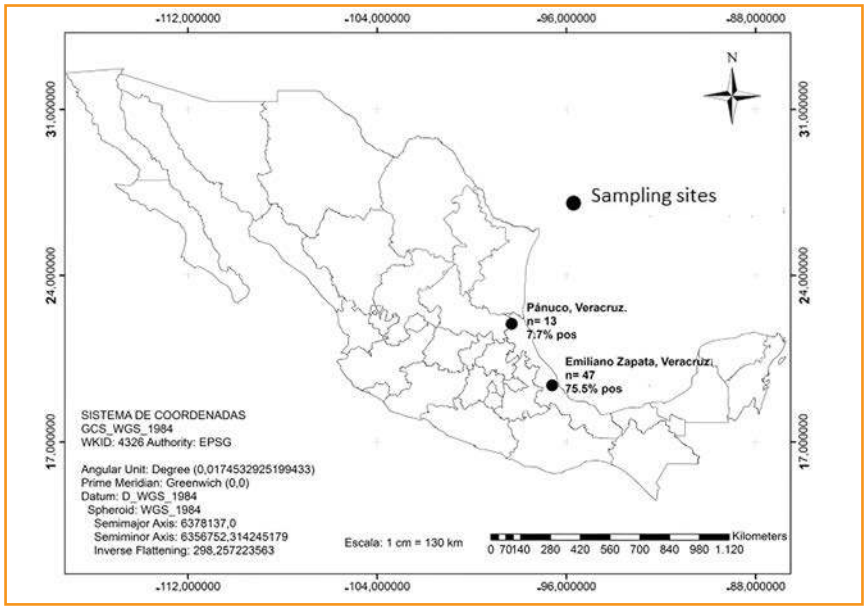


Figure 1. Sampling sites with seropositive bats to *Leptospira* sp. Veracruz, México

Results: The frequency of *L. interrogans* in bats was 66.3% (38/60), with the central zone showing a significantly higher frequency of animals exhibiting signs (χ^2: $p<$ 0.0001). There was no significant effect between the sex of bats and the presence of the disease (χ^2; $p>0.01$).

Discussion and conclusions: A study in Brazil (2009) reported a lower seropositive proportion (7.8%) in bats compared to our findings. The high prevalence of antibody-titers in *Desmodus rotundus* demostrates a high exposure of this bat species against *L. interrogans* and other *Leptospira* species, opening the posibility to consider as a potential host in the central region of Veracruz, a key livestock production area. Our findings highlight the important to stablish a surveillance program on *Leptospira* in wildlife, particularly bats, to clarify the transmission dinamics with livestock.

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SEROLOGICAL FREQUENCY OF *LEPTOSPIRA* SP. IN CATTLE FROM CENTRAL ZONE OF VERACRUZ, MÉXICO

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Introduction: In Mexican livestock are found various infectious diseases that can impact productive and reproductive indicators, highlighting among them leptospirosis, which is a reproductive disease caused by pathogenic bacteria of the genus del *Leptospira* that in the cattle cause an acute or subacute medical condition. This study aims to determinate the serofrequency of *Leptospira* sp. in bovines in central zone of Veracruz, México. A cross-sectional epidemioloical study was carried out in livestock production unit belonging to municipalities of Tlalixcoyan, Manlio Fabio Altamirano y Jamapa pertaining to the center of Veracruz state, during the months of August-October in 2022.

Methods: A sampling was performed to estimate proportions with the program Epi Info with a 95% of CI, population "N" unknown, prevalence of 50% and an error of 5% obtained a "n" = 385 bovines, the selection of the cattle was for convenience. To determine the serofrequency of *Leptospira* was performed MAT test, using nine serovars (*L. interrogans* Hardjo, *L. interrogans* Pomona, *L. interrogans* Hardjo, *L. interrogans* Bratislava, *L. borgpetersenii* Sejroe, *L. borgpetersenii* Mini, *L. borgpetersenii* Ballum, *L. santarosai* Mini and *L. santarosai* Tarassovi). General and specific, as well as confidence intervals of 95% and X^2 were calculated by the statistical program STATA ver 14.

Results: General serofrequency in bovines of the central of Veracruz was 75.5% (229/303; $CI_{95\%}$ 70.3-80.3). Serovars, which had a higher frequency of animals seroreactors by serovars were *L. interrogans* Bratislava with 49.3% and by serovar Hardjo 32.2%. However, the serovar *L. borgpetersenii* Ballum resulted with 24.1% of frequency; and finally, *L. santarosai* the Tarassovi was the most frequent with 31.5%.

Conclusion: The detection of antibodies of *Leptospira* in livestock production units belonging to municipalities of Tlalixcoyan, Manlio Fabio Altamirano y Jamapa. While the frequency of antibodies of *Leptospira* was elevated, this does not mean that performed animals were ill at the time because the test used was MAT and only it detects antibodies, therefore we only have serologically positives.

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CORRELATION BETWEEN PATHOGENIC *LEPTOSPIRA* BURDEN AND SOIL PHYSICOCHEMICAL PARAMETERS IN POORLY SANITIZED COMMUNITIES

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Keywords: Leptospirosis, Environment, *lipL32*, Macronutrients, Micronutrients.

Introduction: Water and soils harbor pathogenic *Leptospira*, serving as infection sources for the transmission of leptospirosis. Few studies, however, have evaluated the influence of physicochemical factors on the environmental burden of pathogenic *Leptospira* species.

Objective: To investigate the correlation between the burden of pathogenic *Leptospira* and physicochemical parameters in soil samples from poorly sanitized urban communities.

Materials and methods: We collected 89 soil samples from three urban settings of Salvador, Bahia, Brazil. We processed the samples, extracted the total DNA, and quantified pathogenic *Leptospira* using real-time quantitative PCR (qPCR) targeting the *lipL32* gene. We analyzed humidity, temperature, macronutrients (total organic carbon - TOC, total nitrogen, phosphorus), and micronutrients (Ca, Cu, Fe, K, Mg, Mn, Na, Zn). We performed the Mann-Whitney U-test and Spearman's correlation analysis to assess significant associations between variables.

Results: 30 (34%) soil samples were positive for *lipL32* qPCR. The percentages of TOC ($p = 0.00$) and total N ($p = 0.00$), and the concentrations of P ($p = 0.01$), Cu ($p = 0.04$), Mg ($p = 0.04$), and Zn ($p = 0.04$) were significantly higher in the positive samples when compared to the negative samples; while Fe ($p = 0.03$) and Ca ($p = 0.00$) were significantly lower. K ($p = 0.68$), Mn ($p = 0.64$), and Na ($p = 0.91$) showed no statistical differences between positive and negative samples. In the correlation analysis, temperature ($R = 0.46$; $p < 0.05$) and humidity ($R = 0.37$; $p < 0.05$) had significant influences on the concentration of pathogenic *Leptospira*.

Conclusion: Humidity and temperature correlated positively with the burden of pathogenic *Leptospira*. Although macro and micronutrients are important for *Leptospira* metabolism, they did not correlate with bacterial burden. Studying physicochemical parameters that influence *Leptospira* burden may help develop future control strategies for leptospirosis.

Funding: Wellcome Trust (grant number 218987/Z/19/Z); Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES; Finance Code 001); Foundation for Research Support of the State of Bahia (FAPESB; BOL0367/2022).

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CLIMATE-DRIVEN EARLY WARNING SYSTEM FOR LEPTOSPIROSIS DYNAMICS IN PACIFIC ISLANDS

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Introduction: Leptospirosis outbreaks are frequent in Pacific islands where tropical climate and lifestyle provide suitable conditions for human infection (1). With challenging clinical and laboratory diagnoses and without effective vaccines, preparedness and prevention remain key to mitigate outbreaks (2). Such strategies imply that epidemics are anticipated months in advance, underlying the need to design operational early warning systems. These systems could focus on the strong association between leptospirosis and climate to produce operational models and inform public health surveillance (3,4).

Material and methods: We adjusted climate-driven machine learning models of leptospirosis dynamics on 7 tropical islands: Fiji, New Caledonia, Tahiti, Futuna, Mayotte, Guadeloupe, and Reunion island. A global seasonal model was first fitted and could be used to estimate leptospirosis seasonal dynamics where the disease is not routinely monitored. Island-specific inter-annual models provided epidemic risk predictions based on climate for islands with known historical dynamics. We tested several explanatory variables based on temperature and precipitation data, including averages, the number of extreme events, and their dispersion over the previous three months. We selected the best models based on mean average error scores obtained in cross-validation.

Results: Our global model was mainly driven by precipitation. The inter-annual dynamics was best estimated with island-dependent models driven by both precipitation and temperature variables. Including variables that describe extreme climate events improved the predictions. The inter-annual dynamic remained difficult to capture in Tahiti. The developed models have been implemented in an experimental dashboard connected to climate data, designed to inform decision-makers on leptospirosis dynamics.

Conclusion: While climate data can be routinely collected, regular and timely access to health data remains a challenge to build operational early warning systems. The model will be further improved with the addition of environment-based risk determinants and through the incremental addition of more leptospirosis surveillance data.

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ASSESSMENT OF *LEPTOSPIRA* SHEDDING IN DOGS AND CATS FROM TWO ANIMAL SHELTERS, SOUTHEASTERN BRAZIL

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Introduction: Animal shelters are favorable environments for the introduction and dissemination of pathogens. They have high population densities of dogs and cats, constant presence of animal waste, animals of unknown origins, and limited resources for treating sick animals. Few studies on leptospirosis have been conducted in animal shelters. Early detection of dogs and cats infected by *Leptospira* in shelters may have public health implications as many people are interested in adopting these animals. The aim of this study was to investigate the presence of dogs and cats shedding *Leptospira* in two animal shelter from Botucatu municipality, Southeastern Brazil.

Material and methods: The studied included 62 asymptomatic animals that were housed in two shelters dedicated to rescuing and caring for dogs and cats. One of them was a non-governmental organization (22 dogs) and the other one was the municipal kennel of Botucatu (29 dogs and 11 cats). Forty-nine dogs were immunized with vaccines against multiple diseases (including leptospirosis), and the vaccinations status of two dogs were unknown. Urine samples were collected either by cystocentesis or natural urination or urethral catheterization. The samples were immediately frozen at -20°C and sent to the laboratory, where they were submitted to molecular diagnostic within 48 hours. Genomic DNA was extracted from the samples using a commercial kit (Cytiva™) according to the manufacturer's instructions. Molecular detection of pathogenic *Leptospira* DNA was performed through a polymerase chain reaction (PCR) using primers directed to the lipl32 gene. Positive and negative controls were included in the reactions. The amplicons were visualized in agarose gel electrophoresis.

Results: All the PCR results were negative. Conclusion: Dogs and cats shedding *Leptospira* were not detected in the shelters studied.

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TEMPORAL AND SPATIAL ANALYSIS OF LEPTOSPIROSIS CASES IN RIO DE JANEIRO, BRAZIL IN BETWEEN 2005-2022

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Keywords: *human leptospirosis, serum microagglutination test, Brazil*

Introduction: Leptospirosis is a neglected disease that poses a persistent concern to human and environmental health, especially among rural and vulnerable regions worldwide. In Brazil, the National Reference Laboratory for Leptospirosis, located in the state of Rio de Janeiro, is responsible to provide microagglutination test (MAT) and real time polymerase chain reaction (qPCR) diagnosis of human suspect cases. In the past years this region is experiencing an increase in the rates of MAT reagent samples. This work aimed to analyze the dynamics and distribution of human leptospirosis cases in Rio de Janeiro state in the past 18 years.

Method: Non-labeled data from MAT-reagent samples from 2005 to 2022 were aggregated and trends were analyzed in each year.

Results: From 2005 up to 2009, Rio de Janeiro experienced an increased number of leptospirosis MAT-confirmed cases, followed by a second increase in between 2013-2019. A new spike in the number of cases was observed in 2022, which suggests the region is entering a new period of increased case numbers. Icterohaemorrhagiae, Australis, Tarassovi and Canicola were the most prevalent serogroups during all time intervals analyzed. Considering that more than 60% of Rio de Janeiro comprises urban regions, the occurrence of cases in urban, rural, and intermediate regions was investigated and for all prevalent serogroups, most cases were in urban.

Conclusion: It is possible to observe a period of high prevalence of cases followed by a period of low prevalence of cases, which is not affected by serogroup, and not by the typology urban/rural of Rio municipalities. More studies are needed to understand the relation of this seasonality with other aspects of the disease, demographics and geographic characteristics.

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ONE HEALTH ASPECTS IN RESEARCH ON THE ROLE OF LEPTOSPIROSIS IN ACUTE UNDIFFERENTIATED FEVER PATIENTS IN UGANDA

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Introduction: Often misdiagnosed as malaria [1, 2], acute undifferentiated fevers (AUF) often lead to inappropriate use of antimicrobials, high morbidity, and mortality, and high healthcare costs [1]. Leptospirosis is increasingly recognized as a major contributor to these AUFs [2, 3, 4, 5, 6, 7, 8] Human-animal contact is thought to be the source of infection to humans, necessitating a One Health approach in its control. We present aspects and advantages of a One Health approach observed in our leptospirosis research among AUF patients in Uganda.

Methods: The study, which started in November 2023, aims to recruit 500 AUF patients from health centres in Hoima district, Western Uganda, over a period of one year. Epidemiological and clinical data are collected and entered into REDCap. Point-of-care diagnostics are validated and used to provide probable diagnoses while blood and urine samples are collected for confirmatory testing using Microscopic Agglutination Test (MAT), real-time PCR and whole genome sequencing. The prevalence of leptospirosis shall be estimated, and the Leptospira species, serogroups and risk factors identified.
One Health approach is integrated into the study through an interdisciplinary team comprising human and veterinary doctors and laboratory experts, and leveraging existing veterinary laboratory for testing human samples, with capacity building occurring between the two disciplines. Further, the MAT panel used in the study is similar to that used in animal studies, permitting comparability of results, and building of the epidemiologic link between humans and animals. The research also identifies the transmission risk between humans, animals, and the environment through a top-down approach, starting with one symptom – a fever.

Implications: The integration of the One Health approach into this study has proven beneficial in cutting costs and time, and strengthened the collaboration among veterinary, human medicine, and laboratory scientists, enabling research on this neglected tropical disease.

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RISK FACTORS ASSOCIATED WITH ANTI-*LEPTOSPIRA* SPP. ANTIBODIES IN HOMELESS PERSONS OF BRAZIL

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Introduction: Leptospirosis is a health concern, caused by pathogenic Leptospira spp. particularly in neglected urban populations. Homelessness is considered one of the most vulnerable populations globally, along with incarceration and refugees. This study aimed to assess Leptospira spp. seroprevalence by the Microscopic Agglutination Test (MAT) and associated risk factors among homeless individuals in São Paulo and São José dos Pinhais, two major cities of Brazil.

Material and methods: In a cross-sectional study, serum samples from 243 homeless people were tested for anti-Leptospira spp. antibodies against 24 different serovars using MAT. A personal questionnaire related to potential risk factors was also administrated. Logistic regression analysis was applied to collected data.

Results: A total of 21/243 (8.6%) individuals tested positive to different serovars including L. interrogans serovars Bratislava, Canicola and Pyrogenes, L. kirschneri serovar Grippotyphosa, and L. meyeri serovar Semaranga. Although flea infestation (p=0.028) and preservative use (p=0.032) were associated with Leptospira spp. seropositivity in São Paulo. No associated risk factor was found in São José do Pinhais.

Conclusion: The study herein has first shown leptospirosis exposure in homeless populations in São Paulo and São José dos Pinhais cities, Brazil, highlighting for *Leptospira* spp. infection in this vulnerable population. Thus, the findings herein have offered a comprehensive approach of leptospirosis to homeless individuals in Brazilian cities, highlighting the urgent demand for targeted interventions and heightened awareness regarding leptospirosis.

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THE ONE HEALTH APPROACH APPLYING TO THE CIRCULATION OF PATHOGENIC LEPTOSPIRES IN A PERI-URBAN REGION IN CÔTE D'IVOIRE, WEST AFRICA

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Keywords: One health, *Leptospira*, Cote d'Ivoire, Serology, Molecular Biology

Introduction: Leptospirosis is a zoonotic bacterial infection caused by pathogenic strains of *Leptospira*, resulting in approximately 1 million cases worldwide each year. The disease cycle involves animals, the environment, and humans, making the One Health approach relevant for its study. In Côte d'Ivoire, Koffi et al. showed that the western region of the country was at high risk of leptospirosis. Studying the circulation of leptospires in this region could provide a better understanding of the disease risks. This work aimed to study the circulation of pathogenic leptospires in the western region of the country using the One Health approach.

Material And Methods: A cross-sectional study was carried out in Bayota, a peri-urban area in western Côte d'Ivoire, from January 2023 to March 2024. As a One Health approach, this study involved humans, animals, and environmental water. Healthy human volunteers and livestock cattle were blood-sampled for anti-*Leptospira* antibodies detection using ELISA and microagglutination test (MAT). Kidneys of live-trapped small mammals and surface water samples (1L) were tested for *Leptospira* DNA using PCR targeting the *lfb1* gene and sequencing. Data were analyzed using EPI-Info 7.2 software.

Results: A total of 338 human sera, 132 bovine sera, 63 small mammal samples (kidneys), and 56 water samples were collected.
Regarding serology, in humans, 4.4% of samples had anti-*Leptospira* antibodies. The main serogroups found were Mini (26.7%) and Icterohaemorrhagiae (20.0%). For cattle, anti-*Leptospira* antibodies were found in 30.3% of samples. The main serogroups found were Sejroe (47.5%) and Australis (30.0%).
Using molecular biology, leptospires were detected in 19.0% of small mammals, of which 91.7% were *L. kirschneri*. In water, only *L. interrogans* was detected.

Conclusion: This study shows high circulation's rates of pathogenic leptospires in both wild and domestic animals and could explain the high risk of leptospirosis of this geographical area.

SOCIO-ENVIRONMENTAL DETERMINANTS OF LEPTOSPIROSIS INCIDENCE IN CARIBBEAN AREA, COLOMBIA

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Background: Human leptospirosis (HL) is a zoonotic disease identified as one of the emerging infectious diseases. Is a multi-factorial infectious disease that is highly associated with both environmental and socioeconomic factors, is most prevalent in tropical and subtropical areas with high rainfall events. Our objective was to determine the spatiotemporal distribution of HL in Caribbean area, Colombia during 2009–2021 and its relationship with the socio-environmental variables.

Methods: In Colombia, HL notifications are mandatory, we estimated incidence and lethality of HL according to subregions and year. To estimate the geographic distribution of HL, we used the terrestrial geographic coordinated system, reports of confirmed cases, a mapping risk of HL was constructed using ArcGis 10.8 Software., annual rainfall, population density and poverty rates for this region of Colombia were used.

Results: During the study period, 3279 HL cases were registered (incidence: 2.585 cases x 100,000) and 106 deaths (lethality 2.3%). In 2010, there was an accumulated precipitation of 5.88 mm, with 592 cases (rate 5.88) and in 2017, a precipitation of 3.99 mm with 204 cases (rate 1.8), in these years it was where the highest report of cases occurred and it was where the greatest rainfall occurred. From 2010 to 2021, a very marked trend of decreasing cases is observed. The HL cases are focused on the subregions of Atlántico, Bolívar and Urabá, which are places with the highest population density.

Conclusions:. The areas with the highest population density and high rainfall events are those with the highest risk of contracting HL, which are mainly urban areas associated with urban slums and extreme poverty and negligible sanitation facilities. The Geographic information systems used were important in analyzing the socioeconomic and environmental risk factors which are fundamental to guide the intervention in public health's politics in Latin-American developing countries.

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DETECTION AND CHARACTERIZATION OF *LEPTOSPIRA* IN SELECTED FARMING COMMUNITIES IN TUGUEGARAO CITY, MUNICIPALITY OF TOLOSA, AND DIPOLOG CITY

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Keywords: leptospirosis, *Leptospira*, farmlands, environment, Philippines

Leptospirosis is a zoonosis common in tropical and subtropical regions, like the Philippines. It is caused by pathogenic *Leptospira*, causing mild to severe infections in humans and animals. Farming has been reported to be a high-risk occupation due to exposure to *Leptospira*-contaminated water and soil.[1,2,3,4] A total of 72 environmental samples (36 soil and 36 water) were collected in selected Philippine farming communities (i.e., Tuguegarao City, Municipality of Tolosa, and Dipolog City). These were characterized according to their climate, temperature, pH, and moisture content for soil, or salinity for water. Isolation of leptospires was done using Korthof's medium with STAFF.[4,5] Isolates were phenotypically characterized by examining growth in temperatures 13, 30, and 37°C,[5] and in culture media with 1M NaCl solution.[6] Isolates were also characterized genotypically through PCR (23S rRNA[7] and nested *fla*B[8]), 16S rRNA sequencing, and phylogenetic analysis. Among the 72 samples, 35 were positive for spirochete growth. Among these, 13 isolates had increased growth in 13°C (non-pathogenic), 35 in 30°C, and 22 in 37°C (pathogenic). Moreover, four isolates converted into spherical forms after a two-hour incubation at 30°C in the 1M NaCl test, suggesting pathogenicity. Thirty-three isolates were confirmed as *Leptospira* via 23S PCR, five were confirmed to be pathogenic through nested *fla*B PCR. Results of 16S sequencing showed the probable identity of two isolates to be intermediate pathogenic *Leptospira* (i.e., *L.wolfii*), 27 as non-pathogenic *(L. montravelensis, L. ellinghausenii, L. yanagawae, L. jelokensis, L. levettii, L. wolbachii*), while six were identical to uncultured bacteria. The presence of *Leptospira* in these farmlands indicates high risk of leptospirosis among workers and residents in and near the farmlands. Therefore, preventive and control measures should be implemented or enhanced. The results contribute to the existing knowledge regarding leptospires in the Philippines and worldwide genomic data of *Leptospira* spp.

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GENETIC DIVERSITY AND ZOONOTIC IMPORTANCE OF UNCULTURED PATHOGENIC *LEPTOSPIRA* SPECIES IN AFRICA: INSIGHTS FROM TANZANIA AND SOUTH AFRICA

Majid K

Supervisors: Mark Moseley, Sandra Telfer, Chris Sutherland And Derek Sloan

This study investigates the distribution, genetic diversity, and zoonotic importance of pathogenic *Leptospira* species in various water systems across Africa, specifically focusing on Tanzania (Kilombero) and South Africa (Cape Town and Johannesburg). Leptospirosis, a neglected environmental zoonotic disease affecting both humans and animals, poses a considerable global health threat. The genus *Leptospira* comprises over sixty species, grouped into four main clades based on pathogenicity: Saprophytic (S1 and S2) and Pathogenic (P1 and P2), each representing distinct lineages with varying levels of virulence. Annually, about 1 million people contract the disease resulting in 300,000 to 500,000 severe cases and 60,000 reported deaths. Higher morbidity rates are predicted in regions with poor disease surveillance like Africa. Despite its prevalence in Sub-Saharan Africa, the distribution and diversity of *Leptospira* spp. are poorly understood due to limited research. The study aims to address this gap by using environmental surveillance and genetic typing to characterise the distribution and diversity of pathogenic *Leptospira* in Kilombero, Cape Town, and Johannesburg, and compare it to other global regions.

Samples from both wet and dry seasons were collected in triplicate from the environment from 25 sites in Johannesburg (n=8) and Cape Town (n=17) river systems, and 7 sites in Kilombero, including 6 water taps (n=6) and a rice paddy (n=1). Soil samples were processed for DNA extraction and screened for *Leptospira* DNA using a semi-nested PCR assay targeting the *secY* gene and a real-time PCR targeting the *lipL32* gene. Positive samples were sequenced to assess the genetic diversity. *Leptospira* DNA was detected at 24/25 sites in South Africa and all 7 sites in Tanzania, but not in every sample within sites. Higher probability of *Leptospira* DNA detection was observed in specific locations such as the middle channel of water taps and outside the ridge at the rice paddy. Preliminary sequencing results demonstrated the presence of pathogenic species associated with human and animal disease and novel, potentially pathogenic *Leptospira* spp., highlighting the wide diversity of potentially pathogenic *Leptospira* spp. in the African environment. This study emphasizes the need for enhanced surveillance and control measures to mitigate public health risks associated with leptospirosis in Africa and beyond.

MAPPING ENVIRONMENTAL ENGINEERING INFRASTRUCTURE IN URBAN SLUMS: A TOOL FOR UNDERSTANDING AND TACKLING LEPTOSPIROSIS TRANSMISSION

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Introduction: Leptospirosis poses a significant public health threat, with over 1 million reported cases and more than 50,000 fatalities annually. It has emerged as a growing concern in urban settings of developing and tropical countries, particularly affecting residents of deprived areas such as slums.

Aim: This work aims to develop and evaluate an innovative tool designed to identify, characterise, and georeference sanitation, storm drainage, and waste management risk factors within urban areas with high leptospirosis burdens.

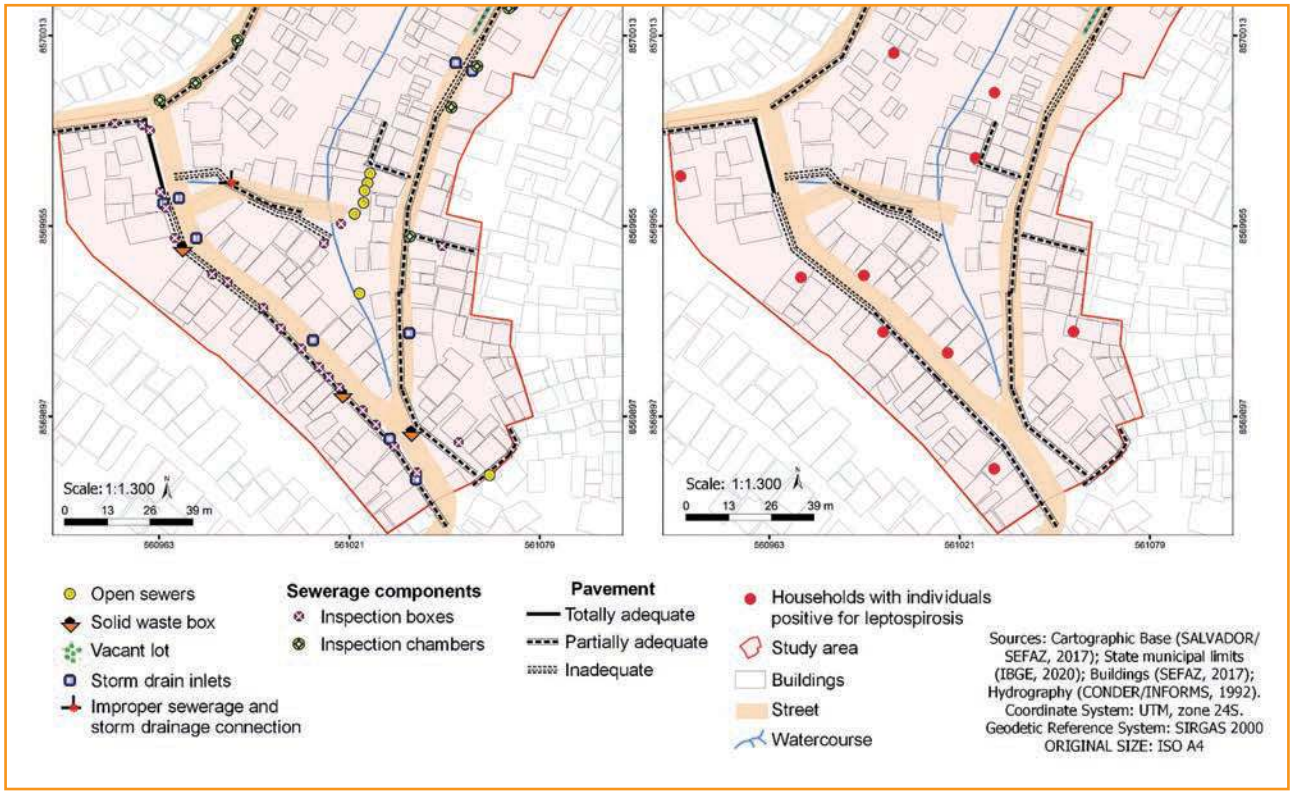


Figure 1 Mapped potential sources of environmental contamination (A - left) and leptospirosis cases (B - right) in one study area (Nova Sussuarana, Salvador, Brazil).

Methods: The tool was implemented and evaluated in four slums in Salvador, Brazil: two intervention areas with planned sewerage works by the state government, and two control areas with similar environmental, socioeconomic, and exposure characteristics but without planned sewerage works. Environmental engineering data were collected through detailed identification, characterisation, and georeferencing using a high-precision Global Navigation Satellite System (GNSS) receiver. Simultaneously, serological exposure to Leptospira of 1,086 residents was evaluated through a microagglutination test. The association between risk factors and human Leptospira exposure was analysed.

Results: A total of 1,215 environmental engineering components were mapped, including those in densely populated areas (Fig 1A). Around 80% of storm drainage components were deemed problematic, and only 19% were considered adequate. Approximately 45% of sewage system components exhibited issues, including open sewers, and waste dumping in streets surrounding households where positive leptospirosis cases were identified. Concurrently, serological results revealed 94 positive cases (8.66%) out of 1,086 tests conducted (Fig 1B).

Conclusion: Capturing the proximity of features such as improper sewerage and storm drainage components, open sewer points, and waste dumping to households presents great potential for leptospirosis prevention. This ranges from understanding the fate of Leptospira in the environment to indicating more targeted investments to tackle leptospirosis transmission in slums. Therefore, this comprehensive approach enables identifying potential sources of leptospirosis contamination.

SPATIAL STUDY ON POTENTIAL CONTRIBUTORS OF LEPTOSPIROSIS IN SELANGOR STATE OF MALAYSIA

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Introduction: Leptospirosis that pose public health concern worldwide is a zoonotic bacterial disease caused by pathogenic spirochetes of the genus Leptospira. The disease is transmitted by direct contact with the Leptospira reservoir animals or indirectly from animal urine contaminated environment. It is endemic in subtropical or tropical countries, often associated with poor urban slum populations, occupation, crowded living conditions, recreational activities where rodents are commonly spotted. In Malaysia leptospirosis is endemic with time-to-time outbreaks post flood. Leptospirosis is more commonly reported in districts of Selangor where recreational forests are open for public. Here, we aimed to determine the spatial distribution and hot spots of leptospirosis and its association with potential drivers such as population, animals, and recreational areas in Selangor districts.

Methods: The disease records for 2011–2021 cases of Leptospirosis were used in the study. Spatial patterns were determined by geographic information systems (GIS). The data on population, animal reservoirs and the recreational areas were obtained from the respective departments.
Results: Spatial heterogeneity was found across the districts, with some displaying greater clusters than others. Kuala Selangor and Kuala Langat exhibited higher rates (4.48 and 4.13 per 100,000 population), whereas Klang district had the lowest (1.03). Hulu Langat, Gombak, and Petaling districts, which have higher population densities, more build-up areas, recreational areas, and more associate with reservoir animals, exhibited higher clusters of leptospirosis. Higher distributions of cases and hot spots were found around Gombak, Hulu Langat, and Petaling, with Sabak Bernam and Sepang areas being dispersed.

Conclusions: From the present investigation, we found that population, recreational areas, and livestock could be categorized as risk factors for leptospirosis. Hence, addressing the population density, recreational areas, and animal association, could serve as areas for targeted interventions and resource optimization, ultimately leading to more effective leptospirosis control and prevention efforts.

A ONE HEALTH PERSPECTIVE ON LEPTOSPIROSIS IN NORWAY: CASE STUDY AND DIAGNOSTIC VALIDATION

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Leptospirosis is considered a rare disease in developed countries of temperate zones, but there is a suspicion of underreporting. This is likely due to low awareness, difficulty in detecting milder cases and chronic infections, and weaknesses in available diagnostic methods. The serological diagnosis of leptospirosis in humans in Norway is currently performed by screening with the commercially available SERION ELISA classic Leptos. As no laboratory in the country offers MAT, samples must be sent elsewhere for confirmatory testing. A bead-based multiplex immunoassay for *Leptospira* serology in humans has been established, and this test showed higher sensitivity than the gold standard MAT. Bead-based suspension array technology (xMap, Luminex) can multiplex up to 500 individual analytes in a single well, using magnetic coated polystyrene beads with distinct fluorescent dye ratios, facilitating simultaneous antibody response measurement to multiple antigens[2]. In our ongoing research project, we are using a similar method for serological detection of *Leptospira* serovars in dogs and humans, looking to validate response against individual serovars to assess the possibility of indicating serogroup-specific results. This need for improved diagnostic methods and greater disease awareness in Norway was highlighted in March 2023 when a previously healthy 11-month-old male dog from Bergen, Norway, was hospitalized due to severe lethargy, anorexia, vomiting, abdominal pain, and fever. Pyelonephritis was suspected, however, a diagnosis of leptospirosis was made eight days after onset of symptoms, with a positive qPCR result on urine (Idexx). MAT showed a positive titer (1:800) for *Leptospira* Icterohaemorrhagiae. The dog had never traveled outside of Norway. Of twelve veterinary clinic employees, five people showed symptoms of leptospirosis. Human samples were tested with ELISA and MAT, but the results were inconclusive. This case study underscores the interconnectedness of human and animal health, but also the diagnostic challenges in understanding and addressing leptospirosis.

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RISK OF LEPTOSPIROSIS TRANSMISSION IN A PERI URBAN AREA IN MADAGASCAR

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Introduction: Leptospirosis is neglected and likely under-reported in Africa, including Madagascar where climatic, socioeconomic, agricultural activities and animal husbandry conditions may facilitate transmission of *Leptospira*. Madagascar has a long tradition of rice production in rural and urban areas where we suspect a high leptospirosis burden in humans; unfortunately, human data are lacking. Our main objective is to decipher the eco-epidemiology of leptospirosis and to identify the various players involved in transmission routes of human infection.

Material and methods: Field investigations were conducted in April 2022 in a periurban floodable plain of Antananarivo characterized by ricefields, hyacinth ponds and crowded human communities who co-habit tightly with domestic animals as well as wild small mammals. Samples were collected from healthy humans (blood, N=363), cattle (urine, N=165) from the same households, small mammals (kidney and urine, N=169) and from the environment (soil/water, N=30). *Leptospira* spp. were screened using bacteriological, molecular tests (PCR) and serological. Bacterial identification was carried out by core genome multilocus sequence typing (cgMLST) and polymorphism of the *lfb1* gene.

Results: Prevalence of pathogenic *Leptospira* in small mammals, cattle and soil was 17.2%, 13.3% and 6.7% respectively. Antibodies IgG were found in 38.3 % of humans by ELISA and and *Leptospira* serogroups were found in 16.7% of humans using MAT with cut off 1:50. Four leptospiral strains were isolated from small mammals and identified as *Leptospira borgpetersenii* serovar Ballum or Arborea. Phylogenetic analyses of *lfb1* retrieved three main groups gathering *L. borgpetersenii* strains from cattle and small mammals specifically.

Conclusion: Pathogenic *Leptospira* circulate in animals and the environment in the slum quarter of Antananarivo and cattle seem important carriers. Our study demonstrates extensive exposition of Malagasy slum inhabitants to pathogenic leptospires. Studies in febrile patients are urgently needed to assess the true burden of acute leptospirosis, as well as to identify the most frequent source of human infection in these highly degraded socio-environments. One Health prevention and control strategies should be developed together with experts and the local population, taking the different reservoirs into account.

HUMAN LEPTOSPIROSIS IN PORTUGAL: A RETROSPECTIVE STUDY FROM 2019 TO 2023

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Introduction: Leptospirosis is a nationally notifiable disease, with an average of 76 cases reported annually between 2017 and 2021, predominantly affecting adult men. This study presents an overview of cases observed during the period 2019-2023 within a convenience sample of the Portuguese population.

Methods: MAT and/or PCR was performed on 628 patient samples, with real-time PCR conducted on 379 samples and MAT on 439 samples. Samples with titre ≥ 100 were defined as positive.

Results: Among those, 55 patients tested positive for Leptospirosis either through PCR and/or serology, with a vast majority (83.6%) being men. Women exhibited a higher average age compared to men (63 and 54, respectively).
11,6% (44/379) of the samples tested for *Leptospira* DNA were positive, 2 CSF, 23 urine and 18 blood samples. A positive result was recorded from a bronchoalveolar lavage fluid sample.
4,8% (21/439) of the serum/CSF samples had antibodies for *Leptospira*. In 38% (8/21) cases specific serogroup agglutination was detected. The most frequent serogroups found were Australis and Louisiana. In 62% of the cases (13/21) no specific serogroup agglutination was observed.
Eight of the 21 positive serology patients had PCR results: 4 were positive (3 urine, 1 blood).
Most cases occurred during autumn and winter, correlating with Portugal rainy seasons, as expected.

Conclusion: This study presents an overview on leptospirosis in Portugal and provides information on possible infecting serogroups. Both serology and PCR were employed for diagnosis, but the majority of cases were diagnosed by PCR. The selection of the right sample/diagnostic method, according to the disease phase, has a relevant importance. Leptospirosis still represents an important health problem, with a considerable number of cases per year in Portugal, and, with the looming threat of climate change, it may become an increasing problem in Mediterranean countries needing greater awareness and surveillance efforts.

COINFECTION OF *LEPTOSPIRA INTERROGANS* AND DENGUE IN A FEMALE PATIENT IN VERACRUZ, MÉXICO

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Introduction: Dengue and leptospirosis are significant diseases that can cause febrile hemorrhagic syndrome, especially in subtropical and tropical areas[1,2].

Material and Methods: We present the case of a 28-year-old woman from Veracruz, Mexico, who was admitted to the hospital with fever, joint and muscle pains, and generalized petechiae. Upon physical examination, the patient was afebrile but in fair overall condition, which prompted serological and bacterial laboratory tests for dengue (complete blood count, dengue NS1 antigen, IgG, and IgM antibodies) and leptospirosis (complete blood count, dark-field microscopy, bacterial isolation from urine and PCR) and positive PRC results were sent to sequence.

Results: The results confirmed dengue infection through serological tests and leptospirosis through the Microscopic Agglutination Test (MAT), bacterial isolation in urine, and PCR and sequencing tests for *Leptospira interrogans*.

Conclusion: The patient received intravenous hydration with 0.9% sodium chloride, a vial of SAMyr (Ademetionine) 500 milligrams, potassium chloride vial at 125 milliliters/hour, and doxycycline, resulting in clinical improvement and subsequent discharge from the hospital. Although dengue and leptospirosis are common in tropical areas like Veracruz, the coexistence of both in a patient is uncommon. Therefore, a comprehensive medical record and laboratory tests are crucial for timely diagnosis and treatment.

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ISOLATION AND CULTURE OF *LEPTOSPIRA* SP. IN RODENTS FROM A LIVESTOCK FARM OF VERACRUZ, MEXICO

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Introduction: Leptospirosis is a disease with worldwide distribution, it´s transmitted in two ways: enzootic cycles with companion animals and wild cycles with wildlife, and the relationship between different species is associated with rodents like disseminators of *Leptospira*. Therefore, in Veracruz, Mexico there are rodents wild and peridomycialids such as *Rattus rattus*, *Rattus norvegicus* and *Mus musculus* associated with maintenance hosts of *Leptospira*, these rodents can be spreaders of *Leptospira* and represent a public health problem[1,2]. The aims of the study were to isolate and identify by PCR the presence of *Leptospira* in synanthropic and wild rodents from a ranch of the Faculty of Veterinary Medicine of the University of Veracruz from Mexico.

Material and Methods: The sampling carried out was a no probabilistic at convenience, and we used Sherman traps in strategic points of the ranch, was captured the rodents, all of them were euthanized under previous sedation and was dissected in University of Veracruz, the number of authorizations was 008/23 from bioethics committee of the University of Veracruz. The specimens were sent at zoology museum of UNAM for identification of the samples. The identifications of the specimens were five differents species: *Mus musculus* (7), *Sigmodon* sp. (12), *Rattus rattus* (1), *Reithrodontomys* sp. (1) and *Peromyscus* sp. (1). Therefore, we obtained 22 samples of kidney tissue (PCR) and urines (cultures) and was performance a final point PCR with gen LipI32, subsequently, the products were sent for sequencing to Macrogen of Korea.

Results: 22 samples of renal tissue were obtained, resulting positive five samples to *Leptospira* sp. and the *Leptospira* identified was *L. borgpetersenii*. The results were: *Mus musculus* (2/22), *Sigmodon* sp. (1/22), *Rattus rattus* (1/22) and *Reithrodontomys* sp. (1/22).

Conclusion: Therefore, it was possible to demonstrate the presence of *Leptospira* in four different rodents' species, as well as a first record in Mexico of *Leptospira* present in a rodent of the *Reithrodontomys* species.

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EFFORTS AIMED AT LONG-TERM CONSERVATION OF "FASTIDIOUS" MICROORGANISMS: CASE *LEPTOSPIRA* SP.

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The proper preservation of microorganisms in biological collections is critical for research purposes. Among these, *Leptospira* isolates are particularly valuable, not only for epidemiological and virulence studies but also because of the difficulties involved in isolating them. The Microorganism Collection with Interest in Animal Health-CMISA, located at AGROSAVIA-Colombia, contains 55 *Leptospira* genus isolates from various sources biological and geographical, preserved in Liquid nitrogen at -196°C. However, preserving *Leptospira* is challenging due to its delicate nature, leading to inconsistent results (Philip et al., 2018). Innovative techniques, such as encapsulation using ionic gelation, show promise for improving long-term preservation methods. Although currently used for delivering leptospiral antigens (Inanlou et al., 2016), in our pilot study, we encapsulated a *Leptospira* isolate using two biopolymers (P1 and P2), a crosslinking agent at different concentrations (0.5%, 1%, and 5%), and four capsule diluents (PBS1, PS2, Ellinghausen-McCullough-Johnson-Harris (EMJH) Medium, and a 50:50 mixture of EMJH medium and PBS1). The capsules were then stored at 4°C, -80°C, -196°C, and room temperature, and viability was assessed using dark-field microscopy at both time zero and two months. Encouragingly, successful encapsulation was achieved using P1 as the polymer, a 1% concentration of the crosslinking agent, and a 50:50 mixture of EMJH medium and PBS1 as the diluent. Importantly, viable *Leptospira* cells were recovered after encapsulation and storage at -80°C and -196°C for two months. This methodology holds promise as a complementary approach to cryopreserving Leptospira, offering potential cost savings by facilitating preservation at -80°C as opposed to the traditional liquid nitrogen storage at -196°C.

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OUTBREAK OF LEPTOSPIROSIS IN SEVERAL GERMAN FEDERAL STATES LINKED TO PET RATS

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Introduction: Leptospirosis, a globally occurring zoonosis that is caused by pathogenic *Leptospira* species, can be transmitted through direct or indirect contact of small skin lesions or mucous membranes with the urine of infected animals. Rodents and other small mammals are an important reservoir for the pathogen.
An outbreak of leptospirosis in several German federal states was linked to the keeping of fancy rats (*Rattus norvegicus* forma domestica) as pets. Serum samples of humans who had been in contact with potentially infected rats and urine of potentially infected rats were investigated by the German consultant laboratory for leptospirosis.

Methods: In total 29 human serum samples and 56 rat urine samples were sent in for investigation. The serum samples were tested using the microscopic agglutination test (MAT) and an in-house enzyme-linked immunosorbent assay (ELISA). Urine samples were investigated by a PCR targeting the *LipL32* gene to detect the presence of pathogenic *Leptospira*. Positive samples were further typed by means of a PCR targeting *secY* and multilocus sequence typing (MLST).

Results: Four of the 29 human serum samples showed positive results using the MAT. Serovar Icterohaemorrhagiae and Copenhageni were detected three times. Serovar Copenhageni alone was detected once.
Four human serum samples showed positive ELISA results for IgG and IgM and three human serum samples for IgM only. A total of 21 samples tested negative in MAT and ELISA.
Nine positive rat urine samples (9/56; 16.07 %) were determined by PCR (*LipL32*). Using PCR (*secY*) and MLST five samples could be typed as *Leptospira interrogans* serogroup Icterohaemorrhagiae.

Conclusion: Contact with rats kept as pets should be given greater consideration as a source of infection in the future, particularly due to the numerous opportunities for contact with the urine of the animals. Furthermore, owners should be advised of the potential risk of infection.

HUMAN LEPTOSPIROSIS IN A REGION OF THE COLOMBIAN CARIBBEAN, 2017-2021 FROM THE "ONE HEALTH" APPROACH

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Keywords: leptospirosis, one health, seroprevalence, risk factor

Introduction: Leptospirosis is the most widely distributed zoonosis in the world with important repercussions on human and animal health. Its study under the "One Health" approach provides an integrative vision that helps to elucidate the relationship between human beings, animals and the environment that favor the presentation of the disease.

Materials and methods: A prospective longitudinal descriptive study was carried out, with non-probabilistic sampling, which included 339 patients suspected of leptospirosis from 2017 to 2021. The cases were confirmed by the Microagglutination Test (MAT) and Polymerase Chain Reaction (PCR). In confirmed cases, samples were taken from animals and the associated environment (soil and water) as a possible source of infection for the detection of Leptospira spp. The 16S rRNA gene amplification products obtained from blood or urine samples were sequenced. MLST was implemented using the *adK, icdA, lipL32, lipL41, rrS2* and *secY* loci in isolates of animal and environmental origin.

Results: The incidence was 19.8% (67/339). In acute cases confirmed by MAT, the most frequently linked serogroups were: Sejroe, Australis and Pomona. The species *L. interrogans and L. borgpetersenii* are associated with acute cases. The presence of *L. interrogans* was evident in environmental samples. Animals of zootechnical interest, companion animals and wild animals were confirmed as renal carriers of *L. interrogans, L. borgspetersenii* and *L noguchii.* Pig ownership, recreational practice or bathing in lakes/lagoons and dams, average temperature of 28°C, flooding 30 days before the onset of symptoms and adolescent and juvenile age were identified as risk factors associated with the presentation of leptospirosis.

Conclusion: The region presents classic risk factors associated with zootechnical production systems and environmental and demographic risk factors emerge that favor the presentation of the disease.

SEROPREVALENCE AND RISK FACTOR ANALYSIS OF HUMAN LEPTOSPIROSIS IN DISTINCT CLIMATIC REGIONS OF PAKISTAN

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Leptospirosis is a global emerging infectious disease of zoonotic importance and large epidemics and epizootics have been reported. A cross survey study was conducted to estimate seroprevalence of human leptospirosis in climatically distinct regions of Pakistan and to identify the risk factors associated with the disease. Blood samples from 360 humans were collected through convenient sampling, 120 from each of three climatically distinct study areas. Serological testing was performed using ELISA kit as per manufacturer's recommendations. The results showed an overall prevalence of 40.83% (95% CI; 35.71-46.11). Statistical analysis showed significant ($P < .05$) differences in leptospiral seroprevalence in three different geographic locations, with highest in humid sub-tropical climatic region (50.83%; 95% CI; 41.55-60.07), followed by semi-arid region (44.16%; 95% CI; 35.11-53.52) and lowest in hot and dry region (27.50%; 95% CI; 19.75-36.40). After multivariate analysis age, gender, exposure to flooding water, source of water usage, disinfection schedule of surroundings and history of cut and wound were found significantly associated with the seropositivity of Leptospira. The present study, first to uncover seroprevalence of human Leptospira in different climatic regions of Pakistan, alarms about effect of climate on prevalence of Leptospira in the region.

ACIDIC pH ALTERS BIOFILM FORMATION AND CELL VIABILITY IN SAPROPHYTIC *LEPTOSPIRA* STRAINS

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Introduction: *Leptospira* encounter a multiplicity of environmental factors when they survive and colonize different environments and hosts. Biofilm formation is a strategy used by bacteria to survive numerous stresses. Despite of that, there is poor knowledge regarding the effect of physicochemical variables in biofilm formation by *Leptospira*.

Objective: To evaluate the effect of different pH ranges on biofilm formation, cell growth and cell viability of *Leptospira*.

Material and Methods: We cultivated *Leptospira biflexa* serovar Patoc strain Patoc 1 (Paris) *and Leptospira limi* (isolated from environmental biofilms) in planktonic and biofilm phenotypes in EMJH liquid medium in pH 7.5 at 30°C. We performed growth, viability and biofilm formation tests under different pH values (5.0; 6.5; 7.5; 8.5): (i) we performed growth curves by counting planktonic cells in dark-field microscopy using a Petroff Hausser chamber; (ii) we grew biofilms in 12-wells polystyrene plates and estimated the biomass by crystal violet staining, followed by optical density quantification at 600 nm; and (iii) we evaluated cell viability of planktonic and biofilm cells through alamarBlue assay.

Results: Cultivation in pH 5.0 resulted in cell death, absence of growth and absence of biofilm formation, for both *L. biflexa* and *L. limi*. Cultivation of biofilms in pH 6.5 reduced biomass ($p<0.0001$) and cell viability ($p = 0.003$) in *L. biflexa*, when compared with pH 7.5. The pH 6.5 modulated *L. biflexa* biofilm formation, causing a 24-hours delay in the mature biofilm stage. Cultivation in pH 8.5 did not show significant difference in biofilm formation for *L. biflexa* and *L. limi*, and there was no difference in cell viability when compared to pH 7.5.

Conclusion: Our results indicate that saprophytic *Leptospira* tolerate alkaline pH and are sensitive to acidic conditions. We also observed that different pH conditions, ranging from acidic to alkaline, modulate biofilm formation for these species.

Funding: This work was funded by the National Council for Scientific and Technological Development (CNPq; grant Universal 425526/2016-0), the Coordination for the Improvement of Higher Level Personnel (CAPES; Finance Code 001) and the Foundation for Research Support of the State of Bahia (FAPESB; BOL0158/2022).

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LEPTOSPIROSIS IN A CANADIAN LANDSCAPE

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The Field Studies section at the National Microbiology Laboratory (Public Health Agency of Canada) is the sole laboratory routinely performing serological and molecular testing for *Leptospira spp.* in humans and animals. As Leptospirosis is not nationally notifiable in Canada, clinical case tracking is difficult. Canada has not shared our Leptospirosis data, diagnostics, nor research internationally. As such, this presentation will detail: i) positive serological and molecular human data from 2019 – current, ii) circulating serovars within Canada, iii) animal surveillance and research work, iv) current diagnostic techniques, and v) proof of concept wastewater source testing. Together, this presentation will demonstrate the landscape of Leptospirosis in Canada, and discuss the need for modern and scalable diagnostics as climatic changes continue, and extreme weather events such as flooding are expected to increase.

EPIDEMIOLOGICAL INVESTIGATION OF LEPTOSPIROSIS IN PUERTO RICO THROUGH HOSPITAL ACTIVE SURVEILLANCE AND COMMUNITY-BASED ANIMAL SAMPLING

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Leptospirosis is endemic to Puerto Rico, affecting people through seasonal increases and outbreaks. Surveillance activities were undertaken to improve the epidemiologic and ecologic knowledge of leptospirosis among acute undifferentiated febrile illness (AUFI) patients and identify exposure risks. Community-based animal surveillance was conducted to estimate prevalence in reservoirs, and investigate associated risk factors and circulating *Leptospira* species.

From 2019-2021, active surveillance was implemented in four hospitals using standardized recruitment and diagnostic methods (PCR and IgM/MAT serology). Animals were sampled from ten areas with a history of human cases and tested using PCR, culture, fluorescent antibody test (FAT), and/or MAT. Patient data was collected from EMR and interviews. Logistic regression models were used to examine risk factors for animals.

Leptospirosis was detected in 4% (16/406) of AUFI patients, who generally were early in the illness (94% were ≤7 days-post-onset) and exhibited a less severe presentation than the 13 routinely detected cases. Overall, leptospirosis cases were 71% male, average age was 43 years old, and the most common presentations included lower back pain (82%), headache (75%), fatigue (71%), vomiting (71%), myalgia (71%), and calf pain (64%). Positivity by PCR/FAT/culture was 62% (32/52) in mice and 43% (18/42) in rats. Seropositivity was 30% (19/63) in cattle, 33% (18/54) in dogs, and 20% (1/5) in cats. *Leptospira* species identified by *secY* sequencing in rodents were *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii*. Successful isolation identified predominantly serogroup Ballum in mice (73%) and Icterohaemorrhagiae in rats (94%). Dogs, cattle, and horses exhibited highest MAT titers to serogroup Icterohaemorrhagiae. Rodent positivity was higher in locations with dog presence (OR=3.8, 95% CI: 1-17). Dog seroprevalence was associated with cat presence (OR=10, 95% CI: 1.2-151). These findings underscore leptospirosis' animal and public health impact in Puerto Rico and the need for further research to identify prevention and intervention strategies.

SESSION 2

NEW CHALLENGES FOR CLINICS AND TREATMENT OF HUMAN LEPTOSPIROSIS

Moderators:
Yupin Suputtamongkol & Robyn A. Stoddard

4TH ELS MEETING
13TH ILS CONFERENCE
BRUSSELS
2-4 SEPTEMBER, 2024

Obstacles in Diagnosing Leptospirosis Clinically and Evidence-Based Treatment Approaches

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Weil's Disease is not challenging to diagnose. Clinicians worldwide, especially those in tropical and subtropical settings or those knowledgeable about tropical diseases, can often diagnose Weil's Disease with a high degree of certainty. However, Weil's Disease constitutes only a fraction of leptospirosis cases. Due to a variety of factors such as the infecting species and serovar, the dose of infection, individual susceptibilities, immune states, previous exposures, and numerous other contributory elements, the clinical presentation of leptospirosis varies widely. The lack of diagnostic tools in resource-poor settings where the disease incidence is high, leads to a diagnostic bias, favoring the identification of cases with classic symptoms and thus causing a publication bias. This overrepresents leptospirosis cases that exhibit classic symptoms of Weil's Disease. Systematic approaches to the clinical description of the disease and community-based studies on leptospirosis transmission indicate that most infections do not result in clinical manifestations and that when the disease is symptomatic, it often presents with non-specific symptoms that mimic other conditions. Patients may develop acute kidney injury, pancreatitis, respiratory failure, myocarditis, or other end-organ failures without jaundice, leading to misdiagnosis of leptospirosis. These clinical presentations often differ by region due to varying circulating serovars. Unless physicians are vigilant in their diagnostic efforts—which may not always be feasible in many settings due to the lack of point-of-care diagnostic tests—leptospirosis is likely to be underdiagnosed, potentially leading to serious complications. The challenge extends beyond diagnosis, as there is uncertainty surrounding the effectiveness of antibiotic therapy during the immune phase of the disease when complications become evident. Additionally, some current practices, like therapeutic plasma exchange, lack a strong evidence base and warrant further investigation. Clinical research on leptospirosis with a focus on patient care is scarce and needs to be prioritized in the tropical disease research agenda.

EPIDEMIOLOGICAL TRENDS AND CASES OF LEPTOSPIROSIS IN PEDIATRIC PATIENTS

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Introduction: Leptospirosis in children can be challenging because the disease has similar clinical presentations to other common tropical diseases, and pediatric patients exhibit a variety of symptoms beyond the clinically known Weil's Disease. This analysis aims to describe cases of leptospirosis in pediatric patients reported by jurisdictions to the Centers for Disease Control and Prevention (CDC) from 2014 to 2020.

Material and methods: Pediatric cases (0 to 19 years old) reported to CDC between 2014-2020 were included in this analysis. The analysis included case surveillance data (demographic data) and supplemental data (clinical presentation, treatment, and risk factors) submitted voluntarily by jurisdictions.

Results: During the selected seven-year period, 99 pediatric cases were reported to the CDC; 40% of reported cases were confirmed, while the remainder were probable. Most pediatric cases reported were male (over 76%) and the majority (85%) of these cases were between 10 and 19 years of age. More cases reported exposure to freshwater or mud than to animals. Forty-five cases required hospital admission, with fever, headache, and myalgia being the most common symptoms in children.

Conclusions: This analysis represents the first-time pediatric leptospirosis cases reported to the CDC have been summarized and presented. It outlines the clinical presentation of leptospirosis and its common exposures, emphasizing the importance of early detection and diagnosis of the disease. These results will assist in raising awareness about this disease amongst clinicians and public health practitioners in the United States, aiding public health officials in disseminating preventative measures to improve disease outcomes among pediatric cases.

UPDATES FOR COCHRANE SYSTEMATIC REVIEWS OF TREATMENTS FOR LEPTOSPIROSIS

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Introduction: Leptospirosis is a zoonotic bacterial disease affecting vulnerable populations. Antibiotics and corticosteroids have been used for the prevention or treatment of disease, in the absence of comprehensive clinical evidence to inform patient management. We present the results of the updated Cochrane systematic reviews and meta-analyses evaluating prophylactic antibiotics, antibiotic treatment, and corticosteroids for leptospirosis.

Material and methods: Randomised clinical trials of any design from an electronic search of 14 databases assessing antibiotics or corticosteroids of leptospirosis were included.
For all reviews primary outcomes for all review were all-cause mortality and serious adverse events; and secondary outcomes included quality of life and non-serious adverse events.

Results: Five trials (2,593 participants) were identified for the review of prophylactic antibiotics, nine trials for the antibiotic treatment (1,019 participants), and four trials (243 participants) for the corticosteroid treatment review.
It is unclear if prophylactic antibiotics (RR 0.15, 95% CI 0.01 to 2.83), antibiotics for treatment (RR 1.57, 95% CI 0.65 to 3.79), or corticosteroids (RR 1.04, 95% CI 0.38 to 2.80) reduce all-cause mortality.
In the review of prophylactic antibiotics and corticosteroids, it was unclear if there is an effect on the proportion of individuals experiencing serious adverse events. There may be an increase in non-serious adverse events (RR 10.13, 95% CI 2.40 to 42.71) with prophylactic antibiotics compared to placebo.
It was unclear if most trials recruited to a full sample size based on a pre-specified primary endpoint. All analyses were judged to have a low-to-very-low certainty of evidence.

Conclusion: Evidence of the benefits or harms of treatments administered for Leptospirosis remains inconclusive and lack rigour.
Harmonised treatment strategies, clinically relevant outcome definitions, and definitive rigorously designed randomised trials to support the use of antibiotics and corticosteroids for leptospirosis are lacking. Future clinical research should focus on these evidence gaps.

INFLAMMATORY PROTEIN PROFILING IN HUMAN LEPTOSPIROSIS: UNVEILING BIOMARKERS FOR SEVERITY

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Background: Leptospirosis infection poses a significant global health burden, yet the underlying immunopathogenesis remains poorly elucidated. To advance our comprehension of the host inflammatory response and to identify potential biomarkers for severe leptospirosis.

Methods: Plasma samples from 88 leptospirosis patients were tested upon admission and categorized into severe (n=44) and non-severe (n=44) groups based on longitudinal clinical and laboratory data. Using the Olink Target 96 Inflammation panel, we measured the expression levels of the 92 inflammatory proteins. Differential Protein Expression Analysis and principal component analysis were employed to compare protein profiles between the two groups. We assessed the discriminatory potential of these proteins as early diagnostic biomarkers for severe leptospirosis.

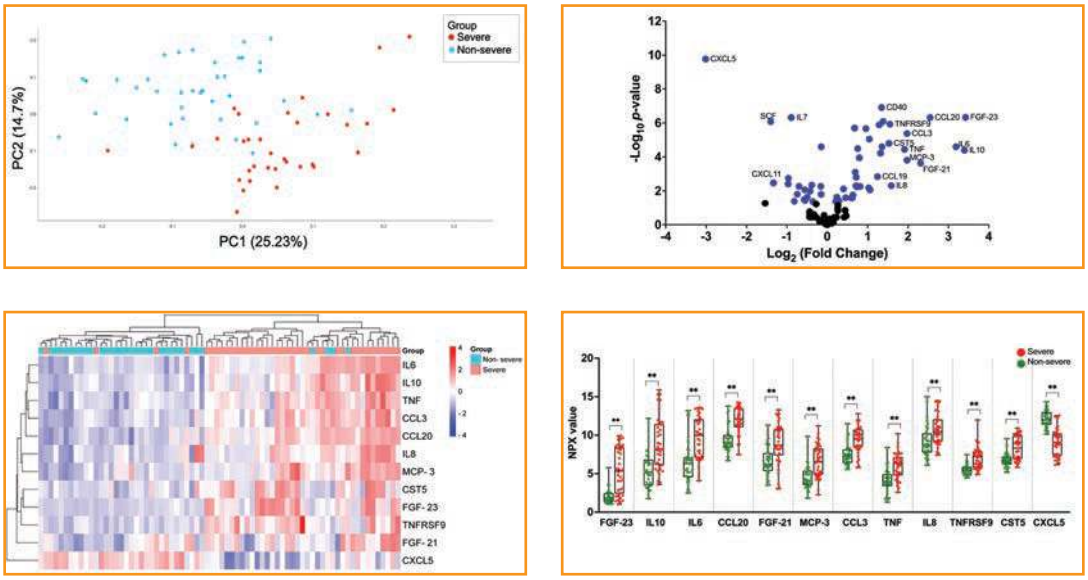


Figure 1 *Proteomic profiling of plasma from severe and non-severe leptospirosis using the Olink Proteomics-96 Inflammation Panel. (A) Principal component analysis demonstrates the grouping of severe and non-severe cases based on PC1 and PC2. (B) The volcano plot compares severe versus non-severe samples, highlighting significantly and non-significantly differentially expressed protein targets in blue and black, respectively. (C) Heatmap shows 12 differentially expressed proteins between severe and non-severe patients. (D) Box plot represents 12 significantly differentially expressed proteins between severe and non-severe patients.*

Results: We identified 12 differentially expressed proteins between the severe and non-severe groups (Fold change ≥1.5 and P-value <0.05). Among these, 11 were up-regulated (FGF-23, IL10, IL6, CCL20, FGF-21, MCP-3, CCL3, TNF, IL8, TNFRSF9, CST5), while 1 was down-regulated (CXCL5). Functional enrichment analysis revealed enrichment in key gene ontology terms such as leukocyte migration, leukocyte chemotaxis, and inflammatory response, along with involvement in several pathways like cytokine-cytokine receptor interaction and IL-17 signaling. Notably, CCL20 and CXCL5 exhibited the highest degree scores in the protein-protein Interaction network, suggesting their potential central role in severe leptospirosis pathogenesis. Moreover, CXCL5, CCL20, FGF-21 and demonstrated an area under the ROC curve of ≥0.80 for discriminating between severe and non-severe cases.

Conclusions: Our findings display distinct plasma proteomic profiles associated with different outcomes in leptospirosis patients, highlighting CCL20, FGF-21, and CXCL5 as novel potential biomarkers for severe leptospirosis. These results offer valuable insights into disease pathogenesis and may pave the way for improved diagnostic and therapeutic strategies.

DISRUPTION OF EPITHELIAL CELL MECHANOSENSING DURING PATHOGENIC *LEPTOSPIRA* INFECTION

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Introduction: A challenging area of basic research is to find a universal cellular pathway to prevent and/or treat leptospirosis caused by genetically diverse pathogenic *Leptospira*. Mechanosensors, such as E-cadherin and Piezo1, located at the plasma membrane are important for the transduction of the mechanical forces into biochemical signals to maintain normal cellular homeostasis. We have previously reported that *L. interrogans* as well as *L. biflexa* induce E-cadherin cleavage, however, only the pathogenic one causes the destruction of the epithelial monolayer by hijacking eukaryotic proteolytic systems [1, 2]. In this study, we hypothesized that *L. interrogans* can also disrupt host cell mechanosensing and focused on Piezo1 to analyze how is affected during leptospiral infection.

Materials and methods: Renal proximal tubule epithelial cells (RPTECs) were cultured in transwells for 14 days to allow monolayer maturation. The transepithelial electrical resistance (TEER) was measured to evaluate epithelial barrier. RPTECs were infected with *L. interrogans* serovar Manilae strain UP-MMC-NIID and fixed with 2% paraformaldehyde for immunofluorescence stainings. Bafilomycin A and MG-132 inhibitors were added before infection or at 8 hours post-infection (p.i.) to analyze their effect on Piezo1 cellular localization.

Results: Confocal microscopy analysis showed that in non-infected RPTECs, Piezo1 localized mainly at the plasma membrane. At 24 p.i., *L. interrogans* induced a decrease of TEER and the mislocalization of Piezo1. Addition of the inhibitors at both time points prevented displacement of Piezo1 from the plasma membrane and monolayer destruction.

Conclusion: Our results showed that the proteasomal hijack by *L. interrogans* is a late event during RPTEC infection. The results suggested a biomechanical crosstalk to preserve epithelial barrier. The manipulation of this crosstalk might be a universal leptospiral target to establish a successful infection.

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D1-39 Tachaboon S

RENAL COLONIZATION AND ACUTE KIDNEY INJURY IN LEPTOSPIROSIS

D1-40 Tran PNT

MICRORNAS AS BIOMARKERS OF SEVERE PULMONARY HEMORRHAGIC SYNDROME IN LEPTOSPIROSIS PATIENTS: A MULTI-OMICS AND MULTI-CENTER STUDY

D1-41 Vernel-Pauillac F

TLR-BASED HOST-DIRECTED STRATEGY TO ALLEVIATE LEPTOSPIRAL INFECTION IN A MOUSE MODEL

D1-42 Yang C-W

ZERO DEATH BY EARLY IDENTIFICATION OF LEPTOSPIROSIS USING RAPID IGM ASSAY AND APPROPRIATE ANTIBIOTICS- A 20-YEAR COHORT

RENAL COLONIZATION AND ACUTE KIDNEY INJURY IN LEPTOSPIROSIS

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Introduction: Despite the kidney being a primary target for *Leptospira* during acute disease, the relationship between renal colonization, urinary shedding of pathogenic *Leptospira*, and AKI remains poorly understood.

Material and methods: A prospective study was conducted, recruiting 201 patients with clinical suspicion of leptospirosis from 15 hospitals in the Sisaket province of Thailand. Among them, 152 were categorized as non-AKI and 49 as AKI based on Kidney Disease Improving Global Outcomes (KDIGO) criteria. Urine samples obtained on the first day of clinical suspicion underwent quantitative PCR targeting the LipI32 gene.

Results: The proportion of positive leptospiruria was significantly higher in the AKI group (44.9%) compared to the non-AKI group (27.6%, p=0.024). Furthermore, there was a trend toward higher leptospiruria levels in the AKI group compared to the non-AKI group (median level of 9,680 vs. 3,265 copies/mL, p=0.07).

Conclusion: These results suggest a potential link between renal colonization, urinary shedding of leptospires, and AKI in leptospirosis patients. Further investigation is warranted to unravel the underlying mechanisms of this association and to understand its implications for the diagnosis and management of AKI in leptospirosis.

Table 1 *Detection of leptospire in urine stratified by AKI status.*

Detection of leptospires in urine by qPCR (n, %)	All patients (n=201)	AKI (n=49)	Non-AKI (n=152)	*p*-value
Negative	137 (68.2)	27 (55.1)	110 (72.4)	0.024
Positive	64 (31.8)	22 (44.9)	42 (27.6)	

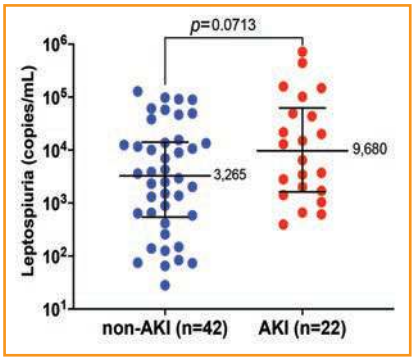


Figure 1 *Leptospiruria levels stratified by AKI status.*

MICRORNAS AS BIOMARKERS OF SEVERE PULMONARY HEMORRHAGIC SYNDROME IN LEPTOSPIROSIS PATIENTS: A MULTI-OMICS AND MULTI-CENTER STUDY

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Introduction: Leptospirosis is among the most widespread zoonoses worldwide. Severe pulmonary hemorrhagic syndrome (SPHS) represents a fatal complication in leptospirosis patients, with poor understanding of the underlying mechanisms and effective biomarkers.

Material and methods: A nested case-control analysis of the blood specimens obtained from two previous multi-center cohorts was conducted. Candidate microRNAs were initially discovered through a global profiling of 800 serum microRNAs in severe non-SPHS and five SPHS patients, then validated in the full sample size. We further conducted a multi-omics analysis incorporating data from transcriptomics and proteomics experiments to identify enriched pathways through which the newly identified microRNAs could regulate.

Results: A total of 28 SPHS and 140 non-SPHS patients were included. Serum microRNA profiling revealed distinct expression patterns between the two phenotypes. From the top 81 significantly expressed microRNAs, seven were selected for validation. Among these, miR-5010-3p and miR-147b had area under the curve (AUC) values of 0.72 (95% CI: 0.62-0.81) and 0.66 (95% CI: 0.55-0.76) for discriminating SPHS. Notably, the two microRNAs could detect SPHS in patients who were yet to manifest chest radiograph shadows at serum collection, and in a subgroup of patients who were recruited on day 2 or earlier of illness, with consistent AUC values. A two-micro-RNA model including miR-5010-3p and miR-548ai substantially increases predictability. Over 1000 differentially expressed molecules were found in each transcriptomics and proteomics layer. Integrated gene-protein pathway enrichment analysis revealed numerous pathways involving immune responses. Among these, tumor necrosis factor signaling, and its downstream pathways were the most significant, with many of its member genes being targeted by miR-5010-3p or miR-147b.

Conclusion: MiR-5010-3p and miR-147b are promising biomarkers of leptospirosis SPHS with robust predictability and biological relevance. Our findings should pave the way for developing miRNA-based prognostic assays and personalized therapeutic strategies for leptospirosis SPHS patients.

A

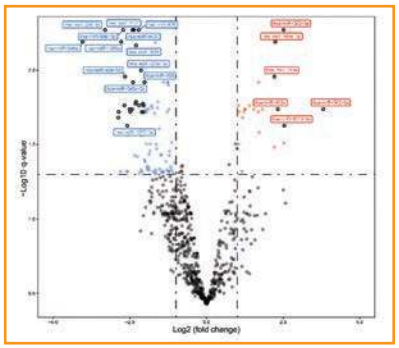


Figure 1A *Global profiling of the serum microRNAs revealed a total of 81 significantly different microRNAs.*

B

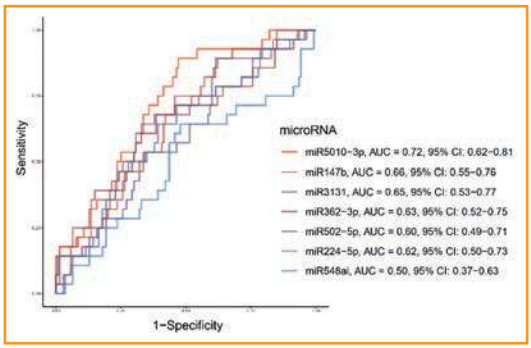


Figure 1B *Receiver Operating Characteristic (ROC) curve analysis confirmed the discriminatory ability of the candidate microRNAs for SPHS progression.*

C

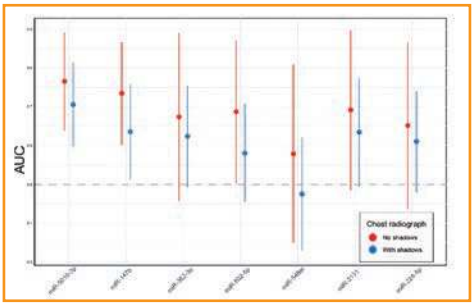


Figure 1C *MiR-5010-3p and miR-147b could predict SPHS even in patient who were yet to have chest radiograph manifestation typical of leptospirosis SPHS at serum collection.*

D

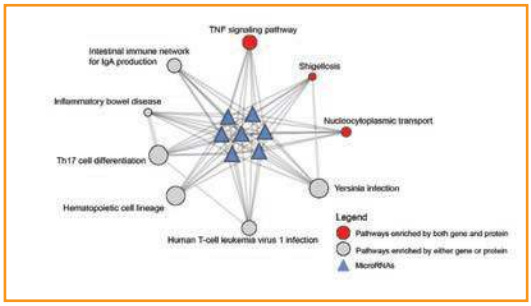


Figure 1D *The integrative gene-protein pathway enrichment analysis revealed TNF signalling pathway as among the most significant pathways.*

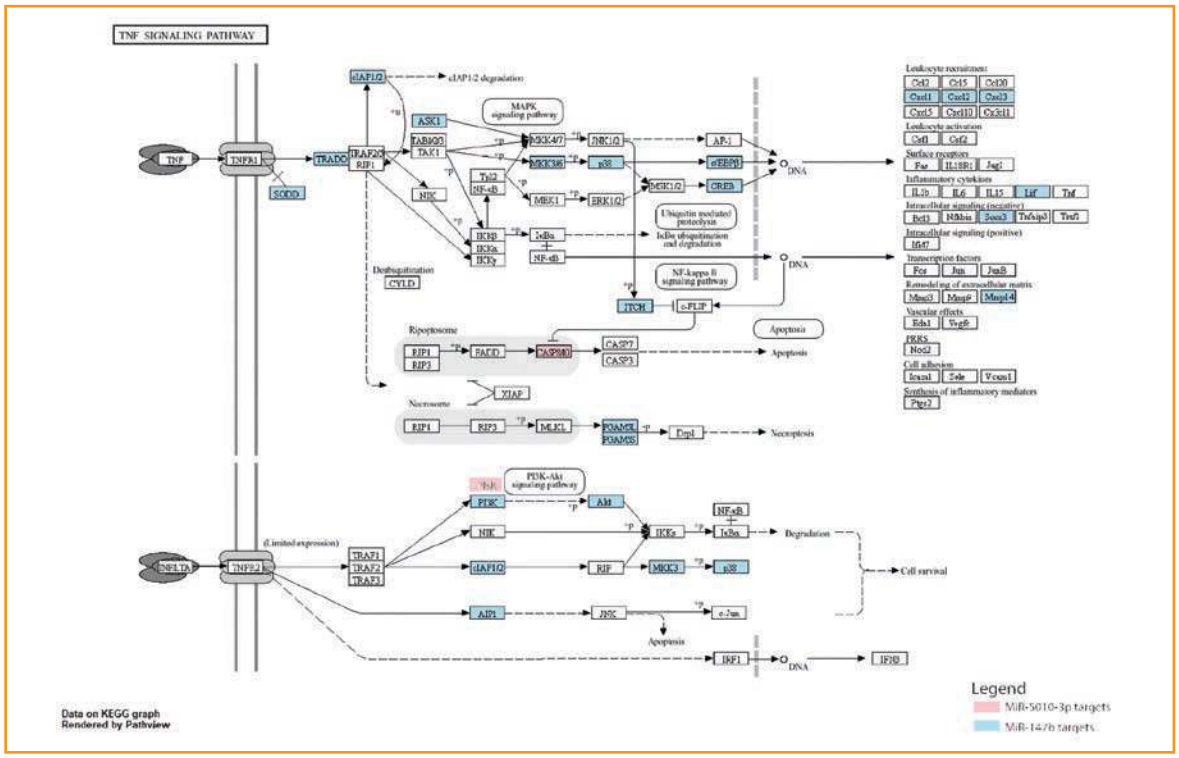


Figure 1E *MiR-5010-3p and miR-147b might regulate TNF signaling and its downstream pathways through a number of target genes.*

TLR-BASED HOST-DIRECTED STRATEGY TO ALLEVIATE LEPTOSPIRAL INFECTION IN A MOUSE MODEL

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Leptospirosis is a neglected and re-emerging zoonosis with a worldwide distribution caused by the spirochete *Leptospira interrogans*. The reservoir of infection includes a variety of mammals that are asymptomatic chronic carriers, excreting the bacteria through urine into the environment. The high number of serovars has hampered the development of a universal, cross-protective human vaccine. Some bacterin-based preventive vaccines have been licensed but these confer only short-term, serovar-specific immunity and are associated with side effects. In animal models, some live attenuated or subunit vaccines proved effective after a lethal challenge but failed to prevent kidney colonization. Innovative strategies are therefore needed to combat leptospirosis.

Our group and others have shown that pathogenic leptospires evade the host immune system by subverting the complement system[1] and recognition by Toll-like (TLR) and NOD receptors[2,3]. We also previously showed that the prophylactic administrations of a dual TLR/NOD agonist induce trained immunity and alleviate leptospirosis[4].

Here, using a murine model of leptospirosis and bioluminescent *Leptospira* strain, enabling bacteria to be tracked by live imaging[5], we investigated the efficacy of another TLR agonist in restoring/complementing host defenses. We assessed its impact on the evolution of the infection, whether as part of a preventive or therapeutic protocol.

This study highlights the interest of TLR agonists to significantly alleviate the renal colonization after a single prophylactic injection. The cellular mechanisms leading to this protective effect are currently being studied and would constitute a promising approach for novel strategies against leptospirosis.

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ZERO DEATH BY EARLY IDENTIFICATION OF LEPTOSPIROSIS USING RAPID IGM ASSAY AND APPROPRIATE ANTIBIOTICS- A 20-YEAR COHORT

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Introduction: Leptospirosis is a prevalent zoonotic disease worldwide; however, it is often under-reported due to its varied symptoms and lack of awareness among healthcare workers in Taiwan.

Material and Methods: A surveillance study was conducted at Chang Gung Memorial Hospital, Taiwan, from September 2003 to December 2023. The study focused on 1281 patients with multiple organ dysfunction syndrome who had an unexplained cause or were clinically suspected of having leptospirosis. A prompt IgM screening test was conducted taking into consideration the infection features of the patients. The diagnosis was subsequently verified using either a microscopic agglutination test or the detection of *Leptospira*.

Results: A total of seventy patients were diagnosed with leptospirosis, representing 5.5% of the overall number of patients with multiple organ dysfunction syndrome. Achieving zero mortality was attainable by administering adequate antibiotics as early as possible during the course of infection. Among those, 17 received treatment with Ceftriaxone whereas 10 were treated with penicillin. Both medications successfully restored kidney function upon admission and treated the systemic illness. The predominant manifestations of leptospirosis were fever, acute kidney injury and jaundice. Since the implementation of the rapid IgM serology assay in 2003, zero deaths have been observed in the group of individuals with leptospirosis. This assay has demonstrated a significantly high level of sensitivity and specificity. Conclusion: Leptospirosis is a significant factor in causing dysfunction in numerous organs in Taiwan, particularly acute kidney injury. Timely recognition of leptospirosis through distinctive symptoms, along with immediate administration of antibiotics, can significantly improve patient outcomes. A rapid IgM serology assay is a suitable screening test for the diagnosis of leptospirosis due to its ease of performance and quick results followed by prompt and effective treatment.

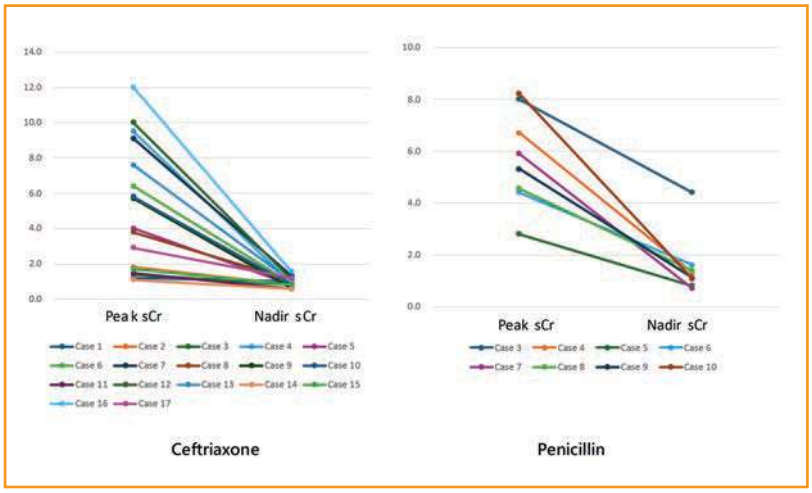


Figure *Effective antibiotic therapy Ceftriaxone vs Penicillin.*

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SESSION 3

ANIMAL LEPTOSPIROSIS IN DOMESTIC AND WILDLIFE SPECIES

Moderators:
Josipa Habus & Cord Heuer

4TH ELS MEETING
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Animal Leptospirosis in domestic and wildlife species

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Lead Scientist on Spirochete Research, Infectious Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States

Leptospirosis, caused by pathogenic species of the genus *Leptospira*, is a zoonotic disease of global significance. Disease transmission to incidental humans and animals is maintained by the persistent colonization and excretion of *Leptospira* from asymptomatic domestic and wildlife reservoir hosts of infection that shed leptospires via urine or the genital tract. More than 40 pathogenic species comprising hundreds of antigenically distinct serovars have been identified to date and knowledge of their interactions with incidental and reservoir hosts is essential to understand the epidemiology of leptospirosis. Reservoir hosts can excrete more than one species/serovar concurrently. Though difficult, isolation of leptospires from reservoir hosts provides a definitive diagnosis: advantages of culture include the recovery of local circulating isolates that can be comprehensively characterized by genome sequencing and serovar typing, used in MAT antigen diagnostic panels, bacterin vaccines, and applied in research to understand pathogenic mechanisms of infection. Our research group has challenged dogma in the field by 1) isolating leptospires from infected animals at 37°C in addition to the conventional 28-30°C to characterize novel species and serovars associated with different animal and environmental reservoirs; 2) using CRISPR strategies to generate mutants in different species and serovars of recent clinical isolates to elucidate pathogenic mechanisms of infection and 3) identifying novel markers of virulence such as circulating foamy macrophages in hamster and deer mice models of leptospirosis. Collectively, results are being used to bring us closer to the goal of achieving efficacious prophylactic measures to reduce acute leptospirosis and asymptomatic disease transmission in domestic and wildlife animal species.

POTENTIAL LEPTOSPIRAL SHEDDING IN VACCINATED AND ASYMPTOMATIC PACK HOUNDS: A ROSPECTIVE STUDY IN 56 DOGS

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Introduction: Asymptomatic carriage of leptospires in dogs may pose a risk of contamination for humans and congeners. The aim of this prospective study was to determine the rate of asymptomatic carriage of pathogenic leptospires in vaccinated pack hounds and to monitor their carriage status over time.

Materials and methods: A total of 56 apparently healthy adult pack dogs were enrolled. They were living in a kennel that was open to a grassy yard, next to forest and ponds, in direct contact to wildlife. All dogs had received a quadrivalent vaccine. Micro-Agglutination Test (MAT) and polymerase chain reaction (PCR) on blood were performed for each dog. PCR was performed on urine from a subset of dogs for which urine could be obtained by free catch (n=32). After eleven months, PCR was repeated on blood and urine from PCR-positive dogs still present in the pack. Positive PCR results were confirmed by conventional 16s rRNA PCR and subsequent typing.

Results: Ten of 32 dogs tested (31%) had positive PCR results on urine (2 with maximal MAT titers of 1:1600 for Australis and 8 with all MAT titers < 1:200). One of 56 dogs (2%) had positive PCR results on blood and undetectable MAT titers. Genomic species were identified in 9/11 cases (*L. borgpetersenii* in 5 cases; *L. interrogans* in 4 cases). Among the whole population, seroreactivities were mainly observed against serogroups Australis and Sejroe. Eleven months later, 8/11 PCR-positive dogs were still in the pack and 2 of them were still PCR-positive on their urine. The dog with a blood-positive PCR at first sampling had left the pack.

Conclusion: This study suggests that pack hounds may shed pathogenic leptospires despite vaccination with a tetravalent vaccine. Excreting DNA is not an evidence of excreting viable bacteria. Further studies including leptospiral urine cultures could clarify the signification of these preliminary results.

MONITORING LEPTOSPIROSIS IN ISRAEL'S WILD FAUNA: A TEN-YEAR STUDY HIGHLIGHTING THE SPREAD OF SEROGROUP POMONA

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Introduction: Since 2009, Israel has intensified Leptospirosis monitoring in wildlife due to the spread of serogroup Pomona.

Materials and Methods: Animals found deceased or captured during population control were tested by microagglutination for antibodies against eight serogroups (positive titers ≥100), and by LipL32 and 16S PCR from the kidneys. *Leptospira* species were identified by secY sequencing.

Results: Seroprevalence between 2009 and 2023 was 4% (n=3,403 samples); and was significantly higher in wild boars (12%, n=770 samples) than carnivores (2%, n=1,186 samples) and other species (1%, n=1,447 samples, primarily wild ruminants (98%) and porcupines). Wild boar seroprevalence peaked in 2017 (24%, n=34 samples), preceding significant leptospirosis outbreaks in humans and cattle[1–3]. Pomona was the major reactive serogroup (85%), with secondary serogroups including Bratislava in wild boars (13%), Canicola and Ballum in carnivores (26% and 22%, respectively), and Sejroe in ruminants (50%). Titers above 400 were predominantly found in wild boars (35% of positive-wild boars), mainly against serogroup Pomona (94% of high-titer wild boars), suggesting recent infections. To date, 17% of kidney samples were PCR-positive (n=168 samples); with wild boars representing 64% of samples, and 28 of 29 PCR-positives. *L. interrogans* was identified in all samples but three, where *L. noguchii* was found. In 2024, one porcupine tested PCR-positive and had high titers for serogroup Pomona.

Conclusion: The results suggest that wild boars may be an important reservoir of pathogenic *Leptospira* spp. from serogroup Pomona, the main serogroup found in domestic animals in Israel; while porcupines' role in leptospirosis epidemiology deserves further investigation. Increasing populations of wild boars and movement due to urbanization may be facilitators of the spread of serogroup Pomona in the country. This study underscores the importance of continuous monitoring and control measures in wildlife as part of Leptospirosis management.

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SEROPREVALENCE OF LEPTOSPIROSIS IN SELECTED LIVESTOCK SPECIES AND THE FIRST REPORT OF *LEPTOSPIRA BORGPETERSENII* SEROVAR TARASSOVI ISOLATED IN THE PHILIPPINES

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Keywords: *Leptospira borgpetersenii,* livestock, the Philippines, rodent, Tarassovi

Leptospirosis is a worldwide zoonosis caused by the pathogenic members of the Genus *Leptospira.* The rat-to-human transmission of *Leptospira* has been widely demonstrated as the common route of transmission in urban areas. However, in rural areas, the contribution of livestock species remains to be elucidated. Hence, this study aimed to examine the seroprevalence of leptospirosis in livestock and wild rodents and to isolate *Leptospira* spp. in livestock and wild rodents.

Serum from cattle, water buffalo, pigs, and wild rodents were collected and examined against 9 *Leptospira* serovars, and urine and kidney samples were collected from livestock and wild rodents, respectively for *Leptospira* isolation.

Seropositivity to at least one serovar was 98.00%, 90.70%, 53.00%, and 51.70% for cattle, water buffalo, pigs, and wild rodents, respectively. Furthermore, there were 2 isolates and 12 isolates from water buffalo urine and wild rodent kidney, respectively. One *Leptospira* isolates from wild rodent kidneys was revealed to be *Leptospira interrogans* serovar Grippotyphosa and both isolated from water buffaloes were *Leptospira borgpetersenii* serovar Tarassovi. However, both RFLP and/or MALDI-TOF-MS and PFGE revealed that all of these isolates are not related to the reference strains used in the study. It is recommended that seroepidemiological study of leptospirosis should be done regularly since the current generation of *Leptospira* vaccine does not confer heterologous and sterile immunity.

Lastly, the isolated *L. borgpetersenii* serovar Tarassovi , to the best of our knowledge, was isolated for the first time in water buffaloes in the Philippines, and is one of the predominant serovar infecting water buffaloes. However, there is no vaccine specifically made to protect water buffaloes or cattle from this serovar. Thus, targeted vaccine development against this serovar is recommended to further improve leptospirosis control and prevention in the Philippines.

OPHTHALMIC MANIFESTATION OF ACUTE CANINE LEPTOSPIROSIS

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Introduction: Leptospirosis is a re-emerging zoonosis with a wide spectrum of clinical signs. Ophthalmological changes, especially uveitis, have been described in humans as a complication of systemic infection. A similar condition, characterised by recurrent episodes of inflammation, is seen in horses in a chronic form, while in dogs, although occasionally described as conjunctivitis, uveitis, retinal haemorrhages and retinal detachment, it is still relatively poorly understood.
The aim of this study was to identify ophthalmological signs in dogs in the acute phase of the disease.

Material and methods: On the first day of admission, a total of six dogs of different breeds and age groups with confirmed leptospirosis underwent a complete ophthalmological examination. Postmortem vitreous and aqueous humour samples were collected from two non-surviving dogs and subsequently tested with MAT and PCR.

Results: None of the dogs examined showed signs of vision impairment, conjunctival changes or corneal damage. Intraocular pressure was normal in five dogs (83.3 %), myosis was observed in three dogs (50 %) and all dogs were flear-negative. Low intraocular pressure with a ciliary flush on the corneal surface was observed in one dog. Retinal haemorrhages were observed in four dogs (66.6%) in one or both eyes without retinal detachment. The optic disc showed no abnormalities. In addition, one dog was found to have a positive post-mortem titre and PCR assay from a vitreous sample.

Conclusion: We have observed a high incidence of various ocular disorders in the acute phase of leptospirosis in dogs. This study contributes to the understanding of ocular manifestations in leptospiral infections and highlights the need for further clinical research and monitoring of ophthalmological pathology with a larger cohort.

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List of poster abstracts

ANIMAL LEPTOSPIROSIS IN DOMESTIC AND WILDLIFE SPECIES

D2-1 Ayral F

CHARACTERISATION OF PATHOGENIC *LEPTOSPIRA* IN INVASIVE RACCOONS (*PROCYON LOTOR*) IN NORTH-EAST AND SOUTH-WEST FRANCE

D2-2 Ayral F

EVIDENCES FOR MAINTENANCE HOST OF PATHOGENIC *LEPTOSPIRA* RELATED TO THE SEROGROUP AUSTRALIS IN HEDGEHOGS (*ERINACEUS EUROPAEUS*) FROM A WILDLIFE HEALTH CENTRE IN NORTHWESTERN FRANCE

D2-3 Barbosa AS

PROTEOLYTIC PROFILE OF *LEPTOSPIRA* STRAINS ON COMPLEMENT SYSTEM AND EXTRACELLULAR MATRIX MOLECULES

D2-4 Benvin I

INSIGHTS INTO CLINICAL MANIFESTATIONS OF *LEPTOSPIRA* SPP. INFECTION IN PET CATS AND ASSOCIATED RISK FACTORS

D2-5 Cohen O

FIRST ISOLATION OF *LEPTOSPIRA* SPP. IN ISRAEL: A CASE OF SEROLOGICAL DISCREPANCY

D2-6 Brihuega B

DETECTION OF LEPTOSPIRES IN WILD ANIMALS IN AQUATIC ENVIRONMENTS OF ARGENTINA

D2-7 Ciuffo C

LEPTOSPIRA INTERROGANS, *L. BORGPETERSENII* AND *L. NOGUCHII* ISOLATED FROM CATTLE SHOW DIFFERENT VIRULENCE CHARACTERISTICS TO ESTABLISH KIDNEY COLONIZATION IN A MURINE MODEL OF SUBLETHAL INFECTION

D2-8 D'Incau M

HIGH *LEPTOSPIRA* SEROPREVALENCE IN FREE-LIVING POND SLIDERS (*TRACHEMYS SCRIPTA*): WHICH EPIDEMIOLOGICAL ROLE DO THEY PLAY?

D2-9 D'Incau M

APPLICATION OF A PROTOCOL FOR CONTROL/ERADICATION OF LEPTOSPIROSIS IN A LARGE DAIRY FARM IN ITALY

D2-10 Delooz L

10 YEARS OF MONITORING OF LEPTOSPIROSIS IN CATTLE IN SOUTHERN BELGIUM

D2-11 dos Santos Ribeiro P

ISOLATION AND CHARACTERIZATION OF LEPTOSPIRES ASSOCIATED WITH EQUINE RECURRENT UVEITIS

D2-12 Dubniewicz K

TRANSCRIPTOME AND PROTEOME ANALYSIS REVEALED KEY PATHWAYS REGULATING PATHOGENIC MECHANISM OF THE LEPTOSPIRA INTERROGANS SEROVAR HARDJO

D2-13 Gomes T

INFECTION BY *LEPTOSPIRA SANTAROSAI* SEROVAR GUARICURA STRAIN VF52 IN HAMSTER ANIMAL MODEL

D2-14 Hahn BL

A MODEL OF HEMATOGENOUS DISSEMINATION OF *LEPTOSPIRA* TO MOUSE TISSUES

D2-15 Shyma VH

SPATIAL EPIDEMIOLOGY AND COMPARATIVE EVALUATION OF DIAGNOSTIC ASSAYS FOR LEPTOSPIROSIS AMONG LIVESTOCK IN NORTHERN KERALA, INDIA

D2-16 Hamond C

ISOLATION OF *LEPTOSPIRA* FROM CATTLE IN THE UNITED STATES

D2-17 LeCount K

LEPTOSPIROSIS IN UNITED STATES ZOOS

D2-18 Magliocca M

FIRST DETECTION OF *LEPTOSPIRA* ST198 RELATED TO *L. INTERROGANS* SEROGROUP AUSTRALIS IN RED FOXES (*VULPES VULPES*) IN NORTHERN ITALY

D2-19 Pardyak L

HOST-BACTERIAL INTERACTIONS LEAD TO VARIED OUTCOMES AMONG PATHOGENIC LEPTOSPIRA SEROVAR HARDJO SPECIES- *IN-VIVO* AND *IN-VITRO* STUDY

D2-20 Putz EJ

PEROMYSCUS DEER MICE AS AN ACUTE RODENT MODEL OF LEPTOSPIROSIS

D2-21 Ristiyanto

ESTIMATION OF BI-INDEX, ABSOLUTE DENSITY OF RATS AND LEPTOSPIROSIS CASES IN CENTRAL JAWA

D2-22 Schmitt KG

LEPTOSPIRA SPP.-SPECIFIC ANTIBODY RESPONSES IN HEALTHY DOGS AFTER VACCINATION AGAINST LEPTOSPIROSIS WITH A 4-SEROVAR VACCINE

D2-23 Selemani M

PATHOGENIC *LEPTOSPIRA* IN RODENTS AND *CROCIDURA* SP: AN EMMEDIATE THREAT TO HUMAN HEALTH IN WETLANDS IN THE KILOMBERO VALLEY, TANZANIA

D2-24 Sohail ML

EVIDENCE OF CLINICOPATHOLOGICAL CHANGES DURING EQUINE LEPTOSPIROSIS

D2-25 Sohail ML

SEROPREVALENCE OF *LEPTOSPIRA* SPP. IN HORSES OF DISTINCT CLIMATIC REGIONS OF PUNJAB, PAKISTAN

D2-26 Soro SD

DYNAMICS OF LEPTOSPIROSIS IN AN URBAN NORWAY RAT (*RATTUS NORVEGICUS*) POPULATION IN DENSELY POPULATED AREA OF LYON AND BORDEAUX (FRANCE)

D2-27 Strutzberg-Minder K

LEPTOSPIRA SEROPREVALENCE IN SOW HERDS IN LATIN AMERICA

D2-28 Tomckowiack C

SEROPREVALENCE OF LEPTOSPIROSIS IN WILD RODENTS ACROSS AN ANTHROPIZED AND FRAGMENTED LANDSCAPE IN SOUTHERN CHILE

D2-29 Vyn CM

SOCIAL AND ENVIRONMENTAL RISK FACTORS FOR CANINE LEPTOSPIROSIS: A SCOPING REVIEW

D2-30 Vyn CM

INVESTIGATING THE INFLUENCE OF SOCIOECONOMIC STATUS ON CANINE LEPTOSPIROSIS IN CANADA

D2-31 Willixhofer D

LEPTOSPIRA ISOLATES FROM DOMESTIC LIVESTOCK ANIMALS AND WILDLIFE IN LOWER AUSTRIA

CHARACTERISATION OF PATHOGENIC *LEPTOSPIRA* IN INVASIVE RACCOONS (*PROCYON LOTOR*) IN NORTH-EAST AND SOUTH-WEST FRANCE

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Leptospirosis is a widespread zoonosis caused by bacteria of the genus *Leptospira*. Although crucial to mitigate the disease risk, basic epidemiological information is lacking, such as the identities of *Leptospira* maintenance hosts. The raccoon (*Procyon lotor*). an invasive species in France, could pose a public health risk if it carries pathogenic *Leptospira*. We investigated the rate and type (selective *vs* unselective) of *Leptospira* carriage in France's two main raccoon populations. Out of the 141 raccoons collected, seven (5%) tested RT-PCR positive (*lfb1* gene) based on kidney, lung, and urine samples when available. Phylogenetic analysis revealed the presence of three different *L. interrogans* clusters. The results suggest that raccoons are more likely accidental hosts and make only a limited contribution to *Leptospira* maintenance.

EVIDENCES FOR MAINTENANCE HOST OF PATHOGENIC *LEPTOSPIRA* RELATED TO THE SEROGROUP AUSTRALIS IN HEDGEHOGS (*ERINACEUS EUROPAEUS*) FROM A WILDLIFE HEALTH CENTRE IN NORTHWESTERN FRANCE

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Leptospirosis is a widespread zoonosis caused by bacteria in the genus *Leptospira*. Although crucial to mitigating disease risk, basic epidemiological information is lacking, such as the identities of *Leptospira* maintenance hosts. Hedgehogs (*Erinaceus europaeus*) are frequently observed near human facilities and are frequently conducted to wildlife rescue centres when found in distress. Thus, they could represent a risk to (veterinary) public health if carrying pathogenic *Leptospira*. We investigated the frequency and diversity of *Leptospira* carriage of hedgehogs present in a wildlife rescue centre between April and June 2022. Among the 72 hedgehogs, 25 (35%) tested RT-PCR positive on urine samples. Typing analysis revealed the presence of a unique *L. interrogans* profile related to the serogroup Australis. The infectious status had no impact on urinalysis parameters. Our results suggest that the hedgehog could be a maintenance host of *Leptospira* in the ecosystem where they thrive.

PROTEOLYTIC PROFILE OF *LEPTOSPIRA* STRAINS ON COMPLEMENT SYSTEM AND EXTRACELLULAR MATRIX MOLECULES

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Young Golden Syrian hamsters and guinea pigs have been widely adopted as experimental animal models for the study of acute leptospirosis. Hamsters, in particular, are also generally used to maintain the virulence of *Leptospira* strains and to evaluate the protective efficacy of vaccines against the disease. Assuming that the hamster is a suitable model to study the pathological characteristics of *Leptospira* infection, the objective of this work was to infect hamsters with virulent, culture-attenuated and saprophytic strains of *Leptospira*, and assess if organ damage resulting from infection correlates with the proteolytic potential of each strain. Hamsters were infected with 10^7 *L. interrogans* serovar Copenhageni strain Fiocruz L1-130, *L. interrogans* serovar Manilae strain L495, *L. interrogans* serovar Canicola strain LO4, *L. kirschneri* serovar Cynopteri strain 3522C (culture-attenuated) or *L. biflexa* serovar Patoc strain Patoc I (nonpathogenic). Leptospires were isolated from kidneys and liver at day 4 (strains L1-130, L495 and LO4) or at day 21 (3522C and Patoc I) and allowed to grow in Ellinghausen-McCullough-Johnson-Harris medium. At passage 2, culture supernatants – previously shown to contain metalloproteases (Silva et al., Front Cell Infect Microbiol, 2018) - were collected after centrifugation, filtered, and incubated with human complement C3b, C4b and C5, and with fibronectin. Cleavage products were evaluated by Western blot with specific antibodies. Gelatinolytic activity was assessed by zymography. Histopathological analysis of lung, liver and kidneys were performed with all animals. According to our results, host molecules were efficiently cleaved in the presence of the three virulent strains supernatants (L1-130, L495 and LO4), with the α chain of C3b, C4b and C5 being more susceptible to degradation by L1-130 proteases. Patoc I did not display any significant proteolytic activity, and 3522C partially cleaved C5 and presented gelatinolytic activity. Correlations between the proteolytic potential of the strains and histopathological findings will be discussed.

INSIGHTS INTO CLINICAL MANIFESTATIONS OF *LEPTOSPIRA* SPP. INFECTION IN PET CATS AND ASSOCIATED RISK FACTORS

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Leptospirosis is a widespread zoonosis with a significant impact on human and animal health. This study aims to provide insights into the clinical features and identify associated risk factors to better understand the involvement of cats in the transmission cycle of leptospirosis.

From December 2022 to December 2023, serum and urine samples were collected from a total of 54 domestic cats presented at the Veterinary Teaching Hospital of the University of Zagreb for various health problems. The microscopic agglutination test (MAT) with 12 pathogenic serovars antigen panel, along with TaqMan real-time (qPCR) and conventional polymerase chain reaction (PCR) targeting the *lipL32* gene, was performed.

Leptospiral antibodies were detected in 10 (18.52%) sera. The most common serogroup was Pomona, followed by Sejroe, Icterohaemorrhagie, Australis, and Javanica. One urine sample (1.85%) was positive for leptospiral DNA by conventional PCR and the cat was diagnosed with acute kidney injury. Statistically significant differences in seropositivity were found between outdoor/indoor and indoor-only cats. Immunocompromised conditions and the presence of another cat in the household increased the rate of seropositivity, and cats with kidney disease showed higher seropositivity rates. Respiratory symptoms and changes in lung structure on radiographs were found to correlate with the presence of leptospiral antibodies, as well as laboratory findings of anaemia, leukocytosis, and thrombocytopenia. In addition, seropositive cats had significantly lower urine-specific gravity than seronegative cats.

The study underlines the seroprevalence of *Leptospira* spp. infection among pet cats, highlighting their susceptibility and association with certain risk factors, as well as clinical and laboratory findings. Moreover, the underdiagnosis and neglect of leptospirosis in cats emphasises the need for increased attention to this disease in veterinary care. Recognising domestic cats as potential carriers of leptospires is crucial, although the zoonotic potential of infected cats, particularly in the case of serogroup Pomona, requires further investigation.

FIRST ISOLATION OF *LEPTOSPIRA* SPP. IN ISRAEL: A CASE OF SEROLOGICAL DISCREPANCY

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Introduction: Epidemiological data on the prevalence of *Leptospira* spp. serovars in animals primarily relies on serological tests. The major reacting antigen in the microscopic agglutination test (MAT) is often assumed to correspond to the infecting serovar. However, recent reports challenge these assumptions[1,2]. We present the first isolates of *Leptospira* spp. in Israel, and report a partial lack of correspondence to the respective serological results in cows.

Material and Methods: Cows from a herd experiencing a high abortion rate were sampled. Serum samples underwent MAT against serovars Pomona, Hardjo, Icterohaemorrhagiae, Canicola, Bratislava, Ballum, Grippotyphosa, and Tarassovi. Urine samples were inoculated in semi-solid EMJH-5FU medium. Isolates were serogrouped by MAT (KVI), serotyped by monoclonal antibodies (AMC, Netherlands), and genotyped by secY and whole-genome sequencing.

Results: All cows exhibited MAT titers exclusively to serogroup Pomona, ranging from 800 to 1600. Seven strains were isolated from eight cows sampled. While five strains were serotyped as Pomona, one was assigned to serovar Canicola and another to Icterohaemorrhagiae, despite lacking corresponding serological titers, including against their own strains. All strains were identified as *L. interrogans* by secY sequencing (ID > 99%). Strains were assigned to novel cgMLST sequence types within Clusters_40 5, 6 and 28, corresponding to *L. interrogans* serogroups Pomona, Icterohaemorrhagiae and Canicola, respectively.

Conclusions: These are the first *Leptospira* spp. strains isolated from animals in Israel. The isolation of non-host-adapted serovars Canicola and Icterohaemorrhagiae from serologically-negative cows challenges the conventional understanding of chronic *Leptospira* infections in cattle. This also highlights the discrepancy between serological predictions and actual infecting serovars, underscoring the necessity for bacterial isolation to accurately assess prevalent serovars.

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DETECTION OF LEPTOSPIRES IN WILD ANIMALS IN AQUATIC ENVIRONMENTS OF ARGENTINA

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Zoonoses are defined as diseases of animals that are transmitted naturally to humans, directly or indirectly. Indirect transmission can manifest itself through the presence of these pathogens in the environment. The vehicle of leptospires is water. Our aim was the serological study was to detect contact with leptospires in wild animals that live in aquifer environments, in oceans and in freshwater environments (rivers, estuaries and wetlands). 141 serum samples were analyzed from *Eubalaena australis (3), Otaria flavescens (19), Arctocephalus australis (18), Mirounga leonine (15), Eunectesnotaeus (20), Myocastor coypus (33), Caimán yacaré 15) and Blastocerus dichotomus (18*). The sera were examined by microscopic agglutination test against Pomona Pomona, Icterohaemorrhagiae Copenhageni, Tarassovi Perepelicin, Castellonis Castellonis, Canicola Hond Utrecht, Hardjo Hardjoprajitno, Wolffi 3705, Grippotyphosa Moska V, Australis Australis, Autumnalis Autumnales, Bataviae Bataviae, Celledoni, Cynopteri Cynopteri, Djasiman Djasiman, Hebdomadis Hebdomadis, Javanica Javanica, Lousiana Lousiana, Mini Mini, Manaho Manaho, Panama Panama, Ranarum Ranarum, Sarmin Sarmin, Shermani Shermani and Bratislava Jez Bratislava. Initial title: 1/25.

We observed 66.6% (2/3) positive whales (*Eubalaena australis*) whit titles:1/50, (*L. weillii* serovar Celledoni and *L. kirschneri* serovar Grippotyphosa); a 11.1% (2/18) positive two-haired fur seals (*Arctocephalus australis*) with title: 1/50-1/100 (L. Mini serovar Mini); a 36.3% (12/33) Coypo (*Myocastor coypus*) with titles: 1/100-1/800 (*L. interrogans* serovar Copenhageni, *L. borgpetersenii* serovar Castellonis); a 20% (3/15) Cayman (*Caimán yacaré*) with titles 1/100-1/1600 (*L. interrogans* serovar Pomona, L. serovar Sarmin), 33% (6/18) Marsh deer (Blastocerus dichotomus) with titles 1/100-1/800 (*Leptospira borgpetersenii* serovar Castellonis, *L. interrogans* serovar Pomona and *L. kirschneri* serovar Grippotyphosa); the 100% (19/19) fur seal (*Otaria flavescens*) were negatives and 100% (15/15) elephant seals (Mirounga leonina) negatives too.

This study demonstrates the contact between the Leptospire and wild animals that live in the oceans, rivers, estuaries and wetlands of Argentina. Knowledge of risk is essential to take appropriate preventive measures.

LEPTOSPIRA INTERROGANS, L. BORGPETERSENII AND *L. NOGUCHII* ISOLATED FROM CATTLE SHOW DIFFERENT VIRULENCE CHARACTERISTICS TO ESTABLISH KIDNEY COLONIZATION IN A MURINE MODEL OF SUBLETHAL INFECTION

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Leptospirosis is an endemic disease in Uruguayan livestock with economic consequences due to abortion, infertility, and death of young animals, in addition to the zoonotic risk. In a recent study, >1000 cows were sampled to characterize the leptospira strains circulating in Uruguayan cattle (1). Approximately 20% of these cows excreted pathogenic leptospires in urine, from which *L. interrogans, L. borgpetersenii* and *L. noguchii* were isolated. Here, we assessed the virulence and immunogenicity of autochthonous bovine *Leptospira* spp. isolates using a murine sublethal infection model that somewhat mimics the *Leptospira* infection cycle in the bovine host.
Adult C57BL/6J mice were challenged intranasally with four different *Leptospira* strains isolated from cattle: *L. interrogans* serogroups Pomona (LIP) and Canicola (LIC), *L. borgpetersenii* serogroup Sejroe (LBS) and *L. noguchii* serogroup Autumnalis (LNA). The infection kinetics in blood and urine was assessed by qPCR targeting the *lipL*32 gene. The serologic humoral response was evaluated by MAT to detect anti-*Leptospira* antibodies. The inflammatory response in lung tissue at 2 days post-infection (dpi) was studied by quantifying pro-inflammatory cytokines expression by RT-qPCR. Renal colonization was assessed at 60 dpi by quantifying the bacterial load by qPCR and isolating viable leptospires using microbiological culture.
Both LIP and LIC were detected in blood and urine, colonized the kidneys and induced a humoral response. LBS and LNA also invaded the blood and induced a humoral immune response but failed to colonize the kidneys. Interestingly, LBS and LNA induced a higher inflammatory response in the lungs compared to *L. interrogans*, suggesting a distinct modulation of the host immune response likely occurring at the site of infection entry. These results, showing that different *Leptospira* species have different abilities to establish chronic renal colonization in mice, highlight the need for further research to understand how different pathogenic *Leptospira* interact with their diverse hosts.

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HIGH *LEPTOSPIRA* SEROPREVALENCE IN FREE-LIVING POND SLIDERS (*TRACHEMYS SCRIPTA*): WHICH EPIDEMIOLOGICAL ROLE DO THEY PLAY?

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Turtles are the reptiles most frequently kept in the houses as pets and, when infected, they may be direct or indirect source of *Leptospira* for other domestic animals and owners while, in the outdoor environment, they may transmit *Leptospira* to other reptiles or mammals which share the same habitat. Investigations on different chelonian species have highlighted seroconversion and/or leptospiral detection with a wide variation in the detected prevalence likely due to the diagnotisc methods, the number of samples, the species and the living environment.
The present study was conducted in in two population in North Italy where 61 pond sliders (*Trachemys scripta spp.*) were blood sampled for serological analysis.
The sera were examined for the presence of antibodies against Leptospira using Microagglutination Test (MAT), according to the recommendations of World Organisation for Animal Health standards (OIE, 2019), with a panel of antigens representing eight serogroups.
Considering that, generally, reptiles have low antibody production, the cutoff titre was set at 1:25.
On the total of the samples, 59 resulted positive for serogroup Tarassovi of which 27 had high titres (≥ 1:800). Five samples showed positivity also for serogroup Icterohaemorrhagiae, one for Australis, and one for Ballum.
Serovars belonging to serogroup Tarassovi are usually related to farm animals, especially swine, but currently they seem to be disappeared from these species. This serogroup has also been found in chelonians and recent serological investigations have found it in different species and environment with different prevalence values.
Although the high seroprevalence found in this study, further investigations are necessary to better understand the role of turtles as shedders of leptospirae and their role in the epidemiology of leptospirosis. Therefore, this serological survey may represent a start point for further investigations through bacterial detection by microbiological or bio-molecular methods.

APPLICATION OF A PROTOCOL FOR CONTROL/ERADICATION OF LEPTOSPIROSIS IN A LARGE DAIRY FARM IN ITALY

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Introduction: In a dairy farm (700 lactating cows, more than 1000 animals in total), not vaccinated for *Leptospira* and with reproductive problems, the presence of serovar Hardjo was diagnosed by serological test (micro-agglutination test, MAT). A protocol to control the infection and limit its economic impact was followed.

Materials and methods: The protocol followed these steps:
- herd serological check (MAT);
- microbiological and RT-PCR test (from kidney, gladder, uterus, and urine);
- antibiotic treatment;
- vaccination;
- periodical check on seronegative animals.

Results: First serological check was carried on more than 40% of the animals and a high sero-prevalence (more than 60% among heifers; more than 20% among lactating cows) was found. About 75% of the seropositive animals showed titres higher than 1/400.
PCR detection gave a positive result while microbiological test was negative. MLST test revealed the presence of *Leptospira* Hardjo sequence type 152.
The antibiotic treatment started, soon after the serological check, on the non-lactating animals and then extended to the lactating cows when drying off. Oxytetracycline by injection was administered.
When available, an imported commercial vaccine was administered according to the manufacturer instruction. A group of 90 seronegative animals was not vaccinated and used as *sentinels* for future serological test.
After vaccination only five sentinels resulted positive at low MAT titres (< 1/400) in three blood sampling at two-month intervals.

Discussion: In Italy control or eradication programmes for bovine leptospirosis are not ongoing neither at national nor at regional level. In case of leptospirosis, following a protocol for control of the infection at herd level is fundamental to reduce the economic impact. Although the protocol here proposed did not eliminate completely the infection, it reduced, considering the serological results, the circulation of the infectious agent. Therefore, further control measures need to be applied as well as the adoption of a vaccination programme.

10 YEARS OF MONITORING OF LEPTOSPIROSIS IN CATTLE IN SOUTHERN BELGIUM

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ARSIA has amongst its goals to monitor diseases in livestock in Wallonia (southern Belgium) and provide help to the breeders to control these diseases, by the means of testing, guidance and implementation of actions and legislations. Leptospirosis, known to have a potential major economic impact on the cattle sector and important zoonotic capacities, is naturally part of these diseases monitored by ARSIA. Its pathology and epidemiology are influenced by the numerous existing serovars and their adaptation to specific hosts. Besides *Leptospira* Hardjo, that is known to be host-maintained, several other serovars infect cattle as shown by the emerging phenomenon of icteric abortions of this last decade in Wallonia.
The control for *Leptospira* sp. in cattle is mainly centered on two sets of measures: (I) the testing of the animals when purchased and moving between two herds and (II) the monitoring of abortion cases.
For the control of purchases, from 2015 to 2023, 100,033 animals have been tested with a commercial ELISA for L. Hardjo (PrioCHECK™, ThermoFisher) ; 1,50% were seropositives.
The monitoring of abortions cases includes the autopsy and testing of aborted fetuses for various pathogens. From 2014 to 2023, 47338 aborted fetuses were subjected to a standardized necropsy. About 1,59% of them were showing suspicious lesions of *Leptospira* infection (yellowish coloration of tissues). There is a strong variation in the observation of these lesions according to the years of monitoring (higher number in 2014, 2021 and 2023). On the suspected fetuses tested by real-time PCR for pathogenic *Leptospira* (VetMAX™, ThermoFisher), 28,36% were positive. For the dams of the suspected fetuses, 49,50% of them were positive with MAT on serum (Sciensano, Brussels), for a least one non-maintenance serovar. In parallel, all the sera of the dams are systematically tested for L. Hardjo when available; 1,56% of the 40465 tested sera were positive. A significant decrease of the seroprevalence is observed over the years.

ISOLATION AND CHARACTERIZATION OF LEPTOSPIRES ASSOCIATED WITH EQUINE RECURRENT UVEITIS

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Keywords: *Leptospira*, Leptospirosis, ERU

Introduction: Equine recurrent uveitis (ERU) is a leading cause of uveitis in horses in Germany and neighboring countries. It is a chronic and painful disease caused by intraocular infection with pathogenic *Leptospira* that leads to blindness if infection persists.

Objective: Identify and characterize isolates of *Leptospira* associated with ERU.

Materials and Methods: Samples of vitreous fluid were collected from horses in Germany that were treated for uveitis by vitrectomy. Samples were tested for *Leptospira* by *lipL32* qPCR and culture. For culture, up to 500 µl of vitreous fluid was inoculated directly into 10 mL HAN media containing 5-fluorouracil (100 µg/mL). Inoculated media was sub-cultured into fresh HAN media, incubated at either 29 or 37 °C with 5% CO_2, and monitored periodically by dark-field microscopy. In some cases, inoculated media was plated on HAN agar plates, which were monitored for colony formation. Recovered isolates were genotyped by whole genome sequencing and serotyped with reference antisera.

Results: Of fifteen horses treated for uveitis, eleven (73%) were positive by qPCR. Of eleven qPCR positive samples, six (54%) were culture positive. Of the six strains recovered in HAN media, three strains were typed as *L. kirschneri* serogroup Grippotyphosa, of which one was serovar Vanderhoedeni and two were serovar Grippotyphosa type Duyster. Additional strains were typed as *L. kirschneri* and *L. interrogans*, both of which were serogroup Australis. A strain recovered on HAN agar plates was typed as *L. interrogans* serogroup Pomona.

Conclusion: Multiple species and serogroups of pathogenic *Leptospira* are associated with ERU in horses in Germany and neighboring countries. Isolation of *Leptospira* provides a definitive diagnosis. The comprehensive characterization of recovered isolates is essential to elucidate pathogenic mechanism of ERU and facilitates epidemiological investigations as well as the development of efficacious bacterin vaccine strategies.

Funding Agencies This research is funded in part by a Doctorate scholarship from the National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES), ORISE (DE-SC0014664), NVSL and ARS.

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TRANSCRIPTOME AND PROTEOME ANALYSIS REVEALED KEY PATHWAYS REGULATING PATHOGENIC MECHANISM OF THE *LEPTOSPIRA INTERROGANS* SEROVAR HARDJO

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Despite the significant risk of animal leptospirosis in almost all regions, the current understanding its molecular mechanisms of pathogen virulence and disease pathogenesis, much remains undisclosed. While ***in vivo*** analysis of the leptospira transcriptome and proteome using rats as an animal model have been documented, a comprehensive comparison of different animal hosts for Leptospira has not yet been undertaken. Endemic infection of sheep with Leptospira serovar Hardjo is now recognized in many countries, and numerous studies have highlighted its economic and clinical significance, especially in relation to reproductive problems. Serovar Hardjo comprises two distinct genotypes, Hardjoprajitno (HP) and Hardjobovis (HB), belonging to the Leptospira species *L. interrogans* and *L. borgpetersenii*, respectively. Their symbiotic host-parasite interaction remaind a conundrum Comparative genomics analysis revealed that 78% of pathogen genes specific to either L. interrogans or L. borgpetersenii remain functionally uncharacterized. Given the overrepresentation of hypothetical genes among those exclusively found in pathogenic Leptospires, it is likely that numerous additional virulence factors await to be discovered.
The objective of the study was to advance our comprehension of Leptospira virulence by identifying genes and proteins involved in pathogen-host interactions. To gain deeper insights into the pathogenesis, we conducted studies using sheep as an animal model. Transcriptomic and proteomic analyses were detected by pairwise comparisons of *in-vitro* culture *vs.* bacteria incubated 24h *in-vivo.* Our results identified a total of 773 transcripts and 497 proteins that were significantly expressed with an adjusted P value of 0.05. The analysis of transcriptome and proteome data yields crucial insights into elements that may be correlated with infection and host adaptation.

Grant 2019/33/B/NZ9/02159

INFECTION BY *LEPTOSPIRA SANTAROSAI* SEROVAR GUARICURA STRAIN VF52 IN HAMSTER ANIMAL MODEL

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Keywords: Bovine Leptospirosis, Uterine Infection, Experimental Infection, *Mesocricetus auratus*.

Introduction: Leptospirosis is a zoonotic disease manifested in ruminants as a reproductive disorder. *Leptospira* from the serogroup Sejroe correspond to the primary worldwide agent in cattle. However, knowledge regarding the pathology of this reproductive disease exhibits gaps.

Objective: To analyze histopathological aspects of *L. santarosai* genital infection in animal model of lethal acute infection (LAI) and non-lethal chronic infection (NLCI) in Golden Syrian hamsters.

Methods: We used *Leptospira santarosai* serovar Guaricura strain 2013_VF52, from Sejroe serogroup, which was previously isolated from a cow. For this purpose, bacteria were intraperitoneally administered to female hamsters aged 6 to 8 weeks. The LAI group was infected with 1.0×10^8 leptospires/mL and was euthanized after four days post-infection, with acute symptoms of lethal infection; whereas the NLCI group was infected with 1.0×10^4 leptospires/mL and survived up to 40 days post infection, when they were euthanized.

Results: Immunohistochemical analysis using anti-*Leptospira* antibodies revealed very different patterns of tissue infection. In the LAI group, we observed that focal spots of infection occurred across all layers of the uterus, with positive staining for *Leptospira* in blood vessels and occasionally in interstitial tissue. On the other hand, in the NLCI group, we found positive focal staining predominantly in two layers of the uterus: the endometrium and the myometrium. Staining for *Leptospira* was most frequently detected in capillaries, with complete or partial obstruction. The uterus of the NLCI group exhibited on H&E staining the classic appearance of homogeneous and eosinophilic globular cytoplasmic inclusions within the endometrial glands that characterize Russell bodies, commonly found in chronic bacterial infections.

Conclusion: This study provides insights into genital leptospirosis in a hamster animal

model, highlighting the complexity of the infection and its histological manifestations in uterine tissue.

Funding: This work was funded by the Coordination for the Improvement of Higher Level Personnel (CAPES, finance Code 001) and the National Council for Scientific and Technological Development (CNPq; grant Universal 423415/2021-2).

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A MODEL OF HEMATOGENOUS DISSEMINATION OF *LEPTOSPIRA* TO MOUSE TISSUES

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Introduction: Rodents are natural hosts of *Leptospira* and shed the organism through their urine into the environment. Humans acquire *Leptospira* through contact with contaminated soil or water. We used an *in vivo* mouse model of hematogenous dissemination to investigate *Leptospira* tissue tropism. We looked at diverse *Leptospira* strains from a range of clades (P1, P2, S1).

Materials and methods: Mice were inoculated intravenously (IV) with 10^8 organisms. Bacteria were allowed to circulate for 20 minutes to 24 hours, after which mice were perfused with saline to wash away unbound organisms. Tissues were collected and analyzed for presence of *Leptospira* by culture and qPCR.

Results: Upon culture of organs, live *L. biflexa sv. Patoc* (S1) was seen via darkfield microscopy at 20 minutes, 3, and 6 hours, but not 24 hours post inoculation while *L. interrogans sv. Manilae (P1+)*was recovered at all time points tested. Live *Leptospira* were found in organs harvested from mice inoculated with *L. langatensi (P2), L. bandrabouensis (S1), L. gomenensis (P1-), and L. licerasiae(P2)* at 1 hr post-inoculation. Strains representing all clades are being tested and results will be available.
Conclusions: This model detected endothelial adhesion of *Leptospira* in several mouse tissues. Clade classification was not a predictor of adhesion to tissues. Future studies will investigate other routes of inoculation, using qPCR to compare bacterial tissue burdens.

Reference

Hematogenous dissemination of pathogenic and non-pathogenic *Leptospira* in a short-term murine model of infection. Surdel, Anderson, Hahn, Coburn. 2022. https://doi.org/10.3389/fcimb.2022.917962

SPATIAL EPIDEMIOLOGY AND COMPARATIVE EVALUATION OF DIAGNOSTIC ASSAYS FOR LEPTOSPIROSIS AMONG LIVESTOCK IN NORTHERN KERALA, INDIA

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Introduction: Leptospirosis, a zoonotic disease caused by Leptospira bacteria, poses significant health and economic burdens globally. Accurate diagnosis in livestock is crucial for disease control and faces challenges. Emerging techniques like Loop-mediated isothermal amplification (LAMP) offer promising alternatives for rapid and accessible detection (Suwancharoen *et al*., 2016). Spatial epidemiology of animal leptospirosis is pivotal for pinpointing high-risk areas and guiding targeted interventions to mitigate disease spread.

Materials and Methods: A study in northern Kerala collected 400 serum, 26 urine, and 167 clinical samples from livestock with various clinical manifestations across six districts. Microscopic Agglutination Test (MAT), staining techniques, Polymerase Chain Reaction (PCR), and LAMP assay were utilized for diagnosis, with efforts made for culture and isolation. Positive MAT cases were mapped using EpiInfo software for spatial analysis.

Results: Analysis of epidemiological data revealed a seroprevalence of 69.5%, 64%, and 51.74% in cattle, goats, and pigs, respectively, with no significant correlation with factors such as sex, breed, age, or farming conditions. The sensitivity of PCR in comparison with MAT was (71.4 % and 34.5%). Specificity of PCR and MAT was (71.2% and 92.2%), where MAT has taken as the reference test. LAMP demonstrated a sensitivity of 75% and specificity of 97.6%, outperforming MAT and PCR in specificity. Kappa statistics showed moderate agreement (0.704) between PCR and LAMP.

Conclusion: The study underscores the prevalence of leptospirosis among livestock in Northern Kerala, emphasizing the role of water sources like spring water in disease transmission. LAMP emerges as a robust diagnostic tool with high specificity, offering simplicity in diagnosis. These findings contribute valuable insights for disease management and control strategies in similar regions, providing clear spatial representations and aiding in the identification of potential hotspots through spatial epidemiology analysis.

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ISOLATION OF *LEPTOSPIRA* FROM CATTLE IN THE UNITED STATES

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Introduction: Bovine leptospirosis can significantly impact production on infected farms by causing infertility, abortions, stillbirths, weak offspring, and decreased milk production and growth rates. Globally, cattle are recognized as a reservoir host for *Leptospira borgpetersenii* serogroup Sejroe serovar Hardjo, which is transmitted via urine, semen, and uterine discharges. However, other species and serovars of *Leptospira* are also associated with infection. Bacterin vaccines can reduce bovine leptospirosis by including serovars associated with this disease. Here, we provide a synopsis of our recent studies to detect shedding and to culture *Leptospira* from bovines to determine the species, serogroups, and serovars associated with bovine leptospirosis in the U.S. from 2018-2023.

Material and Methods: A total of 1233 bovine (cow and bull) urine and 268 semen samples were tested by *lipL32* qPCR or fluorescent antibody testing (FAT). When possible, freshly collected urine was processed for culture with HAN media containing 5-FU. Semen samples were resuspended in 1% BSA prior to PCR, and PCR-positive samples were cultured in HAN media containing 5-FU. The serogroup of each cultured isolate of *Leptospira* was determined by the Microscopic Agglutination Test using a panel of polyclonal rabbit reference antisera. The serovar of selected isolates was determined by monoclonal antibodies. Genome sequencing was performed to determine the species.

Results: Of 1233 urine and 268 semen samples tested by qPCR or FAT, 148 (12%) and 4 (1.5%) were positive, respectively. Isolates cultured from urine include *L. borgpetersenii* serogroup Tarassovi serovar Tarassovi (n=1), *L. borgpetersenii* serogroup Sejroe (n=47), *L. interrogans* serogroup Icterohaemorrhagiae (n=1), *L. interrogans* serogroup Pomona (n=5) and *L. santarosai* serogroup Pyrogenes (n=1). *L. borgpetersenii* serogroup Sejroe was cultured from one semen sample.

Conclusions: Characterization of *Leptospira* cultured from cattle identified diverse species, serogroups, and serovars associated with bovine leptospirosis in the U.S. including novel species, serogroups, and serovars not currently used in bacterin vaccines.

LEPTOSPIROSIS IN UNITED STATES ZOOS

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Introduction: Leptospirosis in zoos is concerning for the health of exotic animals and poses a risk to human health. There is limited information on leptospirosis for exotic animals. In fiscal year 2024, the National Veterinary Services Laboratories (NVSL) saw an increase in suspected clinical cases of leptospirosis in zoo animals without geographical or species correlation.

Materials and Methods: Serum samples were received from three zoos with suspected leptospirosis for microscopic agglutination testing (MAT). Paired serum samples were collected from animals to check for increases in titer. PCR was performed, and results from testing not done at the NVSL were shared.

Results: Zoo 1 received positive PCR results on urine from a red river hog. Subsequent MAT was performed on sera from the PCR positive animal and another housed with it; titers were 1:51,200 and 1:3200, respectively, both to Grippotyphosa. Follow-up *lipL32* qPCR was negative.
Zoo 2 submitted three serum samples from a lemur for MAT over a six-month period. Surveillance MAT five months prior to suspected leptospirosis infection were negative. Samples collected at first signs of clinical illness showed titers of 1:200 to Ballum and Bratislava, and urine was PCR positive. One month after clinical signs, titers of 1:100 to Ballum and Bratislava and 1:12,800 to Grippotyphosa were observed.
Zoo 3 submitted serum for MAT after a Nigerian dwarf goat began displaying clinical signs of leptospirosis. Sera were collected three times at approximately two-week intervals. Titers for Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, and Pyrogenes were consistently below 1:200. Initial titer to Bratislava was 1:800, decreased to 1:400, then negative at 1:100 during this interval. Several weeks after treatment, urine and whole blood were PCR negative.

Conclusions: Determining the infecting serovar through isolation is important to know what to include in future MAT panels and vaccines for exotic species. Isolation, attempted before treatment, increases the chance of recovery.

FIRST DETECTION OF *LEPTOSPIRA* ST198 RELATED TO *L. INTERROGANS* SEROGROUP AUSTRALIS IN RED FOXES (*VULPES VULPES*) IN NORTHERN ITALY

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Leptospires are maintained in nature by subclinical wild and domestic reservoir hosts that serve as source of exposure for wildlife, livestock, companion animals, humans. The most important reservoir host for several *Leptospira* serovars is represented by rodents, but *Leptospira* spp. infection has been documented in accidental hosts as domestic dogs and wild animals such as red foxes (*Vulpes vulpes*). Aim of this study was to investigate the diffusion of *Leptospira* spp. infection in red foxes from Northern Italy. Kidney samples from red foxes culled or found dead or dead in wildlife rescue and rehabilitation centres in Emilia Romagna region from January 2022 to March 2023 were screened for pathogenic *Leptospira* spp. DNA using a SYBR Green real-time PCR assay. The identified leptospires were genotyped using a multi-locus sequence typing (MLST) approach, adopting a scheme based on seven housekeeping genes, and a protocol developed for direct application on DNA extracted from biological samples. Of the 126 red foxes included in the study, two tested positives for *Leptospira* spp. DNA, and the identified bacteria were genotyped by MLST analysis as *Leptospira* sequence type (ST) 198, related to *L. interrogans* serogroup Australis serovar Australis. To date, *Leptospira* ST198 in Italy has been detected exclusively in dogs, hedgehogs, and in a horse. To the best of the author's knowledge, this represents the first report of this variant in the red fox. This finding suggests that *Leptospira* ST198 is widespread in the Italian territory, involving different animal species as maintenance or accidental hosts, and confirms that the red fox may be a reliable sentinel for epidemiological monitoring. Further studies are necessary to understand the role of red fox in the maintenance of this pathogen both in wild and in urban and peri-urban environments.

HOST-BACTERIAL INTERACTIONS LEAD TO VARIED OUTCOMES AMONG PATHOGENIC *LEPTOSPIRA* SEROVAR HARDJO SPECIES- *IN-VIVO* AND *IN-VITRO* STUDY

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Leptospires can theoretically infect any animal species, but host susceptibility and disease spread vary by serovar. Each serovar adapts specifically to its maintenance host, using distinct virulence mechanisms to evade the host's immune system and establish persistent infections. Understanding these host-pathogen interactions is crucial for identifying key factors in pathogenesis and host defense. Our work focused on Leptospira serovar Hardjo, for which cattle and sheep are maintenance hosts . Two genotypes of serovar Hardjo, belonging to different Leptospira species, *L. interrogans* (HP) and *L. borgpetersenii* (HB) have been recognized. The study aimed to clarify their host-pathogen interactions by examining pathogen interactions with host body fluids and macrophages to understand their virulence mechanisms.
To obtain a more faithful representation of how leptospires respond to host-derived signals, cultures of HP and HB strains, using dialysis membrane chambers, were implemented in the peritoneal cavities of sheep. Method based on the reducing capacity of live cells together with differential staining of live and dead cells used for the analyses of *Leptospira spp* viability. Additionally, sheep peripheral blood macrophages (BMs) were utilized to assess the intracellular infection of HB and HP pathogens. Immunofluorescence and flow cytometry were performed to visualize the intracellular fate of leptospires. Real-time RT PCR was used for the analyses of the expression of virulence factors for leptospires.
During the study, divergent survival rates in host body fluids were observed for the two genotypes. HB showed the shortest survival under these conditions. Additionally, although both genotypes successfully infected macrophages, differences in macrophage responses were observed. These variations in macrophage response, along with differences in the expression of the sphingomyelinase gene—a virulence factor produced by pathogenic Leptospires—and the resulting inflammatory responses, may explain the different mechanisms leading to the development of natural infections in animals between HB and HP.

Grant 2019/33/B/NZ9/02159

PEROMYSCUS DEER MICE AS AN ACUTE RODENT MODEL OF LEPTOSPIROSIS

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Leptospirosis is a devastating zoonotic disease affecting humans, companion, and domestic animals. Wild rodents are the primary reservoir hosts of pathogen spread and similarly, laboratory rats and mice are largely asymptomatic. The Golden Syrian Hamster (*Mesocricetus auratus*) is the dominant model for acute disease; they are susceptible to many serovars and can be used to maintain laboratory strains and test bacterin vaccine efficacy. However, hamsters are primarily utilized in survival-based studies, and are limited by a lack of immunology characterizing reagents. *Peromyscus leucopus* (deer mice) are more closely related to hamsters than *Mus musculus* mice and historically have been alternative rodent models for other spirochete diseases such as Lyme disease. This work reports on deer mice as an alternative model of acute leptospirosis. Deer mice were challenged with strains of *L. interrogans* serovar Canicola and *L. borgpetersenii* serovar Arborea by intraperitoneal injection. Upon appearance of severe clinical signs of disease (weight loss, blood on nose/paws, bloody urine, lethargy, etc.) animals were humanely euthanized. Whole blood slides were Giemsa stained and evaluated for differential blood counts. Kidney and liver tissues were cultured and pathogen load determined by *lipL32* qPCR. Immunological tissues were formalin fixed and sectioned for pathology analysis.

Challenged deer mice developed severe clinical signs and were positive for *Leptospira* by culture and qPCR. Males were more susceptible than females to acute disease and contained higher bacterial burdens in tissues. Additionally, deer mice produced circulating foamy macrophages, a marker of disease severity characterized in hamsters, in response to *Leptospira* challenge. This work describes for the first time that deer mice are susceptible to acute leptospirosis. Infected deer mice produce foamy macrophages, display susceptibility differences between sexes, and offer an alternative rodent model for acute leptospirosis.

ESTIMATION OF BI-INDEX, ABSOLUTE DENSITY OF RATS AND LEPTOSPIROSIS CASES IN CENTRAL JAVA

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Keywords: Leptospirosis; Rat ; Density; Central Java; Zoonosis; Indonesia

Background: A study on the determination of rat density indicator by applying the BI-Index formulation and early warning system for leptospirosis control was conducted in Central Java, Indonesia. The objectives of this study were to determine the relative density of rats using the BI-Index and calculate the ratio of leptospirosis in humans and rats.

Methods: The study was conducted from 2018 to 2023. Leptospirosis case finding and rat surveys have been done in Banyumas, Demak, and Semarang, Central Java. The Microscopic agglutination test (MAT) and qPCR were performed to confirm the samples. An absolute density survey of rats using the *Jolly-Seber* method was conducted in the two districts of Banyumas and Demak, while a relative density survey of rats using the BI-Index formula was conducted in Semarang. The BI-Index was calculated using a Geographic Information System. This system was used to digitize land use area and estimate rat territory area (+10 m) based on rat traps.

Result: We collected 382 human blood serum samples and 218 rat blood serum samples. *Leptospira* antibodies were detected in 53 (13.87%) of the positive patients, and 66 rats (22.93%) were positive in the two districts. The estimated absolute density of rats in the study area was 1591 rats, while the average population of the selected hamlets was 882 people in Banyumas and Demak districts, Central Java. The average number of leptospirosis cases was 122 people (13.87% of 882 people) and the average number of leptospirosis-positive rats was 365 rats (22.93% of 1591 rats). Bangkinang serovar was the most common serovar found in humans (37.8%) and rats (48.9%), followed by Bataviae and Ichterohaemorhagiae serovars. The results showed that the ratio of leptospirosis cases in the community with leptospirosis infective rats was 1:3. The ratio of infective rats to healthy rats was 1:12 in Banyumas and Demak districts, Central Java. The average BI-Index value was 0.23 (high) in Semarang City, Central Java.

Conclusion: The abundance of rats and their positivity for pathogenic *Leptospira* spp. in Banyumas, Demak, and Semarang indicated those three regions were endemic to leptospirosis. The BI-Index indicates that rat density in Semarang City is a potential source of leptospirosis transmission. Therefore, rat control measures to prevent leptospirosis transmission are urgently needed.

LEPTOSPIRA SPP.-SPECIFIC ANTIBODY RESPONSES IN HEALTHY DOGS AFTER VACCINATION AGAINST LEPTOSPIROSIS WITH A 4-SEROVAR VACCINE

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Leptospirosis is a serious canine disease with zoonotic potential. Therefore, vaccination against Leptospira spp. is now categorized as core vaccine in many countries. Microscopic agglutination test (MAT) which detects antibodies against different serovars, is considered the diagnostic gold standard for serology. However, this can become positive after vaccination. This study aimed to evaluate how long, against which serovars, and at what level, antibodies persist after vaccination.

A MAT was performed on 582 serum samples from 97 healthy dogs (vaccinated with a 4- serovar vaccine 12 months previously) to detect Leptospira spp.-specific antibodies against 12 serovars of 12 serogroups before re-vaccination with the same vaccine and at 2, 4, 12, 26 and 52 weeks thereafter. A robust linear mixed-effects model was used to evaluate any potential correlation between antibody titers and the dogs' signalment.

Before vaccination, 66/97 dogs had low-level antibodies (1:10 – 1:80) against different serovars. Antibody titers increased during the following 2 to 4 weeks (56/97, maximum titer 1:160, and 57/97, maximum titer 1:320, respectively) and decreased thereafter. Eighty-four dogs developed antibodies (1:10 – 1:320) against all 4 vaccine serovars (Australis, Canicola, Grippotyphosa, Icterohaemorrhagiae). Of these, 76 also developed antibodies against 1 – 7 serovars from non-vaccine serogroups (Autumnalis, Javanica, Pomona, Hardjo, Saxkoebing, Pyrrogenes, Tarassovi). At weeks 12, 26, and 52, 97/97, 92/97, and 71/97 dogs still had antibodies against at least one vaccine serovar. Overall, senior dogs (last 25 % of life expectancy) had significantly higher antibodies ($p<0.05$).
Vaccination with 4-serovar vaccines results in antibody production not only against vaccine serovars but also against serovars from different serogroups. Antibody levels remain high for months after vaccination in most dogs. Regular re-vaccination might increase antibodies in older dogs.

PATHOGENIC *LEPTOSPIRA* IN RODENTS AND *CROCIDURA* SP: AN EMMEDIATE THREAT TO HUMAN HEALTH IN WETLANDS IN THE KILOMBERO VALLEY, TANZANIA

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Introduction: Leptospirosis is a contemporary public health concern in Tanzania, especially in areas with year-round wetness. The disease's existence has been confirmed by several studies done in Tanzania. This investigation examined its prevalence in rodents and *Crocidura* sp. in one of Tanzania's economically significant wetlands, the Kilombero Valley.

Material and methods: Data was collected between February 2022 and November 2023. Animals were recorded in houses, peri-domestic and crop-vegetation. The captured animals were euthanized by Halothane. Blood was collected and sera were extracted and preserved at -20˚C. Kidney samples were collected preserved in 99% ethanol. *Leptospira* was detected using serology though Microscopic Agglutination Test (MAT) and by using PCR assay targeting the *lipL32*, *16S rRNA* and *Sec Y* primer genes. *Leptospira* species identification was done by Melt curve analysis using *lfb1* primer gene and by sequencing.

Results: A total of 993 animals were captured, 962 were used for serology and 375 were selected for molecular identification. About 10.39% of animals tested positive using serology and 8% tested positive with PCR. The prevalence was significantly higher (p=0.001) in the two villages. The habitat with the highest prevalence was peridomestic and crop-vegetation (p=0.037) compared with inside-houses. *Crocidura* sp. had the highest prevalence (p=0.001) using both methods, followed by *Mastomys Natalensis and Rattus rattus*. The matured male population had a higher prevalence (p=0.005) than immature animals in the group. The prevalence was high during the rainy season compared to dry season (p=0.01). The detected *Leptospira* species were *L.borgpetersenii* and *L.kirschneri* as well as serovars *Sokoine, Lora, Hebdomadis* and *Pomona*.

Conclusion: This investigation provides evidence that leptospirosis exists among rodents and *Crocidura* sp in Kilombero Valley, Tanzania. Given that these animals are the most important reservoir hosts of the disease and source of infection for humans, their management deserves more attention to reduce the disease's transmission to humans within the study area.

EVIDENCE OF CLINICOPATHOLOGICAL CHANGES DURING EQUINE LEPTOSPIROSIS

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Leptospirosis is a globally spread zoonotic disease effecting a wide range of animal species and humans. 18 Leptospira positive horses based on ELISA and 9 healthy horses were included in the study. Blood profile, serum biochemistry and mineral profile were analyzed at day 0, 7 and 21 of infection. Results showed significant decrease ($P<0.05$) in red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC) and platelets while total leucocytes count (TLC), neutrophils, eosinophils, basophils, lymphocytes, monocytes, red cell distribution width (RDW) and mean corpuscular volume (MCV) were increased significantly ($P < 0.05$) which indicated hemolytic anemia. While, serum biochemistry showed significant elevation in alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine which indicate renal and liver damage. Concentration of Na, K, Ca, Mg, P, Cu and zinc in serum were significantly decreased ($P< 0.05$), indicating tubular damage in kidneys.

SEROPREVALENCE OF *LEPTOSPIRA* SPP. IN HORSES OF DISTINCT CLIMATIC REGIONS OF PUNJAB, PAKISTAN

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Leptospirosis is zoonotic disease, distributed among many domestic and wild animals. In equines, it causes uveitis, jaundice, and reproductive disorders. A cross-sectional epidemiological study was conducted for the first time in Pakistan to unveil the seroprevalence and to identify associated risk factors of leptospirosis in distinct climatic regions. A total of 384 horse sera (n = 128 from each of three study areas) and data on biologically plausible risk factors along with spatial locations were collected from horses reared in three climatically distinct districts of Punjab, Pakistan. Sera were subjected to double-antigen sandwich enzyme-linked immunosorbent assay to analyze the existence or not of Horse LS-IgG in horses. Chi-square, univariate analysis, and multivariate logistic regression were used to analyze the data. Overall, seroprevalence in Punjab was found to be 33.85% (confidence interval [CI]: 29.13–38.83). Significant statistical difference (P < .05) was observed among prevalence in study areas with highest prevalence in Rawalpindi (40.62%; CI: 32.04–49.66), followed by Lahore (38.28%; CI: 29.83–47.28), and lowest in Bahawalpur (22.65%; CI: 15.73–30.89). Age, gender, living area, herd size, water source, rodents, flooding, feeding management and practices, and usage of animals were the significant risk factors associated with the occurrence. This study is first serological evidence of equine Leptospira and its associated risk factors in climatically distinct regions of Punjab, Pakistan.

DYNAMICS OF LEPTOSPIROSIS IN AN URBAN NORWAY RAT (*RATTUS NORVEGICUS*) POPULATION IN DENSELY POPULATED AREA OF LYON AND BORDEAUX (FRANCE)

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Introduction: Re-emerging bacterial zoonosis with a worldwide distribution, caused by a Leptospira spirochete, leptospirosis is a major public health problem. Rodents, in particular brown rat (Rattus norvegicus), which is abundant in the urban biotope and closely associated with human activities, are the main reservoir of this disease. Several studies have already been carried out on the leptospirosis carriage of urban rats, but most of these did not include all age groups in their analysis. In view of this, this study was carried out to characterise the dynamics of the prevalence of leptospirosis in two populations of urban rats.

Methods: Densely populated areas in Lyon and Bordeaux were selected for this study. In collaboration with a deratting company, animals were captured between January and June 2023 using ferrets. Leptospires were detected by RT-PCR targeting the pathogenic 16S rRNA gene, with an assessment of prevalence by weight category. Positive samples were genotyped using a combination of molecular methods. Statistical modelling was used to evaluate the use of rat and city criteria as predictors of leptospirosis infection.

Results: 507 rats (Rattus norvegicus) were captured, including 406 in Lyon and 101 in Bordeaux, with overall prevalences of leptospires of 50.9% and 28.7% respectively. The heavier rats showed higher infection levels and bacterial loads. None of the 83 embryos from positive females were positive. The circulating strain was Leptospira interrogans Icterohaemorrhagiae Icterohaemorrhagiae, and the probability of infection of rat being positive increased with weight.

Conclusion: This study highlighted the link between *L. interrogans* infection and weight in urban rats, suggesting a need to reconsider traditional control strategies based on trapping and poisoning as part of leptospirosis prevention. Assessing prevalence in the local human population would be a relevant line of research.

LEPTOSPIRA SEROPREVALENCE IN SOW HERDS IN LATIN AMERICA

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Introduction: Leptospirosis is a major threat to public health worldwide [1]. The main sources of infection are rodents and small marsupials, cattle, pigs and dogs [2]. Most animals are subclinically infected, but leptospirosis is also a cause of reproductive loss in pig breeding herds [3]. Recently, a high seroprevalence of *Leptospira* infections in pigs was identified worldwide [4]. Therefore, more recent data on the seroprevalence of leptospires in sow herds in Latin America should be collected and analysed.

Material and Methods: Serum samples were collected from 12 to 90 sows per farm from 36 farms in seven countries in Latin America (Brazil, Colombia, Costa Rica, Guatemala, Honduras, Nicaragua, and Panama) in the period from May to November 2023 (total n=1066, s. Table 1). Sera were analysed by MAT according the manual of WOAH against 11 different Leptospira serovars. A serum with a significant MAT titre ≥ 100 was assessed as positive, a herd if at least one sample showed a positive result. Whenever possible, information on the clinical health status of the sows, parity data and vaccinations against Leptospira were collected.

Table 1 *Number and percentage of farms or sow herds tested serologically positive for* Leptospira *using MAT by country.*

Country	Farms (n)	Pos farms (n)	Pos farms (%)	Prevalence in sow herds (% min)	Prevalence in sow herds (% max)	Serum samples (n)	Pos serum samples (n)	Pos serum samples (%)
Brazil	16	15	93.8	13.3	83.3	478	163	34.1
Colombia	5	5	100.0	6.7	37.3	150	39	26.0
Costa Rica	2	2	100.0	23.3	26.7	60	15	25.0
Guatemala	6	3	50.0	3.3	62.1	174	32	18.4
Honduras	2	2	100.0	76.7	86.7	60	49	81.7
Nicaragua	1	1	100.0	-	83.3	30	25	83.3
Panama	4	3	75.0	12.5	60.0	114	32	28.1
	36	**31**	86.1	3.3	86.7	**1066**	**355**	33.3

n: number; pos: positive

Results: A total of 355 out of 1066 sows (33.3%) tested serologically positive for Leptospira. 31 of the 36 farms tested (86.1%) were positive for leptospires. In positive herds the seroprevalences in the herds ranged from 3.3% to 86.7%. The most frequent serological reactions in MAT with the highest titre were with the serovars Bratislava, Icterohaemorrhagiae, Copenhageni, Grippotyphosa, and Pomona.

Conclusion: Even taking into account the fact that many herds are probably vaccinated against leptospires and that no clear differentiation between infection and vaccination [5] was possible, the analyses of the serological herd profiles show that most of the sow herds are infected with leptospires.

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SEROPREVALENCE OF LEPTOSPIROSIS IN WILD RODENTS ACROSS AN ANTHROPIZED AND FRAGMENTED LANDSCAPE IN SOUTHERN CHILE

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Abstract: southern Chile has experienced significant fragmentation of native forests due to human activities. The impact of this fragmentation on pathogenic leptospira infection in wild rodents required more updated information, since some research suggests that animals inhabiting altered habitats are at higher risk of exposure to these pathogens. This study aims to elucidate how native forest fragmentation affect the exposure to pathogenic leptospira in wild rodents and make clear the complex interplay between anthropogenic activities, wildlife health, and zoonotic risks.

Methods: a total of 266 serum samples were collected from wild rodents in 86 sampling sites, of which 42 were in continuous native forest and 44 in fragmented native forest in Los Ríos and Los Lagos region in southern Chile. These samples were subjected to microscopic agglutination test (MAT) against 8 serovars to determine the dominant serogroups and the overall seroprevalence through the detection of anti-pathogenic leptospira antibodies, comparing exposure between continuous and fragmented native forests.

Results: the study revealed a prevalence of anti - pathogenic leptospira antibodies an overall of 46,6%, with 50,8% in continuous and 49,2% in fragmented forests, with no statistically significant differences. In continuous forests, the dominant serogroups were Serjoe (38,1%) and Autumnalis (38,1%), whereas in fragmented environments, Serjoe (31,1%), Autumnalis (32,8%), and Grippotyphosa (23%) prevailed. Serogroup Grippotyphosa showed differences, being almost three time higher in fragmented environments. No statistically significant differences were observed in the other seven serovars.

Conclusions: the findings suggest a widespread exposure of wild rodents with pathogenic leptospira in southern Chile, with a specific serovar showing varying response to native forest fragmentation. These results underscore the importance of considering forest fragmentation in understanding the epidemiology of leptospirosis in wildlife populations.

This study was supported by the project ANILLO ATE220062 and FONDECYT Postdoctorado 3240238 from ANID.

SOCIAL AND ENVIRONMENTAL RISK FACTORS FOR CANINE LEPTOSPIROSIS: A SCOPING REVIEW

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Introduction: Canine leptospirosis (CL) is a preventable and treatable disease that remains overlooked. Complex interactions between dogs, humans, and the natural environment underscore its One Health significance, providing opportunities for risk factor identification and sentinel surveillance. While some associations with canine signalment have been identified, a broader understanding of upstream factors (e.g., weather, landscape, and socioeconomics) is essential for effective intervention planning. The objectives were to describe current evidence on social and environmental risk factors for CL, discern global trends, and identify gaps in the literature.

Methods: Following PRISMA guidelines for scoping reviews, observational studies on CL were obtained from peer-reviewed and grey literature databases and websites. Variables assessed via formal statistical tests were recorded and categorized into thematic risk factor groups. Risk factors were summarized by the number of unique studies and their direction of association.

Results: Of 3184 articles identified, 66 were included. Most studies examined environmental factors, with eight risk factor groups identified: exposure to animals (n=34), landscape (n=31), water (n=25), outdoor access (n=25), weather and season (n=19), sanitation (n=19), travel history (n=8), and history of leptospirosis (n=2). In comparison, social risk factors were infrequently studied, with only three groups identified: behaviour (n=17), housing (n=13), and socioeconomic status (n=12). Most studies were conducted on populations in higher-income countries, and countries in the Americas.

Conclusion: This work summarizes the current knowledge on risk factors for CL and highlights gaps in the literature. Future work should address knowledge gaps for social risk factors and lower-income countries. Additionally, the weight and quality of evidence was extremely inconsistent, indicating a need for research to follow standard reporting guidelines to improve transparency and increase generalizability to different contexts. This knowledge will support future CL modelling initiatives, leading to improved reporting of social and environmental risk factors that can support evidence-based prevention strategies.

INVESTIGATING THE INFLUENCE OF SOCIOECONOMIC STATUS ON CANINE LEPTOSPIROSIS IN CANADA

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Introduction: Socioeconomic status (SES) can influence risk of leptospirosis in humans and dogs through multiple potential mechanisms, including increasing exposure risk or limiting access to health care. Given historical data limitations, the effects of SES are not well understood, particularly in canine populations. The objective of this cross-sectional study was to explore associations between area-level socioeconomic indicators and canine leptospirosis (CL) across Canada.

Methods: Four socioeconomic indicators were obtained from the Canadian Index of Multiple Deprivation (residential instability, situational vulnerability, economic dependency, and ethno-cultural composition) and linked with CL PCR test results from Canadian veterinary clinics using forward sortation area (FSA) codes. CL data was provided by IDEXX Laboratories Inc. and included the test date and canine signalment. Associations were estimated using a Bayesian hierarchical generalized linear modelling framework, where posterior estimates were approximated via Markov Chain Monte Carlo simulations. Point estimates and 95% credible intervals for each socioeconomic indicator were derived from posterior samples. Convergence was determined by the R-hat statistic (<1.01) and visual inspection of trace plots. Model adequacy was evaluated via posterior predictive checks.

Results: Following deduplication, 10 877 PCR tests were submitted between January 2014 and November 2023, of which 7.2% (n=786) were positive. Submissions originated from 285 FSAs across Canada, primarily from the province of Ontario (n=8088; 74%). Annual number of positive tests ranged from 42-239. Cases consistently peaked during the fall and were lowest during the winter, exhibiting a seasonal pattern. Preliminary models indicate areas with higher economic dependency and ethno-cultural composition have greater risk of CL.

Conclusion: This work explores the important yet overlooked influence of SES on the epidemiology of CL within Canada. Our results will describe if area-level SES influences risk of CL, which can be leveraged to inform prevention efforts.

LEPTOSPIRA ISOLATES FROM DOMESTIC LIVESTOCK ANIMALS AND WILDLIFE IN LOWER AUSTRIA

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Cases of leptospirosis have been known in Central Europe and Austria for decades. The combination of warmer climatic conditions and heavy rainfall events, possibly due to climate change, has made it easier for leptospires to survive in the environment, thereby increasing the risk of infection. Evidence of disease outbreaks in humans and animals in Austria suggests that leptospirosis is an increasing public health concern. Understanding the epidemiology and genetic diversity of Leptospira strains circulating among both domestic livestock animals and wildlife is crucial for effective disease management. Furthermore, serological antibody diagnostics using contain regionally occurring serovars and, as a further step, vaccines which are more effective against local serovars are recommended to prevent leptospirosis. This study aimed to isolate and characterize pathogenic leptospiral strains circulating among domestic livestock (cattle) and wildlife (rodents) in Lower Austria.

Cultivation and Isolation was performed in the laboratory facility of AGES. The characterization was employed by using MAT-serotyping, PCR and whole genome sequencing.

Results revealed the successful isolation of 6 Leptospira strains, three from cattle and three from rodents. The three strains from cattle were characterized by sequencing as *L. borgpetersenii1*. The three rodent strains were characterized by sequencing as *L. kirschneri.*

The identification of Leptospira strains with zoonotic potential highlights the importance of One Health approaches in mitigating the risk of leptospirosis transmission between animals and humans. Furthermore, this study underscores the need for integrated surveillance and control strategies targeting both domestic and wild reservoir hosts to reduce the burden of leptospirosis in Austria. Despite its recognized importance, national comprehensive studies on Leptospira isolates are limited. After cultivation in pure culture, these serovars can be used for serological leptospiral antibody diagnostics (microscopic agglutination test), allowing for specific matching of test results to the regionally occurring serovars.

SESSION 4

IMMUNOLOGY AND PROTECTIVE IMMUNITY

Moderators:
Lourdes Isaac & Catherine Werts

4TH ELS MEETING
13TH ILS CONFERENCE
BRUSSELS
2-4 SEPTEMBER, 2024

Host-Pathogen Interactions in Leptospirosis

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This presentation describes the work of the Leptospirosis Program Project, funded by the National Institute of Allergy and Infectious Diseases, part of the National Institutes of Health, to understand the roles of the pathogen, host, and their interactions that contribute to leptospirosis. This is a multi-institutional collaborative program, with the University of California, Los Angeles, as the lead investigator, and the Pasteur Institute (Paris), Medical College of Wisconsin, and Duke University leading the major projects. Other collaborating institutions include the VA Greater Los Angeles Healthcare System, Cedars Sinai Medical Center (Los Angeles), Kilimanjaro Christian Medical University College (Tanzania), Chulalongkorn University (Thailand), Colombiano de Medicina Tropical (Colombia), Institut Pasteur New Caledonia, and Institut National de la Recherche Scientifique (Canada).

The project examines two Central Hypotheses: **Central hypothesis #1** is that a core set of leptospiral virulence factors have evolved with roles in survival in mammalian host phagocytes, translocation, and dissemination, which are upregulated in response to the host microenvironment. **Central hypothesis #2** is that human inflammatory response pathways drive disease outcomes as shown in vitro (interactions with macrophages and endothelial cells), in animal models (infections in hamsters and mice), and in human field studies.
Some of key unanswered questions we are examining include: 1. What drives gene selection in the evolution of leptospiral host adaptation & virulence? 2. How do leptospires disseminate and invade tissues so rapidly? 3. Why is infection so different in reservoir vs accidental hosts and what enables the long-term persistence in the kidney? 4. Why do some patients have self-limited infection, while others have severe disease? Is it pathogen, host, or both? 6. What is unique about the host response to leptospirosis? 7. How early can patients at risk of severe outcomes be identified?

IN-VIVO HOST-*LEPTOSPIRA INTERROGANS* TRANSCRIPTOMES REVEAL KEY VIRULENCE FACTORS FOR PATHOGEN ADAPTATION AND DISSEMINATION

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Introduction: Pathogenic *Leptospira* lack many classical virulence factors and approximately 35% of their genome encode proteins of unknown function. Their virulence mechanisms and how they subvert the host and colonize tissues remain largely unknown.

Materials & Methods: The dynamic host-*Leptospira* interaction network have been determined through genome-wide transcriptional changes *in-vivo*, in both *L. interrogans* and hamster upon infection. To overcome the challenge of bacterial genome coverage within host tissues, we have developed an enrichment method that allowed to detect expression of 91.8% of *L. interrogans* genes in infected hamsters.

Results: Upon evaluating the temporal host response to *L. interrogans* infection, we observe that only 15 genes were differentially expressed at early infection stage, even though a colonization was already established. This reflects the stealth strategy of pathogenic *Leptospira* to escape innate immune response, possibly triggering acute leptospirosis. At a later stage of infection, we found that 4,143 genes were differentially expressed, with an upregulation of genes involved in cell-cell junctions, cytoskeleton organization and endoplasmic reticulum stress response. Alteration of these pathways were confirmed through *in vitro* studies using human epithelial cells, highlighting that disruption of the cell-cell junction is one of the key features of *Leptospira* pathogenicity. To understand how *Leptospira* induce such disruptions, we investigated the transcriptional profile of *Leptospira* in infected hamsters. We observed that nearly 2,000 leptospiral genes were differentially expressed in infected hamsters compared to the *in vitro* condition. Among the leptospiral genes highly expressed in the host, we identified a gene coding a secreted protein whose inactivation resulted in the loss of virulence in the hamster model. In addition, we demonstrated that its activity is necessary to disrupt cell-cell junctions, promoting the dissemination of *Leptospira* and host colonization.

Conclusion: Overall, this approach allows to explore the *Leptospira*-host interaction dynamics and reveals novel *Leptospira* virulence factors contributing to adaptation and dissemination in the host.

LEPTOSPIRA INFECTION TRIGGERS RENAL SENESCENCE AND LEADS TO SUSTAINED SENESCENCE-ASSOCIATED INFLAMMATORY RESPONSES AND KIDNEY FIBROSIS IN THE HAMSTER

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Keywords: Acute kidney injury (AKI); Cellular senescence; Chronic kidney disease (CKD); Leptospirosis; SASP

Introduction: Leptospirosis, caused by the pathogenic spirochete *Leptospires*, is a pervasive global zoonosis and a re-emerging infectious disease posing a significant public health challenge due to its escalating incidence.
Material and methods: While mice demonstrate resistance to *Leptospira* in experimental animal models, hamsters are susceptible to infection. In this investigation, we successfully established a hamster model of acute *Leptospira* infection. Through observing its physiological symptoms and clinical indicators, we discovered that *Leptospira* infection induces cellular senescence in kidney cells. Seven days post-infection, hamsters displayed symptoms characteristic of acute infection before being euthanized, and blood and kidney samples were collected for analysis. Fluorescence imaging and PCR methods confirmed *Leptospira* colonization in hamster kidney proximal tubules, with clear LipL32 levels indicating *Leptospira* presence.
Results: Senescence markers P16 and P21 exhibited elevated levels in kidney proximal tubules surrounding the infection site. Senescence-associated β-galactosidase (SA-β-gal) activity assay revealed heightened blue coloration in the *Leptospira* infection group, signifying increased SA-β-gal activity compared to EMJH-treated groups. Interestingly, the senescence-associated secretory phenotype (SASP), including CXCL9, CXCL10, CXCL11, CCL17, and IFN-γ, was induced by *Leptospira* infection, with significantly elevated expression compared to the EMJH-treated group. Furthermore, *Leptospira* infection in hamsters promoted kidney fibrosis, evidenced by significantly increased expression of fibronectin and collagen I compared to EMJH treatments.
Conclusion: Our findings demonstrate that *Leptospira* infection induces cellular senescence in proximal tubules, stimulates SASP expression, and contributes to kidney fibrosis in the hamster model.

NO EVIDENCE OF CYTOKINE STORM BUT SIGNS OF ORGAN DYSFUNCTION ASSOCIATED WITH THE DEATH OF MICE UPON ACUTE LEPTOSPIROSIS

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Leptospira interrogans (*L. i*), the most pathogenic *Leptospira* species, can infect all vertebrates and cause leptospirosis. While humans are susceptible to the acute form of the disease, mice are usually resistant and can become chronic renal carriers. However, mice experimentally infected with high doses of *L. i* Manilae L495 develop acute leptospirosis and may die of the infection although the specific cause of their death has not been identified[1]. In this study, we compared the infection of C57BL/6J mice with two strains of *L. i* at the dose of 10^8 bacteria/mouse; *i.e.*, the Manilae L495 strain, known to kill mice 4 to 6 days post-infection and the Icterohaemorrhagiae Verdun strain, responsible for asymptomatic disease in mice. At 3 days post-infection, mice were euthanized, and blood, lung, kidney, liver, spleen, pancreas, and heart were harvested for comparative analyses. Immunohistochemistry and PCR were performed to determine the presence of leptospires in all organs. Cytokine levels were quantified by ELISA and qPCR. Biochemical markers were also assayed. Our results highlight that infection with the two strains caused diverse clinical signs and specific differences in histopathology. Importantly, and at odds to the current view[2], we did not evidence a cytokine storm linked to the death, but instead, signs of organs dysfunction. Overall, our results (i) confirm that leptospires can provoke acute leptospirosis in mice, usually considered as resistant asymptomatic hosts, (ii) affirm the absence of involvement of a "cytokine storm" in the pathophysiology of the disease and (iii) suggest that dysfunction of some organs, overlooked but already observed in human patients hospitalized for acute leptospirosis, could be linked to lethal leptospirosis and subsequent death. In conclusion, we propose a relevant new mouse model to study lethal leptospirosis.

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MOLECULAR AND CELLULAR MECHANISMS OF LEPTOSPIROSIS PATHOGENESIS: CENTRAL ROLE OF VIRULENCE MODIFYING PROTEINS ENCODED BY THE PF07598 DUF1561 GENE FAMILY

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Severe human leptospirosis is characterized by variable combinations of pulmonary hemorrhage, shock, acute kidney injury, jaundice and death. Identifying a toxin that mediates these clinical manifestations of leptospirosis has long been sought but has remained elusive. The *Leptospira* genomic project (Lehmann et al, 2013, PLoS NTDS; Fouts et al, 2016, PLoS NTDS) led to our recent discovery of the PF07598 gene family that encodes the Virulence Modifying Proteins (VMPs). We report that VMPs are exotoxins secreted only by pathogenic *Leptospira*; these proteins are not found in intermediately pathogenic or saprophytic *Leptospira*. The genes encoding the VMPs are expanded in *L. interrogans* compared to *L. borgpetersenii* and *L. santarosai;* PF07598 gene transcription is massively upregulated *In-vivo*. VMPs, highly conserved at the amino acid level (>90-95%), are comprised of two tandemly repeated N-terminal ricin B domains (RBLs) and a C-terminal DNase/toxin domain. We show that recombinant VMPs cause cytopathic effects on HeLa cells mediated by C-terminal DNase activity, confirmed by comparison of wild-type and active site-mutated recombinant VMPs on HeLa cells. Given the high level of VMP amino acid similarity and the challenge of multiple paralog knockdown or targeted gene knockout, we tested and confirmed the hypothesis that vaccination with recombinant, VM proteins protects mice and hamsters from lethal challenge infection. We further examined the central role of VMPs exotoxins in mediating principal manifestations of severe leptospirosis, pulmonary hemorrhage and shock, by testing hypothesis that VMPs cause dose-dependent human primary pulmonary endothelial cell (HPMEC) dysfunction and cell death *in vitro*. The rationale for this hypothesis is that the endothelial cell as a primary target of leptospiral pathogenesis is widely considered to be mechanistically important and toxins play a central role in manifesting the clinical pathogenesis of leptospirosis. The details of mechanisms underlying severe leptospirosis remain unclear, particularly in the pathogenesis of pulmonary hemorrhage, which is accompanied by platelet activation, thrombocytopenia and vascular collapse. Alveolar capillary endothelial cells in the lung have previously been observed to be damaged exhibiting disruption of tight junctions. We used Electric Cell-substrate Impedance Sensing (ECIS) to quantify time-dependent transendothelial electrical resistance (TEER) responses of HPMEC monolayers in response to co-incubation with escalating dose and time of endotoxin-free VMPs. ECIS/TEER and confocal microscopy complementarily confirmed that HPMEC monolayer electrical barrier function and tight junction integrity were disrupted in a dose and time–dependent manner by VMPs. These *in vitro* results complement vaccination studies in demonstrating potential roles in severe leptospirosis. Using our newly developed capture ELISA with anti-VMP monoclonal antibodies, we detected low ng/ml quantities of circulating VMPs in the blood of hamsters infected with *L. interrogans* serovar Copenhageni, further supporting the role of VMPs—the long-sought leptospiral toxin—in leptospirosis pathogenesis. These data are an important foundation for further elucidating the cellular and molecular roles of VMPs in understanding the pathogenesis of human leptospirosis, towards improvement of novel, pan-leptospirosis diagnostic and vaccine strategies.

D2-32 Barbosa LN

VACCINATION WITH A *LEPTOSPIRA* RECOMBINANT PROTEIN CANDIDATE WORSENS THE CLINICAL SIGNS FOLLOWING CHALLENGE IN MICE

D2-33 Brihuega B

STUDY ON THE INTERFERENCE OF VACCINE ANTIBODIES IN THE SEROLOGICAL DIAGNOSIS OF LEPTOSPIROSIS IN CATTLE

D2-34 Cao Y

NEUTRALIZING GUT-DERIVED LIPOPOLYSACCHARIDE AS A NOVEL THERAPEUTIC STRATEGY FOR SEVERE LEPTOSPIROSIS

D2-35 Cao Y

LEPTOSPIRA-SPECIFIC IMMUNOGLOBULIN Y (IGY) IS PROTECTIVE IN INFECTED HAMSTERS

D2-36 Cupillard L

DEVELOPMENT OF A *LEPTOSPIRA* AUSTRALIS EXPERIMENTAL CHALLENGE MODEL USING A VIRULENT CANINE FIELD ISOLATE AND EVALUATION OF THE EFFICACY OF TWO QUADRIVALENT ANTILEPTOSPIRAL VACCINES AGAINST MORTALITY, CLINICAL SIGNS, INFECTION, BACTERIAL EXCRETION, RENAL CARRIAGE, AND RENAL LESIONS

D2-37 Fernandez M

SUBVERSION OF RENAL IMMUNE DEFENSES BY LEPTOSPIRES UPON CHRONIC COLONIZATION IN MICE

D2-38 García F

STUDY OF THE HUMORAL IMMUNE RESPONSE INDUCED BY A POLYVALENT VACCINE IN CATTLE NATURALLY EXPOSED TO *LEPTOSPIRA* spp. INFECTION

D2-39 Gomes-Solecki M

LEPTOSPIRA INDUCED IMMUNE RESPONSES IN THE RESERVOIR HOST BEFORE COLONIZATION OF THE KIDNEY AT 7DPI

D2-40 Haake DA

INDUCTION OF TYPE I INTERFERON RESPONSES BY *LEPTOSPIRA* SPECIES

D2-41 Hartloge C

COMPARISON OF SEROLOGICAL ASSAYS FOR THE DIAGNOSIS OF LEPTOSPIROSIS: IMMUNODOT AND MICROSCOPIC AGGLUTINATION TEST (MAT)

IMMUNOLOGY AND PROTECTIVE IMMUNITY

D2-42 Isaac L

ETIOPATHOGENESIS OF EXPERIMENTAL LEPTOSPIROSIS-ASSOCIATED PULMONARY HEMORRHAGIC SYNDROME IN C3H/HEJ MICE

D2-43 Klein J

DIFFERENTIAL INNATE RECOGNITION IN MURINE AND PRIMARY HUMAN DERMAL FIBROBLASTS UPON LEPTOSPIRAL INFECTION

D2-44 Barbosa LN

OUTCOME OF VACCINATION USING A LIVE-ATTENUATED *LEPTOSPIRA INTERROGANS* VACCINE IN C3H/HEJ MICE

D2-45 Ozuru R

PERCUTANEOUSLY INFECTED *LEPTOSPIRA* INDUCES LIPOLYSIS IN THE HOST ADIPOSE TISSUE

D2-46 Patarakul K

DEVELOPMENT OF mRNA VACCINES ENCODING *LEPTOSPIRA* IMMUNOGLOBULIN-LIKE PROTEIN A PROTEIN AGAINST LEPTOSPIROSIS

D2-47 Werts C

STUDY OF THE JARISCH-HERXHEIMER REACTION IN HUMAN BLOOD AND IN A MOUSE MODEL OF ACUTE LEPTOSPIROSIS

D2-48 Ko Y-C

LEPTOSPIROSIS CHRONIC KIDNEY DISEASE: UNDERSTANDING FROM IMMUNE REGULATION TO RENAL FIBROSIS

VACCINATION WITH A *LEPTOSPIRA* RECOMBINANT PROTEIN CANDIDATE WORSENS THE CLINICAL SIGNS FOLLOWING CHALLENGE IN MICE

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In this study, we characterized an immunogenic *Leptospira* methyl-accepting chemotaxis protein (MCP) identified through a reverse vaccinology approach and tested the protective efficacy of a recombinant fragment in C3H/HeJ mice. The predicted structure of full-length MCP revealed an architecture typical for topology class I MCPs, the most common sensing molecules found in bacteria which direct motility towards favorable environments through sensing chemical cues (1). C3H/HeJ mice were immunized three times with recombinant MCP fragment or sterile PBS (negative control) through intramuscular route. An unvaccinated/unchallenged group was kept as additional negative control. Animals were challenged intraperitoneally with 10^5 pathogenic *L. interrogans*. Immune responses were measured by indirect ELISA and bacterial colonization was assessed using RT-qPCR and bacterial culture. Survival rate and histopathology between groups were compared.

A single dose of vaccine elicited a significant IgG antibody response in immunized mice compared to controls ($P < 0.0001$), especially the IgG1 and IgG2a subclasses. Despite eliciting a robust immune response, the vaccine did not protect mice from disease and renal colonization. MCP vaccinated animals developed clinical signs faster than the control group. The gross and histopathological changes between the MCP vaccinated and control groups were also different.

The lack of protection and exacerbation of clinical signs in this study is intriguing and warrants an investigation to examine the role of this protein in *Leptospira* pathogenesis. It is possible that anti-MCP antibodies may block the MCP signaling cascade, limit chemotaxis and prevent *Leptospira* from reaching its destination, and facilitating *Leptospira* replication in the blood stream leading to septicemia. It is also possible that such antibodies may exist in endemic areas where humans are highly exposed to *Leptospira* antigens and infection might lead to disease enhancement.

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STUDY ON THE INTERFERENCE OF VACCINE ANTIBODIES IN THE SEROLOGICAL DIAGNOSIS OF LEPTOSPIROSIS IN CATTLE

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Keywords: Cattle, ELISA, MAT, Vaccine, Leptospirosis.

A simple whole-cell-based IgG ELISA specific for *Leptospira* spp. was compared with the microscopic agglutination test (MAT) to detect IgG antibody responses to a commercial vaccine in cattle. After initial vaccination (10 cows), specific antibodies to *Leptospira* spp. were detected in 90% of animals by IgG-ELISA and 60% by MAT, whereas after booster, antibodies were detected in 100% and 90% of animals by IgG-ELISA and MAT, respectively. Interferences by vaccine antibodies were evident with both MAT and ELISA serological tests. For the diagnosis of the disease, it would be advisable to use the ELISA method from two and a half months post-vaccination and the MAT method from four months post-vaccination to avoid interference by vaccine antibodies. On the other hand, our results suggest that IgG-ELISA has the potential to be a useful method to evaluate the development of IgG antibodies induced by *leptospira* vaccine.

NEUTRALIZING GUT-DERIVED LIPOPOLYSACCHARIDE AS A NOVEL THERAPEUTIC STRATEGY FOR SEVERE LEPTOSPIROSIS

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Leptospirosis is an emerging infectious disease caused by pathogenic *Leptospira* spp. Humans and some mammals can develop severe forms of leptospirosis accompanied by a dysregulated inflammatory response, which often results in death. The gut microbiota has been increasingly recognized as a vital element in systemic health. However, the precise role of the gut microbiota in severe leptospirosis is still unknown. Here, we aimed to explore the function and potential mechanisms of the gut microbiota in a hamster model of severe leptospirosis. Our study showed that leptospires were able to multiply in the intestine, cause pathological injury, and induce intestinal and systemic inflammatory responses. 16S rRNA gene sequencing analysis revealed that *Leptospira* infection changed the composition of the gut microbiota of hamsters with an expansion of Proteobacteria. In addition, gut barrier permeability was increased after infection, as reflected by a decrease in the expression of tight junctions. Translocated Proteobacteria were found in the intestinal epithelium of moribund hamsters, as determined by fluorescence in situ hybridization, with elevated LPS levels in the serum. Moreover, gut microbiota depletion reduced the survival time, increased the leptospiral load, and promoted the expression of proinflammatory cytokines after *Leptospira* infection. Intriguingly, fecal filtration and serum from moribund hamsters both increased the transcription of *TNF-α*, *IL-1β*, *IL-10*, and *TLR4* in macrophages compared with those from uninfected hamsters. These stimulating activities were inhibited by LPS neutralization using polymyxin B. Based on our findings, we identified an LPS neutralization therapy that significantly improved the survival rates in severe leptospirosis when used in combination with antibiotic therapy or polyclonal antibody therapy. In conclusion, our study not only uncovers the role of the gut microbiota in severe leptospirosis but also provides a therapeutic strategy for severe leptospirosis.

LEPTOSPIRA-SPECIFIC IMMUNOGLOBULIN Y (IGY) IS PROTECTIVE IN INFECTED HAMSTERS

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Leptospirosis, a globally significant zoonotic disease caused by pathogenic *Leptospira*, continues to threaten the health and public safety of both humans and animals. Current clinical treatment of leptospirosis mainly relies on antibiotics but their efficacy in severe cases is controversial. Passive immunization has a protective effect in the treatment of infectious diseases. In addition, chicken egg yolk antibody (IgY) has gained increasing attention as a safe passive immunization agent. This study aimed to investigate whether hens produce specific IgY after immunization with inactivated *Leptospira* and the protective effect of specific IgY against leptospirosis. First, it was demonstrated that specific IgY could be extracted from the eggs of hens vaccinated with inactivated *Leptospira* and that specific IgY can specifically recognize and bind homotypic *Leptospira* with a high titre, as shown by MAT and ELISA. Next, we tested the therapeutic effects of IgY in early and late leptospirosis using a hamster model. The results showed that early specific IgY treatment increased the survival rate of hamsters to 100%, alleviated pathological damage to the liver, kidney, and lung, reduced leptospiral burden, and restored haematological indices as well as functional indicators of the liver and kidney. The therapeutic effect of early specific IgY was comparable to that of doxycycline. Late IgY treatment also enhanced the survival rate of hamsters and improved the symptoms of leptospirosis similar to early IgY treatment. However, the therapeutic effect of late IgY treatment was better when combined with doxycycline. Furthermore, no *Leptospira* colonization was observed in the kidneys, livers, or lungs of the surviving hamsters treated with specific IgY. Mechanistically, IgY was found to inhibit the growth and adhesion to cells of *Leptospira*. In conclusion, passive immunotherapy with specific IgY can be considered an effective treatment for leptospirosis, and may replace antibiotics regarding its therapeutic effects.

DEVELOPMENT OF A *LEPTOSPIRA* AUSTRALIS EXPERIMENTAL CHALLENGE MODEL USING A VIRULENT CANINE FIELD ISOLATE AND EVALUATION OF THE EFFICACY OF TWO QUADRIVALENT ANTILEPTOSPIRAL VACCINES AGAINST MORTALITY, CLINICAL SIGNS, INFECTION, BACTERIAL EXCRETION, RENAL CARRIAGE, AND RENAL LESIONS

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Leptospirosis, the most widespread zoonosis, can cause severe disease in dogs, potentially lethal if not treated. Latest data shows that the most prevalent *L*. spp.-serogroup in Europe is *L*. Icteroheamorrhagiae (53%), followed by serogroups detected in some countries, but not all, like *L*. Australis (13%) (J. Wenderlein et *al*., submitted). We first isolated highly virulent *L*. Australis strains from infected dogs in France (P. Bergamo et *al*., submitted), applying a novel gene-based molecular approach. Then we carried out a selection process both in puppies and adult dogs to select one strain which gave satisfactory results for acute challenge model development (CMD) by intraperitoneal route performed as requested by European Pharmacopeia monograph 0447. This common process of CMD is specific to evaluate antileptospiral vaccines beneficial effects on six different criteria (mortality, clinical signs, infection, bacterial excretion, renal carriage, and renal lesions). All clinical studies have been conducted in compliance with the Animal Procedure Statement #09-09-0001R which was reviewed and approved by the Ethics Committee of Boehringer Ingelheim.

Then we evaluated the efficacy induced by our new licensed quadrivalent antileptospiral vaccine (EURICAN® L4) including four *Leptospira* components (Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis) against our severe *L*. Australis challenge model. Seven to 10-week-old puppies received 2 doses of the vaccine, 4 weeks apart and were challenged with our selected *L*. Australis isolate 2 weeks (OOI) and 12 months (DOI) after the second vaccine injection. Clinical signs, infection, bacterial excretion, renal carriage, and renal lesions were assessed after challenge. The challenge induced multiple severe clinical signs in controls leading to the death or euthanasia of 83% of puppies and 57% of adults. In controls, leptospiremia was detected in all dogs, leptospiruria in 67% of puppies and 86% of adults, kidneys tested positive for *Leptospira* in 83% of puppies and 71% of adults, kidney lesions were observed in 100% of puppies and 86% of adults. In addition, a thrombocytopenia associated with increased concentrations of urea, creatinine, and aspartate aminotransferase was recorded in controls displaying severe clinical signs. In both OOI and DOI studies, none of the vaccinates had clinical signs, no *Leptospira* were detected in blood, urine, and kidney samples, and no kidney lesions were observed. No significant changes in hematological and biochemical parameters from vaccinates were recorded (J. Bouvet et *al*, submitted).

We tested also in a separated study another licensed quadrivalent antileptospiral non adjuvanted vaccine including four *Leptospira* components (Canicola, Ictero-

haemorrhagiae, Grippotyphosa and Australis) against our L. Australis challenge model. Seven to 9-week-old puppies received 2 doses of the vaccine, 4 weeks apart and were challenged with our L. Australis isolate 3 weeks after the second vaccine injection. Clinical signs, infection, bacterial excretion, renal carriage, and renal lesions were assessed after challenge. The challenge induced multiple severe clinical signs in controls leading to euthanasia of 57% of puppies. In controls, leptospiremia was detected in all dogs, leptospiruria in 71% of puppies, kidneys tested positive for *Leptospira* in 71% of puppies, kidney lesions were observed in 71% of puppies. Two vaccinates had severe clinical signs leading to euthanasia, *Leptospira* were detected in blood (100%), urine (57%), and kidney samples (57%), and kidney lesions (57%) were observed in vaccinates. Significant changes in hematological and biochemical parameters from vaccinates were recorded.
L. Australis is one of the most prevalent *Leptospira* strains infecting dogs leading, in natural conditions, to severe life-threatening cases. EURICAN® L4 was shown to induce a quick and long-lasting protection against a severe *L*. Australis infectious challenge, preventing mortality, clinical signs, infection, bacterial excretion, renal carriage, and renal lesions whereas in the similar challenge conditions, the other licensed vaccine tested showed neither reduction of the urinary shedding nor protection against the clinical disease.

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SUBVERSION OF RENAL IMMUNE DEFENSES BY LEPTOSPIRES UPON CHRONIC COLONIZATION IN MICE

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Leptospirosis, caused by pathogenic *Leptospira*, is a re-emerging zoonotic disease prevalent in soil and water, often transmitted through the urine of asymptomatic renal carriers of leptospires. Humans and other mammals can contract the disease through contact with contaminated environments, resulting in mild disease in most cases. However, multi-organ dysfunction syndrome could also occur in human leptospirosis, potentially leading to death. Importantly, recent studies suggest that leptospirosis may be an overlook risk for chronic kidney disease[1].

We developed a mouse model of experimental leptospirosis using bioluminescent *L. interrogans*, which revealed a biphasic disease characterized by an initial acute phase of bacterial dissemination in the bloodstream, followed by lifelong chronic infection localized in the renal tubules[2], provoking mild fibrosis[3]. In endemic regions, humans have also been shown to be asymptomatic chronic carriers of leptospires[4] but whether leptospirosis may affect the renal immune system is unknown.

In this study, we investigated whether asymptomatic leptospiral renal colonization affects the renal innate immune defenses. We used a previously established mouse model of uropathogenic *Escherichia coli* (UPEC) infection[5] to assess the pathophysiological consequences of chronic renal carriage of *Leptospira*.

Therefore, mice chronically infected with leptospires were infected through the retro-urethral route with UPEC. Twenty-four hours post-infection, mice were euthanized, and their kidneys and bladder were analyzed to quantify the UPEC and *Leptospira* loads. Inflammation was assessed by RT-qPCR or ELISA for pro-inflammatory cytokines and chemokines. Leukocyte recruitment and immune cell populations in infected kidneys were studied by purification and cytometric analyses.

The results show that chronic colonization of leptospires modulates the host defense and fragilizes the kidney defense against UPEC. These findings may help to better understand the detrimental consequences of chronic leptospirosis in humans and animals, and potentially, as previously shown[5], give new host-directed therapeutic directions to restore the renal defense.

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STUDY OF THE HUMORAL IMMUNE RESPONSE INDUCED BY A POLYVALENT VACCINE IN CATTLE NATURALLY EXPOSED TO *LEPTOSPIRA* spp. INFECTION

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This study examines Holstein heifers' immune response to a commercial vaccine formulated with eight *Leptospira* spp. antigens. Selected from a farm with 4.5% leptospiruria prevalence in adult cows, sixty heifers were tested negative in two consecutive MATs for 24 serovars (cutoff=50) and urine qPCR analyses. Thirty heifers received the vaccine (Vaccinated Group, VG), while the remaining did not (Control Group, CG). The initial dose was administered at 3-8 months of age followed by three boosters at months 1, 7 and 15 post-first dose. Blood and urine samples were collected monthly over 15 months. Leptospiruria was detected through qPCR and culture (EMJH-medium). MAT measured antibodies against vaccine antigen serovars (homologous), and others circulating in Uruguayan cattle (heterologous) [1]. IgG levels were also assessed by ELISA. Antibody neutralization was evaluated using both *in vitro* assays and *In-vivo* murine infection models. Leptospiruria was observed in both groups, with no significant difference between VG and CG ($p>0.05$). The vaccine showed 20% efficacy in inhibiting *Leptospira* excretion. MAT reactivity in VG sera indicated immunogenicity for seven out of eight homologous antigens. Heterologous antigens showed low MAT seroreactivity, except for *L. interrogans* serogroup Pomona variant Kennewicki, although it was significantly lower than the vaccine homolog *L. interrogans* serogroup Pomona serovar Pomona ($p<0.0001$). IgG levels increased post-boost, indicating a memory humoral response. Vaccine-induced antibodies exhibited *in vitro* neutralization activity against autochthonous variants: *L. interrogans* serogroup Canicola serovar Canicola and *L. borgpetersenii* serogroup Sejroe serovar Hardjo. However, when passively transferred into the murine infection model, antibodies failed to prevent renal colonization by *L. interrogans* serogroup Canicola serovar Canicola and *L. interrogans* serogroup Pomona serovar Kennewicki. In conclusion, while the vaccine elicited a humoral immune response, the efficacy in preventing infection and renal colonization was limited.

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LEPTOSPIRA INDUCED IMMUNE RESPONSES IN THE RESERVOIR HOST BEFORE COLONIZATION OF THE KIDNEY AT 7DPI

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Leptospirosis, a neglected re-emerging enzootic disease, raised serious concerns after recent human and canine outbreaks were reported in the United States. We infected C3H/HeJ mice with pathogenic and non-pathogenic strains of *Leptospira* and analyzed humoral and cellular responses at 7 days post infection, when *Leptospira* interrogans colonizes the kidney and is no longer detectable in blood. Both pathogenic and non-pathogenic *Leptospira* sp. induced IgM and IgG3 in blood at 7 dpi. Flowcytometry data showed that no myeloid or lymphoid cellular response was observed during the non-pathogenic *L. biflexa* infection in spleen and pLN. In contrast, pathogenic *L. interrogans* induced significant recruitment of myeloid and lymphoid cells in the spleen at 7 dpi with an overall increase in phagocytic monocytes, monocyte-macrophage and neutrophils, whereas both dendritic cells and resident-macrophages decreased which is indicative of cell death or transit into peripheral lymph nodes (pLN) to present the antigen. Moreover, pathogenic *L. interrogans* infection led to increase in B cells, helper T cells and its effector phenotypes in spleen and pLN. Interestingly, in contrast to memory phenotypes, effector T cells were increased within helper and cytotoxic T cell subsets in spleen which suggests an adaptive response engagement and activation to prevent pathology.

INDUCTION OF TYPE I INTERFERON RESPONSES BY *LEPTOSPIRA* SPECIES

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Introduction: Leptospirosis is a widespread zoonotic disease that can be potentially fatal in infected humans and animals. The spirochetal bacterium *Leptospira interrogans* is the major causative agent of leptospirosis. Leptospiral lipopolysaccharide and lipoproteins can activate murine TLR4 and TLR2, but the host immune response to *Leptospira* spp. remains poorly defined. The type I interferon (IFN) response plays an important role in T-cell responses, inflammation, and host defense against intracellular pathogens.

Materials and Methods: Bacterial strains: *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 and *Leptospira biflexa* serovar Patoc strain Patoc1. Mice: C57BL/6J served as the genetic background for *cGAS, STING, IFNAR, TRIF, TLR3,* and *TLR4* knockouts. Bone marrow-derived macrophages were exposed to leptospires *in vitro*, gene transcripts were measured by qRT-PCR, and cytokines in culture supernatants were measured by ELISA. Mice were infected by intraperitoneal challenge and burden of infection was determined by measuring the ratio of *L. interrogans* genomic DNA to mouse PPIA by qPCR using primers specific for *lipL32* and *PPIA*, respectively.

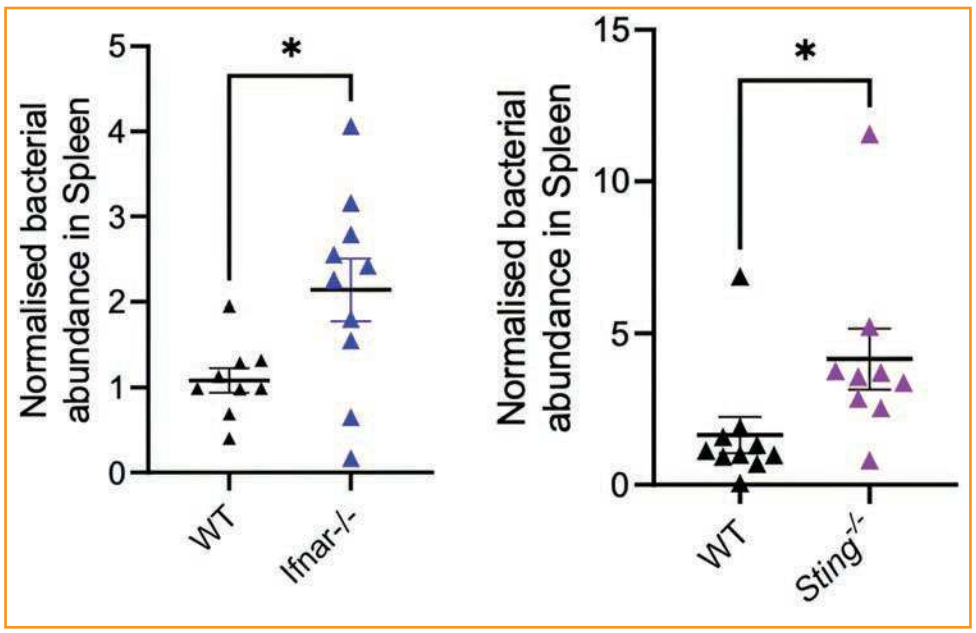


Figure 1 *Bacterialabundance in spleen in knockout mice w.r.t. WT mice and normalized to housekeeping gene (PPIA) on Day 3 post infection with 2x10[8] cfu intraperitoneally. Plotted mean ± SEM p≤0.05 by Unpaired student's t test and Mann-Whitney test.*

Results: We observed that *L. interrogans* induced a robust type I IFN response in both mouse macrophages and human monocytes. The *L. interrogans*-induced type I IFN response correlated with rapid phosphorylation of TBK1 and IRF3. The type I IFN response was induced by both *L. interrogans* and *L. biflexa*, by heat-killed bacteria, and was partially dependent on phagocytic uptake of the bacteria. *In-vivo* studies demonstrated a role of type I IFN response in controlling bacteria loads in the mouse model (Fig. 1). Interestingly, we found the type I IFN response to be dependent on both cGAS-STING and TLR4-TRIF pathways.

Conclusion: These studies demonstrate that *L. interrogans* can drive a potent type I IFN response that appears to require crosstalk between the TLR4-TRIF and cGAS-STING pathways and may contribute to the host defense against the pathogen.

COMPARISON OF SEROLOGICAL ASSAYS FOR THE DIAGNOSIS OF LEPTOSPIROSIS: IMMUNODOT AND MICROSCOPIC AGGLUTINATION TEST (MAT)

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Leptospira serologic assays can be difficult to interpret given timing of symptom onset and development of antibodies. From 1998 to 2014, 1544 serum specimens were received at the Centers for Disease Control and Prevention (CDC) for *Leptospira* serology testing and confirmation by the microscopic agglutination test (MAT) and the IgM ImmunoDOT test, including samples from 36 states, Washington D.C., and Puerto Rico. The MAT panel consisted of up to twenty different serovars with the overall result interpretation determination based on the highest reported serovar titer. 875 specimens were reported to be borderline/supportive or positive/confirmatory by at least one test. 668 specimens had information available to calculate the days post onset of symptoms, of which 433 were borderline/supportive or positive/confirmatory by at least one test. Of the 211 specimens that were negative by IgM ImmunoDOT, *L. interrogans* serovar Bratislava, *L. kirschneri* serovar Grippotyphosa, and *L. interrogans* serovar Djasiman most often had supportive or confirmatory titer levels by MAT. Results suggest that these serovars are at higher risk of being missed by the IgM ImmunoDOT screening method.

ETIOPATHOGENESIS OF EXPERIMENTAL LEPTOSPIROSIS-ASSOCIATED PULMONARY HEMORRHAGIC SYNDROME IN C3H/HEJ MICE

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Leptospirosis is a neglected disease caused by *Leptospira*. Most infected individuals (90%) are asymptomatic or present mild symptoms common to other acute febrile illnesses. Some patients may develop a severe form known as Leptospirosis-Associated Pulmonary Hemorrhagic Syndrome (LPHS), characterized by aggressive clinical evolution and high lethality (~50%). The etiopathogenesis of LPHS is still poorly understood, and very little is known regarding the contribution of the immune response to local tissue damage. Additionally, previous studies by Croda *et al.* (2010) have shown the deposition of C3 from the Complement System in the alveolar septa of LPHS-patients. In this study, we infected male and female C3H/HeJ mice (TLR4$^{-/-}$) – susceptible to leptospirosis - with distinct doses of pathogenic *L. interrogans* Fiocruz L1-130. We observed a lower survival rate associated with greater severity of pulmonary hemorrhage and jaundice in infected males compared to infected females. Next, we inoculated C3H/HeJ male animals with sublethal and lethal doses of L1-130 to evaluate the circulating leukocyte populations on different days of infection. We observed a reduction in the lymphocyte population (at 7 dpi) and an increase in granulocyte numbers (at 3 dpi and 7 dpi). In parallel, infected mice had fewer circulating platelets at 3 dpi and 7 dpi compared to non-infected animals. Hemolytic assay and C5a concentration (ELISA) were performed to analyze the Complement System activation in the serum and the bronchoalveolar lavage samples, respectively. A decreased Complement System hemolytic activity and lower C5a levels were observed in infected mice compared to negative controls. The local deposition of Complement System proteins has been investigated in the lung tissue of infected mice by immunohistochemistry analysis. Based on these findings, we suggest a potential immunomodulatory role of this pathogen in the immune response during LPHS, which may contribute to pathological aggravations. Further experiments will be performed to better understand the variation in leukocyte populations and the involvement of Complement System in LPHS.

DIFFERENTIAL INNATE RECOGNITION IN MURINE AND PRIMARY HUMAN DERMAL FIBROBLASTS UPON LEPTOSPIRAL INFECTION

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Leptospirosis is a worldwide neglected and reemerging zoonosis caused by *Leptospira interrogans*, which are pathogenic spirochetes that infect their hosts by crossing the skin and mucosa barrier without creating local inflammation. Fibroblasts are spindle-shaped cells that constitute the major cell population of the connective tissue and are therefore found in the dermis and lamina propria. They play an important role in wound healing and contribute to the synthesis and remodeling of the extracellular matrix (ECM). The ECM is a well-known adhesion site for leptospires. Fibroblasts have recently emerged as immune cells able to activate and modulate innate immune responses upon a pathogenic stimulus. To date, the immune response of fibroblasts is poorly studied even though they represent the first defense barrier that *Leptospira* cross upon natural infection.

Upon infection, the innate immune system recognizes a microbial associated molecular pattern (MAMP) (such as lipopolysaccharide (LPS), lipoproteins, etc.) by a pattern recognition receptor (PRR) (e.g. Toll-Like receptors (TLRs) or NOD receptors). Our group previously showed that leptospires are stealth bacteria evading different PRR recognition in macrophages[1], therefore limiting the inflammation[2].

In this study, we investigated the response of skin murine and human primary fibroblasts[3] to leptospiral infection and stimulation with TLR/NOD receptor agonists. Our results show a (i) differential recognition of leptospires and TLR agonists between mouse and human fibroblasts and (ii) production of chemokine but an unexpected absence of pro-inflammatory cytokines production in primary fibroblasts.

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OUTCOME OF VACCINATION USING A LIVE-ATTENUATED *LEPTOSPIRA INTERROGANS* VACCINE IN C3H/HEJ MICE

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Introduction: Live-attenuated *Leptospira* mutant vaccines could be interesting alternatives to overcome the limitations associated with vaccines composed by killed whole-cell available against leptospirosis [1-3]. In this study, we tested a laboratory adapted pathogenic *Leptospira* field isolate as a vaccine candidate [4].
Methods. C3H/HeJ mice were immunized twice subcutaneously with $5x10^7$ live-attenuated *Leptospira* vaccine or PBS+Alhydrogel®, and an unvaccinated/unchallenged group was kept as additional negative control. Animals were challenged by intraperitoneal route with 10^5 pathogenic *L. interrogans*. Immune responses were measured by Indirect ELISA and vaccine efficacy was evaluated by survival rate, bacterial culture, RT-qPCR and histopathology.

Results: A single dose of the live-attenuated *Leptospira* vaccine elicited significant IgM and IgG antibody responses in immunized mice compared to negative controls ($P < 0.0001$), including levels of the subclasses IgG1 ($P < 0.0001$), IgG2a ($P = 0.0009$) and IgG3 ($P < 0.0001$). No significant difference in protection was observed between live-attenuated *Leptospira* vaccine (87.5%) and the PBS+Alhydrogel® control groups (37.5%). *Leptospira* DNA was detected in kidneys, lungs, liver, spleen and heart of all immunized animals. Renal colonization was further confirmed by positive *Leptospira* cultures obtained from kidneys of 7/8 animals immunized with live-attenuated vaccine.

Conclusion: Despite eliciting a robust immune response, our live-attenuated *Leptospira* vaccine candidate did not protect mice from *Leptospira* infection and renal colonization. Our study reinforces the need to improve the understanding of the nature of protective immune responses against leptospirosis, especially the role of cellular immune response.

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PERCUTANEOUSLY INFECTED *LEPTOSPIRA* INDUCES LIPOLYSIS IN THE HOST ADIPOSE TISSUE

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Introduction: We previously reported that pathogenic *Leptospira* colonizes the subcutaneous adipose tissue neighboring the injection sites *In-vivo* [1] and induces lipolysis in murine adipocytes *in-vitro* [2]. Because the primary carbon sources of leptospires are fatty acids and glycerol [3, 4], which are the components of triglycerides, this seems a reasonable strategy. This study investigated the detailed interaction between pathogenic *Leptospira* and host adipose tissue both *in-vitro* and *In-vivo*.

Materials and methods: Live or heat-killed (56°C, 30 min) *L. interrogans* serovar Manilae strain L495 were co-cultured with differentiated 3T3-L1 adipocytes (the murine-derived adipose progenitor cell line) in the ratio of 100 to 1. After 72 hours of co-culture, total RNA was extracted from the cells for subsequent transcriptomic analyses. 8-week-old male C57BL/6J mice were subcutaneously injected with the 10^7 of L495 in the inguinal region. Subcutaneous adipose tissue was collected four days later for histopathological analysis using hematoxylin and eosin staining.

Results: 3T3-L1 adipocytes co-cultured with live L495 showed transcriptomic down-regulation related to lipid metabolism. The disruption of signaling pathways involved in lipolysis was particularly suggested. The similar lipolysis observed previously *in-vitro* also occurred in mice adipose tissue, indicating that percutaneously infected *Leptospira* affects the host lipid metabolism.

Conclusion: The percutaneous infection of *Leptospira* induced lipolysis in the subcutaneous adipose tissue of mice. The exact impact of this lipolysis on the pathogenicity and proliferation of *Leptospira* remains uncertain at present and is a primary focus for future study. If the fatty acid profile produced by this lipolysis is specific to *Leptospira* infection, these fatty acids could serve as specific leptospirosis biomarkers.

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DEVELOPMENT OF mRNA VACCINES ENCODING *LEPTOSPIRA* IMMUNOGLOBULIN-LIKE PROTEIN A PROTEIN AGAINST LEPTOSPIROSIS

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Leptospirosis, a global zoonotic disease caused by pathogenic *Leptospira* species, affects nearly a million people annually and has a fatality rate exceeding 10% in severe cases. Vaccination of at-risk populations is a key strategy for the prevention and control of infectious diseases. However, current leptospirosis vaccines have not achieved complete protection. Messenger RNA (mRNA) vaccines, successfully utilized against viral infections like COVID-19, have emerged as a promising platform for addressing a broader range of pathogens. In this study, we developed mRNA vaccine constructs encoding the C-terminal domains 7-13 of leptospiral immunoglobulin-like protein A (LigAc), implicated in leptospiral pathogenicity during infections. The mRNA vaccine constructs consisted of either the native sequence or a codon-optimized sequence of LigAc, with adjustments to the guanine and cytosine content. After transfection into HEK293T cells, both mRNA constructs were non-toxic and resulted in the secretion of the LigAc protein into the culture supernatant. The expressed LigAc protein exhibited a higher molecular weight than expected, which normalized to the expected size upon deglycosylation, indicating post-translational glycosylation. The expression of LigAc increased in a time- and concentration-dependent manner. Notably, the codon-optimized construct exhibited higher protein expression levels than the native sequence. The constructs demonstrating superior expression will be further modified and tested as vaccine candidates in animal models to evaluate their immunogenicity and protective efficacy against leptospirosis. This study offers valuable insights into the advancement of mRNA-based vaccines against leptospirosis, paving the way for future protective strategies.

STUDY OF THE JARISCH-HERXHEIMER REACTION IN HUMAN BLOOD AND IN A MOUSE MODEL OF ACUTE LEPTOSPIROSIS

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Leptospirosis is a neglected and re-emerging zoonosis with a worldwide distribution caused by pathogenic *Leptospira*. Most cases are mild or asymptomatic but multi-organ failure may occur in severe cases of human leptospirosis, leading to death in 10 % of cases. The pathophysiology of leptospirosis is poorly understood. Antibiotics are active to combat *Leptospira* if administered early, but the diagnostic is complicated, and patients admitted at the ICU already exhibit severe symptoms and antibiotics are not always helpful.

Leptospira are stealth bacteria escaping several innate immune pathways[1]. Our group showed that pathogenic leptospires evade the human and mouse immune system and recognition by Toll-like (TLR) and NOD receptors, therefore limiting the inflammation[1-3]. Moreover, we recently showed in mice that no cytokine storm is involved in lethal leptospirosis (Papadopoulos, unpublished data). Yet, hospitalized patients with leptospirosis can suffer from a detrimental inflammatory syndrome following antibiotic treatment called the Jarisch-Herxheimer (JHR) reaction[4].

In this study, we aimed to understand the causes of inflammation occurring in patients with leptospirosis. We will test our hypothesis that JHR happens in case of administration of antibiotics targeting the bacterial cell wall of *Leptospira*, potentially releasing inflammatory compounds in the circulation.

We used our recently developed JHR models, using human blood from healthy volunteers and a mouse model of lethal leptospirosis, to study the inflammation triggered by different regimen of antibiotics used in New Caledonia. Our objective is to identify the leptospiral compounds responsible for the inflammation, the leukocytes involved in inflammation as well as the Toll-like Receptor (TLR) or other receptors involved.

This study should help to better understand the JHR reaction that may aggravate patients with leptospirosis, elucidate the paradox of *Leptospira* being stealth bacteria but provoking inflammation, as well as helping MDs in the choice of antibiotics to avoid adverse inflammatory effects.

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LEPTOSPIROSIS CHRONIC KIDNEY DISEASE: UNDERSTANDING FROM IMMUNE REGULATION TO RENAL FIBROSIS

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Introduction: *Leptospirosis* is often overlooked as an infectious disease. Research shows that *Leptospira* prefers to live in the kidneys, escaping the host cellular immune response. The association between leptospirosis and chronic kidney disease remains uncertain. This study aimed to elucidate why the immune system is unable to clear *Leptospira* after developing immunity following infection, leading to kidney fibrosis.

Methods: Mouse splenocytes were cultured ex vivo and subsequently infected with pathogenic and nonpathogenic *Leptospira* strains to simulate cellular immune responses. Analysis of helper T cell subtypes Th1, Th17, and Tregs-specific markers using flow cytometry. In an animal infection model, 7-week-old C57B6/L mice were infected with *Leptospira* after intraperitoneal injection of anti-CD25 antibodies to deplete Tregs and induce immune imbalance. We evaluated the animals' immune response and expression of renal fibrosis markers.

Result: Ex vivo research of cellular immune responses in mouse splenocytes revealed that pathogenic *Leptospira* induced the differentiation of splenocytes into the helper T cell subtypes Th1, Th17, and Treg. Furthermore, the polarization of helper T cell subtypes was higher than that induced by non-pathogenic *Leptospira*. In infected animal models, depletion of Tregs led to the loss of immunosuppression, which enhanced the immune response for *Leptospira* clearance and improved renal fibrosis.

Conclusion: Pathogenic Leptospiral infection leads to polarization of Tregs in host to play a key role in immune regulation. This mechanism enables the host to suppress the immune response, thereby conferring the ability of *Leptospira* to escape immunity. Therefore, pathogens can safely persist in the kidney and cause renal fibrosis.

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SESSION 5

RODENT-BORNE HAEMORRHAGIC FEVERS

Moderators:
Arent Zbigniew

4TH ELS MEETING
13TH ILS CONFERENCE
BRUSSELS
2-4 SEPTEMBER, 2024

Emerging infections in the global village: the rodent and mosquito borne hemorrhagic infections

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Introduction

Infectious diseases are still - and even increasingly - an important health risk, not only in resource limited and/or less developed countries, but also increasingly in developed countries. The illusion that infectious diseases are virtually out of the world we have left behind more than 30 years ago. This illusion could arise from an underestimation of the complex dynamics between infectious diseases, people and their environment and yet also an overestimation of knowledge, knowledge obtained by science. Epidemiology of infectious diseases and the challenge to find sustainable solutions in terms of prevention and management need to be seen in the scope of the Planetary Health concept.

Emerging virus infections; clinical aspects and epidemiology

Exotic [viral] infections are virus infections that traditionally occur mainly in the tropics and subtropics. Characteristic of this group of infections is that they circulate permanently, endemic, or present themselves in smaller or larger outbreaks, with an epidemic or even pandemic character. With the change in climate, environment and animal reservoirs the distribution area and epidemiology of these virus infections is changing rapidly. And thus we cannot exclude the possibility that a number of these infectious diseases will occur also in middle and northern European countries in the [near] future.
We are confronted with a rapid change in mosquito populations on the European continent with a change in the epidemiology of [viral] mosquito borne infections e.g. West Nile virus [WNV] and dengue virus [DF] infection. Beside the mosquito borne infections the rodent-borne infections is a relevant group, containing pathogens with a major clinical impact in different areas. There is a need for sufficient rodent control programs, knowing that the rodent populations in many European countries is growing, which means an increased risk for rodent-borne infections e.g. leptospirosis and hanta virus infection.
From the clinical perspective the [viral] hemorrhagic infections and the [viral] infections causing meningo-encefalitis are among the most presented clinical features.

Haemorrhagic infections; pathogenesis

Leptospirosis and hanta virus infections belong to the hemorrhagic fevers, among which also ebola and dengue virus. What is the current knowledge about epidemiology, what do we know about pathogenesis, what are the common pathways and options for prevention and intervention in this complex and dynamic pathogenetic cascade?

INVESTIGATING NORWEGIAN URBAN RATS AS CARRIERS FOR *LEPTOSPIRA* AND SEOL VIRUS

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Rodents are recognized as hosts of a range of zoonotic agents that may present a serious threat to public health, and especially rats are known to have spread pathogenic microorganisms across the globe. Humans have strived to reduce risk of disease and damage from rats for centuries, leading to the modern pest control industry. During the last decade, some rat-borne zoonotic diseases such as the rodent-borne hemorrhagic fevers have (re-)emerged in Europe, including infections from *Leptospira* bacteria and Seoul virus. Leptospirosis was occasionally detected in dogs and humans in Norway 70-80 years ago, but the introduction of antimicrobial agents and rodent control made the disease disappear the decades to follow. However, there have been more recent reports of suspected leptospirosis amongst dogs, pigs and humans, but there are no comprehensive data on the distribution of pathogenic *Leptospira* and potential maintenance hosts in Norway. Nothing is known about the presence of Seoul virus in rats in Norway. In this study, DNA and RNA were extracted from 96 rats collected by pest control companies and frozen until further processing. DNA from kidneys (n=96) was analyzed by qPCR for pathogenic *Leptospira,* and RNA from lungs (n=80) was analyzed by RT-qPCR for Seoul virus. 9 kidney samples tested positive for *Leptospira* (9.4%). Sequencing of the *secY* gene on 6 of the positive samples identified *Leptospira interrogans,* which were further typed using MLST 7L scheme as sequence type ST17. All samples tested negative for Seoul virus. The presence of pathogenic *Leptospira* in rats in Norway deserves further investigation, with molecular typing helping to shed light on possible links to animal and human disease.

RODENT-BORNE HAEMORRHAGIC FEVERS

D2-49 Abazaj E

DIFFERENTIAL DIAGNOSIS OF LEPTOSPIROSIS AND HEMORRHAGIC FEVER WITH RENAL SYNDROME IN ALBANIAN PATIENTS DURING 2023

D2-50 Obiegal A

POTENTIAL INFLUENCING FACTORS OF LEPTOSPIRA PREVALENCE IN SMALL MAMMALS FROM GERMANY

D2-51 Silpasakorn S

A TWO-DECADE EXPERIENCE: SIMULTANEOUS DIAGNOSIS OF LEPTOSPIROSIS, SCRUB TYPHUS, AND MURINE TYPHUS USING AN INDIRECT IMMUNOFLUORESCENT ASSAY PANEL IN THAILAND

DIFFERENTIAL DIAGNOSIS OF LEPTOSPIROSIS AND HEMORRHAGIC FEVER WITH RENAL SYNDROME IN ALBANIAN PATIENTS DURING 2023

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Keywords: Leptospirosis, hemorrhagic fever with renal syndrome, acute renal failure, differential diagnosis

Introduction: Hemorrhagic fever with renal syndrome (HFRS) and leptospirosis (LS) are known in Albania as two endemic zoonotic illnesses spreading from animals to people. Acute renal failure (ARF) is one of the many common clinical syndromes of LS and HFRS diseases. This research aims to identify the common features of both HFRS and LS and their relationship with ARF.

Material and methods: We analyzed all data provided within the web-based infectious diseases surveillance system tested for both LS and HFRS during 2023. Considering the two illnesses have similar clinical presentations and some epidemiological characteristics in all cases, especially those with ARF, they are tested concomitantly for both pathogens. Specific ELISA assays measuring anti-IgG and anti-IgM antibodies have been used during the analyses.

Results: Out of all suspected patients referred to IPH, approximately 8.3% and 23.2% out of 69 tests were respectively positive for anti-IgG/IgM, HFRS, and LS, respectively. The average patient age was 45.7±9.3, with a range of 27 to 73 years old, with no differences among both diseases. After different diagnoses, one case that was referred to as suspected for HFRS turned out to be a LS-positive case, and one case that was suspected for LS turned out to be a positive case for HFRS. Fever, headache, muscle soreness, vomiting, and tiredness were the most common clinical manifestations for both diseases, with no significant differences. Only one positive case of HF and five positive cases of LS developed common signs of ARF and high levels of bilirubinemia. Severe LS and HFRS were significantly associated with borderline urine production of 400 mL/24 hours and bilirubin levels of 18 mg/dL ($P<.005$ and $P=.003$, respectively). There are no significant differences between their occupations (drivers, mechanics, builders, and pensioners), while most of the patients reported the presence of rodents in their work environments or residences.

Conclusion: Because of the increased presence of different types of rodents in workplaces and homes, LS and HFRS have displayed life-threatening severe forms in recent years, accompanied by a broad range of clinical symptoms, including renal involvement. While no significant differences were found for their clinical forms, the medical staff, particularly physicians and public health professionals, should have a

high index of suspicion for both diseases to make an early differential diagnosis and start the right treatment and other important control interventions.

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POTENTIAL INFLUENCING FACTORS OF *LEPTOSPIRA* PREVALENCE IN SMALL MAMMALS FROM GERMANY

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Abstract: Leptospirosis is a globally prevalent zoonotic disease, causing over one million human cases annually. Transmission typically occurs through direct contact with infected animals or indirect exposure to contaminated water or soil. This meta-analysis synthesizes findings from multiple studies assessing the prevalence of *Leptospira* spp. in small mammal species within Germany, evaluating factors such as seasonality, geographical variation, and demographics on *Leptospira* occurrence. Additionally, the distribution of *Leptospira* genomospecies and sequence types (ST) was investigated. A total of 6017 kidney samples from small mammals, representing 11 species, were analyzed using real-time PCR targeting the lipl32 gene. Positive samples underwent further analysis to identify *Leptospira* genomospecies and determine the ST. The overall prevalence of *Leptospira* was 7.5% (95% CI: 0.0684 - 0.0817). *Leptospira kirschneri* was detected across almost all small mammal species. Notably, bank voles exhibited a higher infection rate with L. interrogans (83.3%) compared to *L. kirschneri* (11.5%) and *L. borgpetersenii* (5.2%). The detected MLST sequence types included ST24, ST110, ST117, and ST197. Prevalence fluctuations may be due to extreme climatic events such as droughts and floods. Although human leptospirosis cases in Germany remain low, this study underscores the presence of pathogenic *Leptospira* spp. in small mammals, highlighting a potential infection source for humans. Clinicians should recognize this risk for better management of leptospirosis.

A TWO-DECADE EXPERIENCE: SIMULTANEOUS DIAGNOSIS OF LEPTOSPIROSIS, SCRUB TYPHUS, AND MURINE TYPHUS USING AN INDIRECT IMMUNOFLUORESCENT ASSAY PANEL IN THAILAND

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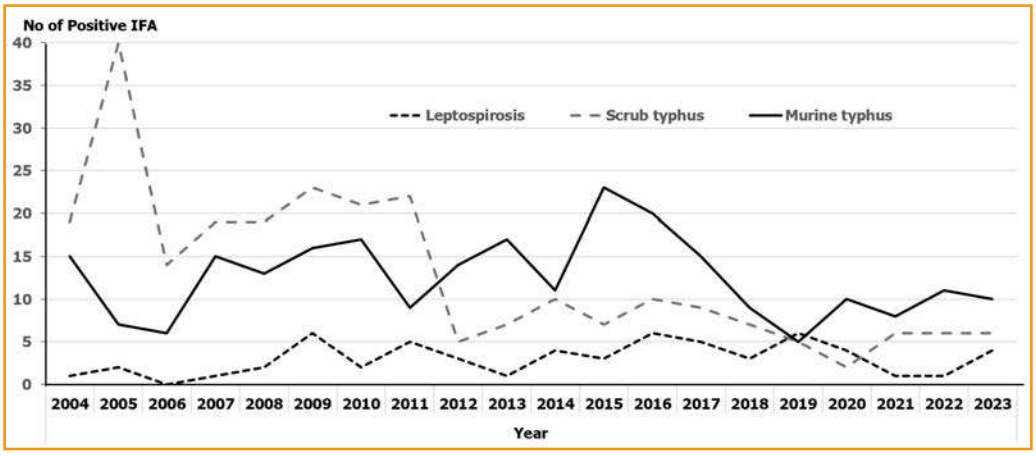
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Introduction: Leptospirosis, scrub typhus and murine typhus are common causes of acute undifferentiated fever (AUF) in Asia, including Thailand. Diagnosing these infections typically relies on serological tests. Since 2001, Siriraj Hospital in Bangkok, Thailand, has utilized an indirect immunofluorescent assay panel for the simultaneous detection of IgM and IgG antibodies to diagnose leptospirosis, scrub typhus, and murine typhus (IFA profile) in AUF patients.

Methods: We retrospectively analyzed the results of 10,971 IFA profiles conducted on adult AUF patients (age > 18 years) at Siriraj Hospital between 2004 and 2023. A positive result was defined as an antibody titer exceeding 1:400 or a fourfold rise in titer between paired serum samples.

Results: We performed a median of 718 (min 343, max 928) tests per year, and results were reported within two working days. The overall positive rate for leptospirosis, scrub typhus, or murine typhus was 4.3%, ranging from 2.5% to 16.3% over the study period (as shown in Figure below). The data suggests a stable incidence of leptospirosis, a decrease in scrub typhus cases, and a potential rise in murine typhus over time.



Conclusion: The IFA profile demonstrated its utility in managing hospitalized AUF patients with leptospirosis, scrub typhus, or murine typhus. However, the need for faster and potentially more sensitive diagnostic tests for AUF remains an important area for future development.

4TH **ELS** MEETING
13TH **ILS** CONFERENCE

DAY 3

BRUSSELS
2-4 SEPTEMBER, 2024

SESSION 6

GENETICS, MOLECULAR EPIDEMIOLOGY AND GENOMICS

Moderators:
Mathieu Picardeau & Renee Galloway

Using "omics" approach to study *Leptospira*

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High-throughput molecular methods referred to as "omics" have increased our capability to study bacterial pathogens. The most commonly used methods include genomics, transcriptomics, proteomics, and metabolomics and other recent applications such as lipidomics, metagenomics, and epigenomics are emerging. The Omics applications provide a comprehensive understanding of a biologic system under investigation. We have been applying genomics, transcriptomics, proteomics, and metabolomics approaches to study various aspects of *Leptospira* and some of these activities are summarized in this abstract. During an extensive genome sequencing project, we used short and long read genome sequencing to generate hybrid assembly of several *Leptospira* genomes and tested it usefulness for serovar and strain identification. Immunoproteomic profiling of *Leptospira* related peptides resulted in identification of several previously unknown proteins of interest to use as vaccine and diagnostic candidates. Application of *Leptospira* transcriptome sequencing using Direct cDNA and Direct RNA sequencing protocol, identified potential presence of polyadenylation of mRNA and several previously unannotated transcripts in *Leptospira*. Prokaryotic mRNA polyadenylation is an underrecognized concept which may have significant impact on interpretation of prokaryotic transcriptome. The Direct RNA sequencing also opens the opportunity to identify the presence of previously unknown transcripts. Our metagenomic approach to study *Leptospira* in environmental samples revealed usefulness of culture enrichment followed by metagenomic sequencing to effectively detect abundance and diversity of *Leptospira spp*. Using untargeted metabolomics, we were able to detect differences in extracellular and intracellular metabolites from *in vitro* cultures of *Leptospira*. In conclusion, the "Omics" methods described above are powerful tools to devise a system approach to study *Leptospira*. However, these methods result in substantial amounts of data and challenges exist in experimental design, data analysis, data management and meaningful data interpretation.

ADAPTATION OF *LEPTOSPIRA* TO OXIDATIVE STRESS AND ITS IMPLICATION FOR THE EMERGENCE OF PATHOGENICITY

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Reactive oxygen species (ROS), such as superoxide ($^{\cdot}O_2^-$) and hydrogen peroxide (H_2O_2), are natural byproducts of oxygen metabolism produced by aerobic organisms. They are also produced as part of the host innate immunity in response to microorganism invasion. These highly reactive molecules exhibit bactericidal activity, and consequently, microorganisms have evolved defense mechanisms to counteract ROS-induced oxidative damage.

While all leptospires are exposed to endogenously produced ROS, only pathogenic species are well-equipped to withstand the oxidative stress encountered inside a host, and ROS defenses are indispensable for their virulence. Notably, pathogenic *Leptospira* spp., but not saprophytes, possess a periplasmic catalase involved in reducing extracellular H_2O_2. However, pathogenic *Leptospira* spp. lack any superoxide dismutase (SOD) that could enzymatically scavenge superoxide and how they cope with this ROS is unknown.

By using comparative genomics and phylogenetic analyses, we could show that the whole repertoire of oxidative stress-related scavenging enzymes and regulators is different across the *Leptospira* genus, suggesting that the oxidative stress response has evolved with pathogenicity in *Leptospira* genus. Notably, we could demonstrate that a SOD was present in the Last Leptospiral Common Ancestor (LLCA), maintained in saprophytes clades, but lost upon the emergence of host-adapted *Leptospira* clades. In addition, transcriptomic and proteomic approaches revealed that adaptation to superoxide relies on a metabolic reprogramming involving cysteine, methionine, and leucine biosynthesis pathways. Finally, we have compared the adaptive response against oxidative stress in saprophytic and pathogenic *Leptospira* spp. and our findings support an evolutionary scenario in which free-living leptospires, which have become well-adapted to a highly oxygenated and superoxide-producing environment, transitioned towards pathogenicity by switching to a greater tolerance to host-produced hydrogen peroxide.

TRANSCRIPTOMIC ANALYSIS OF *LEPTOSPIRA* FORMING BIOFILM REVEALS ADAPTATIONS TO STARVATION AND GENERAL STRESS WHILE MAINTAINING VIRULENCE

Davignon G, Pietrosemoli N, Benaroudj N, Soupé Gilbert ME, Turc E, Picardeau M, Guentas L, Goarant C, Thibeaux R

Leptospira interrogans navigates a dual existence: surviving in environmental reservoirs and infecting mammalian hosts. *Leptospira* biofilm formation is posited as a crucial survival strategy in environmental contexts. Understanding the dynamics between biofilm lifestyle and pathogen virulence is therefore likely pivotal and has been hypothesized to be a key mechanism by which pathogenic *Leptospira* complete their life cycle. To further explore this survival strategy, our study focused on elucidating the biological state of pathogenic *Leptospira* within biofilms, particularly aiming at uncovering the adaptations and regulatory mechanisms that are involved in such complex microenvironments. In order to determine the transcriptional profile of pathogenic *Leptospira* in biofilm, we compared the genome-wide gene expression in a late biofilms (21 days old) with that in exponential planktonic cultures (5 days old), revealing a pronounced transcritpomic shift. While genes linked to motility, energy production, and metabolism were downregulated, those governing stress response, metal ion defense, and redox homeostasis showed a significant upsurge, hinting at a tailored defensive strategy against oxidative stress in late biofilms.

A standout finding was the heightened expression of the *csoR*, *copZ*, and *copA* locus, integral to copper ion stress response in other bacterial genera, suggesting a unique adaptation to metal-induced stress. Despite a reduced metabolic state in biofilms, disruption swiftly restored metabolic activity. Crucially, bacteria either in mature biofilms or disrupted thereof retained virulence in a hamster infection model, defying the notion that biofilm maturation abolishes pathogenicity.

In summary, our study illustrates *Leptospira's* adaptive equilibrium in biofilms: minimizing cellular energy expenditure as a means to conserve resources, potentially aiding in withstanding stresses while maintaining its infectious edge. These insights are crucial for understanding the survival strategies of *Leptospira*, revealing that a biofilm lifestyle may confer an advantage in maintaining virulence. This understanding is vital for managing leptospirosis across both environmental reservoirs and mammalian hosts.

A NOVEL CRISPR-PRIME EDITING STRATEGY FOR SINGLE-NUCLEOTIDE RESOLUTION MUTATIONS IN *LEPTOSPIRA* SPP.

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INTRODUCTION: The development of efficient mutagenesis techniques for *Leptospira* spp. is critical to elucidate pathogenic mechanisms of leptospirosis. The CRISPR/Cas9 system consists of the expression of endonuclease Cas9, which interacts with single-guide RNA sequences (sgRNA) and a protospacer adjacent motif (PAM) NGG, to create double-strand breaks (DSBs) in the genome. However, DSBs are lethal to *Leptospira*. Alternatively, the CRISPR prime editing (PE) strategy is capable of deletion, insertion, and base substitutions without introducing DSBs. CRISPR-PE comprises a nickase Cas9 (nCas9) genetically linked with an engineered reverse transcriptase (RT), alongside a modified sgRNA (PEgRNA) containing an extended 3' end in which a primer binding sequence (PBS) and a reverse transcription template (RTT) are present.

MATERIAL AND METHODS: A plasmid expressing the nCas9 and reverse transcriptase fusion protein was constructed (pMaOriPE). For PEgRNA, the *lipL32* promoter was used, followed by a 20-nt protospacer, the nCas9 scaffold, a 3' extension comprising a 13-nt RTT sequence and a 13-nt PBS. RTT sequences were designed to disrupt the NGG motif and create frameshift mutations. Plasmids were delivered to several species and serovars of *Leptospira* spp. by conjugation with *E. coli* β2163. nCas9-RT and target protein expression were evaluated by immunoblotting. Mutations were confirmed by sequencing. The virulence of mutant phenotypes was evaluated in the hamster model of leptospirosis.

RESULTS: CRISPR-PE was first validated in *L. biflexa*: delivery of plasmids targeting either 1 or 2 nucleotide deletions or insertions in the β-*galactosidase* gene resulted in colonies containing knock-out (KO) mutant cells confirmed by sequencing. Application of CRISPR-PE with different serovars of *L. interrogans* and *L. borgpetersenii* resulted in a 100% KO mutant recovery targeting *lipL32*. LipL32 mutants were virulent in the hamster model of infection.

CONCLUSIONS: CRISPR-PE is a versatile and efficient tool to introduce single-nucleotide resolution mutations in multiple species and serovars of pathogenic *Leptospira*.

RFB CLUSTER BARCODING TO ASSIGN SEROVAR OF *LEPTOSPIRA* P1+ SPECIES

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Leptospirosis is a zoonotic disease caused by several species of pathogenic *Leptospira* and transmitted to humans through exposure of soil or water contaminated with the urine of an infected animal. Our groups have expanded, and more elaborate phylogenetic analyses resulted in a comprehensive new genome-based classification scheme. We have also described the genomic features of a specific sublineage of *Leptospira* called $P1^{+}$ or $P1_{hv}$ (for high virulence), being the species that diverged after a specific node of evolution and are most often associated with severe infections in humans and animals. Nevertheless, it is still unknown whether all $P1_{hv}$ species and subspecies share similar animal reservoirs, their geographical distribution, and the symptoms they cause or not. This epidemiological gap is due to difficulties in culturing the bacteria and identifying species or strains by complex serological techniques. Accurate species/clones identification is critical for epidemiological, phylogenetic, and diagnostic purposes. Over the years, *Leptospira* classification has gained in complexity, whereby it is not only identified at the species level, but also separated into serogroups (n=26), and further subdivided into serovars (n=300), based on lipopolysaccharide (LPS) antigenic relatedness. The situation is even more complex as there is total absence of correlation between genospecies and serovars (i.e. the same serovar can be found in different species). Using a bioinformatics tool that we have recently developed, called "Annotator" we can create "barcodes" representing the presence and absence of certain genes in a given species. We realized that these barcodes could be used to determine the serovar of an isolate with certainty. In fact, we recently showed that the analysis of the gene content of the LPS *O*-antigen–encoding cluster, or *rfb* cluster, correlates with *Leptospira* serovar and serogroup identity. We also sequence several dozens of genomes representing serovar never sequenced. The final objective of this collaborative project is to build and test a software with a graphical and easy-to-use user interface, which will allow the identification of species, serovars and plasmids of different *Leptospira* spp.

List of poster abstracts

GENETICS, MOLECULAR EPIDEMIOLOGY AND GENOMICS

D3-1 Bellinati L

UPDATING THE CHARACHTERIZATION OF *LEPTOSPIRA* SPP. INFECTING SYMPTOMATIC DOGS: A POSSIBLE ROLE OF INTERMEDIATE STRAINS?

D3-2 Foltran BB

CHARACTERIZATION OF LEUCINE-RICH REPEAT PROTEINS OF *LEPTOSPIRA INTERROGANS*

D3-3 Caimi K

COMPARATIVE ANALYSIS OF HUMAN ISOLATES OF LEPTOSPIRA INTERROGANS RESPONSIBLE OF SEVERE PULMONARY HEMORRHAGIC SYNDROME (SPHS)

D3-4 Handayani F

RODENT-BORNE DISEASES (PLAGUE, LEPTOSPIROSIS, RICKETTSIOSIS, AND HANTAVIRUS INFECTION) IN PASURUAN, EAST JAVA INDONESIA

D3-5 Gomes T

PHYSIOLOGICAL ADAPTATIONS OF *LEPTOSPIRA BIFLEXA* IN BIOFILM FORMATION

D3-6 Hamond C

REAL TIME PCRWHIGH RESOLUTION MELTING ANALYSIS FOR PATHOGENIC *LEPTOSPIRA* SPP. IDENTIFICATION

D3-7 Krairojananan P

DIVERSE CIRCULATING GENOTYPES OF PATHOGENIC *LEPTOSPIRA* IN THAILAND

D3-8 Kumar M

INSIGHT INTO THE ASSOCIATION OF ClpC CHAPERONE WITH THE CASEINOLYTIC PROTEASE OF PATHOGENIC *LEPTOSPIRA*

D3-9 Llanes A

GENETIC RELATEDNESS AMONG LEPTOSPIRA SEROVARS AND THEIR *RFB* LOCI

D3-10 Barbosa LN

IN-VITRO GROWTH AND METABOLOMIC PROFILE OF *LEPTOSPIRA INTERROGANS* UNDER DIFFERENT CONDITIONS

D3-11 Ozuru R

GENOME-SCALE METABOLIC NETWORK RECONSTRUCTION REVEALS TEMPERATURE-DEPENDENT GLYCOMETABOLISM SHIFTS OF PATHOGENIC *LEPTOSPIRA*

GENETICS, MOLECULAR EPIDEMIOLOGY AND GENOMICS

D3-12 Picardeau M

INSIGHTS INTO THE EVOLUTION AND PHENOTYPIC TRAITS OF PATHOGENIC *LEPTOSPIRA*

D3-13 Schuler EJA

CHARACTERIZATION OF CDAA: THE UBIQUITOUS LEPTOSPIRAL DIADENYLATE CYCLASE AND SOLE SOURCE OF C-DI-AMP

D3-14 Silva IRM

A NOVEL PUTATIVE PROTEIN OF *LEPTOSPIRA INTERROGANS* THAT BINDS TO ENDOTHELIAL CELLS *IN-VITRO*

D3-15 Sohail ML

EVIDENCE OF CLINICOPATHOLOGICAL CHANGES DURING EQUINE LEPTOSPIROSIS

D3-16 Staples M

LEPSERICO: WORKING TOWARDS GENETIC TYPING OF LEPTOSPIRA SEROVARS

D3-17 Steinrigl A

FIRST DETECTION, ISOLATION AND FULL GENOME SEQUENCING OF *LEPTOSPIRA LICERASIAE* IN AUSTRIAN SWINE

D3-18 Stone NE

OVERLAP OF PATHOGENIC LEPTOSPIRA SPP. IN SAMPLES OBTAINED FROM HUMANS, DOGS, AND THE ENVIRONMENT IN PUERTO RICO

D3-19 Surdel MC

DEFINING ENDOTHELIAL CELL INTERACTIONS OF PATHOGENIC AND SAPROPHYTIC *LEPTOSPIRA*

D3-20 Teixeira AF

STRUCTURAL, FUNCTIONAL AND IMMUNOGENIC CHARACTERIZATION OF TWO PROTEINS OF *LEPTOSPIRA INTERROGANS* CONTAINING FECR DOW MAINS

D3-21 Fernandes LGV

CHARACTERIZATION AND TARGET PREDICTION OF SMALL RNAS FROM *LEPTOSPIRA BORGPETERSENII* SEROVAR HARDJO STRAINS JB197 AND HB203

D3-22 Wongsawat E

METAGENOMIC NEXT-GENERATION SEQUENCING: A WAY FORWARD FOR UNDERSTANDING *LEPTOSPIRA* DIVERSITY IN HUMAN LEPTOSPIROSIS IN THAILAND

UPDATING THE CHARACHTERIZATION OF *LEPTOSPIRA* SPP. INFECTING SYMPTOMATIC DOGS: A POSSIBLE ROLE OF INTERMEDIATE STRAINS?

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Introduction: Dogs are highly susceptible to the infection by pathogenic leptospires and act as a sentinel for the environmental risk to humans. This study aimed to update the data on genotypes of leptospires affecting symptomatic Italian dogs examined between 2020 and 2023.

Material and methods: Organ, urine, and/or whole blood samples were collected from 821 symptomatic Italian dogs for diagnostic purposes. All samples were subjected to a Real-time PCR (rtPCR) targeting the 16S rDNA gene of pathogenic and intermediate leptospires (1). Isolation (2) was attempted on samples from 211 dogs. Positive samples underwent genotyping by Multilocus Sequence Typing (MLST) analysis (3).

Results: Out of the 821 symptomatic dogs, 149 resulted positive in rtPCR. Additionally, isolation was successful in 7 rtPCR-positive and 3 rtPCR-negative animals. Overall the molecular positivity was 18.5% (95%CI: 15.9-21.3), with MLST analysis succeeding in 53.6% of cases. The 4 distinct Sequence Types (STs) identified correspond to the species *Leptospira interrogans* (ST17, ST198 and ST24) and *Leptospira kirschneri* (ST117). Among strains isolated from rtPCR-negative animals, one resulted as ST17, while the other two were identified as the intermediate species *Leptospira inadai* by 16S DNA gene sequencing.

Conclusion: This study highlights the importance of characterizing circulating leptospires by molecular methods. We confirm the circulation of known STs, a result consistent with findings from previous studies (4). Moreover, we report for the first time the isolation of *Leptospira inadai* in symptomatic dogs, a strain sporadically described in human cases (5). Further investigations are needed to evaluate its circulation, pathogenicity in dogs and potential zoonotic implications.

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CHARACTERIZATION OF LEUCINE-RICH REPEAT PROTEINS OF *LEPTOSPIRA INTERROGANS*

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Introduction: Leptospirosis is a global zoonosis caused by pathogenic bacteria of the genus *Leptospira*. Comparative genomic studies between pathogenic and saprophytic species of *Leptospira* have shown significant differences in the number of genes encoding proteins with Leucine Rich Repeat (LRR) domains, suggesting a potential role in virulence. LRR proteins have been reported to be involved in bacterial host-pathogen interactions, invasion, and stimulation of host immune response.

Methods: DNA sequences coding for the LRR proteins LIC11051 and LIC11505 were selected, *in silico* analyzed and cloned into pAE expression vector. *Escherichia coli* was transformed with plasmids, and recombinant protein expression was carried out by addition of IPTG. Bacterial cell fractions were analyzed by SDS-PAGE and protein purification was performed by metal affinity chromatography; immunogenicity was evaluated in BALB/c mice immunized with of each recombinant protein. The animals were bled, the collected sera used for ELISA titration and native protein detection in different fractions of *L. interrogans* cells and supernatant. Recombinant proteins interactions with host macromolecules were evaluated by ELISA.

Results: Recombinant proteins were expressed in the soluble fraction in all *E. coli* strains tested, with the best yield achieved in BL21 Star (DE3) pLysS. Proteins rLIC11051 and rLIC11505 were successfully purified and both were immunogenic in BALB/c mice with titers of 160,000 and 640,000, respectively. Both Recombinant proteins displayed significant interaction with plasminogen, whereas rLIC11505 had a broad spectrum of ligands, binding also to plasma fibronectin, vitronectin, collagen 1 and glycosaminoglycans. Both native proteins counterparts were detected in whole cell lysates of *L. interrogans*, SDS-soluble membrane proteins and in secreted fraction.

Conclusions and future directions: rLIC11051 and rLIC11505 are immunogenic proteins and able to interact with host components. Functional characterization of their role in the pathogenicity of leptospirosis are currently under investigation.

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COMPARATIVE ANALYSIS OF HUMAN ISOLATES OF *LEPTOSPIRA INTERROGANS* RESPONSIBLE OF SEVERE PULMONARY HEMORRHAGIC SYNDROME (SPHS)

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Leptospirosis is a zoonosis worldwide distributed caused by spirochetes of *Leptospira* genus. Human disease span a wide range of non-specific symptoms such as fever, myalgia and headache that goes from a subclinical infection to a severe syndrome with hemorrhages and failures in different organs. In recent years, it has been demosntrated the increase of severe pulmonary hemorrhagic syndrome (SHPS) associated with Leptospirosis. We characterized here three human isolates (YCC1, YCC2 and YCC3) of *L. interrogans* that were responsible of human SHPS. The isolates were obtained from serum samples of patients with proven diagnosis of Leptospirosis and with a fatal outcome. The growth rate of the three strains was higher than that of the reference strain, reaching the exponential phase in the first week of growing. Hamster model was used to study the strains. Animals infected with the three strains died between 7 and 15 days post-infection. We observed micro-hemorrhages in the lungs, and we could recover one of the strains from lungs but no from urine or kidney. The strains belong to serovars Icterohaemorrhagiae (YCC1), Pomona (YCC2) and Canicola (YCC3) that generally circulate in humans, and Multilocus Sequence Typing showed that the strains belong to genotypes of major circulation in Argentina: ST47 ST52 and ST3. These genotypes are closely related to the serovars mentioned. We have also obtained the whole genomic sequences. A preliminary phylogenetic analysis showed that YCC1 grouped with other human strains that caused SHPS, while YCC2 and YCC3 grouped with strains that cause mild leptospirosis. These results indicate that even the strains belong to serogroups and genotypes frequently found in human population in Argentina, they presented a higher degree of virulence with tropism to lungs. These findings makes necessary to depeen in the molecular characteristic of these strains in order to find possible targets for differential diagnosis.

RODENT-BORNE DISEASES (PLAGUE, LEPTOSPIROSIS, RICKETTSIOSIS, AND HANTAVIRUS INFECTION) IN PASURUAN, EAST JAVA INDONESIA

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Keywords: *Leptospira*; Rodents Borne Diseases; Enzootic plague; Indonesia.

Background: Rodent-borne diseases are important to public health, as rats carry 70 worldwide known rodent-borne diseases. Pasuruan has been recognized as an enzootic area of plague infection. However, other rodent-borne diseases (RBDs) have not been reported. This study aims to reveal rodent-borne diseases of plague, leptospirosis, rickettsiosis, and hantavirus infection in both patients and rodents in the plague foci areas.

Material and Methods: This was a cross-sectional study, conducted from May to June 2024. We collected the serum of acute fever patients in Public Health Centers and the District Hospitals. lipL32-probe-qPCR was performed for pathogenic *Leptospira*; serology by IFA-IgG anti-*R.typhii* for seroprevalence of murine typhus continued by PCR; nested PCR for *Orthohantavirus infection*. The pla-caf1 PCR for *Y.pestis* detection was carried out only in the rats' fleas for plague evidence. We collected the kidneys, lungs, and ectoparasites of *Rattus rattus spp.* and *Suncus murinus* trapped in settlement areas, gardens, and forests.

Results: We collected 136 acute fever patients samples between 18 to 75 years old with the occupation mostly farmers. The 9 of 136 (6,6%) were confirmed leptospirosis, 11 of 73 (15,06%) hantavirus infection, and 17,9% positive serology of murine typhus (Rickettsiosis), whereas molecularly the *R. typhii* detected only 3 out of 89 patients (3.3%). Likewise, we obtained evidence that those RBDs infected the animal reservoirs and vectors. A total of 119 from 107 rats and 12 small mammals' kidneys were detected 7.6% (9/119) contained pathogenic *Leptospira spp.* However, none of the fleas ectoparasites revolved positive for *Y. pestis*, and 8 of 79 of the rats' lung samples (10%) were positive for hantavirus.

Conclusion: We confirmed for the first time leptospirosis, rickettsiosis, and hantavirus infection in the plague foci area, Pasuruan. Awareness of the clinicians and control measures for rat populations should be implemented to avoid underdiagnosis and under-reported of diseases.

PHYSIOLOGICAL ADAPTATIONS OF *LEPTOSPIRA BIFLEXA* IN BIOFILM FORMATION

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Keywords: generation time, cell length, cell viability.

Introduction: Bacteria adapt by means of genetic and metabolic changes to different conditions in the environment or host. Biofilms may form as a result of these adaptations.

Objective: To analyze physiological aspects of *Leptospira biflexa* during biofilm formation.

Methods: We cultivated *L. biflexa* in biofilm (BIOF) and planktonic (PLANK) phenotypes. We conducted growth curves, calculated the generation times (GT) and specific growth rates. We observed cells by dark-field microscopy (20 fields per sample) and measured cell lengths (µm) at each timepoint of the curves (4, 8, 12, 24, 36, 48, 60, 72, 84, 96, and 120 hours). We then evaluated cell viability in biofilms by cultivating BIOF on glass slides (for 24, 36, 48, 72 and 96 hours), staining with FilmTracer™ LIVE/DEAD® Biofilm Viability Kit and acquiring SYTO® 9 green and PI red fluorescence signals.

Results: During the log phase, the GT of BIOF (5.24 h ± 0.08) was significantly shorter than PLANK (6.21 h ± 0.30) ($p=0.03$) and the specific growth rate was significantly higher for BIOF (0.132 h^{-1} ± 0.002) than for PLANK (0.112 h^{-1} ± 0.005) ($p=0.02$). The maximum cell concentration achieved was ten times lower in BIOF (~2E+08 cells/mL) than in PLANK (~2E+09 cells/mL). Leptospiral length diminished with cultivation time for both phenotypes. We observed an increase in the proportion of live cells in mature biofilms, but also observed cell death during this stage.

Conclusion: We showed that *L. biflexa* presented physiological alterations in the biofilm phenotype, marked by a faster growth. Early cell death during biofilm formation may contribute to elimination of public goods (as extracellular DNA) that will build the biofilm's architecture. The saprophyte *L. biflexa* stands out as a model for the study of leptospiral biofilms. We believe these results will contribute to a better understanding of *Leptospira* physiology and biology in different phenotypes.

Funding: This work was funded by the National Council for Scientific and Technological Development (CNPq) grant Universal 425526/2016-0 and the Coordination for the Improvement of Higher Level Personnel (CAPES), Finance Code 001.

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REAL TIME PCR-HIGH RESOLUTION MELTING ANALYSIS FOR PATHOGENIC *LEPTOSPIRA* SPP. IDENTIFICATION

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Keywords: *leptospirosis, high resolution melting (HRM), genotyping.*

Introduction: Leptospirosis is an important zoonotic disease widespread worldwide. The etiological agent, *Leptospira* spp., is a highly heterogeneous bacterial genus divided into pathogenic and saprophytic species. The high-resolution melting (HRM) analysis was already described as an important tool for *Leptospira* spp. typing at the species and subspecies levels. The present study aims to evaluate the performance of real time PCR-high resolution melting (qPCR-HRM) analysis, targeting the housekeeping gene *mre*A, in the identification of pathogenic species of *Leptospira* spp. isolated from human cases of leptospirosis.

Method: 18 reference strains belonging to five *Leptospira* pathogenic species (*L. interrogans*, *L. kirshneri*, *L. noguchii*, *L. borgpetersenii* and *L. santarosai*) and 6 strains isolated from Brazilian human blood cultures were initially selected for this study. Human isolates were previously identified at species level by 16S DNA sequencing. All the strains were obtained from the *Leptospira* Collection/IOC/Fiocruz. Primer pairs *mre*A-1 and *mre*A-2 were designed based on the *in-silico* analysis of the core genomes of these species deposited at the GeneBank. Amplification reactions were performed with 2X Type-it HRM PCR Kit (Qiagen) using the Rotor Gene-Q (Qiagen) instrument. HRM analysis was obtained using the software Rotor Gene Q Series version 2.3.2.(Qiagen).

Results: The qPCR-HRM assay using the primer pair *mre*A-2 was able to successfully distinguish *L. interrogans, L. kirshneri* and *L. noguchii*, although with low accuracy, and improvements in our qPCR-HRM assay are still necessary. On the other hand, primer pair *mre*A-1 produced distinct melting curve profiles for *L. borgpetersenii* and *L. santarosai* (Tm 84.4 and 85.6°C, respectively). This is an important finding since *L. santarosai* was already described as one of the predominant species in many countries from Central and South America.

Conclusion: qPCR-HRM assay we designed could be a simple tool for species identification, especially considering the prevalence of *L. santarosai* in Brazil.

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DIVERSE CIRCULATING GENOTYPES OF PATHOGENIC *LEPTOSPIRA* IN THAILAND

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Introduction: Leptospirosis, caused by bacterium *Leptospira*, is a significant global zoonotic disease disproportionately impacting vulnerable populations in tropical areas. In Thailand, the population often experiences high levels of morbidity and mortality associated with the disease. Among the known animal reservoirs of *Leptospira*, rodents play a crucial role in disseminating the pathogen across endemic regions. However, the diversity of pathogenic *Leptospira* genotypes circulating in Thailand remains poorly understood.

Materials and Methods: To address this knowledge gap, we conducted a comprehensive leptospirosis surveillance program in rodent populations collected between 2011 and 2020. This initiative yielded the isolation of 101 *Leptospira* strains from the captured rodents. For further characterization, these isolates underwent molecular analysis using 16S rRNA gene sequencing and multilocus sequence typing (MLST) scheme 1.

Results: The majority of pathogenic strains identified were *Leptospira interrogans* isolates (75.2% (n=76)) compared to *L. borgpetersenii* (24.8% (n=25)), suggesting that *L. interrogans* may be a more dominant reservoir-adapted strain in the studied rodent population. Furthermore, a greater level of genetic diversity was observed within *L. interrogans,* with 23 distinct sequence types (STs) identified compared to only two STs found in *L. borgpetersenii*.

Conclusions: Our findings highlight the continued circulation of *L. interrogans* ST34, a known outbreak strain, across Thailand. The wider variety of circulating *L. interrogans*, STs particularly in the southern regions, suggests a more complex epidemiological picture in these endemic areas. Further investigation into these diverse genotypes is necessary to understand their potential impact on diagnostics, vaccine development, and public health efforts.

INSIGHT INTO THE ASSOCIATION OF ClpC CHAPERONE WITH THE CASEINOLYTIC PROTEASE OF PATHOGENIC *LEPTOSPIRA*

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The role of bacterial caseinolytic protease-chaperone complexes in removing misfolded and aggregated protein substrates is crucial. Caseinolytic proteases and chaperones have garnered considerable attention as targets for antibacterial action after their direct relationship with bacterial viability and virulence was proven in Gram-positive and Gram-negative bacteria. In the spirochete *Leptospira interrogans*, Clp-chaperones (ClpX, ClpA, and ClpC) associate with two isoforms of LinClpP (ClpP1 and ClpP2), a serine protease. LinClpC, a Class-I chaperone in *L. interrogans*, possesses two active ATPase domains separated by a middle domain. Using size exclusion chromatography, ANS dye binding, and dynamic light scattering analysis, it has been revealed that LinClpC undergoes nucleotide-induced oligomerization. Despite being equally capable of binding to LinClpP1 and LinClpP2, LinClpC cannot independently form an active protease complex with a single LinClpP isoform. However, combined with LinClpP1P2 heterocomplex, it creates a functional proteolytic machinery capable of degrading β-casein or FITC-casein without energy dependency. The protease activity of the LinClpCP1P2 complex increases with ATP or ATPγS, which promotes the functional oligomerization of LinClpC. Interestingly, acyldepsipeptides (ADEP1) antibiotic activates the LinClpP1P2 protease activity more strongly than LinClpC. This study explores the complex association of hetero-tetradecamer LinClpP1P2 with its corresponding ATPase. It reveals new insights into the structural reorganization of LinClpP1P2 facilitated by the presence of the chaperone LinClpC, ultimately leading to protease activity. Thus, a better and more elaborate understanding of individual Clp components of *Leptospira* will pave the way for new antibacterial targets.

GENETIC RELATEDNESS AMONG *LEPTOSPIRA* SEROVARS AND THEIR *RFB* LOCI

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Leptospira species are traditionally classified into serovars using costly and tedious serological assays. The serovar classification is based on the antigenic composition of the lipopolysaccharide (LPS), which is synthesized in part by enzymes encoded in an operon called *rfb* locus. Here, we generated chromosome-level genome assemblies for 17 *Leptospira* serovars from a microscopic agglutination test (MAT) panel routinely used in epidemiological studies. We then compared the newly sequenced genomes and the corresponding *rfb* loci among them and with external reference genomes for the same or closely related serovars. Despite the reasonably good genetic relatedness among specimens belonging to the same *Leptospira* species, evidence at the genome-wide level did not entirely agree with the traditional serovar classification for some of these specimens. For instance, our specimen classified as serovar Mankarso was found to be genetically closer to that of serovar Pyrogenes than to those from serogroup Icterohaemorrhagiae. Likewise, the specimen classified as serovar Autumnalis, which together with Djasiman belong to the Autumnalis serogroup, was found to be closer to the Canicola and Pomona specimens. Conversely, comparison of *rfb* loci was more consistent with the underlying serogroup classification, with the *rfb* loci of Mankarso and Djasiman specimens showing high sequence similarity to those from serovars Icterohaemorrhagiae and Autumnalis, respectively. Our findings suggest the occurrence of horizontal gene transfer events involving the *rfb* locus, not only between different *Leptospira* species but also between strains belonging to the same species. The complex events governing the evolution of the *rfb* locus may result in inconsistencies in genomic, phylogeographic and evolutionary studies, with a direct impact in the epidemiology of this neglected bacterial pathogen. Our results highlight the importance of improving *Leptospira* classification system based on genetic markers for species and lineage identification, as well as the *rfb* locus for serovar relatedness.

IN-VITRO GROWTH AND METABOLOMIC PROFILE OF *LEPTOSPIRA INTERROGANS* UNDER DIFFERENT CONDITIONS

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Introduction: Metabolome profiling is a powerful tool to establish broad-spectrum metabolites and their intermediates in a biological system. This study aimed to establish the pattern of *Leptospira* growth and to identify the profile of metabolites associated to it, highlighting the differences related to *in-vitro* growth conditions that may be associated with the alteration of leptospiral virulence.

Methods: We compared *Leptospira interrogans* growth in two different EMJH media compositions (commercial EMJH, in-house EMJH) at 28 °C and 37 °C, and the *Leptospira* densities were measured daily. Bacterial pellets, culture supernatants, and sterile EMJH media (controls), were used for metabolites' extraction and untargeted metabolic profiling by Mass Spectrometry (UHPLC-HRMS).

Results: We observed a distinct pattern of *Leptospira in-vitro* growth between the conditions tested. Cultures achieved the highest densities of 10^8*Leptospira*/ml in both EMJH media. The cultures using commercial EMJH medium at 37 °C that did not support *Leptospira* growth. Our preliminary results identified 100 metabolites from *Leptospira* cultures in commercial EMJH medium at 28 °C. Metabolites' abundances were statistically different between *Leptospira* pellet, culture supernatants and the control ($p \leq 0.01$ and $p \leq 0.05$). The metabolite profiles of *Leptospira* cultures using in-house EMJH, an improved version of the EMJH medium [1], has been currently evaluated as this medium supported *in-vitro* growth of *Leptospira* at 37 °C.

Conclusions: Our results demonstrated that *Leptospira* cultivated under different *in-vitro* conditions display distinct patterns of growth. Additionally, preliminary data of metabolomic profiling evidenced differences in intracellular and extracellular metabolites' abundances. Several *in-vitro*-associated factors, such as media composition and temperature, are very important external stimuli for leptospiral growth, which we believe can influence the viability and virulence of pathogenic species.

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GENOME-SCALE METABOLIC NETWORK RECONSTRUCTION REVEALS TEMPERATURE-DEPENDENT GLYCOMETABOLISM SHIFTS OF PATHOGENIC *LEPTOSPIRA*

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Introduction: *Leptospira* proliferates most actively at approximately 30°C in culture [1]. Given that *Leptospira* is a free-living organism capable of surviving in the environment, it is logical to have an optimal cultivation temperature lower than the internal temperature of the host body, approximately 37°C. However, considering *Leptospira* can colonize within a host, it is likely that the metabolic activities during proliferation in the environment differ from those within the host body [2,3]. This study aimed to elucidate the differences in the metabolic systems of *Leptospira* among various temperature using genome-scale metabolic network reconstruction methods.

Materials and methods: We generated the genome-scale metabolic network model of *L. borgpetersenii* serovar Hardjo strain JB197 using Reconstructor [4]. Next, we integrated public transcriptome data into the model to better reflect the actual metabolic activity observed in JB197 with RIPTiDe [5]. We selected the data from GSE168507 [6], in which JB197 was incubated at different temperatures (29°C and 37°C).

Results and discussion: Metabolic simulation results indicated that the growth yield of JB197 decreased at 37°C compared to 29°C, supporting the notion that the optimal cultivating temperature for *Leptospira* is around 30°C. Subsequently, we identified metabolic reactions that distinctively characterize the two temperature-dependent metabolic models. Notably, reactions within the TCA cycle and those related to sugars and amino acid metabolism were prominent in differentiating the models. Further analysis revealed that in the 37°C model, unlike in the 29°C culture, there was an increase in conversion from glucose 6-phosphate to fructose 6-phosphate (F6P) and fructose 1,6-bisphosphate to F6P (Fig. 1). These findings suggest that *Leptospira* undergoes a metabolic shift at 37°C, intensifying the synthesis of F6P, a precursor for the biosynthesis of aromatic amino acids[7].

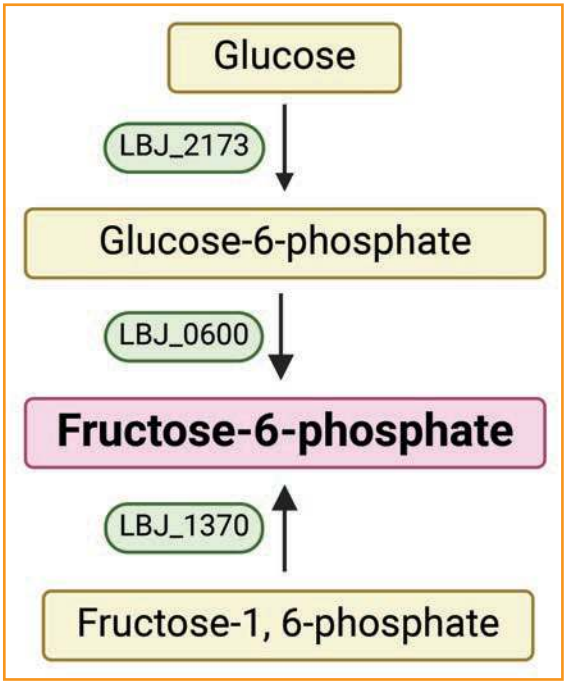


Figure 1 *Changes in glycometabolism that occur in* L. borgpetersenii *strain JB197 strain when cultured at 37°C. These reactions are expected to be very infrequent or absent at 30°C. The label 'LBJ_XXXX' refers to the specific enzyme in the strain JB197 that catalyzes each depicted reaction.*

This metabolic shift could lead to the identification of novel biomarkers and the development of new therapeutic approaches within the host.

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INSIGHTS INTO THE EVOLUTION AND PHENOTYPIC TRAITS OF PATHOGENIC *LEPTOSPIRA*

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Introduction: *Leptospira* is a highly heterogeneous bacterial genus and leptospires are ubiquitous bacteria found as free-living saprophytes or as pathogens that can cause disseminated infections, from asymptomatic carriage in rats to lethal acute infection in both humans and animals. Despite leptospirosis being a re-emerging zoonosis, little is known about the ability of the etiologic agent to adapt to different hosts and cause disease.

Material and methods: We studied the *in-vivo* (hamster and macrophage infections and survival in serum) and *in-vitro* (metabolic activity and survival in water) phenotypes of 5 representative P1+ species (*L. interrogans, L. weilii, L. noguchii, L. mayottensis, and L. santarosai*), 4 representative P1- species (*L. adleri, L. gomenensis, L. tipperaryensis,* and *L. yasudae*), 2 representative P2 species (*L. licerasiae* and *L. fluminis*) and the saprophyte *L. biflexa*.

Results: Combining genome analysis and phenotyping studies of representative species and mutant strains, we show that only a small group of species have the ability to evade the host immune system and cause disease.

Conclusion: Taken together, our findings define a new vision on *Leptospira* pathogenicity, identifying virulence attributes associated with clinically relevant species, and provide insights into the evolution and emergence of these life-threatening pathogens.

CHARACTERIZATION OF CDAA: THE UBIQUITOUS LEPTOSPIRAL DIADENYLATE CYCLASE AND SOLE SOURCE OF C-DI-AMP

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Pathogenic *Leptospira* participate in complex disease dynamics involving diverse vertebrate hosts, as well as transient environmental persistence. Such a lifestyle requires comprehensive stimulus sensing mechanisms, such as two-component regulatory systems, alternative sigma factors, heat shock proteins, and cyclic nucleotides. Cyclic di-GMP, ppGpp, and cAMP are well-known examples of cyclic nucleotides which have been demonstrated in *Leptospira* to act as second messenger molecules, modulating motility, biofilm formation, metabolism, and virulence. Cyclic di-adenosine monophosphate (c-di-AMP), synthesized from two molecules of ATP, is employed as a second messenger molecule in diverse Bacteria and Archaea; however, this system has yet to be investigated in *Leptospira*. Bioinformatic analyses of reference genomes from 72 pathogenic and saprophytic *Leptospira* species revealed that all encode a single, putative CdaA-type diadenylate cyclase (EC 2.7.7.85). Percent amino acid identities of this diadenylate cyclase between P1 and P2, S1, or S2 isolates were modest on average (73%, 53%, and 54%, respectively); however, across all 72 strains, the "DGA" and "GXRHRXA" active site motifs were 100% conserved. The presence of these motifs, shown in other organisms to be essential for enzymatic activity, suggests that *Leptospira* are capable of synthesizing c-di-AMP from ATP *de novo*—an important finding, as diadenylate cyclases are the only source of c-di-AMP. Recombinant CdaA (LIC10844), lacking the three N-terminal transmembrane domains, was capable of synthesizing c-di-AMP from ATP *in-vitro*, in the presence of only Mn^{2+} or Co^{2+} divalent cations. Size exclusion chromatography revealed that LIC10844 formed enzymatically-active homodimers. Site-directed mutagenesis of residues within the putative active and ATP-coordination sites abrogated enzymatic activity. This study is the first to characterize the structure and functional residues of a diadenylate cyclase in *Leptospira*. As eukaryotes do not produce or utilize c-di-AMP, this system could serve as a novel target for antibiotics.

A NOVEL PUTATIVE PROTEIN OF *LEPTOSPIRA INTERROGANS* THAT BINDS TO ENDOTHELIAL CELLS *IN-VITRO*

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Introduction: Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* spp.. It is transmitted through contact with contaminated soil or water. Due to their location, outer membrane proteins may induce host immune response. In this sense, this work aims to assess the role of a novel putative lipoprotein, LIC13355, in *Leptospira* pathogenicity.

Methods: The gene LIC13355 was amplified from *Leptospira interrogans* sorovar Copenhageni strain L1-130 by PCR and cloned into pAE vector. Its heterologous expression in *Escherichia coli* strains was assessed by SDS-PAGE and Western Blotting, the recombinant protein was purified using IMAC. Its secondary structure was determined by circular dichroism. Protein interaction with host's extracellular matrix, plasma components, and endothelial cells was evaluated by ELISA or Western Blotting.

Results: Sequencing analysis reveals that the gene LIC13355 was successfully cloned in pAE vector. Tests using *E. coli* as expression host under variable conditions showed that the protein is expressed in its insoluble form. Inclusion bodies were solubilized in urea and refolded with decreasing urea concentration in HisTrap nickel column, with subsequent elution. Fractions containing higher concentration of the purified protein were dialyzed in Tris-HCl buffer. Circular dichroism analysis indicates that the protein structure is composed of 31,6% alfa-helix, 21,8% of beta-sheet and 42,5% other structures. In ELISA assay, rLIC13355 bound to laminin, type I collagen, cellular and plasmatic fibronectin, and plasminogen, exhibiting dose-dependent behavior. rLIC13355 was able to capture plasminogen from human plasma, and convert into plasmin. rLIC13355 bound to Ea.hy926 and HMEC-1 in monolayer and suspension cells, indicating affinity to extracellular matrix and cell surface components. Binding to Ea.hy926 cells also presented dose-dependent behavior, with an estimated Kd of 24.

Conclusions: rLIC13355 is most probably a novel leptospiral adhesin that can participate in different steps of the infection.

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EVIDENCE OF CLINICOPATHOLOGICAL CHANGES DURING EQUINE LEPTOSPIROSIS

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Introduction: Leptospirosis affects a wide range of mammals, humans, and even a few poikilothermic animal species. In Pakistan, serological studies of equine leptospirosis have reported a prevalence of over 40%, but no study has ever been conducted towards molecular detection of *Leptospira* in horses. Material and Methods: Blood samples from 128 horses were screened using ELISA and 41 positive samples were examined for the presence of leptospiral DNA using specific primers for 16S rRNA gene. Results: Out of 41 tested samples, 20 samples were found to be PCR-positive, revealing a fragment of 306 bp after gel electrophoresis. Sequencing and phylogenetic analysis of positive samples revealed circulation of pathogenic *Leptospira* spp. in Pakistani horses. No evidence of circulation of intermediate species was found in this study. Conclusion: This study reports the first molecular evidence of equine leptospirosis in Pakistan and lays ground for further research in this area. It also confirms the efficiency of 16S rRNA for the diagnosis of equine leptospirosis.

LEPSERICO: WORKING TOWARDS GENETIC TYPING OF LEPTOSPIRA SEROVARS

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The World Health Organisation Collaborating Centre for Reference and Research on Leptospirosis is a Queensland Health Leptospirosis Reference Laboratory located in Brisbane, Australia and provides Leptospirosis diagnostic testing and support for Australia and the Western Pacific Region. Laboratory capabilities include serology using the Microscopic Agglutination Test (MAT), diagnostic PCR, culture, and isolation of leptospires. For all isolated *Leptospira* spp. the likely infecting serovar is inferred using reference antisera through a "reverse MAT" technique. Additionally, the laboratory has developed protocols for which all isolated *Leptospira* spp. are whole genome sequenced.

The laboratory holds a complete pathogenic *Leptospira* reference culture collection in addition to an extensive catalogue of *Leptospira* spp. isolated from patient specimens nationally. Whole genome sequencing of this culture collection is currently underway, with initial focus on circulating Australasian serovars, currently underrepresented in genomic *Leptospira* databases worldwide. Analysis of these genomes is shaping the development of 'LepSerico' (*LEPtospira* SERotyper *in silICO*); an application devised to predict isolate serovar through analysis of gene variants. Here we describe the progress, validation, and future directions of this project.

FIRST DETECTION, ISOLATION AND FULL GENOME SEQUENCING OF *LEPTOSPIRA LICERASIAE* IN AUSTRIAN SWINE

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Introduction: Leptospiraceae are a diverse family of spirochetes, of which many are involved in infectious diseases of animals and humans. Currently, at least 69 species belong to the genus *Leptospira*. By genetic analysis, 4 major subclades can be distinguished, of which two (P1 and P2) have pathogenic potential in man and animals. Among domestic animals, leptospirosis is widely distributed, as judged by frequent detection of anti-*Leptospira* antibodies. Nevertheless, local leptospiral diversity in domestic animals is often poorly understood, which is in part also due to difficulties in isolating the agent. Here we describe the incidental detection of *Leptospira (L.) licerasiae* in an Austrian pig.

Material and methods: During an animal experiment, a urine sample from an uninfected contact pig was found to give rise to spirochaetal growth in EMJH-STAFF medium. Electron microscopy, PCR, Sanger sequencing, MAT-serotyping and whole genome sequencing were employed to characterize the strain.
Results: PCR and serotyping confirmed that the strain cultured from the unchallenged contact pig was clearly different from the challenge strain used in the animal experiment. Sequencing established that the strain isolated from the healthy contact animal belonged to the species *L. licerasiae.*

Conclusion: The known geographical range of *L. licerasiae* was hitherto limited to tropical/subtropical areas. Rodents and wild swine living in these areas have been suggested to act as hosts of this pathogen which also exerts limited pathogenicity in humans. This is the first description of *L. licerasiae* isolated from a domestic pig in Europe. This suggests that the distribution of *L licerasiae* is considerably broader than previously known and raises interesting questions about potential implications of this species in leptospirosis of domestic animals in Europe as well as its zoonotic potential.

OVERLAP OF PATHOGENIC *LEPTOSPIRA* SPP. IN SAMPLES OBTAINED FROM HUMANS, DOGS, AND THE ENVIRONMENT IN PUERTO RICO

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Introduction: Leptospirosis in humans and animals is most often caused by multiple species of *Leptospira* that belong to the pathogenic P1 clade, which is further subdivided into two main phylogenetic subclades, hypervirulent (P1+) and subvirulent (P1-). Members of the P1+ subclade cause most leptospirosis cases in humans and dogs but are rarely detected in the environment; the virulence potential of the P1- subclade is not fully understood. In Puerto Rico, an increase in human leptospirosis occurred in the wake of 2017 hurricanes Irma and Maria.

Materials and Methods: To better understand the diversity of *Leptospira* in Puerto Rico, we analyzed multiple genes from *Leptospira*-positive environmental and clinical samples to 1) determine *Leptospira* species and assign genotypes, 2) identify mixed genotypes/co-infections, and 3) assess species overlap among sample sets. Eighty-six soil and water samples from 15 sampling sites positive for P1 clade pathogenic *Leptospira* spp. were amplified at eight loci with a nested-PCR approach, followed by deep amplicon sequencing (AmpSeq), and 107 clinical samples from humans (n=91) and dogs (n=16) collected in Puerto Rico were sequenced using a larger panel of 42 loci.

Results: The environmental samples were comprised mostly of mixtures of P1- species sequences, although *L. interrogans* (P1+ clade) DNA was detected as a minor component at 6/15 sites (40%). In contrast, the clinical samples were entirely comprised of species in the P1+ clade (*L. interrogans*, *L. kirschneri*, *L. santarosai*, and *L. borgpetersenii*); none contained DNA from the P1- clade. Four co-infections were identified in the 107 clinical samples: two from humans (2.2%) and two from dogs (12.5%).

Conclusions: Our findings reveal overlap among clinical and environmental P1+ *Leptospira* spp. in Puerto Rico and suggest that environmental *Leptospira* in the P1- clade did not contribute to leptospirosis in these humans and dogs. AmpSeq facilitated identification of mixtures and detection of *L. interrogans* in environmental samples.

DEFINING ENDOTHELIAL CELL INTERACTIONS OF PATHOGENIC AND SAPROPHYTIC *LEPTOSPIRA*

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Introduction: Leptospirosis is an important cause of morbidity and mortality worldwide. Disease severity ranges from asymptomatic colonization to multiorgan dysfunction and hemorrhage (i.e. Weil's disease). Leptospirosis is caused by pathogenic gram-negative spirochetes of the genus *Leptospira*. *Leptospira* spp. are classified into pathogenic (P1, P2) and saprophytic (S1, S2) clades.[1] In this study, we evaluated strains from each clade to define their interactions with endothelial cells *in-vitro* and *in-vivo*.

Materials and methods: Representative strains from each clade were selected that have full genome sequences available.[1] Each strain was tested for their ability to alter VE-cadherin (an important protein in adherens junctions between endothelial cells) by immunofluorescence microscopy, and adhesion to endothelial cells quantified by qPCR, and binding to mouse tissues in a hematogenous dissemination model quantified by qPCR.[2]

Results: High virulent P1 strains disrupt VE-cadherin at endothelial cell junctions. Strains bind *in-vitro* to endothelial cells and *in-vivo* to mouse tissues to varying extents. These phenotypes do not correlate between assays, or with pathogenicity as defined by the clades.

Conclusions: We have determined that *Leptospira* strains interact with endothelial cells with varying phenotypes. Specifically, VE-cadherin disruption, endothelial cell adhesion, and tissue adhesion do not correlate for each strain, and do not correlate based upon their clade classification. Taken together this defines that *Leptospira*-endothelial cell interactions are complex processes. Future work will define the mechanisms by which *Leptospira* interact with endothelial cells, including evaluation of candidate adhesins in their role in these interactions.

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STRUCTURAL, FUNCTIONAL AND IMMUNOGENIC CHARACTERIZATION OF TWO PROTEINS OF *LEPTOSPIRA INTERROGANS* CONTAINING FECR DOMAINS

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Introduction: *Leptospira interrogans* is the major causative agent of leptospirosis, a wordwide spread zoonosis. Its outer membrane proteins are interesting study targets, as they can interact with host molecules. FecR domain containing proteins are associated with ferric dicitrate ion transport in *Escherichia coli* [1]. Although ferric ion transport pathway is not well elucidated in *Leptospira* spp., FecR proteins have already been described as *L. interrogans* adesins[2]. This study aims to characterize structurally, functionally, and immunologically two *L. interrogans* FecR proteins, LIC10902 and LIC20185.

Methods: The coding sequences (CDS) of the target-proteins were analyzed *in silico* by public webservers, amplified by PCR from *L. interrogans* serovar Copenhageni genome, cloned into pAE vector and expressed in *E. coli* BL21 Star (DE3) pLysS. Recombinant proteins were purified by affinity chromatography and their secondary structures were evaluated by circular dichroism (CD). Proteins' ability to bind host molecules and immunogenicity in BALB/c mice were analyzed by ELISA.

Results: The CDS were cloned, expressed and purified successfully. CD determined both proteins to have β-sheets as their predominant secondary structure. Proteins were immunogenic producing high antibody titer in BALB/c mice. LIC10902 was able to interact with plasminogen, plasmatic fibronectin and complement molecules C8 and C9. LIC20185 interacted with plasminogen and C9. Both proteins bound to glycosaminoglycans (GAGs) and were able to interact with integrins αVβ3, α2Bβ3 and α8. Moreover, LIC20185 interacted with the α5β1, αVβ8, αVβ6, αVβ5 and αVβ1 integrins.

Conclusion: The results show that these recombinant proteins are very versatile, interacting with several host components, suggesting an important and diverse putative role on leptospiral pathogenesis. Immunoprotective activity of these proteins in hamsters is currently undertaking.

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CHARACTERIZATION AND TARGET PREDICTION OF SMALL RNAS FROM *LEPTOSPIRA BORGPETERSENII* SEROVAR HARDJO STRAINS JB197 AND HB203

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Introduction: Bovine leptospirosis causes abortion and other costly reproductive problems that seriously impact animal health. In cattle, the disease is primarily caused by *Leptospira borgpetersenii* strains belonging to serogroup Sejroe, serovar Hardjo.[1] Despite high genetic similarity, the *L. borgpetersenii* sv. Hardjo strains JB197 and HB203 differ in disease severity in a hamster model with JB197 causing severe acute disease and HB203 causing asymptomatic chronic disease.[2] Small noncoding RNAs (sRNAs) are involved in regulating bacterial gene expression, often through direct interaction of the sRNA with an mRNA transcript. While sRNAs have been identified in *L. biflexa* and *L. interrogans*, they have not been characterized in *L. borgpetersenii*.[3,4,5] The disparity in virulence between JB197 and HB203 may in part be explained by differences in sRNA activity.

Materials and methods: Using the recently developed HAN media, which supports culture of many fastidious *Leptospira* strains *in-vitro* at 29 °C and 37 °C, our group recently compared the transcriptomes of JB197 and HB203 grown at these two temperatures.[6,2] From this dataset, unannotated sRNAs were manually identified by inspecting the read alignments. The CopraRNA2 software, which leverages genomic similarity combined with RNA folding kinetics,[7] was modified to accept additional user input and was used for *in silico* identification of sRNA targets. A subset of sRNAs were also validated using northern blotting.

Results: A total of 268 previously unannotated sRNAs were identified. Among the predicted targets of the sRNAs were *lipL32* and *sphC*, which respectively encode the most abundant leptospiral surface protein and a sphingomyelinase associated with virulence. Northern blotting also validated other sRNAs of interest including NC80, a putative sRNA previously found to be upregulated by *L. interrogans* sv. Copenhageni Fiocruz L1-130 *in-vivo*.

Conclusion: This project expands knowledge of *Leptospira* sRNAs and further explores their roles in gene regulation and provides novel characterization of *L. borgpetersenii* which is largely underrepresented in the field.

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METAGENOMIC NEXT-GENERATION SEQUENCING: A WAY FORWARD FOR UNDERSTANDING *LEPTOSPIRA* DIVERSITY IN HUMAN LEPTOSPIROSIS IN THAILAND

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We performed metagenomic next generation sequencing (mNGS) to identify known and novel pathogens from 533 patients with acute undifferentiated febrile illness (AUFI) diagnosed between 2018 and 2021 at Siriraj Hospital, Bangkok, Thailand. Reads were taxonomically classified by DiVir pipelines. mNGS identified four *Leptospira* species: *L. interrogans*, *L. borgpetersenii*, *L. mayottensis*, and *L. santarosi*. A 34-year-old male with *L. interrogans* also had a positive serological test. *L. borgpetersenii* in a 28-year-old male showed high read count and nearly 100% alignment to *Leptospira borgpetersenii* serovar Ballum. A 20-year-old female with *L. santarosi* had a history of heavy alcohol consumption and died from multiorgan failure. Details on all patients' clinical presentations and discharge diagnosis are provided in Table 1.

	Case No1	Case No2	Case No3	Case No4
Presenting symptoms: - fever, day - headache, myalgia* - nausea/vomiting/diarrhea* - hypotension*	 1 - /+ -/-/+ +	 - -/- -/-/+ +	 3 -/+ -/+/- +	 4 +/+ +/+/+ +
Laboratory results: - Hct, % - WBC, cu.mm. - Platelets, x103/cu.mm.	 32.9 4,870 24	 24.6 27,460 132	 34.9 4,420 38	 34.5 8,770 80
- PT/activated PTT, sec	15.8/36.3	42.2/112.6	13.4/26.7	14.5/34.7
- Total bilirubin/Alb/glob, mg/dl - AST/ALT, U/L - Urea/ Cr, mg/dl	1.9/3.3/2.8 105/65/169 28.1/2.75	50.7/2.2/2.5 272/88/342 85.6/14.1	3.5/3.9/3.4 555/84/232 13.6/0.98	1.3/2.9/3.2 38/44/199 9.2/0.93
Discharge diagnosis	Aute kidney injury, unknown cause	Acute liver failure, chronic alcoholic liver disease, sepsis	Alcoholic liver disease, acute viral infection	Acute febrile illness
mMGS result	*L. interrogans*	*L. santarosai*	*L. mayottensis*	*L. borgpetersenii*

** + present, - not present*

In summary, mNGS offers promise for diagnosing human leptospirosis and providing genotypic information. More mNGS data is needed to understand the diversity of *Leptospira spp*. in Thailand, informing better epidemiological and control strategies.

SESSION 7

RECENT ADVANCES ON DIAGNOSTIC APPROACHES

Moderators:
Nattachai Srisawat & Paula Ristow

4TH ELS MEETING
13TH ILS CONFERENCE
BRUSSELS
2-4 SEPTEMBER, 2024

Clinical Challenges in early diagnose human leptospirosis and prediction disease severity: an unmet need

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Leptospirosis is a zoonotic disease that affects global health with over one million cases and 58,900 deaths annually. The disease is caused by the pathogenic spirochete *Leptospira* spp. which can adapt to a broad spectrum of mammalian hosts and environments. The clinical signs and symptoms of leptospirosis share similarities with other infectious diseases such as dengue, sepsis, and malaria, making it difficult to diagnose.

One of the key barriers in reducing the impact of leptospirosis is the lack of affordable sensitive diagnostic tools. There are currently standard methods recommended by the WHO. The first is the microscopic agglutination test (MAT), a serological-based diagnosis method. Although the MAT is accurate, it requires a skilled technician, well-equipped laboratory, and is time-consuming. The second is dark field microscope diagnosis from sample cultures collected from the patient's blood at the early stage of *Leptospira* spp. infection. However, because *Leptospira* spp. is a slow-growing bacterium, results can take several weeks. The third technique is the use of polymerase chain reaction (PCR), a nucleic acid detection method that is faster and more accurate. The quantitative polymerase chain reaction (qPCR) is an improvement of the PCR that is widely used as the primary diagnostic procedure. The drawback of qPCR is the high-cost of equipment and the lack of availability in every hospital, especially in rural areas where most leptospirosis cases occur. The WHO also recommends the enzyme-linked immunosorbent assay (ELISA) for antibody detection. However, antibody levels are often low during the first week after infection. The development of a novel diagnostic tool that can detect pathogenic *Leptospira* spp. early, providing rapid and accurate results and is not cost-prohibitive to resource-limited hospitals and clinics is essential for public health. Therefore, testing at the point of care is ideal for resource-constrained settings and still unmet need .

Recently, numerous studies have been conducted to investigate point-of-care tests for leptospirosis inlcuidng Loop-Mediated Isothermal Amplification, anti-*leptospira* IgM rapid diagnostic tests (RDTs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas12a system that has shown promising results in nucleic acid detection. The CRISPR/Cas12a system relies on a crispr RNA (crRNA) which acts as a targeting system for the effector function of the Cas12a enzyme to recognize and cleave specific DNA targets. After CRISPR/Cas12a detects its target and cleaves it, the collateral cleavage activity is activated resulting in the fluorescent reporter being cleaved from the quencher and creating a detectable fluorescent signal. For this reason, RPA preamplification combined with the CRISPR/Cas12a detection system can be used for diagnostic screening in a limited-resource setting without the need for specialized instruments.

IMMUNOGENICITY STUDY OF THE OUTER MEMBRANE PROTEIN LIPL41 FROM IRANIAN ISOLATES OF *LEPTOSPIRA INTERROGANS* APPLICABLE FOR A PROMISING DIAGNOSTIC ELISA TEST

Golab

Background: Leptospirosis is a widespread zoonotic disease caused by *Leptospira interrogans* which affects humans in both developing and developed countries. It is commonly prevalent in tropical, subtropical including Iran, resulting in high mortality and morbidity annually. Transmission occurs via contaminated soil or water. It is also a major financial burden. The clinical syndromes alternate from mild symptoms to severe cases, so the diagnosis remains challenging. Recently, many researchers have focused on the outer membrane proteins (OMPs) as potential targets in diagnostic kits and subunit vaccines. Lipl41, considered as one of the most important OMPs is only found in pathogenic species and expressed during infection. The objective of this research was to analyze the LipL41 from *Leptospira interrogans* isolates in Iran, produce a recombinant protein and evaluate its immunogenicity in mice.

Methods: Bioinformatic studies of Lipl41 sequences from prevalent local pathogenic serovars and comparison to other countries were performed. On the basis of polymorphisms one local dominant pattern was selected and expressed in pET32a+ vector and purified by denaturation. Immune responses were evaluated by indirect ELISA in mice.

Results: Sequence analysis showed 96.3-100% similarity among local isolates. Multiple Alignment identified 20 different amino acid substitutions, 8 of which belonged to Iran. The recombinant LipL41 (rLipL41) demonstrated high expression levels and was successfully purified in an insoluble form with the yield of 107.78 mg/L. The administration of rLipL41 with adjuvant led to robust specific antibody titers as well as high levels of IFN-γ in comparison to the control groups ($P < 0.05$); however, the level of IL-4 was similar to the controls.

Conclusions: In conclusion, rLipL41 could stimulate specific immune responses effectively, resulting in both humoral and cell-mediated responses. It may have potential as a candidate for diagnostic test.

PROTEOMIC SIGNATURES IDENTIFY ACUTE LEPTOSPIROSIS AND ELUCIDATE IMMUNE AND INFLAMMATORY RESPONSE PATHWAYS IN HUMAN HOSTS

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Introduction: Despite microbiologic advances, etiologic diagnosis of acute febrile illnesses (AFI) at the time of clinical presentation remains challenging. Blood proteomics-based classification models show tremendous promise for improving diagnosis of AFI as well as providing critical information on the pathophysiology of host responses during acute infection.

Methods: We performed targeted proteomics via Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) on acute-phase blood samples (plasma) collected from pediatric and adult patients with AFI from a multinational cohort (n=189, predominantly from Sri Lanka, Nepal and Kenya). This cohort included patients with acute and largely uncomplicated Leptospirosis as well as routine bacterial infections (*Staphylococcus* spp, *Streptococcus* spp, *Enterobacteriaceae*), rickettsial disease (*Orientia* and *Rickettsia* spp) and viral illness (dengue, influenza). We utilized linear sparse logistic regression models to define proteins whose expression levels in peripheral blood best classify acute Leptospirosis.

Results: The proteomic elements most dysregulated in leptospirosis compared to other AFI are enriched for elements representing inflammation, complement activation, and coagulation cascades. Some of the most dysregulated proteins include IgGFc-binding protein, Leukocyte immunoglobulin-like receptor A3, complement Factor H, serum amyloid A, Kallikrein, and Fibrinogen. From these data we developed a 15-peptide Leptospirosis-specific proteomic signature which differentiated Leptospirosis from other bacterial and viral infections with an area under the receiver operating characteristic (auROC) of 0.81. Interestingly, host proteomic responses to rickettsial illness (predominantly scrub typhus) showed the most overlap with acute leptospirosis and accounted for most of the diagnostic misclassifications, demonstrating a high degree of similarity in the nature of the host response to these two pathogens.

Conclusion: These data demonstrate that novel host-derived multi-analyte proteomic biomarker can accurately discriminate leptospirosis from other causes of acute febrile illness. Additionally, analysis of the peripheral blood proteome offers insight into the inflammatory and coagulopathic pathophysiology of acute Leptospirosis in human hosts.

OBJECTIFICATION OF EVALUATION CRITERIA IN MAT USING DEEP LEARNING

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Introduction: Microscopic Agglutination Test (MAT) is the standard definite diagnosis for leptospirosis. We proposed an agglutination rate prediction method that mimics how technicians estimate the actual diagnosis (Nakano et al., ILS. 2022). Although the method achieved human-equivalent estimation accuracy, its accountability regarding the evaluation criteria was unclear. To address this issue, we propose a method objectifying the evaluation criteria in MAT.

Material and Methods: We made a dataset from the microscopic images taken for our previous study (Nakano et al., ILS, 2022). We applied object-level annotations for each image containing the objects' shape and category. In this study, we defined three categories;
free *leptospira* as *leptospira*, connected *leptospira* including agglutination as a clot, and obstacles such as contaminated stuff as an outlier. With this annotation, we know the number of *leptospira* for each image.

Figure 1 shows the proposed method. Our method first detects all objects in microscopic images and categorizes each one. Next, it computes the agglutination rate from the number of *leptospira*. We utilized the dataset for training and validation and performed performance analysis.

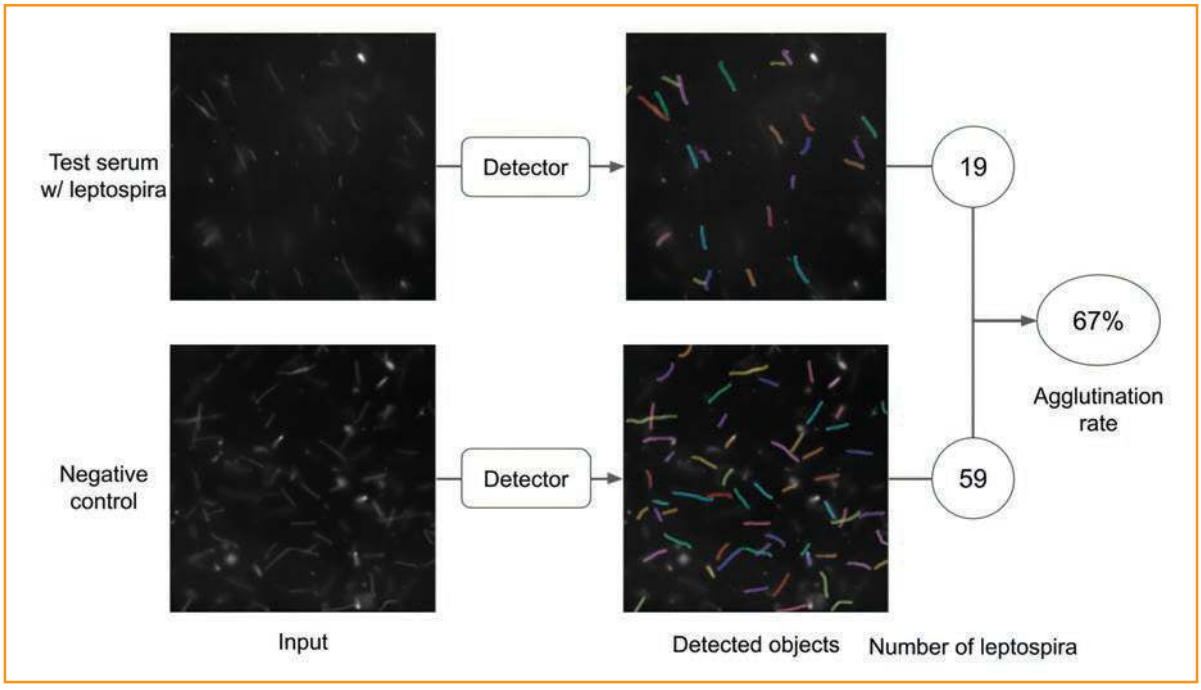


Figure 1 *The flow chart of the proposed agglutination rate estimation method.*

Results: The predicted values had a positive correlation to the ground truth. The prediction error was unstable, with lower error for higher agglutination rates and higher error for lower agglutination rates. This was due to the accuracy of object detection. The pictures of lower agglutination rates had more *leptospira*, and therefore, the object detection error affected the agglutination rate estimation much.

Conclusion: This study used a deep learning technique to objectify the evaluation criteria in MAT. When detecting *leptospira* on microscopic images, we can count the number of *leptospira* and calculate the agglutination rate with simple arithmetic operations. In this presentation, we would like to discuss prospects, including implementing user-friendly software.

DEVELOPMENT AND VALIDATION OF A COMPETITIVE ENZYME-LINKED IMMUNO SORBENT ASSAY (cELISA) FOR THE DETECION OF *LEPTOSPIRA* SEROVAR GRIPPOTYPHOSA ANTIBODIES IN CATTLE SERA

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Introduction: Bovine leptospirosis in Belgium has been associated with outbreaks caused by *Leptospira* Grippotyphosa and Australis serovars[1]. The infection is underestimated in cattle, due to the low efficiency of the current diagnostic techniques. The gold standard for serological diagnosis, the Microscopic Agglutination Test (MAT) is complex and time-consuming, which makes the test not suitable for large sample set testing. To curb these drawbacks, a *Leptospira* serovar Grippotyphosa specific cELISA assay was developed.

Methods: Flat-bottomed 96-well plates were coated with a monoclonal capture antibody specific for this serovar. In a separate plate, serial dilutions of the test serum, along with positive and negative controls, were incubated with inactivated *L. kirschneri* Grippotyphosa Moskva V. Next, the serum/antigen mixture was challenged to a sandwich ELISA using identical antibodies for capture and detection, with the detection antibody conjugated to horseradish peroxidase (HRP). After incubation with the HRP substrate, the reaction was halted and optical density measured at 492 and 620 nm[2].

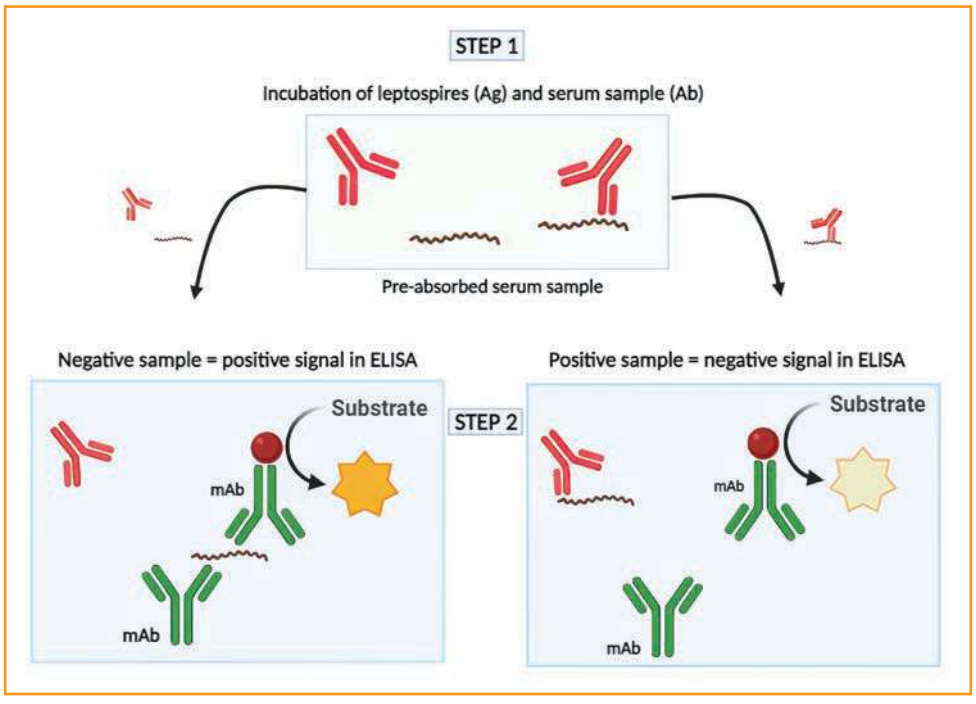


Figure *Graphical representation of the cELISA method.*

Results: The Grippotyphosa specific cELISA was validated according to following validation parameters: repeatability (Coefficient of Variation (CV=2,84), limits of quantification (for sera at MAT 1/100 concordance was at 83,3%), dilutional linearity (estimated %inhibition range: 10,30–85,53 and estimated MAT-titer: 1/58–1/1170; with R^2=0,97), diagnostic specificity (100%; tested against *L.* Hardjo (n=20), *C. burnetii* (n=19) and *Brucella* (n=13) positive cattle sera) and diagnostic sensitivity on retrospective samples (>80%).

Conclusions: In conclusion, a sensitive and specific cELISA for the measurement of the Grippotyphosa leptospiral antibodies was developed. This platform could be used in the context of large screening testing in cattle sera, i.e. during outbreaks. As the conception is multi-species, the validation of this cELISA could be extended for its use in other animal species.

Hence, an equivalent cELISA has been implemented for the *Leptospira* serovar Australis which will be ready for use pending the validation of the test.

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D3-23 Brihuega B

USE OF THE C-TERMINAL CODING REGION OF LIGB FOR THE MOLECULAR DIAGNOSIS (LigBct PCR) OF BOVINE LEPTOSPIROSIS IN SERUM

D3-24 Brihuega B

OPTIMIZATION AND VALIDATION OF LAMP TECHNIQUE FOR DE DETECTION OF EPTOSPIRAL DNA

D3-25 Brihuega B

IN SILICO EVALUATION OF LIPL32 PEPTIDES FOR ANTIGEN CANDIDATES IN LEPTOSPIROSIS DIAGNOSTIC

D3-26 Chaurasia R

IN-VIVO EXPRESSION OF LEPTOSPIRAL VIRULENCE MODIFYING PROTEINS IN RHESUS MACAQUES AND HUMANS: IMPLICATIONS FOR EARLY DIAGNOSIS OF LEPTOSPIROSIS

D3-27 de Koff EM

EVALUATION OF TWO RAPID IMMUNODIAGNOSTIC TESTS (*LEPTOSPIRA* VIRCLIA AND LEPTOCHECK-WB) FOR THE DIAGNOSIS OF HUMAN LEPTOSPIROSIS

D3-28 Dinhuzen J

EVALUATION OF REAL-TIME PCR TARGETING THE LIPL32 GENE IN BLOOD AND URINE FOR THE EARLY DETECTION OF LEPTOSPIROSIS

D3-29 Maneewatchararangsri S

DIAGNOSTIC PERFORMANCE OF COMMERCIAL IGM/G LATERAL FLOW IMMUNOCHROMATOGRAPHIC TEST KITS FOR DIAGNOSIS OF LEPTOSPIROSIS DURING AN OUTBREAK IN THAILAND

D3-30 Monti G

DIAGNOSTIC ACCURACY OF AN IMMUNOMAGNETIC SEPARATION-PCR ASSAY TO DETECT PATHOGENIC *LEPTOSPIRA* spp. IN URINE FROM DAIRY CATTLE, USING A BAYESIAN LATENT CLASS MODEL (BLCM)

D3-31 Prachongsai I

DETECTION OF *LEPTOSPIRA* spp. FROM LEFT-OVER SAMPLES USING NOVEL PROBE-BASED MULTIPLEX QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

D3-32 Shruthi G

RAPID LEPTO MEDIA: A POTENTIAL IN EARLY DETECTION AND CULTURING OF *LEPTOSPIRA INTERROGANS*

D3-33 Stoddard RA

EVALUATION OF A REAL-TIME PCR TO DETECT FOUR *LEPTOSPIRA* SPECIES IN HUMAN CLINICAL SPECIMENS

USE OF THE C-TERMINAL CODING REGION OF LIGB FOR THE MOLECULAR DIAGNOSIS (LigBct PCR) OF BOVINE LEPTOSPIROSIS IN SERUM

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Argentina is an endemic country with epidemic outbreaks of leptospirosis. In livestock, reproductive failures are knowing. A recent study considers that *Leptospira* is the second most common abortigenic pathogen and is responsible for more than US$ 7 million of losses in the cattle and dairy industry, respectively (1). In this context, it is essential to have sensitive and specific tools in order to effectively control the disease the spread. Molecular tools are widely used in different biological samples
In this work, LigBct PCR which amplifies a 756 bp region of the C-terminal end of the conserved LigB protein (2) (ligbct) was used to detect leptospiral DNA in bovine serum.
We used 134 bovine sera from the Leptospirosis Laboratory of INTA Castelar (OMSA Reference Laboratory) which arrived with a suspected diagnosis of Leptospirosis. All sera were classified by MAT (Microagglutination Test) as positive (n: 89) or non-detectable ND (n: 45) with a cut-off point equal to 1/200. LigBct PCR was performed according to the protocol standardized by Saraullo et al (3). LigBct PCR was able to detect leptospiral DNA in 78.65% of positive sera, and in 42.22% of sera classified as ND by MAT. In the face of positive titers by MAT, remanent leptospiral DNA in the bloodstream can still be detected by LigBct PCR. However, leptospiral DNA was also detected in 42.22% of samples where antibodies were ND by MAT. High MAT titers infer infection as opposed to lower titers or ND, often without visible symptomatology. Animals may not present symptoms compatible with leptospirosis, have low or undetectable titers with TMA, but be carriers and disseminate the bacteria through urine infecting the environment, other animals, and humans; therefore, the present technique could be serve to detect carrier animals. It is considered that LigBct PCR could be a complementary tool to detect leptospiral DNA in serum. The development of new diagnostic methods is of utmost importance in order to carry out an adequate diagnosis of leptospirosis in Argentina and thus contribute to the reduction of the great economic losses associated with this important zoonotic disease.

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OPTIMIZATION AND VALIDATION OF LAMP TECHNIQUE FOR DE DETECTION OF LEPTOSPIRAL DNA

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Leptospira spp. is the second most important abortifacient pathogen in livestock production in Argentina, responsible for the loss of millions of dollars in the beef and dairy industry in the Pampas region (Cantón et al., 2022). It is crucial to have diagnostic tools that allow simple and effective detection of the agent, in order to implement necessary measures to prevent spread of the pathogen and mitigate economic and public health consequences. The aim of this study was to develop a method for detecting leptospiral DNA based on loop-mediated isothermal amplification (LAMP) technique, designed for application in low-complexity laboratories: Lepto-LAMP. Molecular target of Lepto-LAMP was *rrs* gene (Koizumi et al., 2012). After optimization, Lepto-LAMP incorporated calcein as an indicator and presented high analytical sensitivity (10 pg of DNA per reaction, or 0.1; 1 and 10 leptospires/mL in bovine serum, urine, and kidney macerate, respectively). Lepto-LAMP specifically detected DNA from pathogenic and intermediate strains of *Leptospira* spp. In a trial using hamsters (*Mesocricetus auratus*) as a model of animal leptospirosis, Lepto-LAMP allowed for the detection of a greater number of positive samples in infected animals compared with end-point PCR *lipL32*. Both molecular diagnostic techniques showed substantial agreement according to Cohen's Kappa coefficient. Employing clinical samples (serum, urine, and organs) from domestic and wild animals, Lepto-LAMP demonstrated high diagnostic sensitivity (100%, 95% CI:93.6–100%) and high diagnostic specificity (96.6%, 95% CI: 95.2–97.7%). Lepto-LAMP had a lower implementation cost compared to the end-point PCR, making it more accessible for low-complexity laboratories. The use of Lepto-LAMP for the diagnosis of animal leptospirosis has great potential to complement the microscopic agglutination test (MAT) as it can be implemented in most non-specialized laboratories and provides a short time to obtain results (between 75–90 minutes from DNA extraction to final result reading).

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IN SILICO EVALUATION OF LIPL32 PEPTIDES FOR ANTIGEN CANDIDATES IN LEPTOSPIROSIS DIAGNOSTIC

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Leptospirosis is a zoonotic infectious disease caused by the spirochete of the genus *Leptospira.* The gold-standard technique for diagnosis is the microscopic agglutination test, however, this serological test is complex and time consuming, and requires specific equipment, highly trained staff, and the live cultures of reference strains of *Leptospira* for use as antigens. It is for this reason that the search for antigenic candidates for immunodiagnostics takes on significant importance. LipL32 is an outer membrane lipoprotein that is exclusively found in pathogenic leptospires, highly conserved and immunogenic. In this study, *in silico* analysis of the amino acid sequence of LipL32 was carried out with the aim of finding peptides that are good candidates for diagnostic use, bound to gold nanoparticles. First, amino acid sequences of lipL32 were searched in Genbank and conservation in pathogenic leptospires (Pomona and Hardjo) was compared between them, using the MUSCLE clustal platform. The signal peptide region was determined by DTU health tech as it is ruled out as an antigen. Remaining amino acid sequence was evaluated in different predictors of epitopes (EMBOSS, Antibody Epitope Prediction, SVMTrip, Predicted Antigenic Peptides, ABC Pred). Once the results of the predictors were obtained, they were compared with each other. The peptides that matched in most of the predictors were selected and evaluated with PepCalc for molecular weight and water solubility.

Although the peptide KKLLVRGLYRISFTTYK was only matched in two softwares, it

Table 1 *Ranking of candidate peptides.*

Sequence	Length	Position	Molecular Weight	Solubility	Software Matching
LPSLKSSFVLSE	12 aa	26	1306.5 g/mol	Poor water solubility	5
LFPPGIPGVSPL	12 aa	222	1193.43 g/mol	Poor water solubility	5
VKTLLPYGSVINYYGYVK	18 aa	47	2077.42 g/mol	Poor water solubility	4
AYYLYVWIPAVIAEM	15 aa	79	1802.14 g/mol	Poor water solubility	4
KKLLVRGLYRISFTTYK	17 aa	202	2086.52 g/mol	Good water solubility	2

would be the best candidate as it has good water solubility. This characteristic is essential for its use in conjunction with gold nanoparticles.

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IN-VIVO EXPRESSION OF LEPTOSPIRAL VIRULENCE MODIFYING PROTEINS IN RHESUS MACAQUES AND HUMANS: IMPLICATIONS FOR EARLY DIAGNOSIS OF LEPTOSPIROSIS

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Accurately assessing global burden of disease due to leptospirosis as well as early diagnosis underscores the need for a reliable diagnostic biomarker. We recently reported a novel paralogous PF07598 gene family encoding ≥12 distinct paralogs, so-called Virulence Modifying Proteins (VMPs). Members of the PF07598 gene family are only found in group I pathogenic *Leptospira*, and are massively upregulated *in-vivo*. VMPs are comprised of two tandemly repeated N-terminal ricin B domains (RBLs) and a C-terminal DNase/toxin domain. Vaccination with VM proteins protects mice and hamsters from lethal challenge infection. Here, we tested the hypothesis that VMPs are expressed *in-vivo* as evidenced by antibody recognition after leptospiral infection. Serum from Rhesus macaques (n=3) that were experimentally infected with *L. interrogans* serovar Copenhageni, and 222 leptospirosis–confirmed/suspected Sri Lankan patient samples, were evaluated by VMP ELISA. Rhesus macaques had anti-VMP IgM antibodies at day 4 after infection; IgG-specific anti-VMP antibodies were present as early as days 4-8. In Sri Lankan humans with leptospirosis, IgG antibodies outperformed qPCR, MAT, and culture for the early diagnosis of leptospirosis. In 222 patients, 26.6% were qPCR-positive and 16.0% were probable cases. MAT confirmed 21.7% positivity, with 19.0% reactive (titer<400) and 41.4% non-reactive cases. These results confirm that VMPs are expressed *in-vivo* in monkeys and humans and demonstrate that anti-VMP antibodies arise early in infection. Leptospiral VMPs are a promising antigen for early diagnosis of leptospirosis.

EVALUATION OF TWO RAPID IMMUNODIAGNOSTIC TESTS (*LEPTOSPIRA* VIRCLIA AND LEPTOCHECK-WB) FOR THE DIAGNOSIS OF HUMAN LEPTOSPIROSIS

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Introduction: The diagnosis of leptospirosis is serologically confirmed by the microscopic agglutination test (MAT) and/or enzyme-linked immunosorbent assay (ELISA). However, these tests are labor intensive and require specialized laboratories and technicians, which increases turnaround time and may pose a barrier for clinicians to request microbiological confirmation of leptospirosis. Timely diagnosis is important for adequate antibiotic treatment of the infection as well as source and contact tracing by public health services We tested the clinical performance of two rapid and easy to perform immunodiagnostic tests.
Material and methods: *Leptospira* VIRCLIA (Vircell, Granada, Spain) and Leptocheck-WB (Zephyr Biomedicals, Goa, India) were performed on 50 sera from serologically confirmed leptospirosis patients (by MAT and/or ELISA; 25 with disease duration ≤10 days and 25 with disease duration >10 days) and 50 sera serologically negative for leptospirosis (by ELISA).

Results: For *Leptospira* VIRCLIA, we found an overall sensitivity of 88% and specificity of 94%. The sensitivity was high regardless of disease duration, with a sensitivity of 84% for patients who had been ill ≤10 days and 92% for patients who had been ill for >10 days. For Leptocheck-WB, we found an overall sensitivity of 76% and specificity of 100%. Leptocheck-WB had a higher sensitivity for patients with a disease duration ≤10 days than for patients with a disease duration >10 days (92% vs. 60%; chi-squared test, p=0.020). The overall sensitivity increased to 96% with a specificity of 94% when results from *Leptospira* VIRCLIA and Leptocheck-WB were combined.

Conclusion: The diagnostic accuracy of *Leptospira* VIRCLIA and Leptocheck-WB was high, especially when performed in combination. Both tests can detect presence of antileptospiral IgM at an early stage of the disease. This yields new opportunities to improve the accessibility of the diagnostic workflow of leptospirosis, facilitating epidemiologic monitoring and timely diagnosis of acute infections.

EVALUATION OF REAL-TIME PCR TARGETING THE LIPL32 GENE IN BLOOD AND URINE FOR THE EARLY DETECTION OF LEPTOSPIROSIS

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Introduction: Early detection leptospirosis infection is still challenging. Molecular diagnostics, which are faster and more sensitive compared to culture analysis, can identify *Leptospira* infection before antibodies become detectable. The objective of our study is to assess real-time (q)PCR techniques using blood and urine samples collected from patients displaying symptoms suggestive of leptospirosis.

Material and methods: A prospective multicenter cross-sectional study enrolled 663 patients with suspected leptospirosis from hospitals in northeastern and southern Thailand. Whole blood and urine samples underwent qPCR assays targeting the *Lipl32* gene. Reference tests included positive microscopic agglutination tests (MAT), blood qPCR, or blood culture. Diagnostic accuracy was evaluated on enrollment day and stratified by symptom onset.

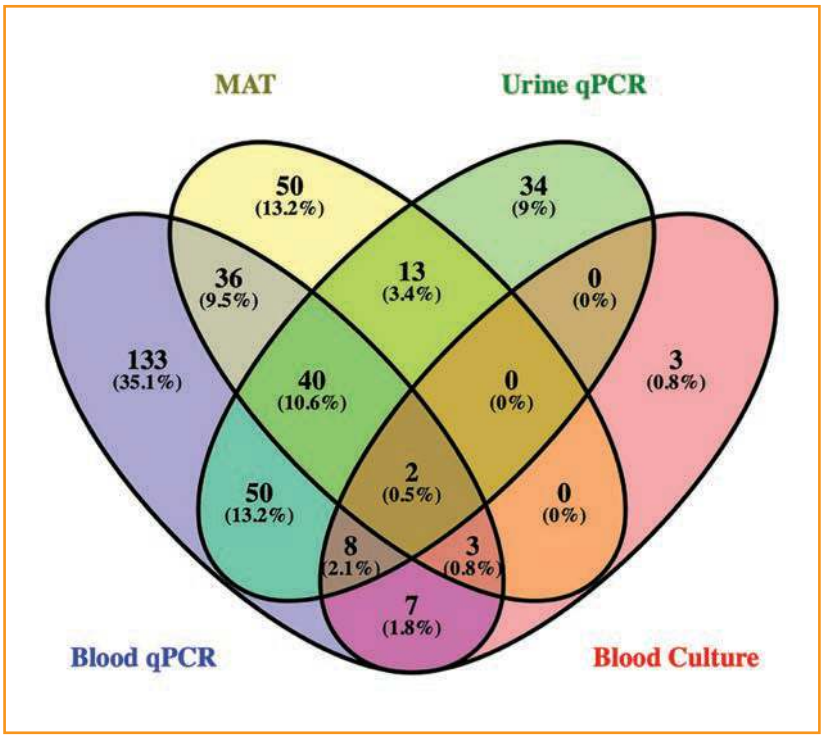


Figure 1 *The Venn diagram represented the number of positive cases diagnosed by each method.*

Table 1 *Diagnosis accuracy at different days after the fever onset (DOF).*

Tests	Overall				DOF 1-3 (n=414)				DOF 4-7 (N=182)				DOF>7 (N=22)			
	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
MAT	42	100	100	61	39	100	100	61	50	100	100	61	44	100	100	72
Culture	7	100	100	50	9	100	100	51	4	100	100	46	NA	NA	NA	NA
Blood qPCR	81	100	100	83	84	100	100	86	72	100	100	74	67	100	100	81
Urine qPCR	33	89	77	55	32	91	79	56	32	86	74	50	44	77	57	67
Combined qPCR Blood and Urine	84	89	90	84	88	91	91	88	77	86	88	75	67	77	67	77

Results: Overall, the positivity rates for MAT, blood culture, blood qPCR, and urine qPCR were 22%, 4%, 42%, and 22%, respectively. Combining blood and urine qPCR significantly raised the positive rate to 49% ($p<0.001$, compared with blood qPCR alone), with 14% exclusively detected by urine qPCR. The estimated sensitivity of qPCR on blood (81%) and combined blood and urine qPCR (84%) was higher than that of MAT (42%) and culture (7%). Test performance categorized by the day after symptom onset showed that the combined blood and urine qPCR consistently displayed an increase in sensitivity, reaching a peak of 88% within the first 3 days of fever onset, compared to blood qPCR alone (84%).

Conclusion: Integrating urine qPCR for leptospires detection may improve diagnosis of acute leptospirosis.

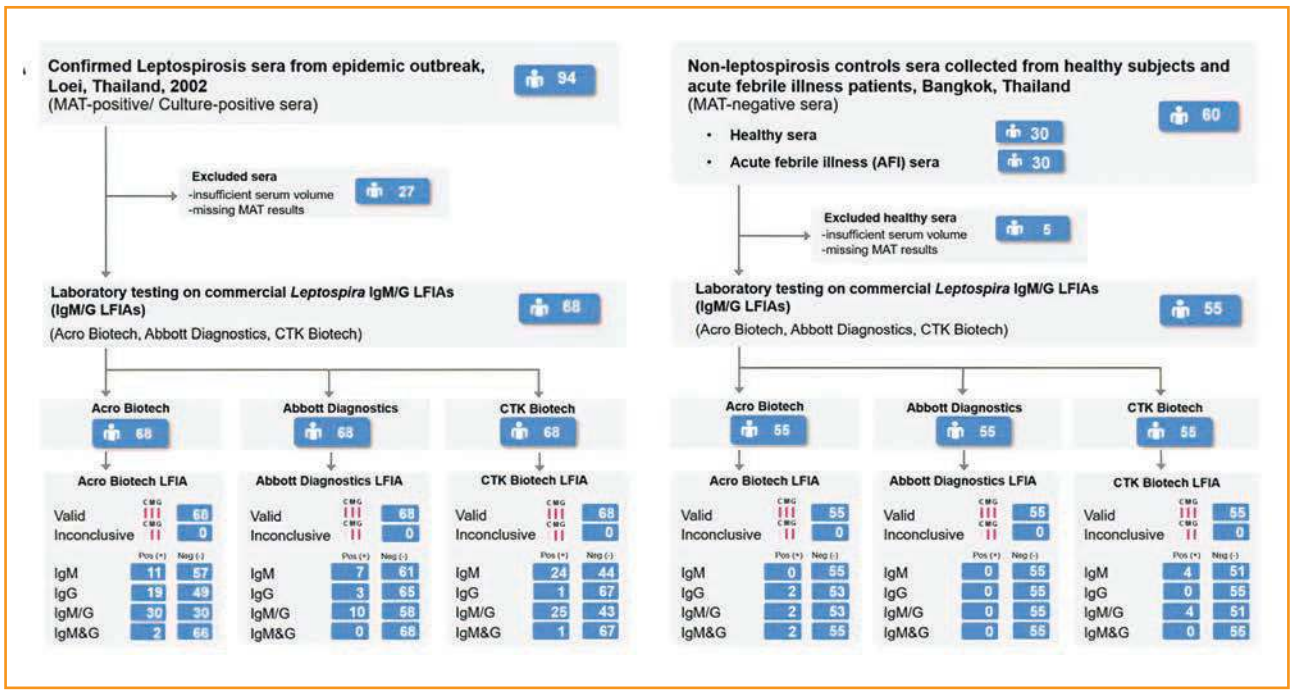
DIAGNOSTIC PERFORMANCE OF COMMERCIAL igM/G LATERAL FLOW IMMUNOCHROMATOGRAPHIC TEST KITS FOR DIAGNOSIS OF LEPTOSPIROSIS DURING AN OUTBREAK IN THAILAND

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The present study, a case–control study was conducted to assess diagnostic test's accuracy and the clinical validity of commercial *Leptospira* IgM/G LFIA test kits, including the Onsite *Leptospira* IgG/IgM Combo Rapid Test kit, Bioline™ *Leptospira* IgG/IgM Rapid test, and *Leptospira* IgM/IgG Rapid test, for diagnosing leptospirosis compared with reference MAT and culture and IgM/G ELISA. Commercial *Leptospira* IgM/G LFIAs demonstrated overall sensitivity and specificity of 44.1% (95% CI: 32–56.6), 96.3% (95% CI: 87.4–99.5) for Acro Biotech, 14.7% (95% CI: 7.2–25.3), 100% (95% CI: 93.5–100) for Abbott Diagnostics, 36.7% (95% CI: 25.3–49.3), and 92.7% (95%CI: 84.7–97.9) for CTK Biotech tests. Clinical validity of the IgM/G LFIAs good for ruled-in disease was demonstrated by minimal to moderate changes of positive predictive values of 86.2%–100% but weak negative predictive values of 48.6%–58.2%. The LFIAs detect IgM/G antibodies as early as the days of postonset of fever 1–3 of 17%–22% with limited sensitivity of 15%–47% on paired-sera. Sensitivities increased to 50%–80.9% with improved performance metric (f1 score) of 0.81 (diagnostic odds ratio [DOR]:79.5, precision: 0.82 and 0.95, K: 0.73) for the Acro Biotech test, 0.57 (DOR: 81.4, precision:1.0 and 0.89, K: 0.54) for Abbott Diagnostics, and 0.59 (DOR: 12.7, precision: 0.60 and 0.89,



K: 0.45) for CTK Biotech on MAT[3]1:400 sera and slightly increased at 20%–52.5% on paired-sera compared with ELISA. IgM/G LFIAs have demonstrated poor sensitivity and could be used to for acute diagnosis in suspected patients in addition to the reference MAT. The diagnostic performance of rapid diagnosis tests on local sera, including leptospirosis (Loei, 2002), is essential data to allocate the tests in the diagnostic pathway under disease outbreak and for improving accuracy of leptospirosis diagnosis and management in Thailand.

This study is supported by Specific League Funds from Mahidol University, 2022 to S. Maneewatchararangsri.

DIAGNOSTIC ACCURACY OF AN IMMUNOMAGNETIC SEPARATION-PCR ASSAY TO DETECT PATHOGENIC *LEPTOSPIRA* spp. IN URINE FROM DAIRY CATTLE, USING A BAYESIAN LATENT CLASS MODEL (BLCM)

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Introduction: Leptospirosis infection is often not diagnosed, due to the lack of a cost-effective test for samples with low urinary bacterial loads. As most infectious animals are asymptomatic carriers, the main test's aim was to detect the presence of leptospires in urine, as evidence of an active infection, for surveillance. The combination of Immunomagnetic separation (IMS) and PCR has been reported to increase PCR's specificity and sensitivity for detecting target DNA.
The study aimed to assess the diagnostic accuracy of a qPCR coupled with IMS step (IMS-qPCR) against the qPCR without using IMS, without a gold standard.

Material and methods: A cross-sectional study on 40 commercial dairy farms from southern Chile was used to obtain 863 urine samples from lactating cows. *Leptospires* were detected using a qPCR targeting the *LipL32* gene, with or without an IMS phase (Tomckowiack *et al.,* 2021). Herds had varying levels of leptospirosis prevalence to ensure the identifiability of BLCM. Diagnostic performance was assessed using a conditional-dependence BLCM; applied to determine the infectious status of leptospirosis in naturally infected dairy cattle, based on two tests and three populations without gold-standard (Wang *et al.*, 2017).

Results: IMS qPCR sensitivity (Se) was 95.7% (95% Probability Interval (PI) = 85.0; 99.4) and specificity (Sp) = 98.0 (95% PI = 96.1; 99.4), indicating that it is more sensitive than qPCR (Se = 69.7 (95% PI = 59.2; 79.0); median difference = 25.2 (Monte Carlo Error (MCE) = 10.2); and Sp = 98.8 (95% PI = 97.6; 99.5), median difference = 0.8 (MCE = 2.1).

Conclusion: Our study showed that IMS-qPCR is a more accurate diagnostic test for detecting pathogenic *Leptospira* spp. in cow urine than conventional qPCR. It is recommended for use as it allows for detection of naturally infected and infectious animals that would otherwise go undetected.

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DETECTION OF *LEPTOSPIRA* spp. FROM LEFT-OVER SAMPLES USING NOVEL PROBE-BASED MULTIPLEX QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

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Introduction: Leptospirosis is a common infectious disease in several tropical countries. Laboratory investigations are crucial for diagnosis of leptospirosis. This study aims to evaluate clinical utility of *leptospira* diagnostic tests including hydrolysis probe-based multiplex real-time PCR and specific IgM detection for patients in a rural endemic area.

Materials and methods: Specific hydrolysis probes and primer sets were designed to simultaneously detect the *LipL32* gene and *16S rRNA* of *Leptospira* spp. PCR had been evaluated previously in another cohort. Blood samples obtained from suspected cases of leptospirosis at Phrom Khiri (rural district) Hospital during January-February (endemic season) in 2024. EDTA whole blood was collected within 24 hours of symptom onset. DNA was extracted using the Qiagen DNA Blood Mini Kit. Probe-based qPCR was performed using the BioRad CFX96 real-time thermal cycler. *Leptospira*-specifc IgM was performed using rapid immunochromatography test (LEPkit).

Results: Blood samples were collected from 117 suspected cases of leptospirosis, among which 33 patients (28%) were IgM-positive, whereas 87 patients (72%) were IgM-negative. Among 33 IgM-positive cases, only 16 cases (48%) were positive by real-time PCR. Among 87 patients with negative IgM, second blood samples could be obtained from 17 patients for repeated diagnostic tests. Interestingly, 5 out of 17 patients (29.41%) showed IgM seroconversion in second specimens, and PCR was positive in all of these 5 patients.

Conclusions: The results suggest that this probe-based qPCR is useful for diagnosis of leptospirosis. The specificity of the IgM rapid test in this endemic area is still questionable because about half of patients with IgM-positive were PCR-negative even at the early stage of infection. The combination of gene detection and serologic testing is recommended to increase diagnostic accuracy. PCR and commercial serologic tests from different companies should be further evaluated in larger clinical samples.

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RAPID LEPTO MEDIA: A POTENTIAL IN EARLY DETECTION AND CULTURING OF *LEPTOSPIRA INTERROGANS*

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Leptospirosis is one of the few challenging infections to diagnose early, as it presents itself with an array of symptoms such as myalgia, fever, headache, jaundice etc. Leading to misdiagnosis most often due to the overlapping symptoms with other infections. Depending on the severity of infection Leptospirosis is found to have a mortality rate between 5-15%, which is huge considering the impact it poses on the livestock. Early identification and timely administration of therapeutics plays a critical role in prevention of deaths due to leptospirosis.

Microbial culturing is a gold standard for identification of infectious pathogens but conventional culture medias cannot be sought to identify *Leptospira* at an early stage, as *L. interrogans* is a fastidious organism and can take more than 2 weeks for positive culture results. Hence other faster detection methods such as PCR test and serological evaluation are used to diagnose Leptospirosis. But one of the major setbacks of these tests are the fact that the sensitivity of these tests is suboptimal, resulting is false negative results. Hence, to mitigate these setbacks we have developed a rapid growth media selective for growth of *Leptospira*, which not only gives the results within 24 hours but also can be used even with inoculum size of 10^4 cells, resulting in disease detection at the early on set, thereby facilitating timely treatment which, in turn decreasing the mortality rate.

EVALUATION OF A REAL-TIME PCR TO DETECT FOUR *LEPTOSPIRA* SPECIES IN HUMAN CLINICAL SPECIMENS

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Diagnosis of leptospirosis can be challenging due to the difficulty and length of time it takes to culture the bacteria, the limited time *Leptospira* can be detected in blood (one week after symptom onset) and urine (intermittently shed) by PCR, and the complexity of the reference serology assay, the microscopic agglutination test (MAT). It is even more difficult to perform epidemiologic investigations since culture is rarely done and neither the diagnostic PCR for *lipL*32 nor MAT can determine the infecting species. A real-time PCR assay was developed for detection of four different *Leptospira* species in animal tissues (Ferreira et al. 2014). We validated the assay for use with human clinical samples to aid in epidemiologic investigations for leptospirosis. *Leptospira interrogans*, *L. kirschneri*, *L. borgpetersenii*, and *L. noguchii* were grown in EMJH to the equivalent of 0.5 McFarland and then diluted 10^{-1} in PBS. A 1:25 dilution of this stock was made in PBS and human clinical matrices (sera, urine, whole blood, and synthetic CSF). Serial dilutions were done in each matrix down to 10^{-7}. Extractions were done in triplicate using the RSC Whole Blood DNA Kit on the Maxwell Promega platform. Real-time PCR was first done targeting the *lipL*32 gene and then on the species-specific PCR assay targeting the appropriate species. The limit of detection (LOD) was lower in PBS compared to clinical matrices. The LOD was approximately 10-fold higher for all the species-specific PCR assays compared to *lipL*32 in all matrices. Overall, the assay targeting *L. noguchii* performed the poorest with a much higher LOD 10-100 time higher compared to the other species-specific PCR assays.

AUTHOR INDEX

4TH ELS MEETING
13TH ILS CONFERENCE
BRUSSELS
2-4 SEPTEMBER, 2024

A

Abazaj E 197
Abazi E 47
Acain AKL 76
Acosta-Jamett G 155
Adamu S 81
Agampodi S 97, 256
Agudelo-Flórez P 89
Aguilar Domínguez M 60
Ahmed A 142
Ahmed AA 148, 232
Ajima N 118
Alameh M-G 186
Alinaitwe L 45, 70
Alla L 47, 197
Almeida E 217
Alves Fiorani F 68
Amamura TA 182
Anderson P 234
Anderson PN 140
Anderson T 94, 142, 143
Andrade García F 62
Andrade L 64
Andrade López AN 86
Andrade-García F 59
Andrade-López AN 59
Andreasen CC 146
Año CEMZ 76
Arent ZJ 137, 145
Arévalo A 177
Arévalo AP 131
Armand C 54
Atherstone C 49, 98, 181
Aturinda CJ 45
Auffret A 50
Avelar KES 69, 219
Ayral F 50, 54, 116, 125, 126

B

Bagó Z 158
Bahn P 45, 88
Bakinahe R 246
Balassiano IT 69, 219
Balboni A 144
Ballados González GG 85, 86
Ballard R 233
Barbosa AS 127
Barbosa C 138
Barbosa Carvalho N 91
Barbosa LN 169, 184, 224
Bastaraud A 83
Batisse-Bertrand J 54
Batista Domingues LT 68
Batista MS 69
Battilani M 144
Baum M 117
Bauza-Herrera R 59
Bayles DO 135, 142
Beer Ljubić B 128
Bellinati L 213
Belmain S 149
Benaroudj N 162, 206, 207, 227
Benoit E 152
Benschop J 34, 58
Benvin I 119, 128
Berenstein M 117
Berg MG 238
Bergamo P 173
Bergmann M 148
Berke O 156, 157
Bernard M 54
Bertasio C 133, 144, 213
Bezerra Nobrega R 79
Bigeard C 50
Bino S 47, 197
Biondo AW 35, 51, 53, 72
Bipin KC 141
Blum S 117, 129
Bodinayake C 243
Bodoarison ZI 83
Bodura Göksu A 194
Boggiatto P 146
Böhmer MM 88
Boneca IG 108, 164, 175
Botman J 126
Boucraut C 37
Bourgeois RM 233
Bourhy P 37, 50, 73, 83
Bouvet J 173
Brangulis K 169
Brauneisen P 54
Brihuega B 130, 170, 251, 253, 254
Brun K 38
Bualuang P 263
Burns MA 231
Busch JD 233
Buschiazzo A 177, 209
BuschiazzoA 131

C

Cadano JS 76
Cagliero J 187
Caimi K 215
Calderón-Rangel A 89
Camp P 135, 142
Campagna SR 224
Campo RDC 55
Cao Y 57, 171, 172
Carmona Buendía M del P 85
Carvalho N 64
Casà G 133
Cassel SL 179
Castro-Cordero A 89
Cavazzani Baena C 68
Ceglie L 213
Celona KR 233
Chang C-C 163
Chaparro-Rodríguez M 87
Chatron N 152
Chaurasia R 165, 256
Chavoix C 38
Chen X 171
Cherel D 50
Chiani Y 215
Chio RPI 55
Chou L-F 109, 163, 189
Christopher CJ 224
Ciuffo C 131, 177
Cloherty GA 238
Coatsworth H 93
Cobos-Villalobos I 59
Coburn J 140, 234

Cohen O 129
Collel M 153
Collins-Emerson J 58
Collins-Emerson JM 34
Cook EAJ 45
Cordeiro Kalabric L 91
Coriano N 94
Correia de Carvalho G 217
Costa F 64, 79, 91, 138, 147
Coullin K 206
Courrol DS 127
Coutrier F 216
Cremonese C 64, 79
Crispo M 131, 177
Cruz Romero A 59, 62, 85, 86
Cruz-Barrera M 87
Cruz-Romero A 60
Cuba Gaspar R 35
Cupillard L 173

D

D'Incau M 132, 133
Dagbo KC 209
Dahlke Machado C 53, 72
David-Watine B 183
Davignon G 207
de Abreu Nogueira J 68
de Castro WAC 53
de França DA 51
De Jesus SKI 76
de Koff EM 257
de la Paz Santangelo M 215
de Melo Jurema Guimarães J 91
De Silva D 243
DeBord K 49
Decors A 50
Deepa PM 141
Delattre D 37
Delooz L 134
Diao L 172
Diescher S 88
Dinhuzen J 100, 105, 106, 258
Djelouadji Z 116
do Couto AC 72
Dobigny G 83
Doline FR 51
Domingues OJ 35
Dondi F 144
Donizete Menozzi B 68
dos Santos Ribeiro P 64, 135, 217
Dosso M 73
Douchet L1 66
Drewes S 199
Dreyfus A 33, 70, 83
Duarte Neto AN 182
Dubniewicz K 137, 145
Dürr S 45

E

Edwards T 99
Ekpo P 200, 263
Espinaze M 149
Esteban M 130, 170, 251, 253, 254
Eyre MT 64

F

Fabrega A 223
Facile V 144
Fakatika T 38
Fanton d'Andon M 175
Farace P 215
Farinhas JH 51
Fasching E 158
Fauveau C 54
Fávero GM 35
Fayne BN 169, 184
Fernandes L 135
Fernandes LGV 142, 208, 214, 233, 236
Fernandes LVG 146
Fernandez M 175
Ferrari N 132
Ferreira dos Santos PE 64
Fitz D 128
Foltran BB 214
Fontana DL 153
Fornazari F 51, 68
Fournier-Chambrillon C 125
Freise J 199
Fujiki S 185

G

Gaffuri A 133
Gallina L 144
Galloway R 49, 94, 233
Galloway RL 265
Galván Macuixtle L 59
Gamboa-Prieto J 59
García F 131, 177
Garcia JCM 76
Garcia KS 64
Gardon S 54
Gaspar JP 235
Gastal G 177
Gatti M 133
Gautrelet M 125
Gayani Tillekeratne L 243
Genthon P 38
Giannitti F 131
Giraud-Gatineau A 162, 227
Girond F 66
Goarant C 38, 66, 207
Goldstein D 153
Gomes T 91, 138, 217
Gomes-Solecki M 178
Gonçalves da Silva AM 127, 182
Gonçalves Machado G 79
Gong L 172
Gourlay P 126
Govan R 38
Graham RM 231
Grant LE 156, 157
Greca Jr H 72

Grégoire F 134
Grindstaff AL 98
Gros J 116
Groud K 125, 152
Guentas L 207
Guillois Y 50
Gulvik CA 265
Gunckel-Grillon P 38
Gupta S 179
Gurgul A 137

H

Haake DA 161, 179
Habuš J 119, 128
Hađina S 119, 128
Hahn BL 140, 234
Hall CM 233
Haluch A 143
Hamer M 130, 170, 251, 254
Hamer, M 253
Hammerl JA 45
Hamond C 69, 94, 135, 142, 146, 177, 208, 219
Hanagal Subbaraya S 264
Handayani F 147, 216
Hansen SF 194
Hanzen Pinna M 68
Hardy D 164
Harran E 50
Harran E1 125
Harrison L 209, 227
Hartloge C 181
Hartmann K 148
Heading E 231
Heinemann MB 182
Heinmüller P 88
Herbreteau V 66
Heuer C 34
Hicks J 135
Hicks JA 142
Hiromatsu K 185, 225
Hoffmaster AR 233
Hoona JJ 45
Hori AY 51
Howard G 79
Hsu S-H 109, 163, 189
Huang C-T 109, 189
Huete SG 206
Hugonnard M 116
Hussein Gasem M 216
Hutton S 233

I

Imholt G 199
Isaac L 182
Ishii K 185

J

Jacob J 199
Jardine CM 156, 157
Jasielczuk I 137
Jiménez-Velásquez CS 87
Jocson CT 55
Johne A 88
Johnson M 234
Juillot F 38
Junaedi A 216

K

Kainiu M 38
Kalempa Panazzolo 35
Kankya C 45
Katakweba AAS 149
Katamba Bugeza J 45
King R 117
Kirabo AV 70
Klein J 183
Kmetiuk LB 35, 51, 53, 72
Ko ER 243
Ko Y-C 109, 189
Kobba K 70
Kodjo A 50
Koel MC 257
Koffi KS 73
Koffi S 83
Kolling GL 225
Kota M 197
Kouassi KS 73
Krairojananan P 221
Kumar M 222
Kumari S 222
Kundu S 178
Kuntz G 50
Kureta E 197
Kurihara Y 185
Kurukulasooriya R 243
Kuwahara S 185

L

Lacbayen YLF 55
Lagares A 74
Lagunes-Morales S 62
Lamorde M 70
Langoni H 35, 51, 68, 72
Lapid R 117
Lattar V 152
Le Dréan E 50
Le Guern A-S 216
Le Guyader M 116, 126
Leal M 155
Lecot L 116
LeCount K 94, 142, 143
Lee N 99
Leekitcharoenphon R 186
Lefebvre S 152
Lejas C 50
Lequeux G 50
Lessa A 138
Li C 163
Llanes A 169, 184
Libera KC 156
Lilenbaum W 138
Lima JA 91
Limothai U 100, 105, 106, 258
Limsiaco MLN 76
Lindroth E 221
Lindstedt EEH 194
Linn-Peirano SC 169, 184
Linsuwanon P 221
Lisboa R 35

Littlejohn S 58
Liu J 171
Liu Y-C 109
Llanes A 223
Lockhart B 177
Loomer C 93
Lorioux C 37
Lozano S 74
Lubega A 45
Lucchese L 213
Luge E 88
Lund H 82
Lund HS 82
Lv T 172

M

Madero A 254
Madesh S 169, 184
Magliocca M 144
Mahillon J 29
Majani Kivali V 45
Majid K 78, 149
Makundi RH 149
Mambe P 73
Maneewatchararangsri S 260
Mangeas M 66
Manita C 84
Maraes Oliveira MV 64
Marchione S 213
Marconi RT 228
Mariet J-F 37
Marques BM 153
Marques da Silva TR 217
Martinez M 130, 251, 253, 254
Martinez ML 170
Martínez-Ibarra E 89
Martinković K 128
Masbud ZIA 55
Massawe AW 149
Matias R 84
Matos R 84
Matsunaga J 179
Matt B 54
Maurić Maljković M 128
Mayer-Scholl A 45, 199
Mayito J 70
Mazzotta E 213
McClain MT 243
Meite S 73
Mende DR 232
Méndez-Ojeda ML 60
Menkes C 66
Metz J 88
Michel P 246
Mikejevs Lorga AC 182
Mingala CN 118
Moctezuma Diaz LM 86
Mojčec Perko V 119, 128
Monari E 144
Monot M 162
Monti G 35, 36, 51, 53, 72, 262
Moore H 49
Moreno Sanchez P 68
Moretti A 132
Mori M 246
Morningstar-Shaw B 143
Moseley M 149
Mueller M 94
Mukadi P 99
Mulyono A 147, 216
Munoz-Zanzi C 94
Murata R 118
Murphy HM 157
Museux K 37
Myrmel M 194

N

Nagahawatte A 243
Nakano R 244
Nally JE 94, 115, 135, 142, 146, 208, 233, 236
Nascimento ALTO 214, 229, 235
Nascimento Silva AM 79
Natale A 213
Nathan J 138
Ndawula EC 70
Neela VK 81
Negron ME 49, 98
Neris RLS 69, 219
Neves F 64
Nicolosi C 231
Nieves C 209, 227
Nilsson VI 82
Nisa S 34, 58
Nisimyan T 117
Nóbrega J 68
Nogueira Paz L 68
Nunes AL 138

O

O'Grady N 243
Obata F 225
Obiegala A 199
Objois T 38
Ochoa-Valencia JL 62
Olivares T 155
Oliveira A 91, 138
Oliveira Santana J 79
Olsen S 146, 208
Onuma M 118
Ortiz M 233
Oulhen RM 38
Oyamada Y 244
Ozuru R 185, 225, 244

P

Palmer M 146
Palmer MV 208
Palombarini de Oliveira AT 68
Papadopoulos S 108, 164, 187
Papin JA 225
Pardyak L 137, 145
Pascal Fournier 125
Passos S 64
Patarakul K 186
Patel DT 228

Perdomo Y 131
Pereira Figueira C 138, 217
Perharić M 119, 128
Périanin A 108
Peutot A 125
Pfeffer M 199
Phoompoung P 238
Picardeau M 37, 38, 162, 206, 207, 209, 227
Pierce R 165
Pietrosemoli N 207
Pilard MA 50
Pivette M 50
Pizzaia Henrique AL 68
Plichta V 119
Pober J 165
Polzer D 158
Prachongsai I 263
Prado LG 127
Puca E 197
Pucci N 232
Putz EJ 142, 146, 236

Q

Qyra S 197

R

Rached A 152
Rahelinirina S 73, 83
Rajeev S 169, 184, 205, 223, 224
Rajerison M 83
Rakotoharinome VM 83
Rakotomanana F 83
Ramery E 126
Ramos T 84
Ramos TMV 219
Ramos Vazquez JR 85, 86
Ramos-Vazquez JR 59
Randriamparany T 83
Reis MG 138
Reller ME 243
Renjifo-Ibañez MC 87
Revilla-Fernandez S 158
Reyes A 74
Ribeiro P 91, 138
Richomme C 125
Richter MH 45, 88
Richter S 232
Ristiyanto R 147, 216
Ristow P 64, 91, 138, 217
Rivera Gutierrez R 94
Rivera-Garcia S 233
Rocha Soares SA 64
Rodrigues da Silva L 182
Rodrigues J 84
Rodrigues NJL 51
Rodriguez-Campos S 82, 194
Rodríguez-Rodríguez V 89
Roesel K 45
Romero-Salas D 60, 62
Rozental T 117, 129
Rubach MP 243
Runge M 199
Ruxrungtham K 186

S

Saathoff M 199
Safari D 216
Sahl JW 233
Salgado M 155, 262
Salguero-Romero JL 62
Salina A 68
Samartino L 130
Samartino Luis 170
Sampaio Pinto M 68
Sampieri M 144
Sanchez C 130, 170
Sánchez C 251, 253, 254
Sanchez Hernandez ME 153
Sánchez Montes DS 60, 85, 86
Sánchez-Montes DS 62
Santana JAS 219
Santana JO 64
Santana-Propper E 233
Santiago Ramos LJ 94
Santiago S 64
Santos CM 127
Saraka D 73
Sarassari R 216
Saraullo V 130, 170, 251, 253, 254
Schafer IJ 49, 94, 98
Schindler M 232
Schmidt E 199
Schmitt KG 148
Schuler EJA 228
Scott Weese J 156, 157
Sebastian I 102
Sekawi Z 81
Selemani M 149
Selmaoui-Folcher N 38
Serna-Lagunes R 60
Shakya R 194
Shimizu A 185
Shruthi G 264
Shyma VH 141
Sibim AC 53
Siemińska I 137
Silpasakorn S 200, 238
Silva IRM 229
Silva KCFA 69, 219
Silva MC 69
Sivasankaran SK 236
Slater JJ 157
Smith C 99
Soares A 37
Soebandrio A 216
Soeiro S 84
Sohail ML 90, 150, 151, 230
Sokolova M 34
Soleng A 194
Soltero F 233
Soni N 147
Sonoda T 225
Soonthornworasiri N 260
Soro SD 152
Sossai Possebon F 68
Soupé Gilbert ME 207
Soupé-Gilbert M-E 38
Springer Browne A 142
Srisawat N 100, 105, 106,

241, 258
Staples M 231
Stasko JA 146
Steinparzer R 158, 232
Steinrigl A 158, 232
Stoddard R 181
Stoddard RA 265
Stollberg KC 88
Stone NE 233
Straubinger RK 148
Štritof Z 119, 128
Strutzberg-Minder K 153
Stuber T 135, 142
Suarez Galaz A 85
Subharat S 34
Sullivan M 231
Suputtamongkol Y 200, 238
Surdel MC 140, 234
Sutterwala FS 179
Szmatoła T 137

T

Tachaboon S 100, 105, 106, 258
Taddei R 144
Tantibhedhyangkul W 200, 263
Techawiwattanaboon T 186
Teixeira AF 214, 229, 235
Telfer SE 149
Terrasson J 54
Terrusi A 144
Thao G 234
Thenon N 125
Thibeaux R 38, 207
Thornton N 233
Tian Y-C 163
Tibbs-Cortes B 146
Tibbs-Cortes BW 142, 208, 236
Tichit M 164
Toft B 82
Toma C 102
Tomckowiack C 155, 262
Toot K 143
Torres Castro MA 85
Torres-Higuera L 87
Tourniaire O 125
Tramier C 38
Tran PNT 106
Trombert-Paolantoni S 37
Tsai C-Y 189
Tubalinal GASP 118
Turc E 207
Turk N 119, 128

U

Ullerich A 153
Ulrich RG 199
Unterweger C 232
Urbani L 144
Urioste V 177

V

Vallee E 34
van der Linden H 129, 135, 142, 232
van Elzakker EPM 257
van Gorp ECM 193
Van Nimmen M 246
Vasconcelos L 91
Veldman T 243
Veríssimo G 64, 138
Vernel-Pauillac F 108, 164
Veyrier FJ 209, 227
Vilanova J 38
Vilas Bôas D 138
Villanueva MA 118
Villanueva SYAM 55, 76
Vinetz JM 165, 256
Vyn CM 156, 157

W

Wagner DM 233
Wainaina M 45
Waitt B 93
Waldner K 158
Wasuworawong K 221
Weiner Z 181, 233
Weiner ZP 265
Weiss SL 238
Weissman D 186
Werts C 108, 164, 183, 187
WERTS C 175
Wigati M 147, 216
Wilkinson DA 34
Willixhofer D 158, 232
Wilson PR 34
Wollanke B 135
Wongsawat E 200, 238
Woods CW 243

X

Xie X 57, 171, 172
Xu J 157

Y

Yamaguchi J 238
Yamashiro T 102
Yang C-W 109, 163, 189
Yang H-Y 109, 189
Yartrak J 263
Yasir A 94
Yoshimura M 185, 225
Yupiana Y 34

Z

Zablotski Y 148
Zar Win T 99
Zarantonelli L 131, 177
Zečević I 119, 128
Zeiss C 256
Zhang S 57, 171
Zhang W 57, 171, 172
Zinini F 37
Zulkafli Z 81



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