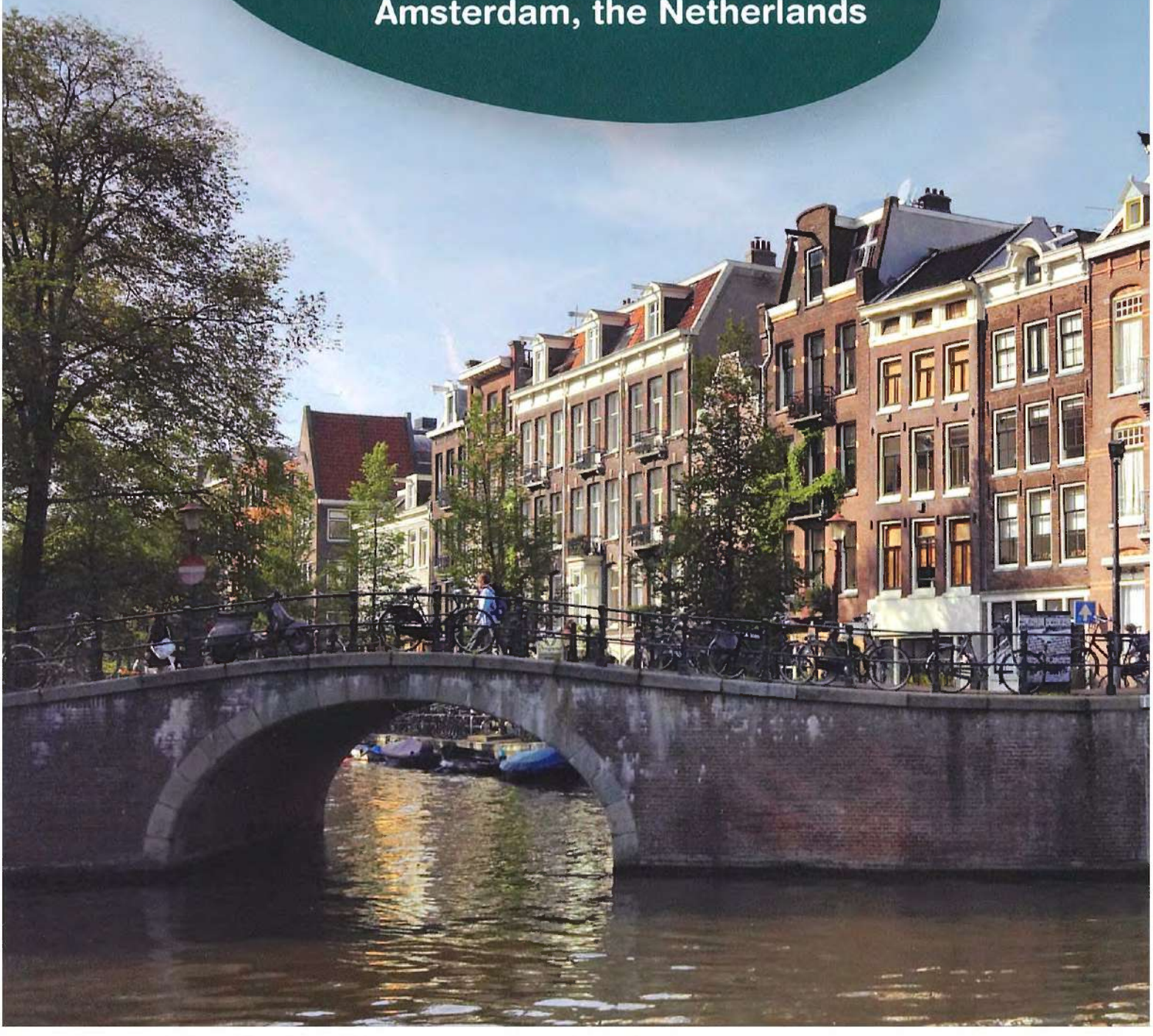
Program

2nd ELS meeting on leptospirosis and other rodent borne haemorrhagic fevers

16 - 18 April 2015

Royal Tropical Institute (KIT)
Amsterdam, the Netherlands



Errata Program 2nd ELS meeting on leptospirosis and other rodent born haemorrhagic fevers

Page 5

09.45 title will be:
Leptospirosis Incidence and Risk Factors in Meat Workers in New Zealand - *Comparison between Standard Generalized Linear Modelling and Additive Bayesian Network*

10.45 Key note Yulia Ananyina, Russian Academy of Medical Science, Russia
Ecology of Leptospires: pregenomic and postgenomic era
CANCELLED

New

10.15 Workshop on Micro Agglutination Test for the diagnosis of animal leptospirosis
A need for consensus?
Marcella Mori (CODA-CERVA) and Zorée DJELOUADJI (VetAgro group).

Page 6

15.00 Pathogenic *Leptospira* spp. causing aseptic meningitis in Sudanese children
Nada Abdelghani Abdelrahim, Sudan Medical and Scientific Research Institute, Sudan
CANCELLED

Page 7

12.15 Speaker will be **Artur Jablonski** instead of Jacek Zmudki

Page 8

14.45 Characterization of the genus *Leptospira* isolates from rodents captured in Angola
Elsa Fortes Gabriel, Universidade Nova de Lisboa, Portugal
CANCELLED

Dear Colleagues and Friends,



On behalf of the organizing committee it is my pleasure to welcome you to the 2nd Scientific Meeting on Leptospirosis and other rodent-borne haemorrhagic fevers of the European Leptospirosis Society (ELS2015). Following the first meeting in Dubrovnik in 2012, we are proud to now host this triennial meeting in the Netherlands, Amsterdam, 16-18 April 2015.

The European Leptospirosis Society (ELS) was formed in 2012 in order to promote knowledge on leptospirosis and related haemorrhagic fevers in the region. While the society primarily is intended for members from Europe, Africa and the Middle East, the meeting welcomes everyone who is interested in the subject and, indeed, we are happy to have registrations from all over the world.

This meeting is of particular interest as we currently see an increasing distribution of leptospirosis cases both in humans and animals as well as hantavirus infections in various European countries, notably in 2014. One might argue that this, at least for part, is due to the ongoing climate change with increasing occurrence of warm periods and excessive rainfall in the continent and thus that the rising incidence is not a unique phenomenon but a start of a new trend.

ELS2015 includes lectures of eminent keynote speakers and provides the opportunity to present and discuss many issues on leptospirosis and related subjects. The participants will get benefit from networking with colleagues from different background, for research as well as exchange of the experiences and new ideas in management and controlling the disease.

We recommend and encourage your attendance to the satellite symposium "Transmission Routes of Infections" on Thursday evening, which has state-of-the-art presentations of several cutting-edge scientists on this well-associated and very interesting subject.

There is more than science. Amsterdam is an interesting city to visit and in April tulips will be flowering - a visit to the Keukenhof is recommended.

I wish you all a very fruitful meeting and a pleasant stay in Amsterdam.

Rudy Hartskeerl

President of ELS

Table of contents

Welcome by the President of ELS Rudy Hartskeerl 1

Table of contents 2

Organising Committee and Scientific Committee 3

Program 4

Satellite symposium Transmission routes of infections 9

Oral presentations Thursday 16 April Pathophysiology and clinical aspects 10

Oral presentations Friday 17 April Epidemiology and ecology 19

Oral presentations Friday 17 April Diagnostics and molecular aspects 30

Oral presentations Saturday 18 April Veterinary aspects 36

Oral presentations Saturday 18 April Immunology and vaccine development 51

Poster presentations Thursday 16 April Pathophysiology and clinical aspects 59

Poster presentations Friday 17 April Epidemiology and ecology 63

Poster presentations Friday 17 April Diagnostics and molecular aspects 70

Poster presentations Saturday 18 April Veterinary aspects 82

Poster presentations Saturday 18 April Immunology and vaccine development 94

List of participants 97

Sponsors 101

Timely abstracts have been screened for nomenclature and when appropriate adapted to the instructions that can be found on the ILS homepage: Genus and species must be italicized, with the serovar name not italicized and with an upper case first letter. For example *Leptospira interrogans* serovar Icterohaemorrhagiae.

Organising committee

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Senior Scientist, Royal Tropical Institute
Executive member ELS

Eric van Gorp
Professor of clinical virology, Erasmus MC
Executive member ELS

Rudy Hartskeerl
Head Leptospirosis Reference Centre, KIT Biomedical Research
President ELS

Chantal Reusken
Senior Scientist Public Health Virology
Department of Viroscience, Erasmus MC

Marga Goris
Coordinator Leptospirosis Reference Centre, KIT Biomedical Research
Executive member ELS

Joke W. B. van der Giessen
Senior scientist Center Zoonoses & Environmental Microbiology, National Institute for Public Health and the Environment (RIVM)

Eric Klaasen
Executive member ELS
Senior Project Leader R&D MSD Animal Health

Erik Sickmann
Managing director
Sickmann Congres Organisatie

Scientific committee

Yulia Ananyina
Gamaleya Institute for Epidemiology and Microbiology, Russia

Eric van Gorp
Erasmus MC, The Netherlands

Heikki Henttonen
Natural Resources Institute Finland, Finland

Eric Klaasen
MSD Animal Health

Ake Lundkvist
Uppsala University, Sweden

Alemka Markotic
Hospital for infectious diseases Dr. Fran Mihaljevic, Croatia

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Jarlath Nally
University College Dublin, Ireland

Mathieu Picardeau
Institut Pasteur, France

Nicoletta Ponti
Instituto Zooprofilattico Sperimentale della Sardegna, Italy

Nenad Turk
University of Zagreb, Croatia

Jaap Wagenaar
University of Utrecht, The Netherlands

Congress secretariat Sickmann Congres Organisatie
www.els2015.eu
info@els2015.eu
+31 6 4569 3847

Venue Royal Tropical Institute
Mauritskade 63
1092 AD Amsterdam

Thursday 16 April

10.00 Registration, poster sessions
12.00 Board meeting ELS
14.00 Opening
Rudy Hartskeerl, President ELS
Jaap van Dissel, Director of the RIVM Centre for Infectious Disease Control, RIVM

Pathophysiology and clinical aspects (14.15 – 15.45 hr)
Chair: Rudy Hartskeerl, KIT Biomedical Research, the Netherlands
Jiri Wagenaar, Erasmus MC, the Netherlands
14.15 **Key note Mathieu Picardeau, Institut Pasteur, France**
What makes *Leptospira interrogans* a pathogen?
14.45 Effect of puumula hantavirus infection on the proteome and hemostatic function of Human Umbilical Vein Endothelial Cells
Marco Goeijenbier, Erasmus MC, the Netherlands
15.00 Clinical manifestation of severe leptospirosis in Dr. Soetomo Hospital Surabaya, Indonesia
Usman Hadi, Dr Soetomo Hospital - Airlangga University, Indonesia
15.15 Regulation of cytokine response in leptospirosis: a possible role of suppressor of cytokine signaling
Mariko Matsui, Institut Pasteur de Nouvelle-Calédonie, France
15.30 Differentiation of severe leptospirosis from critical phase dengue infections
Narmada Fernando, University of Colombo, Sri Lanka

15.45 Coffee break, poster sessions (15.45 – 16.15 hr)

Pathophysiology and clinical aspects (16.15 – 17.30 hr)
16.15 **Key note Ben Adler, Monash University, Australia**
Leptospiral genomics: how does it relate to taxonomy, pathogenesis and immunity?
16.45 Combined in vivo and in vitro analysis of endothelial cell activation in severe leptospirosis shows pathogen and immune mediated endothelial cell activation and association with disease severity
Marco Goeijenbier, Erasmus MC, the Netherlands
17.00 Human leptospirosis in Sardinia from 2005 to 2014
Nicoletta Ponti, Istituto Zooprofilattico Sperimentale della Sardegna, Italy
17.15 Pulmonary involvement during leptospirosis
Edmond Puca, Nobel Ilac, Albania

17.30 Welcome reception (17.30 – 18.30 hr)

18.30 Satellite symposium Transmission routes of infections (18.30 – 21.30 hr)
Chair Eric van Gorp, Erasmus MC, The Netherlands

Friday 17 April

Epidemiology and ecology (8.30 – 10.15 hr)

Chair Chantal Reusken, Erasmus MC, the Netherlands
Nicoletta Ponti, Istituto Zooprofilattico Sperimentale della Sardegna, Italy

08.30 **Key note Georgies Mgode, Sokoine University of Agriculture, Tanzania**
Leptospirosis: commonly forgotten in Africa

09.00 Leptospirosis outbreak in strawberry harvesters in Germany
Anne Mayer-Scholl, Federal Institute for Risk Assessment, Germany

09.15 Livestock and human leptospirosis occurrence and control in New Zealand
Peter Wilson, Massey University, New Zealand

09.30 Transmission and mixed infections in a diverse reservoir community: *Leptospira* in Madagascar
Mark Moseley, University of Aberdeen, United Kingdom

09.45 Leptospirosis in New Zealand
Anou Dreyfus, University of Zurich, Switzerland

10.00 Strenghtening Local and Regional Health Systems for improved Leptospirosis Surveillance: Lessons from an epidemiological study of three Regencies in East Java
Bianca van Bavel, Trinity College Dublin, Ireland

10.15 Coffee break, poster sessions (10.15 – 10.45 hr)

Epidemiology and ecology (10.45 – 12.45 hr)

10.45 **Key note Yulia Ananyina, Russian Academy of Medical Science, Russia**
Ecology of Leptospires: pregenomic and postgenomic era

11.15 Estimating the economic impact of leptospirosis on public health and livestock farming in New Zealand
Cord Heuer, Massey University, New Zealand

11.30 Swedish city rats - a potential health threat
Tanja Strand, Uppsala University, Sweden

11.45 Monitoring of rodents and the rodent-borne pathogens *Leptospira* and hantavirus in the Netherlands
Joke van der Giessen, RIVM, the Netherlands

12.00 **Key note Heikki Henttonen, Natural Resources Institute Finland, Vantaa, Finland**
Eco-epidemiological research on rodent-borne pathogens in Finland

12.30 ELS member meeting (12.30 – 13.30 hr)

12.30 Lunch and poster sessions (12.30 – 14.30 hr)

Diagnostics and molecular aspects (14.30 – 15.30 hr)

Chair Peter Wilson, Massey University, New Zealand
Muhammad Hussein Gasem, Diponogoro University, Indonesia

14.30 **Key note Marga Goris, Royal Tropical Institute, the Netherlands**
Diagnostic tests for human leptospirosis

15.00 Pathogenic *Leptospira* spp. causing aseptic meningitis in Sudanese children
Nada Abdelghani Abdelrahim, Sudan Medical and Scientific Research Institute, Sudan

15.15 Global Leptospirosis Environmental Alert Network (GLEAN): mission and activities
Mathieu Picardeau, Institut Pasteur, France

15.30 Coffee break, poster sessions (15.30 – 16.00 hr)

Diagnostics and molecular aspects (16.00 – 17.00 hr)

16.00 **Key note Ake Lundkvist, Uppsala University, Sweden**
Hantavirus diagnostics

16.30 Diagnostic accuracy of a Recombinase Polymerase Amplification Assay for the detection of pathogenic *Leptospira*
Ahmed A. Ahmed, KIT Biomedical research, the Netherlands

16.45 Molecular and antigen detection tests for leptospirosis: a systematic review of diagnostic test accuracy for the Cochrane Collaboration
Bada Yang, KIT Biomedical research, the Netherlands

17.00 Poster sessions (17.00 – 18.00 hr)

18.00 Conference dinner on canal boat (18.00 – 20.30 hr)

Saturday 18 April

Veterinary aspects (8.30 – 10.45 hr)

Chair Eric Klaasen, Executive member European Leptospirosis Society, the Netherlands
Joke van der Giessen, RIVM, the Netherlands

08.30 **Key note Katrin Hartmann, Ludwig-Maximilians-Universität, Munich, Germany**
Canine and feline leptospirosis

09.00 Diagnostic performance of the Test-itTM *Leptospira* Canine IgM Lateral Flow ELISA in the early diagnosis of acute canine leptospirosis
Corinne Gloor, University of Bern, Switzerland

09.15 Distribution of pathogenic *Leptospira* serogroups in dogs from Lima, Peru
Juan Jose Siuce Moreno, Universidad Nacional Mayor de San Marcos, Peru

09.30 Canine leptospirosis in Croatia - epizootiological and clinical features
Josipa Habuš, University of Zagreb, Croatia

09.45 Prevalence of *Leptospira* serovar Hardjo infection in aborted foetuses and stillbirths
Zbigniew Arent, Agri-Food and Biosciences Institute, United Kingdom

10.00 A review of leptospirosis research in farmed deer in New Zealand
Peter Wilson, Massey University, New Zealand

10.15 Unusual patterns of pathogenic *Leptospira* spp. Infection in animal hosts from Northern Tanzania
Kathryn Allan, University of Glasgow, United Kingdom

10.30 Reproductive disorders and leptospirosis: a case study in a mixed units farm (cattle and swine)
Marcella Mori, VAR-CODA-CERVA, Belgium

10.45 Coffee break, poster sessions (10.45 – 11.15 hr)

Veterinary aspects (11.15 – 13.00 hr)

11.15 **Key note Nenad Turk, University of Zagreb, Croatia**
Leptospirosis in Europe - neglected disease of natural focus

11.45 A nationwide survey of pathogenic leptospires in urine of cattle and buffaloes by Loop-mediated isothermal amplification (LAMP) method in Thailand, 2011 – 2013
Duangjai Suwancharoen, National Institute of Animal Health, Thailand

12.00 A leptospirosis overview on wild fauna in Sardinia, Italy
Maria Nicoletta Ponti, Istituto Zooprofilattico Sperimentale della Sardegna, Italy

12.15 A serological survey of selected *Leptospira* serovars in wild boar in Poland
Jacek Zmudzki, National Veterinary Research Institute, Poland

12.30 **Key note, Jaap Wagenaar, University of Utrecht, the Netherlands**
Past, current and future surveillance of leptospirosis in animals in the Netherlands

13.00 Lunch, poster sessions (13.00 – 14.00 hr)

Immunology and vaccine development (14.00 – 15.30 hr)

Chair Ben Adler, Monash University, Australia
Mathieu Picardeau, Institut Pasteur, France

14.00 **Key note Byron Martina, Erasmus MC, the Netherlands**
The host response to hantaviruses: a delicate balance between resolution and pathology

14.30 Large epidemiological investigation about leptospirosis within the non-rodent wildlife in the French landscapes
Benoit Combes, Entente de Lutte Interdep Contre les Zoonoses, France
Zorée Djelouadji, VetAgro Sup, France

14.45 Characterization of the genus *Leptospira* isolates from rodents captured in Angola
Elsa Fortes Gabriel, Universidade Nova de Lisboa, Portugal

15.00 Mannose-binding lectin inhibits motility of *Leptospira*
Jun Xu, Tohoku University, Japan

15.15 Efficacy of EURICAN® DAPPi2-L3 against leptospirosis
Lionel Cupillard, MERIAL, France

15.30 Coffee break, poster sessions (15.30 – 16.00 hr)

Immunology and vaccine development (16.00 – 17.30 hr)

16.00 **Key note Jarlath Nally, National Animal Disease Center, Iowa, USA**
Leptospira regulate protein expression during persistent infection of reservoir hosts

16.30 Safety and efficacy of a new octavalent combined Erysipelas, Parvo and *Leptospira* vaccine in gilts against *Leptospira* serovar Pomona associated disease and foetal death
Ton Jacobs, MSD Animal Health, the Netherlands

16.45 Leptospirosis severity association with the humoral immune response
Roshan Niloofa, University of Colombo, Sri Lanka

17.00 A Mouse model of *Leptospira* infection designed for the development of anti-shedding drugs or vaccines
Maria Gomes-Solecki, University of Tennessee Health Science Center, USA

17.15 Superoxide production by differentiated HL-60 cells interacted with *Leptospira*
Narmada Fernando, University of Colombo, Sri Lanka

17.30 Closure and farewell drink

Satellite symposium Transmission routes of infections
Thursday 16 April

18.00 Registration

18.30 Opening, Prof.dr. Eric C.M. van Gorp, Erasmus MC
Introduction on transmission routes and examples of recent outbreaks

18.40 Wildlife-borne infections
Dr. Joke W.B. van der Giessen, RIVM

19.00 Foodborne infections [Norovirus]
Prof.dr. Marion P.G. Koopmans, Erasmus MC

19.20 Vectorborne [sandfly] infections [Leishmaniasis]
Dr. Pieter P. van Thiel/ Dr. Ed E. Zijlstra, Rotterdam Centre for Tropical Medicine

19.40 Bloodborne infections
Prof. dr.Hans Zaaijer AMC, Sanquin

20.00 Break

20.30 Waterborne infections
Prof. dr. Ana de Roda Husman, RIVM

20.50 Airborne infections [MersCoV]
Dr. Bart Haagmans, Erasmus MC

21.10 Vector borne infections [Malaria]
Prof.dr. Robert Sauerwein, Radboudumc

21.30 Closure

ORAL PRESENTATIONS Thursday 16 April
Pathophysiology and clinical aspects

What makes *Leptospira interrogans* a pathogen?

Mathieu Picardeau
Institut Pasteur, France

Our current understanding of the virulence mechanisms and more generally the biology of pathogenic *Leptospira* remains largely unknown. This hindrance is partly due to a lack of efficient genetic tools available for use in pathogenic *Leptospira* spp.

We have recently developed genetic tools for the pathogen *L. interrogans*. These advances, together with genome sequencing and post-genomic technologies, have now made it possible to study virulence determinants in *L. interrogans*. In this way several avirulent mutants were generated by random mutagenesis.

Leptospira researchers have focused on identifying outer membrane proteins that could account for the disease that manifests in susceptible hosts. While progress has been made, there has yet to be a single definitive study that demonstrates a "true" virulence determinant. To gain a global understanding of the composition of the *L. interrogans* exoproteome and to elucidate how these proteins contribute to disease pathogenesis, we identified exoproteins under various *in vitro* culture conditions mimicking infection using label-free quantitative mass spectrometry. Our results suggest that the majority of exoprotein encoding genes from pathogenic *Leptospira* are dedicated to heterotrophic processes with potential moonlight functioning in disease pathogenesis. Proteins of unknown function, which represent the majority of pathogen-specific proteins (presumably involved in unique virulence mechanisms), were underrepresented. Thus, disease manifesting from *Leptospira* infection likely results from a combination of primary and potentially secondary functioning of exoproteins and the host immune response to these proteins, rather than "true" virulence proteins such as toxins and effectors.

Our findings bring novel insights into the molecular basis of leptospiral pathogenesis. Future research should focus on how these proteins function within the host to acquire nutrients and/or how they interact with host components.

Effect of puumala hantavirus infection on the proteome and hemostatic function of Human Umbilical Vein Endothelial Cells

Marco Goeijenbier[1], Simone S. Schuller[2] , Joost C.M. Meijers[3,4], Rudy A. Hartskeerl[5], Jiri F.P. Wagenaar[1,5] Marga G.A. Goris[5] , Jeroen Roose[1,6] , Albert D.M.E. Osterhaus[1,6] ,Eric C.M. van Gorp[1*] , Byron E.E. Martina[1,6*], Jarlath Nally[7*]

1.= Department of Viroscience, Erasmus MC, Rotterdam, CE 50 301S, The Netherlands, 2= Vetsuisse Faculty, University of Bern, Bern, Switzerland, 3.= Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands,4.= Department of Plasma Proteins, Sanquin Research, Amsterdam, the Netherlands,5.= Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam, the Netherlands,6.= ARTEMIS One Health Research Institute, Utrecht, The Netherlands,,7.= Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, Ioaw, United States of America *data is subtracted from multiple papers; these authors equally contributed to the data presented in this abstract

Background: Puumala virus (PUUV) infection causes over 5000 cases of hemorrhagic fever in Europe annually and can influence the hemostatic balance extensively. Infection might lead to hemorrhage, while a recent study showed an increased risk of myocardial infarction during or shortly after PUUV infection. The mechanism by which this hantavirus influences the coagulation system is unknown. Therefore we aimed to elucidate mechanisms explaining alterations seen in primary and secondary hemostasis during PUUV infection by using two different approaches.

Methods: By using low passage PUUV isolates to infect primary isolate endothelial cells we decided to first study alterations in the regulation of primary- and secondary hemostasis, by using on cell platelet aggregation and functional clotting assays and by measuring the release of fibrinolysis regulators. Subsequently we used a helicopter view approach and studied differences in protein abundance, by using 2D-DIGE analysis. We analyzed the complete proteome of PUUV infected HUVECs compared to non-infected cells and cells infected with a bacteria that causes a comparable disease in human (*Leptospira interrogans* serovar Copenhageni).

Results: Our main finding was an activation of secondary hemostasis due to increased tissue factor expression leading to increased thrombin generation in a functional assay. Furthermore we showed that during infection platelets adhered to HUVECs and subsequently specifically to PUUV virus particles. The binding of platelets to PUUV particles could be blocked by incubtation with polyclonal PUUV serum. Furthermore, PUUV infected HUVECs produced more plasminogen activator inhibitor type-1 (PAI-1) compared to controls. The PAI-1 produced in this model formed complexes with vitronectin, increase its half-life. The use of 2D-DIGE analysis provided insight into the contribution of endothelial cell responses during PUUV infection. Comparative proteome analysis revealed five specific proteins down regulated after infection while ten proteins showed an increase in protein abundance.

Conclusions: Platelets bind to PUUV infected endothelial cells and PUUV particles in the absence of endothelial cells. Furthermore, the procoagulant changes in PUUV patients could be the result of increased thrombin generation due to an increase in tissue factor expression on infected endothelial cells. Furthermore, we identified at least five down regulated and ten upregulated proteins by 2D-DIGE as candidates for further pathogenesis - or biomarker studies. This combined approach gives a unique insight into the early host response of endothelial cells during infection with PUUV.

Clinical manifestation of severe leptospirosis in Dr. Soetomo Hospital Surabaya, Indonesia

Usman Hadi
Dr. Soetomo Hospital-Airlangga University, Surabaya, Indonesia

Introduction: Leptospirosis is an important zoonotic disease and distributed worldwide, rhodent and other wildlife animal commonly considered to be important epidemiological carriers. In Indonesia leptopsirosis still endemic, some time appear out break in several area. Indonesian ministry of health reported the incidence of this disease around 0.5 / 100,000 population in the year 2008.
Material and method: Review severe cases of patients admitted in the Dr. Soetomo Hospital Surabaya.
Result and conclusion: While in the Dr. Soetomo Hospital in the year 2014 there were 34 cases suspected having leptospirosis with mortality rate 50%. In this paper have been reported 2 cases of severe *leptospira*, with cardiac involvement in the form of myocarditis and in the other patient revealed pulmonary haemorrhage and acute respiratory distress. Both patients can be cured with antibiotics and supportive therapy.
Conclusion: Leptospirosis is still big problem in Indonesia, outbreak of this disease usually followed by severe manifestation which is high mortality.

Key words: Zoonosis, Severe leptospirosis, Indonesia
Corresponding author: Usman Hadi, e-mail usmanhadi@sby.centrin.net.id

Regulation of cytokine response in leptospirosis: a possible role of suppressor of cytokine signalling

Flavien BOUCHET, Emilie BARSAC, Sophie GEROULT, Cyrille GOARANT, Mariko MATSUI
Institut Pasteur de Nouvelle-Calédonie

Introduction: Leptospirosis is a worldwide zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. The clinical presentation of the human cases is highly variable ranging from flu-like symptoms to severe forms with multiple organ failure. This clinical polymorphism could be due to the variability of the immune response of the host. One of the main differences between human monocytic cells (THP-1) and murine macrophages (RAW 264.7) lies in the fact that *Leptospira* lipopolysaccharide activate THP-1 via a Toll-Like Receptor(TLR)2-dependent mechanism, while RAW 264.7 are activated by a TLR2- and TLR4-dependent mechanism. In addition, the anti-inflammatory cytokine interleukin (IL)-10 is rapidly and massively overexpressed in resistant animal models as mice, while it was lately regulated in susceptible model. Suppressor of Cytokine Signalling (SOCS) constitutes a family of 8 proteins identified as inhibitors of numerous cytokine regulation pathways by a negative feedback control, and their involvement, especially SOCS1 and SOCS3, was shown in the regulation of immune and inflammatory processes during infectious diseases. Knowing that proteins of the SOCS family are regulated by IL-10 and by the activation of Toll-like receptors (TLR) pathway, pathophysiology of leptospirosis may be correlated with differential regulation of cytokines in response to IL-10 and SOCS proteins. **Materials and Methods:** Using *in vitro* immortalized cell line models, we evaluated the expression profiles of the cytokines tumor necrosis factor (TNF-)α, IL-1β, IL-6, IL-10, and SOCS1 and SOCS3 proteins produced by RAW 264.7 murine macrophages as compared to THP-1 human monocytes PMA-activated in response to virulent leptospires *L. interrogans* serovar Icterohaemorrhagiae strain Verdun. For this purpose, Reverse Transcription-quantitative PCR techniques were used. **Results:** Our results show a differential regulation of cytokines in time depending on the human or mouse cell line. Early overexpression of the anti-inflammatory mediators IL-10, SOCS1 and SOCS3 was observed in murine macrophages in response to virulent leptospires. This contrasts with their later regulation in the human cells found along parallel massive overexpression of pro-inflammatory cytokines TNF-α and IL-6. **Conclusions:** These results also suggest a regulatory role of SOCS1 and SOCS3 proteins in the early phase of the inflammatory response, probably related to the signalling pathway of TLRs. A deeper understanding of the immunopathogenesis of leptospirosis in humans is highlighted here.

Corresponding author's: Mariko MATSUI mmatsui@pasteur.nc

Differentiation of severe leptospirosis from critical phase dengue infections

Narmada Fernando[1], Roshan Niloofa[1], Lilani Karunanayake[2], H Janaka De Silva[3], Senaka Rajapakse[4], Sunil Premawansa[5], Shiroma Handunnetti[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka

[2]Department of Microbiology, Medical Research Institute, Sri Lanka

[3]Faculty of Medicine, University of Kelaniya, Sri Lanka

[4]Tropical Medicine Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka

[5]Faculty of Science, University of Colombo, Sri Lanka

Leptospirosis is one of the commonest zoonotic infections in the world, and has a wide geographic distribution worldwide. Leptospirosis and dengue contribute to a significant proportion of hospitalized cases of febrile illnesses in endemic areas. Differentiation of the severe form of leptospirosis (SL) from the critical illness of dengue (DC) is important as both forms are fatal and also as their management is different. Leptospirosis cases are misdiagnosed as dengue in endemic regions, especially during outbreaks of dengue and are thus not appropriately treated. As clinical features do not reliably differentiate the two disease conditions, the objective of this study was to identify oxidative stress biomarkers in SL and to evaluate the potentiality of these biomarkers in differentiating SL from DC. Microscopic agglutination test based laboratory confirmed and clinically characterized patients (SL: n=60) and age and gender matched, dengue non-structural Protein 1 (NS1) positive dengue patients during critical phase (DC: n=30) were recruited for this study. Serum protein carbonyl (PC) levels were measured using a commercially available protein carbonyl content assay kit according to the manufacturer's guidelines and lipid peroxide (LP) level was measured in FOX 2 method. SL patients had significantly high PC levels (20.32 ± 10.6 µm/ mg protein) and LP levels (16.56 ± 23.3 µM) compared to than those of DC patients (5.8 ± 2.75 µm/ mg protein and 0.91 ± 2.27 µM respectively) ($p < 0.005$). ROC curves of PC levels and LP levels between SL & DC with high area under the curves (AUC) (93 % and 76 % respectively) demonstrated that both serum PC and LP levels can be used as oxidative biomarkers of SL over DC ($p < 0.001$). Using the cutoff value of 6.94 µm/ mg protein in serum PC and 0.21 µM in serum LP, a test had 97 %, 60 % sensitivity and 81 %, 86% specificity in differentiating severe leptospirosis patients from dengue patients in critical phase ($p<0.001$). Concluding, both serum PC and LP levels may be suitable and reliable biomarkers for differentiation of severe form of leptospirosis from critical phase dengue infections.

This work is financially supported by National Research Council (NRC-12-077)

Corresponding Author's name : Narmada Fernando

Email address : narfernando@gmail.com

Leptospiral genomics: how does it relate to taxonomy, pathogenesis and immunity?

Ben Adler
Department of Microbiology, Monash University, Australia.

The availability of leptospiral genome sequences has allowed comparative genomics analyses of pathogenic, saprophytic and intermediate species. These studies have identified a set of core leptospiral genes and suggested that intermediate species are more closely related to pathogenic species, but retain the enhanced metabolic capacity of saprophytic species. However, sequence analyses of multiple isolates suggests that the concept of a strict species may be flawed and instead suggest a continuum within the three complexes, pathogens, intermediates and saprophytes.

Genes of unknown function are over-represented in the pathogen-specific gene subsets, suggesting pathogenic mechanisms in leptospirosis are unique to *Leptospira*. Likewise, whole genome transcriptomics studies have found that genes upregulated under simulated *in vivo* conditions generally have no defined function. The advent of methods for constructing defined transposon mutants in pathogenic *Leptospira* has allowed for the first time an investigation of specific virulence factors involved in acute disease. These studies have now identified several essential virulence factors, including LPS, motility, catalase, heme oxygenase, the stress proteins ClpB and HtpG, the uveitis-associated protein LruA, and the OmpA-like protein Loa22. Also of interest has been the finding that many previously predicted virulence genes are not essential for the ability of *Leptospira* to cause disease, consistent with the notion of a degree of functional redundancy for virulence-associated genes.

At least 25 leptospiral proteins, in recombinant form, have been shown to adhere to a range of mammalian cellular components. In many cases, the same protein was found to bind to multiple host components. However, unequivocal genetic evidence for a role in virulence is lacking for almost all of these putative adhesins. A high throughput screen of transposon mutants has allowed the identification of virulence genes of unknown function and has also identified for the first time genes required for renal colonization of the carrier host. Many leptospiral proteins in recombinant form have been assessed for their ability to elicit protective immunity, with limited success to date.

Combined *in vivo* and *in vitro* analysis of endothelial cell activation in severe leptospirosis shows pathogen and immune mediated endothelial cell activation and association with disease severity

Marco Goeijenbier[1], Mohammed H. Gasem[2], Joost C.M. Meijers[3,4], Rudy A. Hartskeerl[5], Ahmed Ahmed[5], Marga G.A. Goris[5] ,Simone S. Schuller[6], Jarlath Nally[7], Byron E.E. Martina[1,8], Albert D.M.E. Osterhaus[1,8] ,Eric C.M. van Gorp[1] ,Jiri F.P. Wagenaar[1,5,9]

1.= Department of Viroscience, Erasmus MC, Rotterdam, CE 50 3015, The Netherlands, 2= Dr. Kariadi Hospital, Department of Internal Medicine Faculty of Medicine, Diponegoro University, Semarang, Indonesia., 3.= Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands,4.= Department of Plasma Proteins, Sanquin Research, Amsterdam, the Netherlands,5. = Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam, the Netherlands,6.= Vetsuisse Faculty, University of Bern, Bern, Switzerland,7.= Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, Ioaw, United States of America,8= ARTEMIS One Health Research Institute, Utrecht, The Netherlands,9.= Department of internal medicine, Erasmus MC, Rotterdam, CE 50 3015, The Netherlands

Background: Leptospirosis is an infectious disease of global importance. Patient presentations can vary from mild non-specific symptoms up to a very severe disease with multi organ failure and widespread haemorrhage. Previous studies concluded that one of the most accurate prognostic factors of mortality in leptospirosis is the occurrence of bleeding. Therefore, we investigated endothelial cell activation in relation to bleeding manifestations in severe leptospirosis patients using an *in vivo, in vitro, in vivo* study design.

Methods: A retrospective cohort study was conducted on cases of severe leptospirosis included in the Dr. Kariadi hospital in Indonesia. Blood was taken on day of admission and during follow up. Firstly endothelial cell activation was evaluated by sE-selectin and Von Willebrand factor levels. Secondly we made use of an *in vitro* endothelial cell model assessing endothelial cell activation during infection with life, virulent *Leptospira interrogans* serovar Copenhageni. Results from the *in vitro* work lead to the hypothesis of immune activation as a contributing factor to endothelial cell dysfunction. This was tested *in vivo* by the levels of T cell associated activation and cell damage markers: soluble IL2 receptor (sIL-2r) and the soluble Fas ligand (sFasL).

Results: The sE-selectin and vWF levels were strongly elevated on admission compared to the controls. In a subgroup analysis between patients that survived and patients that did not, sE-selectin (203 ng/ml vs. 157 ng/ml) was significantly higher. Further analysis *in vitro* showed increased endothelial cell VWF after infection with *L.interrogans*. E-selectin and ICAM-1 did not seem to increase during the first 48 hours after infection *in vitro* when compared to infection with non-virulent *Leptospira* control. Analysis of the Infection kinetics revealed the presence of intracellular leptospires with a strong suggestion of replication. In *vivo* analysis showed increased levels of sFasL and sIL-2r both strongly associated with mortality. Furthermore sIL-2r levels were increased in patients that developed bleeding and significantly correlated to length of hospital admission.

Conclusion: Markers of endothelial cell activation and immune related cell death are highly elevated in patients suffering from severe leptospirosis and endothelial dysfunction could very well be the result of both pathogen and immune related causes.

Human Leptospirosis in Sardinia from 2005 to 2014

[1]Piredda I., [1]Palmas B., [1]Noworol M., [1]Falchi A., [2]Campus M., [1]Ponti M.N.

[1]Istituto Zooprofilattico Sperimentale della Sardegna ~ Sassari ~ Italy

[2]Ospedale SS. Trinità ~ Divisione Malattie Infettive ~ Cagliari ~ Italy

Keywords: Leptospirosis, pathogenic, Human

INTRODUCTION: Leptospirosis is a zoonotic disease caused by spirochetes belonging to different pathogenic species of the genus *Leptaspira*. It is a contagious disease, transmitted through water or soil contaminated with urine of animal hosts containing many pathogenic spirochet[es]. The clinical spectrum can range from an asymptomatic, subclinical infection to a fatal hepatorenal syndrome. The aim of this report is to review the epidemiological status of human leptospirosis in Sardinia in the last ten years.

MATERIALS AND METHODS: From January 2005 to April 2014 the following were tested: 395 samples of human sera with microscopic agglutination test (MAT), 27 urine specimens with bacteriological culture, and 46 whole blood and urine samples with molecular analysis. The assigned serum reactivity for MAT titer was ≥1:20 and the titer ≥1:160 was defined positive in accordance with parameters set forth by KIT in Amsterdam. Whole blood and urine samples were tested by using a serial dilution technique in liquid EMJH medium. Blood and urine samples were examined for the presence of *Leptospira* DNA with a polymerase chain reaction (PCR) using a pair of primers LigA and LigB to amplify 16S rRNA gene.

RESULTS: 34 (8,6%) of 395 examined samples by MAT showed a reactive titer for *Leptospira* antibodies (Ab). The presence of Ab against L. icterohaemorrhagiae was confirmed in 25 samples, Ab against serovar copenhageni in 12 samples, and Ab against serovar bratislava in 7 samples. Seven cases of leptospirosis were diagnosed over the last three years. The titers increased rapidly from 1:320 to 1:2560. 8 cases out of 395 (2%) were hospitalized with a diagnosis of leptospirosis. Their major clinical manifestations at admission included fever, chills, headache, and myalgia. Jaundice with acute renal failure and thrombocytopenia was only present in two cases diagnosed as Weil's disease. 8,7% of 46 whole blood and urine samples tested positive in the molecular approach. No isolation of *Leptospira* was obtained in 27 samples examined with bacteriological culture. The data obtained in this research study illustrate the spread of serovar Bratislava in humans according to the official data.

DISCUSSION AND CONCLUSIONS: The MAT and PCR are the two techniques used to confirm the diagnosis of leptospirosis. The culture requires three months of incubation and does not allow for quick diagnosis. The MAT detects late anti-leptospires antibodies and a second blood sampling is necessary to confirm leptospirosis. Clinical and epidemiological observation along with PCR are essential for early diagnosis. Suitable preventive measures ought to be adopted in order to control the disease.

Pulmonary involvement during leptospirosis

Edmond Puca[1], Arben Pilaca[1], Gentian Stroni[1], Elda Qyra[1], Silvia Bino[2], Erjona Abazaj[2], Eugena Tomini[2], Luljeta Alla[2], Entela Puca[3], Pellumb Pipero[1], Dhimiter Kraja[1].

1. Service of Infection Diseases, University Hospital Center, Tirana, Albania
2. Institute of Public Health, Tirana, Albania
3. Amerikan Hospital, Tirana, Albania

Leptospirosis is a zoonosis, caused by *Leptospira* species. The spectrum of human disease is extremely wide, but the involvement of lunch has a bed prognosis.

Purpose: To asses the pulmonary manifestations during leptospirosis.

Material and Methods: From 107 cases serologically confirmed with positive IgM ELISA for leptospirosis during January 2009- December 2014 in our hospital, we included 42.99 (46 patients) presented with respiratory symptoms.

Results: All patients were males. Mean age at the time of diagnoses were 43.8 years old with 14.8±DS. The time from the first symptoms until they presented to hospital was 6.3 days. Signs and symptoms were: jaundice in 93.47%, conjunctival suffusion in 86.9%, myalgia in 82.6%, oliguria in 71.7%, temperature with average 38.7°C in 67.3% of cases, breathlessness in 54.3%, cough in 45.6%, haematuria in 41.3%, headache in 30.4%, abdominal pain in 32.6%, chills in 28.2%, chest pain in 20.6%, vomiting in 21.7%, cough with hemoptosis in 21.7%, and diarrhea in 10.6% of cases. Acute respiratory distress syndrome was presented in 19.5% of cases. In 71.7% were confirmed pathological manifestation by chest X-ray including: bilateral bronchoalveolar infiltrations in 52.1% and infiltrative massive pneumonia in 19.5%. Mortality in patients with pulmonary involvement was 18.2%.

Conclusion: Pulmonary involvement in leptospirosis was present in 42.99% of patients, so leptospirosis should be considered in the differential diagnosis of patients with febrile illnesses associated with pneumonitis and respiratory failure, especially in the presence of hemoptysis or other signs of pulmonary hemorrhage. We can't explain why all patients with pathologic manifestation were males. Envolvement of lunch in leptospirosis is a prognostic factor for case fatality.

Keywords: leptospirosis, pulmonary, chest X-ray.

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Leptospirosis: commonly forgotten in Africa

Georgies F. Mgode
Pest Management Centre, Sokoine University of Agriculture, P.O. Box 3110, Morogoro, Tanzania

ABSTRACT
Africa has the highest mean annual incidence of leptospirosis in the world (95.5 people per 100,000 individuals). The incidence in Western Pacific is 66.4, Americas (12.5), South East Asia (4.8) and Europe (0.5). Awareness of leptospirosis disease among medical personnel, animal health personnel and the general public is largely lacking in Africa despite this higher incidence. It is neither routinely diagnosed in Africa nor considered as a causal of fevers/illness in humans. Some of major factors limiting awareness and consequently hindering the diagnosis of leptospirosis in Africa include lack or unavailability of leptospiral specimens for teaching in medical and veterinary schools, little access to updated *Leptospira* and leptospirosis information and unavailability of live local *Leptospira* strains for successful diagnosis using the gold standard microscopic agglutination test (MAT). This paper reports on the burden of leptospirosis in humans and diverse animal species in Tanzania; the common *Leptospira* strains from diverse reservoir hosts for potential scaling-up diagnosis of this disease in Tanzania and across African where this disease is totally forgotten. Also outlines potential malpractices that likely obscure the effects of leptospirosis in Africa.

* Correspondence: gmgode@suanet.ac.tz; gfmgode@hotmail.com

Leptospirosis outbreak in strawberry harvesters in Germany

Anne Mayer-Scholl[1], Martin Runge[2], Leonhard Hamschmidt[3], Berthold Lüsse[3], Jona Freise[2], Joachim Ehlers[2], Karsten Nöckler[1], Carolin Knorr[2], Barbara Keller[2], Salla Toikkanen[4,5], Johannes Dreesman[4]

[1] Federal Institute for Risk Assessment, Berlin, Germany

[2]Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg/Hannover, Germany

[3]Landkreis Oldenburg Public Health Department, Wildeshausen, Germany

[4]Governmental Institute of Public Health of Lower Saxony, Hannover, Germany

[5]European Programme for Intervention Epidemiology Training, European Centre for Disease Prevention and Control, Stockholm, Sweden

In industrialized countries such as Germany, travelling abroad, recreational activities linked to freshwater exposures and residential exposures such as gardening and owning pets are major risk factors for human leptospirosis. Due to the modernisation of farming practices outbreaks associated with agricultural exposure risks seemed to have minor importance. In 2007, the first major leptospirosis outbreak linked to field work was reported in Germany since the 1960s. Here we report a further outbreak among predominantly Polish strawberry pickers in Germany in 2014.
Between June and August 2014, 45 leptospirosis cases among strawberry pickers working on two different farms were officially registered in the German federal state Lower Saxony. Leading symptoms were sudden onset of high fever (>38.5°C), generalized body/muscle pain and an increase of renal or liver enzymes. 47% of the patients were hospitalised. For eight cases laboratory evidence for leptospirosis was reported, all other patients had similar working exposure and characteristic symptoms, which was regarded as sufficient evidence for notification as leptospirosis. Sera from a subset of patients were tested by microscopic agglutination test (MAT) and IgM and IgG ELISA. Two patients had a positive IgM ELISA result, and in one patient both the IgM and IgG ELISA were positive. In all three cases the MAT was negative. Further, the patient which had displayed the earliest onset of symptoms was tested positive in both IgM and IgG and had an antibody titer of 1:200 against *L. kirschneri* serovar Grippotyphosa in the MAT. Two patient's urine or EDTA blood could be analyzed by *lipl32* PCR and *secY* sequencing. In both patients *L. kirschneri* was identified by *secY* sequencing.
To identify the potential outbreak source 64 mice, voles and shrews were trapped on one of the strawberry fields and tested by real time PCR, *secY* sequencing and multi locus sequence typing (MLST). In 67% of the rodent kidneys *Leptospira*-specific DNA was detected by *lipl32* PCR. The infecting species was identified as *L. kirschneri* by *secY* sequencing and as ST 110 by MLST.
Further outbreak investigations included an analysis of the climatic conditions. As at the time of the outbreak the strawberry season was drawing to a close and the seasonal workers returned to their home country, further potential leptospirosis cases were traced from this outbreak by the Polish National Institute of Public Health and a case control study was started to identify risk factors.
Similar to the outbreak in 2007, the winter preceding the outbreak in 2014 was unusually mild and the summer was warm with heavy rain events. Due to the ongoing climate change, with mild winters and wet summers, more agriculturally associated outbreaks can be expected in future. Therefore, a set of information measures was agreed upon in order to raise the awareness of farmers, workers and general practitioners about the disease. As further prevention measures the appropriate personal protective equipment and rodent control are subjects under discussion.

Livestock and Human Leptospirosis Occurrence and Control in New Zealand

Peter R Wilson, Cord Heuer, Jackie Benschop, Julie M Collins-Emerson
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, PB 11222, Palmerston North New Zealand 4442.

Notified human leptospirosis cases/year for the past 20 years in New Zealand ranged from 60–100 with a current rate of 2.5/100,000 population. Occurrence is highest in farmers (~55% of cases) and meat workers (~20% of cases) A recent study showed the incidence rate of exposure in sheep abattoir workers to be 11.1% p.a.). Notification data over the past 12 years show a reduction in the proportion of cases attributable to Hardjo, a small increase attributable to Ballum, and a steady proportion attributable to Pomona, Tarassovi and Copenhageni. Seroprevalence studies (any serovar) showed 12% (n=582) in meat workers, 4.7% (n=277) in veterinarians, 4.1% (n=173) in farmers and 0% (n=302) in veterinary students. Sero-surveys showed 97% of sheep flocks (n=161) and beef herds (n=118) and 76% (n=98) of farmed deer herds, and 58% (n=2308) of beef cattle, 51% (n=3,361) of sheep and 34% (n=1,992) of deer were positive (MAT titre ≥48) to Hardjo and/or Pomona. Within herd/flock seroprevalence ranged up to 100%. Survey data suggest annual incidence of veterinary-confirmed/suspected clinical leptospirosis in pastoral livestock is 2.6% (n=118) in beef cattle, 1% in deer (n=98) and 0.6% (n=161) sheep herds/flocks suggesting clinical incidence is under-estimated. Vaccination is practised by an estimated 95% of dairy farmers, and reported 18% (n=118), 5% (n=98), and 0.6% (n=161) of beef, deer and sheep farmers, respectively. Vaccination studies in already naturally infected deer and beef herds showed a reduction in urine shedding rate of 44% and 38%, respectively, after approximately nine months. Zero shedding was observed in individual sheep and deer after a similar period when vaccinated at a young age, prior to natural exposure. When data for sheep flocks exposed before and after vaccination was combined, overall vaccine efficacy against shedding was 86.3% [CI: 63.6%-94.8%]. In dairy cattle, urine shedding (using PCR) was found in 30% of herds (n=44) and 13% of cows in positive herds, with the risk of shedding in adult cows being six times higher when vaccination started at six months of age compared with three months or less ($p = 0.04$). The number of human notified cases dropped from ~800 to 200 p.a. in the early 1980s, concurrent with widespread adoption of vaccination in dairy and pig herds and a concerted awareness campaign targeting at-risk groups about the disease and its preventive measures. Other indirect evidence supports that this reduction was due to vaccination. Data suggest that improved vaccination programmes in dairy and vaccination of sheep, cattle and deer would likely be an effective means for further reducing livestock-associated human cases in New Zealand.

Corresponding author: Professor Peter Wilson. Email: P.R.Wilson@massey.ac.nz

Transmission and mixed infections in a diverse reservoir community: *Leptospira* in Madagascar

Mark Moseley | PhD researcher
University of Aberdeen | r01mhm13@abdn.ac.uk

Introduction

Madagascar is one the world's biodiversity hotspots, with numerous endemic and introduced small mammal host species that may act as reservoir hosts for *Leptospira*. Epidemiological and ecological data for leptospirosis in Madagascar are scarce. Four pathogenic *Leptospira* spp. (*L. interrogans, L. kirschneri, L. bargpetersenii* and *L. borgpetersenii* group B) have been identified circulating in small mammals. Distinct associations between host genera and *Leptospira* spp. have been noted, with no evidence of transmission between endemic and introduced small mammals. Using molecular typing techniques we aimed to test whether host specificity and lack of transmission are supported in areas where endemic and introduced small mammals share the same habitats.

Materials and Methods

Small mammals were captured at 20 rural and 5 forest sites within a landscape scale study area. *Leptospira* detection was undertaken on kidney extracts using a *16s* TaqMan qPCR. Genotyping was performed by sequencing of 300nt *lfb1* and 500nt *secY* gene fragments. Species specific primers for resolution of mixed infections were designed based on the *lfb1* sequences. Alignment, evolutionary model testing and phylogenetic tree construction was performed using MEGA 6.0.

Results

The prevalence of renal carriage ranged between 12% and 55% depending on host species, and mixed infections were detected in at least 25% of positive samples. Contrary to previous studies, *L. interrogans* was detected in three subfamilies of endemic small mammals. Moreover, *L. borgpetersenii* was detected only in introduced small mammals. *L. kirschneri* and *L. borgpetersenii* group B was predominantly detected in endemic small mammals although *L. borgpetersenii* group B was also detected in a black rat (*Rattus rattus*).

Conclusions

We provide evidence for the transmission of *Leptospira* between reservoir host species and a high prevalence of mixed infections. In Madagascar, black rats are the most abundant and widespread small mammal species. Currently, it is assumed that black rats only transmit *L. interrogans*. However, we found that black rats may be important reservoir hosts for a variety of *Leptospira* spp., including species previously thought to be restricted to endemic small mammals. Moreover, the high prevalence of mixed infections suggests that the prevalence of some types of *Leptospira* may have been dramatically underestimated. Mixed infections provide an environment for recombination and the emergence of novel genotypes which has important implications for the evolution and epidemiology of *Leptospira*.

Leptospirosis in New Zealand

A. DREYFUS[1&2*] and M. PITTAVINO[3], C. HEUER[4], J. BENSCHOP[4], P. TORGERSON[1], R. FURRER[3]

(1) Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

(2) EpiCentre, Institute of Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

(3) Institute of Mathematics, University of Zurich, Zurich, Switzerland

(4) Institute of Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

Introduction: Leptospirosis burdens New Zealand's (NZ) rural communities with most cases occurring in farmers and meat workers. In a recent cohort study in four sheep slaughtering abattoirs in NZ, sera were collected bi-annually from 384 meat workers and tested by the Microscopic Agglutination Test for *Leptospira interrogans* sv Pomona and/or *L. borgpetersenii* sv Hardjobovis. The annual infection risk (incidence) was 12%. Significant risk factors for new infection in the generalized linear model (GLM) were worker position, abattoir and time worked in the meat industry. The objective was to compare GLM with Additive Bayesian Network (ABN) results, analysing risk factors for Pomona infection in meat workers.

Methods: In a cohort study in four sheep slaughtering abattoirs in New Zealand, sera were collected bi-annually from 384 meat workers and tested by Microscopic Agglutination for *Leptospira interrogans* sv Pomona. ABNs are a form of graphical modeling which generalize the usual GLM to multiple dependent variables. In a first step we identified the best fitting model using the marginal likelihood metric and in a second step we adjusted this model for any over-fitting applying a parametric bootstrapping approach. The marginal posterior 95% confidence intervals of the log odds ratio for each parameter were estimated and compared with the GLM results.

Results: Significantly associated with Pomona were "Worker position" and "Abattoir" (GLM) and "Worker position" (ABN). The odds of Pomona infection (OR, [95% CI]) was highest at stunning and hide removal (ABN 41.0, [6.9 - 1044.2]; GLM 56.9, [6.5-496.6]), followed by removal of intestines, bladder, and kidneys (ABN 30.7, [4.9 - 788.4]; GLM 28.8, [3.3-252.4]). Facemasks/glasses did not have a protective effect.

Conclusions: ABN confirmed the main outcome from GLM. The advantage of ABN is the modelling of relationships between all variables, revealing more about key features of complex disease systems.

**Author to whom correspondence should be addressed; Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 270, 8057 Zurich, Switzerland, Office +41 44 635 90 51, Mobile +41 78 895 46 90, E-Mail: adreyfus@vetclinics.uzh.ch*

Strengthening Local and Regional Health Systems for Improved Leptospirosis Surveillance: Lessons from an epidemiological study of three Regencies in East Java, Indonesia

Bianca van Bavel[a], Fiona Larkan[a], Jarlath E. Nally[b] & Armand Purwati[c]

[a] Centre for Global Health, Trinity College, Dublin, Ireland, *vanbaveb@tcd.ie*

[b] School of Veterinary Medicine, University College Dublin, Ireland

(Agricultural Research Services, United States Department of Agriculture)

[c] Dr. Soetomo Teaching Hospital, Institute of Tropical Diseases, Airlangga University, Surabaya, Indonesia

Introduction: Despite being one of the most widely distributed zoonotic infections worldwide, leptospirosis has a disproportionally concentrated burden of infection in tropical climates and low- and middle-income settings. Inaccurate reporting of the current incidence of leptospirosis in Indonesia poses an issue to current public health and disease surveillance systems. The application of a One Health approach, integrating diverse forms of expertise and knowledge, is an important step towards improving future leptospirosis identification and awareness within these vulnerable populations.

Methods: The study applied a cross-sectional survey to explore key environmental and social exposures associated with leptospirosis across nine targeted sample sites in the Regencies of Surabaya, Sampang and Gresik East Java, Indonesia. A total of 275 household questionnaires were collected and used for analysis. Odds ratios and binomial logistic regression models were used to explore specific associations and variations at the household level. Given the stratified sampling applied, a generalised linear mixed model analysis was performed using both fixed and random effects to adjust for any variation at the level of location. Both Irish and Indonesian ethical committee approval was obtained.

Results: Results revealed a history of leptospirosis in 30 respondents, with reported prevalence ranging from 0-36.8% across the nine sample sites. Potential sources of exposure that demonstrated significant associations ($p<0.05$) with the reporting of leptospirosis were: living in flood prone areas (OR: 4.06; 95% CI: 1.50—10.98), recent history of in-house flooding(OR: 4.43; 95% CI: 1.90—10.33), living in close proximity (<20m) to refuse deposits (OR: 2.57; 95% CI: 1.15—5.74), having limited access to piped or canalized running water (OR: 2.28; 95%CI: 1.05—4.96), occupational farming (OR: 2.76; 95% CI: 1.23—6.18), and using alternative sources of water for domestic use (artesian wells, rivers and collected rain water) (OR: 3.33; 95%CI: 1.49—7.45). Presence of household animals, gender, type of residence, and reported flood contact, did not demonstrate significant associations with case history ($p > 0.05$). The majority of all respondents reported some element of daily exposure, be it direct or indirect, to known leptospirosis reservoirs; 240 reported living less than 20 metres from an open sewage system and 242 reported seeing rodents daily both inside and around the home.

Discussion & Conclusion: This study found that the opportunity of household exposure is created through layers of overlapping environmental and social determinants. In both rural and urban community settings, these findings support the need to address certain environmental and social determinants prevalent in these targeted populations; such as improving access to safe water, adequate sanitation and waste management. Furthermore, the results of this study highlight local resource limitations and infrastructural deficiencies, suggesting the need to strengthen Eastern Javanese regional health systems through more centralised reporting and improved surveillance. Using an inclusive and holistic approach to health across species, disciplines, nations and contexts, is recommended for the effective detection and future prevention of leptospirosis burden within these communities.

ECOLOGY OF *LEPTOSPIRES*: PREGENOMIC AND POSTGENOMIC ERA

Ananyina Yu.
Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia

Leptospirosis is known as a water-borne zoonosis, its agents playing an important role in human and animal infectious pathology worldwide. Over many decades of the pregenomic era a large body of evidence was obtained in research implemented in natural and made-man foci of leptospirosis in various geographical areas. During this period the basic natural cycles of leptospires were clarified. The major and additional reservoir host species for most of them were established. In field observations and experimental studies leptospires of different species and subspecies taxons (serovars) were shown to display not only substantial diversity in pathogenic potential, but also ecology. They appeared to be highly variable in terms of host specificity (specific mammalian host range), selective tropism to renal and nervous tissues as well as ability to compete and persist in vivo as well as under environmental conditions. These findings provided the background for developing surveillance and control leptospirosis stategies, both national and international being used so far.

Before the development of molecular techniques, *leptospira* research was largely reliant on the methods of identification and isolation. This traditional approach indeed should still be used. Meanwhile, the benefits of ever-improving molecular technologies, including whole-genome sequencing and metagenomics, are apparent.

Leptospires are known to colonize diverse natural habitats: from water, marine, and soil environments (*Leptospiro* biflexa sensu lato) to a wide range of animal reservoir hosts (*Leptospira* interrorgans sensu lato). The ecological features and in some cases even the origin of the *leptospira* strains belonging to the so-caled "intermediate" species are still to be elucidated. The unique ecological diversity of leptospires gives grounds to consider them a perfect model for research in the field of comparative microbial ecology, phylogeny, probing the structures of environmental microbial communities as well as revealing fine mechanisms of pathogenicity.

Future studies targeted on comparative genomics of leptospires differing in their mode of existence as well as revealing epidemiological and clinical implications of their biodiversity are mostly needed.

Corresponding author: Yulia.V.Ananyina, Gamaleya Research Institute for Epidemiology and Microbiology, Gamaleya str.18, 123098, Moscow, Russia

E-mail: ananyina@gamaleya.org

Estimating the economic impact of leptospirosis on public health and livestock farming in New Zealand

C Heuer, J Sanhueza, J Collins-Emerson, J Benschop, E Vallee, PR Wilson
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Priv Bag 11222, Palmerston North, New Zealand

In New Zealand, human leptospirosis ranks highest among notified human diseases transmitted from animals (annually 2.5 per 100,000), despite being at least 10 to 15-fold under-reported. *Leptospira* spp. are endemic in NZ, highly prevalent (>50% seropositive) in domestic sheep, deer, beef, dairy cattle and working dogs, prevalent in wildlife, causing production loss and disease in animals. About 25-30% of adult sheep, beef cattle and deer in New Zealand are known to shed *Leptospira* in urine at any point in time and thus exposing humans to infection. The six most common serovars found in notified human cases (*L. borgpetersenii* sv. Hardjobovis, Ballum, Tarassovi, and *L. interrogans* sv. Pomona, Canicula, Copenhageni) also infect a large number of animal hosts including domestic livestock and wildlife species. The disease predominantly affects the rural workforce (farmers, abattoir workers, sheep shearers, veterinarians etc.). Annual infection rates based on seroconversion of workers in 8 abattoirs were 9% (range 5-19%). They were higher when processing sheep (12%) than cattle (4%). One fifth (20%) of these infections was associated with moderate signs of illness, and 3% workers reported severe clinical flu-like disease. Every year, 2% sheep abattoir workers were 4.4 days (standard deviation 8.0 days) absent from work due to mild-moderate clinical signs and 0.6% suffered serious clinical leptospirosis lasting several weeks. Computer simulation suggested that outbreaks following a flood may impact on the human disease burden for about 10 years after the outbreak. Despite most dairy farmers vaccinating their herds for preventing human exposure since the 1990s, a recent pilot study suggested that 30% herds, and within them 13% dairy cows, continue to shed *leptospira* in urine, possibly due to an ineffective use of vaccines. Surveillance data from 2008-13 appear to confirm the transmission from dairy cows to humans in that 68% of farmers notified with leptospirosis were involved in dairy farming.

Prevalence, incidence and illness estimates and demographic data were aggregated to approximate the economic impacts of leptospirosis on the public health of human populations and on the productivity of livestock farming. To the author's knowledge, this is the first time that the full population impact of a zoonosis has been evaluated in a comprehensive approach covering both public health and livestock productivity with detailed differentiation of four production systems (sheep, beef, dairy, deer). While input parameters were based on findings from population based studies, informed assumptions were made about the exposure of not previously investigated rural and urban population strata. Analyses are currently undertaken and results will be available prior to the ELS meeting in April 2015.

Swedish city rats – a potential health threat?

Tanja Strand[1,2], Karin Sundström[1,2], Jenny Verner-Carlsson[1,2], Mare Löhmus Sundström[3], Annette Backhans[4], Thomas Persson-Vinnersten[5], Katarina Wallmenius[6], Jay Lin[7], Olga Katargina[1,2], Olivia Borg[2], and Åke Lundkvist[1,2]

[1]Zoonosis Science Center, Dept. of Medical Biochemistry and Microbiology, Uppsala University, Sweden

[2]Public Health Agency of Sweden

[3] Institute for Environmental Medicine, Karolinska Institute, Sweden

[4]Dept. of Clinical Sciences, Swedish University of Agricultural Sciences

[5] Anticimex, Sweden

[6]Division of Clinical Microbiology, Dept. of Medical Sciences, Uppsala University, Sweden

[7]Dept. of Virology, Immunobiology and Parasitology, National Veterinary Institute, Sweden

The number of rats rapidly increases in our cities and are well-known carriers of various microorganisms pathogenic to humans like plague (*Yersinia pestis*), *leptospira* and Seoul hantavirus (SEOV). SEOV causes hemorrhagic fever with renal syndrome (HFRS) expressed mainly by fever, fatigue and kidney problems. SEOV is found mainly in rats in China and Southeast Asia, but has recently also been found in European wild rats in France, Belgium, England and Holland. Very little is at present known about any potential pathogens carried by Swedish city rats. In this study we are investigating potentially dangerous microorganisms, like Seoul hantavirus, Hepatit E and *leptospira*, carried by wild brown rats (*Rattus norvegicus*) in Sweden. Together with actors in the pest control industry, and own trapping efforts, we have collected hundreds of wild-caught rats as well as droppings from rats, mainly from different Swedish cities. Rats have been dissected and organs such as spleen, kidneys and brain are being analysed. The methods for analyses of previously known microorganisms in the collected rat tissues are predominantly ELISAs, immunofluorescence assays (IFA), neutralization tests (NT), RT-PCR, Q-PCR, genome sequencing, and cell culture isolation. We are searching for various pathogenic and zoonotic viruses, bacteria and protozoa and we will present the first results of our analyses. The project will clarify whether wild rats in Sweden can be a threat to human health, regarding a selection of pathogens.

Corresponding author: Tanja Strand. Tanja.Strand@imbim.uu.se

Monitoring of rodents and the rodent-borne pathogens *Leptospira* and hantavirus in the Netherlands

Miriam Maas1, Ankje de Vries1, Katsuhisa Takumi1, Chantal Reusken2, Arno Swart1, Mark Engelsma3, Dick Bekker4, Rudi Hartskeerl5, Joke van der Giessen1,3

1 Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM)

2 Erasmus University Medical Center, formerly Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM)

3 Central Veterinary Institute, Wageningen University Research, Lelystad

4 Bureau van de Zoogdiervereniging, Nijmegen

5 National Reference Laboratory for Leptospirosis, KIT Biomedical Research, Amsterdam

Corresponding author: Joke van der Giessen

In a report on prioritized emerging zoonoses, published in 2010, rodents and rodent-borne pathogens were identified as a potential public health risk. One of the conclusions was that little was known about this risk and it was recommended that a surveillance system would be developed to study rodents and rodent-borne pathogens in the Netherlands. The National Institute for Public Health and the Environment was asked to develop and coordinate this surveillance system, to allow early detection of emerging zoonoses and to reveal trends in time, which may be used for early warning and risk mapping of relevant rodent-borne zoonoses. As a first step, rodents and rodent-borne pathogens were listed and prioritized for the Netherlands. Based on the resulting rodent host and pathogen combinations, recommendations were given for a surveillance system.

Four rodent host-pathogen combinations were identified as priority combinations for the Netherlands. These included: *Myodes glareolus* and Puumala-hantavirus; *Microtus arvalis* and Tula-hantavirus; Seoul virus and *Leptospira interrogans* in *Rattus norvegicus* and *R. rattus*. Separate surveillance systems were designed for the voles and rats, as these species have different natural habitats and human attitudes towards them differs (i.e. rats live close to humans and are considered pest animals). The surveillance systems assess both the rodent-borne pathogens and the rodent population dynamics of prioritized rodent host-pathogen combinations.

The surveillance system was initiated in 2014. Data on rodent populations is collected in various ways, ranging from notifications of municipalities (rats) to the development of a model to associate mast production of trees with rodent populations (bank voles). Pathogens of rats and voles are studied in specific (high-risk) areas. Though pathogen-detection has already given new insights on the circulation of pathogens in rodent-populations in the Netherlands, such as infection with leptospires in a high percentage of rats, the surveillance system will need to be in place for several years before data on trends in rodent populations can be combined with pathogen information to allow for risk mapping of zoonoses.

Eco-epidemiological research on rodent-borne pathogens in Finland

Heikki Henttonen
Natural Resources Institute Finland, Vantaa, Finland; Heikki.Henttonen@luke.fi

Hantaviral epidemiological patterns are related to the dynamics of reservoir rodent species. In temperate Europe masting is the important driver of hantaviral epidemics (mostly due to Puumala virus, PUUV) and it has been suggested that warm summers induce masting. In contrast, in boreal northern Europe, like Finland, specialist predation is thought be the main driver of bank vole (*Myodes glareolus*) fluctuations. Consequently, the underlying top-down or bottom-up causes of rodent fluctuations are different depending on the biome. In the large collaborative EU projects EDEN and EDENext our aim was to understand the human epidemiology of hantaviral disease between boreal and temperate Europe, in different climatic conditions. In Finland, in addition to short-term fluctuations (3-4 y) in rodent dynamics, there can be long-term trends, superimposed on the shorter "cycles", possibly due to the climatic changes, as exemplified e.g. by the results of the national long-term monitoring of vole fluctuations in Finland.

We documented clear seasonal differences in the transmission dynamics of PUUV between these two biomes, affecting also seasonality of human epidemics. Transmission of PUUV among bank voles in Finland is seasonal and highest seroprevalences are found in old overwintered voles in late spring – early summer, also in low-density years. Highest densities of positive voles are found in late autumn – early winter, with a 1-2 month time lag to the following peak in human epidemic. In wild voles, once infected, shedding lasts for life time, and these results differ clearly from lab experiments.

We have further documented the role of landscape patterns (homogenous taiga) in rodent/virus dispersal, and differences in the presence or absence of host threshold densities for the PUUV occurrence. In addition, temperature and moisture affect the virus survival outside the host, affecting indirect transmission geographically. Because there are no vectors in hanta transmission, the virus survives quite long in the environment. Long shedding and long survival are obvious adaptations to cyclic very low phases in host density. Geographic differences in the immunogenetics of reservoir rodents can affect their susceptibility.

These results are essential for human risk evaluation with regard to both long-term and seasonal occurrence of PUUV in the environment. In conclusion, it is important to realize that within the same host/virus system, *biome specific hanta epidemiologies* occur, which highlights the importance of geographically comparative studies on PUUV/NE in Europe, or in any host/pathogen system.

In addition to the research on our main object, PUUV and the bank vole, we have studied also hantas in insectivores, and other rodent-borne viruses, like cowpox, LCMV and Ljungan. Long-term monitoring data on human tularemia, vole cycles and climate suggest that peaks in human tularemia take place in summer a year after the vole peak (i.e. rodent decline year) but the weather of the epidemic summer determines the magnitude of human peak. Both warm/dry and rainy/cold summers reduce the number of human cases, probably because negative impacts on mosquito emergence.

ORAL PRESENTATIONS Friday 17 April
Diagnostics and molecular aspects

Diagnostic tests for human leptospirosis

Marga Goris
Coordinator Leptospirosis Reference Centre
Royal Tropical Institute, KIT Biomedical Research, Amsterdam, the Netherlands
WHO/FAO/OIE and National Collaborating Centre for Reference and Research on Leptospirosis

Leptospirosis a protean disease; there is a great variety of *Leptospira*, causing great variation in the clinical picture. This makes the diagnosis challenging and therefore a laboratory is essential to confirm the diagnosis. The conventional tests include culture, MAT and ELISA. These require skills and do form the basis to establish a laboratory case definition.

An increasing number of Rapid Diagnostic Tests (RDTs) are becoming available, based on serology. Though these can be used in most places, still confirmation by conventional tests is required. Moreover RDTs have shown variation in performance through time due to insufficient Quality Control and subjectivity in reading.

Serological tests are particular sensitive in late acute and convalescent phase of leptospirosis which is too late for effective antibiotic treatment. Novel molecular tests increasingly provide diagnostic results in an early acute phase since antigen is detected. This presentation focuses on conventional methods and RDTs and emphasizes the need for the formulation of a valid laboratory case definition for each geographical region.

Pathogenic *Leptospira* spp. causing aseptic meningitis in Sudanese children

Nada A. Abdelrahim,[1*] Imad M. Fadl-Elmula,[2] Ahmed Ahmed [3] and Rudy A. Hartskeerl [3]

1\ Department of Medical Microbiology, Sudan Medical and Scientific Research Institute, University of Medical Sciences and Technology, Khartoum, Sudan

2\ Department of Pathology, Program of Medical Laboratory Sciences, Nahda College, Khartoum, Sudan

3\ WHO/FAO/OIE, Leptospirosis Reference Centre, KIT Biomedical Research, Amsterdam, The Netherlands

Overview: Leptospirosis has been recognized as an important emerging global public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries. It is a major cause of human morbidity and mortality worldwide. Leptospirosis is a zoonotic disease that is severely overlooked and that has not or only scarcely been investigated in most African countries, including Sudan. The clinical presentations are diverse, ranging from undifferentiated fever to fulminant disease; one of these is leptospiral meningitis. This form of leptospirosis can easily be confused with other causes of aseptic meningitis. Among other techniques, PCR is known to be the most sensitive tool for diagnosing leptospirosis, especially during the early stages, where all other tests often fail.

Objectives: The aim of this study was to investigate the involvement of pathogenic *Leptospira* spp. in Sudanese children suspected of meningitis using clinical and molecular methods.

Methodology: This is a cross-sectional laboratory-based study. A total of 153 CSF samples were collected randomly from patients attending a reference paediatric hospital; *Omdurman Hospital*, during a ten months period. All patients were children less than 15 years, and were provisionally diagnosed as having meningitis on admission. Demographic and clinical data were retrospectively collected. Conventional laboratory investigations were performed for the estimation of CSF glucose, proteins and total white cell count as well as *in vitro* bacterial culture. DNA was extracted using QIAamp® DNA Mini Procedure and amplification was done by quantitative SYBR® Green I Real-Time PCR.

Results: Out of the 153 CSF specimens, 9 (6%) were positive for pathogenic *Leptospira* spp. with an average melting temperature (Tm) for all positive strains and duplicates of 80.5±0.8 °C (Skewness 0.86; Kurtosis 0.83), and median thermal cycle (Ct) of 37 with a range of 36.9 to 38.1. All patients were males less than 4 years old (7 infants and 2 toddlers). Typical conventional laboratory findings; i.e. CSF turbidity and pleocytosis, normal or reduced glucose levels and normal or elevated protein levels, were demonstrated in the CSF of 5 (55.6%) patients. All patients (100%) presented with febrile seizures, 55.6% had vomiting and stiff neck, and 25% had bulging fontanel. The majority (66.7%) presented in summer; this period extends from Mars to July. In addition to the leptospiral DNA, other pathogens were identified in the CSF of two patients; one is *Streptococcus pneumoniae* and the other is Human Herpes Virus type-6.

Conclusion: We demonstrated leptospiral DNA in 6% of children suspected of meningitis, suggesting *Leptospira* as the causative agent. From this, we deduce that leptospirosis might be common in Sudan. Further, we anticipate that a significant proportion of cases of non-viral hepatitis jaundice, non-malarial febrile illnesses, non-dengue and non-typhoid fevers are actually due to leptospirosis. In addition, several positive leptospiral PCRs were identified in CSF samples showing normal cellular count. Mixed microbial aetiologies were also observed. We recommend the inclusion of leptospirosis in the differential diagnosis of CNS infections and other febrile illnesses in Sudan. Molecular techniques can play a vital role in the rapid diagnosis of infectious diseases; these should be used routinely to support the clinical decision.

The Global Leptospirosis Environmental Action Network (GLEAN): mission and activities

Mathieu Picardeau
Institut Pasteur, France

The Global Leptospirosis Environmental Action Network (GLEAN) is a consortium devoted to the fight against leptospirosis. In collaboration with other international initiatives, GLEAN adopts a multi-disciplinary and multi-sectorial approach and builds upon the leptospirosis efforts that have already been undertaken.

The GLEAN mission is to reduce the impact that leptospirosis outbreaks have on communities throughout developing countries through providing cost-effective, implementable and sustainable solutions.

In striving to attain its mission, GLEAN pursues two main objectives:

(i) coordinate the acquisition and use of knowledge with respect to disease prediction, prevention, detection and intervention through four working groups Detect, Predict, Intervene and Prevent; and

(ii) translate relevant research findings into operational tools for affected communities and countries as well as for organizational actors.

The GLEAN initiative was developed in 2011 to provide direction and coordination to fill the many gaps in leptospirosis knowledge with the ultimate goal of translating the research findings into operational guidance for communities and countries affected by leptospirosis outbreaks.



Hantavirus diagnostics

Åke Lundkvist
Uppsala University, Sweden

Hantavirus diseases are zoonoses. The causative viruses, within the genus Hantavirus of the Bunyaviridae family, are harbored by small mammals as reservoir hosts and transmitted to humans mainly via virus-contaminated aerosolized excreta. In humans, they cause febrile diseases, named "Hemorrhagic Fever with Renal Syndrome" (HFRS) in Asia and Europe (case fatality <0.2-12%) and "Hantavirus Cardiopulmonary Syndrome" (HCPS) in the Americas with case fatality rates of up to 35–50%. The pathogenesis of hantavirus disease is characterized by changes in blood coagulation, vasodilatation and disturbances in the barrier function of the capillaries, resulting in extravasion of blood and inflammatory processes in the affected organs.

The diagnostics of hantavirus infections is usually based on serological methods, such as ELISA, immunofluorescence or immunoblot. In almost all cases, virus-specific IgM can be detected at the onset of clinical symptoms. The IgM titers disappear usually after a few months. The appearance of IgG is usually slightly later and might in rare cases be significantly delayed. A common diagnostic problem is the detection of non-specific IgM, leading to false conclusions. In general, however, the IgM ELISA is the best single method for diagnosis during the acute course of disease. In Asia, Europe, and North America, rapid immunochromatographic assays have been developed. However, due to significant serological cross-reactions between the various hantaviruses, these tests do not allow typing of a patient or rodent host serum. In addition, some commercially available tests suffer of low specificity and/or sensitivity. The focus-reduction neutralization test (FRNT) and its variants is the only serological test, which allow specific recognition of the infecting virus species. Neutralizing antibodies are directed against the viral envelope proteins and are less cross-reactive than the antibodies directed against the nucleocapsid protein. Unfortunately, FRNT is both time- and labor-consuming, and requires a BSL-3 safety laboratory. In addition, the FRNT is unable to identify new viruses, which are not part of the virus collection investigated. At least some of the disadvantages of the FRNT can be overcome by the usage of recombinant pseudotype viruses bearing hantavirus envelope glycoproteins.

The hantavirus genome can be rapidly detected by reverse transcription (RT)-PCR, in clinical and rodent samples such as blood, saliva or organ fragments. Based on a conserved region of the hantaviral L-segment, a genus-wide "Pan-Hanta-RT-PCR" has been developed which enables the detection of established as well as novel hantaviruses. The real-time RT-PCR is a rapid and sensitive test that allows the efficient diagnosis of hantavirus infections. However, PCR has an important limitation: the test is only positive if viremia is still present, meaning only in the very early phase of illness. Furthermore, some human infections are often so shortlived or lowly viremic (e.g. PUUV), that the virus often can be missed, even with the most sensitive PCRs. In summary, the combination of serologic tests, such as IgM/IgG ELISAs and FRNT, with RT PCRs is the desirable approach for the laboratory diagnosis of hantavirus infection. For virus typing and identification of new viruses, characterization of viral nucleotide sequences is the method of choice.

Diagnostic accuracy of a Recombinase Polymerase Amplification Assay for the Detection of Pathogenic *Leptospira*

Ahmed A Ahmed, Hans van der Linden and Rudy A. Hartskeerl
KIT Biomedical Research, WHO/FAO/OIE and National Leptospirosis Reference Centre, Amsterdam, The Netherlands

Leptospirosis is an infectious disease caused by pathogenic species of the genus *Leptospira*. Difficult to diagnose in the clinic and laboratory, leptospirosis is severely underdiagnosed and thus neglected. However, with recent increasing incidence rates worldwide, mainly due to climate change, flooding, natural disasters, population growth and urbanization, leptospirosis is recognized as one of the major re-emerging diseases threatening public and animal health.
Because its clinical manifestations mimic many diseases, high quality diagnostic tools are necessary to confirm clinically suspected cases of leptospirosis. Laboratory diagnosis of *acute* leptospirosis mainly relies on DNA-based techniques, such as real-time PCR and LAMP. Recently, a novel isothermal recombinase polymerase amplification assay (RPA) has been developed and its analytical and diagnostic sensitivity and specificity have been estimated. This study analysed 43 clinical samples (serum and ETDA blood) from Dutch patients suspected of leptospirosis. With culture result as reference standard, the diagnostic accuracy of our RPA assay was determined. We find a diagnostic sensitivity (DSe) and specificity (DSp) of 86% (95% CI, 42 to 98%) and 94% (95% CI, 81 to 99%), respectively.

Molecular and antigen detection tests for leptospirosis: a systematic review of diagnostic test accuracy for the Cochrane Collaboration

Bada Yang1, Sophia G. de Vries1, Benjamin J. Visser1, Ingeborg M. Nagel2, Marga G. A. Goris3, Mariska M. G. Leeflang4, Martin P. Grobusch1, Rudy A. Hartskeerl3

1Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands

2Medical Library, Academic Medical Center, Amsterdam, Netherlands

3KIT Biomedical Research, Royal Tropical Institute (KIT), Amsterdam, Netherlands

4Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam, Netherlands

Background: Early diagnosis of leptospirosis may contribute to the effectiveness of antimicrobial therapy and early outbreak warning. Molecular and antigen detection tests, such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) have the potential for early diagnosis of leptospirosis. To date, no systematic review has yet been conducted to assess the diagnostic accuracy of these tests.

Search methods: We will search a total of 16 electronic databases including MEDLINE (Ovid SP), EMBASE (Ovid SP), The Cochrane Library and regional databases from inception to present. We will not apply restrictions to language or periods.

Selection criteria: Cross-sectional studies and diagnostic case-control studies of tests that makes use of molecular (nucleic acid) and antigen detection methods in patients suspected of systemic leptospirosis will be included. As reference standards, we will consider microscopic agglutination test (MAT) alone or in a composite reference standard with culturing or other serological tests. Studies will be excluded if the controls are healthy individuals or when there is insufficient data to calculate sensitivity and specificity.

Data collection and analysis: Data from each study will be independently extracted by at least two reviewers. Risk of bias assessment will be done with the revised Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2). We will calculate study-specific values for sensitivity and specificity with 95% confidence intervals and pool the results in a meta-analysis if appropriate. As possible sources of heterogeneity, we will explore: endemicity, timing of index test, timing of reference test, choice of reference test, study design and the brand of the test. Studies with high risk of bias will be excluded in sensitivity analyses.

Results: The results of our review are pending and will be presented during the meeting.

ORAL PRESENTATIONS Saturday 18 April

Veterinary aspects

CANINE AND FELINE LEPTOSPIROSIS

Katrin Hartmann
Ludwig-Maximilians-Universität, Munich, Germany

Leptospirosis is common in dogs, but rare in cats. Both can shed leptospires in the urine, and thus, serve as source for human infection.

Canine Leptospirosis

While infection in dogs was most commonly caused by *Leptospira spp.* of the serogroups Canicola and Icterohaemorrhagiae (at least when relying on MAT results) in early times, today most infections are caused by other serovars, including Grippotyphosa, Australis, Sejroe, and Pomona. Leptospirosis is considered a seasonal disease with higher numbers of diseased dogs during summer and autumn. Affected dogs are more likely to live in the proximity of outdoor water, swimming, or drinking from outdoor water sources, and exposure to wildlife. Canine leptospirosis is a multi-systemic disease, affecting mainly kidneys and liver, but also other organs, such as lungs, spleen, endothelial cells, uvea/retina, skeletal and heart muscles, meninges, pancreas, and the genital tract. In recent years, leptospiral pulmonary haemorrhage syndrome (LPHS) is seen in dogs, as a very severe form with high mortality.

Feline Leptospirosis

Exposure to several serogroups was identified in cats, including serogroups Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, Hardjo, Autumnalis, Ballum, and Bratislava. Antibody prevalence in cats varies from 0 to 35%. Cats are more likely to become infected by feeding on animals harbouring *Leptaspira* spp. than *via* contaminated water. Cats can also shed leptospires in their urine.

Although cats can be infected with leptospires, clinical signs are rarely described. Experimental infection of cats results in leptospiraemia and leptospiruria, but only mild disease with histologic evidence of renal and hepatic inflammation. A few studies in pet cats report an association between *Leptospira* spp. infection and clinical signs, and cats with kidney disease are more likely to have antibodies against *Leptospira* spp. and to shed leptospires in their urine.

Prevention of Transmission

Dogs and cats can serve as reservoir and potential source of infection for humans. Transmission can occur as a result of direct contact with reservoir hosts or indirectly via environmental contamination. In USA, leptospiral DNA was detected by PCR in urine of 8% of dogs, while in Ireland, 7% of 525 dogs shed *Leptospira* spp. In southern Germany, 3 of 200 (1.5%) healthy dogs tested positive in urine PCR.

The exact risk of exposure of humans by infected dogs and cats is unknown. Presence of *Leptospira* antibodies in veterinary staff working in a teaching hospital with high leptospirosis case load and pet owners exposed to dogs with leptospirosis is uncommon. Still, appropriate precautions should be taken. Owners should be instructed to wash hands after handling their pet and to wear gloves when cleaning up urine spills using routine household disinfectants.

Vaccination of dogs is important as vaccines not only reduce the risk of clinical disease but also of *Leptospira* spp. shedding. The "old" vaccines containing serovars of serogroups Canicola and Icterohaemorrahgiae induce only partial immunity to heterologous serogroups. Recently, new vaccines containing three or four (Icterohaemorrhagiae, Canicola, Grippotyphosa, and Bratislava) serovars have become available in European and are recommended.

Diagnostic performance of the Test-it™ *Leptospira* Canine IgM Lateral Flow ELISA in the early diagnosis of acute canine leptospirosis.

C.Gloor, T. Francey, A. Schweighauser, S. Schuller
Small animal clinic, Vetsuisse Faculty, University of Bern, Länggassstr. 128, 3012 Bern (Switzerland)

Acute canine leptospirosis is a potentially life-threatening zoonotic disease. Prompt diagnosis is essential in order to appropriately treat infected dogs and prevent transmission to humans and other animals. Rapid patient side assays can be helpful tools in the clinical decision making. Aim of this retrospective study was to determine the diagnostic performance of the commercially available Test-it™ *Leptospira* Canine IgM Lateral Flow Assay (LeptoELISA) in the early diagnosis of leptospirosis.

Dogs presented to a referral center between January 2013 and December 2014 with a diagnosis of acute kidney injury (AKI) and a clinical suspicion of acute leptospirosis were included in the study. A diagnosis of leptospirosis was based on the presence of one positive microscopic agglutination test (MAT) titre (≥1:800 for nonvaccinal serovars, ≥1:3200 for vaccinal serovars) or evidence of seroconversion (≥4 fold increase in MAT titres in paired serum samples). 5 dogs, who died early on, were included without meeting above criteria because of a very strong clinical and clinicopathological suspicion. A diagnosis of leptospirosis was excluded in dogs with negative MAT titer in 1 or 2 samples and a convincing alternative diagnosis. In all dogs LeptoELISA and MAT were performed within 24 h of initial presentation.

101 dogs were included in the study (leptospirosis n=75; control n=26). LeptoELISA was clearly positive in 48/75 (64%) of dogs with leptospirosis. Weak positive results were seen in 16/75 (21%) of dogs with leptospirosis and 3/26 (11.5%) of control dogs. These 3 control dogs had been vaccinated less than 4 weeks previously. If all positive results were considered, the LeptoELISA had a sensitivity of 84 %, a specificity of 88%, a positive predictive value (PPV) of 95% and a negative predictive value (NPV) of 66%. If weak positive tests were considered negative, sensitivity and NPV decreased to 63% and 48% respectively, but specificity and PPV both increased to 100%. MAT with a cutoff of 1:400 had a sensitivity of 75 %, specificity of 100 %, NPV of 58 % and PPV of 100 %. Positivity rates of the LeptoELISA were 17% at a MAT titer of ≤ 1:100, 31% at 1:200, 57% at 1:400 and 1:800 and 88% at 1:1600. However at a MAT titre of ≥1:3200 only 58% were positive by the LeptoELISA. Isolated batches of the LeptoELISA were defective with poor absorption of the running buffer into the blotting paper and unreliable results.

In conclusion, the LeptoELISA shows a good clinical performance and a similar accuracy to that of the MAT. False positive results can occur in recently vaccinated dogs. Lower positivity rates with high MAT titres (≥1:3200) could be explained by an antibody switch leading to decreasing IgM concentrations in the face of high IgG. The correct interpretation of this ELISA can be difficult if the reaction is only weakly positive. Quality assurance procedures need to be improved before this assay can be widely recommended.

Corresponding author: Simone Schuller (simone.schuller@vetsuisse.unibe.ch)

DISTRIBUTION OF PATHOGENIC *LEPTOSPIRA* SEROGROUPS IN DOGS FROM LIMA, PERU

SIUCE M., JUAN[1,2]; CALLE E., SONIA[1]; PINTO J., CHRIS;[1];PACHECO S. GABRIELA; SALVATIERRA R., GUILLERMO[1];SEDANO S. ANDRE.

[1] MICROBIOLOGY AND PARASITOLOGY VETERINARY LABORATORY- FACULTAD DE MEDICINA VETERINARIA - UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS.

[2] E-mail: jsiucem@gmail.com

Leptospirosis is an important bacterial zoonosis on worldwide. Dogs and other animals can be infected and suffered the disease which dogs can act as a reservoir for human infection. The infection is caused by any of the more than 250 pathogenic serovars from 25 pathogenic serogroups of *Leptospira* spp. The aim of this sero-epidemiological study was to examine the pathogenic serogroups of *Leptospira* spp. circulating in domestic dogs in Lima. 305 serum samples from dogs were selected who had presumptive clinical diagnosis of Leptospirosis, over 6 months old, unvaccinated or more than six months since the last vaccination against Leptospirosis. The samples were submitted by veterinarians to Microbiology and Parasitology Veterinary Lab from Universidad Nacional Mayor de San Marcos. The Microagglutination test (MAT) was performed, establishing titles ≥ 1/100 seroreactors as seropositive using reference strains of the 25 pathogenic serogroups, including the new serogroup Iquitos, serovar Varillal, Var 10 strain, isolated and reported human outbreaks in the Peruvian Amazon. 177 seropositive samples (58.03%) were detected. Seropositives were reactors against 18 serogroups, the the predominant serogroup was Iquitos (46/305; 15.08%), followed by Tarassovi (37/305; 12.13%), Canicola (37/305; 12.13%), Australis (14/305; 4.59%), Icterohaemorrhagiae (13/305; 4.26%), Pomona (12/305; 3.93%), Mini (10/305; 3.28%) and Ballum (8/305; 2.62%). Besides, 31/305 (10.16%) were co-agglutinations. There were no seroreactors to serogroups: Bataviae, Celledoni, Hebdomadis, Lousiana, Panama, Ranarum, and Sarmin.

Keywords: Dog, leptospirosis, *leptospira*, MAT, serovar, serogroup.

Canine leptospirosis in Croatia – epizootiological and clinical features

Josipa Habus, Zrinka Stritof, Vesna Mojcec, Zoran Milas, Matko Perharic, Kresimir Martinkovic, Vilim Staresina, Ljubo Barbic, Vladimir Stevanovic, Suzana Hadina, Nenad Turk

Department of Microbiology and Infectious diseases with Clinic, Faculty of Veterinary Medicine University of Zagreb, Croatia

Leptospirosis is a well-known zoonotic infection with worldwide distribution. Due to the increased incidence and number of outbreaks recorded during the past few years leptospirosis is classified as a (re)emerging zoonosis that presents a significant problem both in human and in veterinary public health. Additional concerns in veterinary medicine have been raised after recent reports from mainland Europe that indicated an altered epizootiological situation of canine leptospirosis. The aim of this study was to determine the incidence of canine leptospirosis, prevalence of the presumed infective serovars and to analyse main epidemiological and clinical features among clinically ill dogs in Croatia.

A retrospective analysis of archive data obtained from NR Laboratory for leptospires and Clinic for infectious diseases was conducted. During a period of four years (January 2010 to October 2014), 311 canine sera were submitted to the Laboratory for leptospires, Faculty of Veterinary Medicine, University of Zagreb. Using a microscopic agglutination test (MAT) with cut-off point of 1:800, isolation procedures and/or PCR - 67 (21,5%) positive animals were detected. The most prevalent presumed infective serovars, in decreasing order, were Pomona (33,3%), Icterohaemorrhagiae (26,7%), Grippotyphosa (21,7%) and Bratislava (11,7%) and infection was mainly detected in large breed male dogs from suburban and urban areas. Detailed clinical data of 27 dogs that were presented at the Clinic for Infectious diseases was additionally analysed. Most common presenting clinical signs included lethargy (89%), anorexia (85%), vomiting (70%), muscle weakness (63%), icterus (59%) and abdominal pain (59%). Major haematological and biochemical abnormalities were azotemia (93%), thrombocytopenia (70%), bilirubinemia (70%), anaemia (59%) and leukocytosis (56%). Although acute renal and hepatic failures were the most frequent clinical manifestations in dogs with leptospirosis a very high percentage of dogs with pulmonary involvement (41%) was also detected. Eight dogs were euthanized because of financial reasons connected with the high costs of intensive care while survival rate in treated animals was 63%. Negative outcome was mainly associated with leptospirosis pulmonary haemorrhagic syndrome (LPHS) and/or severe acute kidney injury (AKI).

In this study, an increasing overall incidence of canine leptospirosis was detected, as well as increasing incidence of severe cases with high mortality due to severe AKI and pulmonary involvement. In addition, results showed that most infections were caused by serovars not covered by the currently used vaccines raising questions concerning its efficacy in preventing leptospirosis in dogs.

Corresponding author: Josipa Habus, jhabus@vef.hr

Prevalence of *Leptospira* serovar Hardjo infection in aborted foetuses and stillbirths

Zbigniew Arent, Colm Gilmore, William A. Ellis
OIE Leptospirosis Reference Laboratory, Veterinary Sciences Division, AFBI, Belfast, Northern Ireland

Leptospirosis is an economically important infectious disease of domestic livestock. Renal infection is the most common microbiological sequel of the disease in most animal species but in the case of host maintained infections, such as serovar Hardjo infection in cattle (both type Hardjobovis and Hardjoprajitno) persistent genital tract infection is commonplace. These infections are mostly subclinical in cattle and sheep, but can be associated with reproductive problems such as abortion, stillbirth and infertility. These symptoms occur weeks after infection and are usually seen when a dam is infected for the first time during pregnancy.

In maintenance host infections, particularly serovar Hardjo infection in cattle and sheep or Bratislava infection in pigs, infected animals often have a poor antibody response to infection. Often at the time of abortion, antibody titers may be low or negative. Demonstration of the organism in tissues of the aborted foetuses is the most definitive diagnostic test. Previous studies have shown the value of immunofluorescence as a diagnostic tool in cases of leptopsiral abortion.

The aim of the present study was the diagnosis of *Leptospira* Hardjo infection in aborted bovine and ovine foetuses or stillborn calves and lambs that were submitted for postmortem examination between 2004-2014. Lung, kidney, adrenal glands and placenta specimens from 6521 bovine and 2800 ovine cases were examined during this time. Leptospires were demonstrated in 774 (11.0%) bovine and 399 (14.3%) ovine foetuses.

These results indicate that L. Hardjo infection remains a significant factor in the etiology of abortion in domestic ruminants. The decline of the prevalence of these infections in the last four years can be justified by effectiveness of eradication methods and in particular vaccination.

A Review of Leptospirosis Research in Farmed Deer in New Zealand

Peter R Wilson, Cord Heuer, Jackie Benschop, Julie M Collins-Emerson
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, PB 11222, Palmerston North New Zealand 4442.

Many *Leptospira* serovars are reported in deer species globally. This paper reviews research into *Leptospira borgpetersenii* sv. Hardjobovis and *L. interragans* sv. Pomona which infect farmed deer in New Zealand (NZ) causing risk to animals and people. A 2004 sero-survey (110 herds) showed 82% seropositive, with Hardjobovis (H) in 77% and 60.8%, and Pomona (P) in 20% and 8.4% of herds and deer, respectively. Concurrent H and P titres occurred in 16.4% of herds and 6.6% of deer and within-herd seroprevalence was up to 100%. A 2009 sero-survey (99 herds) showed 76% positive, 60% with H and 49% with P, and 59% and 47% of deer H and P positive, respectively. Approximately 2% of farms/year experience clinical leptospirosis, often as outbreaks. Infection rate is highest between 3-12 months of age, but varies with climate. Deer managed with sheep and cattle are at higher risk of infection, and evidence exists for cross-species transmission. A SYTO9 real-time PCR was developed and validated. Sensitivity and specificity on kidney was 85% and 98.8%, respectively, and on urine 96.7% and 100%, respectively. Estimated urine *Leptospira* shedding was 3.7×10^3 to 1.7×10^6 cells/ml. Shedding may persist for at least 10 months. Vaccinated adult hinds in a herd with dual H and P infection had a higher weaning rate (97 vs. 88%, p=0.04). Another study (n=5 herds) showed a 5.7% higher mean weaning rate in vaccinated primiparous hinds (p=0.05) in H infected herds. Weight of 9-month-old deer either H and/or P sero-converting or urine shedding during the previous six months was 3.7 kg lower (p=0.37) than negative deer. A vaccine study (n=3 H infected farms) showed weight gain to 12 months was 12 – 26g/d higher in vaccinated deer (p<0.05), depending on seroprevalence in controls. A sero-survey of workers in two deer abattoirs showed 5% and 6%, and 19% and 11% positive to P and H, respectively (overall 17% and 19%). Workers potentially in contact with urine were at higher risk. Vaccination with a dual H and P vaccine resulted in a serological response (Microscopic Agglutination Test) in deer similar to that in cattle. Colostral interference was not detected when vaccinated at 3-months-of-age. Efficacy in reducing urine shedding and kidney culture in infected herds was 44% and 55%, respectively (p<0.001). Vaccination prior to natural exposure resulted in no shedding in urine compared with 12% and 56% of controls in two H positive herds, a risk difference of -24.5% (p=0.0012). Vaccination is estimated to be cost-effective for growth if seroprevalence is ≥~20%, and for reproduction if the difference in weaning rate is ≥~1.3%. Evidence shows leptospirosis infection is common in NZ farmed deer, principally affecting growth and reproduction, and likely to be economically significant on a majority of farms. Humans are at risk of leptospirosis from deer. Long-term vaccination is likely to be an efficacious and cost-effective means for prevention.

Corresponding author: P.R.Wilson@massey.ac.nz

Unusual patterns of pathogenic *Leptospira* spp. infection in animal hosts from northern Tanzania

Kathryn J. Allan[1], Jo E.B. Halliday[1], Tito Kibona[2], Ryan Carter[1], Marga Goris[3], Rudy Hartskeerl[3], Venance P. Maro[2], Rudovick R. Kazwala[4], Julius Keyyu[5], John A. Crump[2,6] & Sarah Cleaveland[1].

[1]University of Glasgow, Glasgow, UK; [2]Kilimanjaro Christian Medical Centre, Moshi, Tanzania; [3]KIT Biomedical Research, Amsterdam, The Netherlands; [4]Sokoine University of Agriculture, Morogoro, Tanzania; [5]Tanzania Wildlife Research Institute, Arusha, Tanzania; [6]University of Otago, Dunedin, New Zealand.

Corresponding author: Kathryn.Allan@glasgow.ac.uk

Acute leptospirosis is the cause of illness in 8% of febrile hospital admissions in the Kilimanjaro Region of northern Tanzania. However, little is known about the sources, transmission routes or risk factors for human leptospirosis in this region. To help tackle these knowledge gaps, we performed a field study to identify pathogenic *Leptospira* spp. in rodents, cattle, sheep and goats, and to investigate the role of these animals in the epidemiology of *Leptospira* in northern Tanzania. We performed cross-sectional surveillance of peri-domestic rodents in 12 randomly selected villages within the catchment area of the two study hospitals (Moshi Urban and Rural Districts). Rodents were live-trapped in 250 study households using Sherman and Tomahawk traps and humanely euthanized. Samples of kidney were collected and tested for pathogenic *Leptospira* spp. using *lipL32* quantitative PCR (qPCR). Kidneys from ruminant livestock (cattle, sheep, and goats) were sampled opportunistically from five slaughter-slabs and one abattoir in the Moshi Urban District and were also tested by *lipL32* qPCR. Culture media (EMJH-5'FU) was inoculated with kidney tissue from a subset of rodents and cattle and monitored for *Leptospira* growth. A total of 393 rodents were trapped in two trapping seasons in 2013 and 2014. qPCR (n=393) and culture (n=100) results were negative for all rodents. Results were verified by confirmatory testing of a random subset of samples at an international reference laboratory. In contrast, pathogenic *Leptospira* spp. was detected in 22 (6.3%) of 348 cattle kidneys, and *L. borgpetersenii* isolates were obtained in four cases (n=100). One isolate was serologically typed as *L. borgpetersenii* serovar Hardjo (serogroup Sejroe). Pathogenic *Leptospira* infection was also detected in the kidneys of two (5.0%) of 40 goats and one (2.5%) of 40 sheep. Rodents are thought to be a major source of *Leptospira* infection for people around the world. However, unusually our results indicate that ruminant livestock may be more important than rodents in the epidemiology of leptospirosis in northern Tanzania. Work is ongoing to explore the molecular and serological diversity of *Leptospira* spp. in linked human and animal populations in northern Tanzania, and to investigate key risk factors for human infection.

REPRODUCTIVE DISORDERS AND LEPTOSPIROSIS: A CASE STUDY IN A MIXED UNITS FARM (CATTLE AND SWINE)

Mori M.*1, Raïssa Bakinahe1, Jo Maris2, Ellen de Jong2, Marylène Tignon3, Brigitte Cay3, Martine Marin1, Damien Desqueper1, David Fretin1, Isabelle Behaeghel4

1Bacterial Zoonoses of Livestock, CODA-CERVA, Brussels, Belgium

2Dierengezondheidszorg Vlaanderen, Lier, Belgium

3 ENZOREM, CODA-CERVA, Brussels, Belgium

4Epidemiology and surveillance, CODA-CERVA, Brussels, Belgium

Reproductive disorders comprise a plethora of clinical manifestations spanning from infertility to abortions that can be associated to single or more complexes dynamics implicating among others infective agents. Various reports present leptospirosis as one possible cause of reproductive disorders in production animals. However, because of its epidemiology, the wide range of susceptible and reservoir hosts, the intricate ecosystem, limitation in cultivating the germ, make this disease difficult to diagnose in animals as such, particularly in case of chronic infections.

Here, a case-study in a mix-species farm with documented reproductive disorders in the cattle unit is described. A long calving internal was the hallmark among the problems observed in cows. Some cows presented high titre of antibodies against *Leptospira* serogroup Sejroe, but the overall in-herd prevalence was low. The in-herd prevalence of leptospirosis in the second unit (sows) was high, but it would have been not observed at standard cut-off threshold dilution (1:100). In this unit, the most prevalent serogroups was Autumnalis. The final direct confirmation of *Leptospira* maintenance in the farm was obtained through detection of *leptospira* DNA in an abortive swine litter (6 out of 7 piglets). The most affected organs were spleen (4/7) and kidneys (3/7). Despite the fact that a common causative bond between the two animal units was not highlighted some factors such as drinking from the same water source and the use of manure prepared with the swine slurry might suspect for a possible cross-contamination between the two units. This report aims to highlight that leptospirosis need to be considered by the veterinary practitioners in the differential diagnosis of reproductive disorders and abortions and wants to re-open a debate on the setting of threshold dilutions for different animal species.

*corresponding author: Marcella.Mori@coda-cerva.be

Leptospirosis in Europe – neglected disease of natural focus

Nenad Turk
University of Zagreb, Croatia

Leptospirosis continues to be a significant zoonosis in Europe. Although Central and Eastern European countries, according to official data, demonstrate the highest incidence of animal leptospirosis in Europe, the surveillance and control of this re-emerging zoonosis is still insufficient. Traditional disease surveillance system often involves reports of laboratory-confirmed cases to public health agencies resulting underestimation of zoonotic infections such as leptospirosis. While there are considerably disparities between the levels of awareness of animal leptospirosis importance in different countries/regions, the underlying limitations are mainly common to all and they only differ by degree.

Changes in epizootiology and epidemiology of leptospirosis within natural foci areas due to anthropogenic influence on ecosystem necessitate thorough studies of ecological specific features of causative agents, reservoirs and vertebral hosts reflecting their adaptation to various natural geographical and social conditions. The attention should be focussed on geographical variability of leptospires as a causative agent, changes in populations of rodent reservoirs, climate changes, monitoring of natural territorial complexes and scientifically grounded prognosis for leptospirosis within natural foci.

In this way, control of animal leptospirosis in Europe involves not only the duties of the veterinarians but also a multidisciplinary approach of other relevant experts supported by adequate health and surveillance networks; awareness of the evolution of the incidence of animal leptospirosis is important to determine the relative risks in international trade and replacing carrier animals as well as in highlighting areas that should move at the epicentre of enhanced disease surveillance and control. For a disease for which a lot is still to be learned on its pathogenesis, treatment and prevention principles, understanding of leptospirosis as a natural focus disease with all its specificities may allow for enhanced policy of allocation European finances for its control. It is up to the leptospirologist to dedicate their time on the issue.

A nationwide survey of pathogenic leptospires in urine of cattle and buffaloes by Loop-mediated isothermal amplification (LAMP) method in Thailand, 2011-2013

Suwancharoen D[1,*], Limlertvatee S[1], Chetiyawan P[1], Tongpan P[1], Sangkaew N[1], Sawaddee Y[1], Inthakan K[1], Wiratsudakul A[2]

[1] National Institute of Animal Health, Department of Livestock Development, Bangkok, Thailand

[2] The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand

Leptospirosis is one of important zoonotic diseases in Thailand. The disease was first recognized in 1942 and has been endemic in the country to present. However, the role of animals especially livestock species in the spread of leptospirosis is still poorly understood. A cross-sectional nationwide survey was conducted during 2011-2013 to determine prevalence of leptospirosis in cattle and buffaloes and to examine associations between uropositivity and geographical locations, species, sex and age. Urine samples from 3,157 cattle and 487 buffaloes (3,644 animals) were collected from 25 of 77 provinces across Thailand. The 16S rDNA of pathogenic *Leptospira* was detected using Loop-mediated isothermal amplification (LAMP) method. A total of 347 [9.5%; 95% confidence interval (CI), 8.6 – 10.5] animals were uropositive and the highest uroprevalence was found in lower northern region (24.8%, 95% CI 20.8 – 29.1) followed by upper northeastern region (17.6%, 95% CI 13.9 – 21.8). Uroprevalence for beef cattle, dairy cattle and buffaloes were 11.4% (95% CI 9.6 – 13.4), 9.3% (95% CI 7.3 – 11.7) and 10.6% (95% CI 7.9 – 13.8), respectively. Males were 2.19% (95% CI 1.63 – 2.96) more likely to be uropositive than females. Exclusively small sample sizes from animals younger than one yerar of age, the uroprevalence increased with increasing age. Further study particularly in high prevalence areas was required to determine the role of cattle and buffaloes as reservoirs of human leptospirosis.

Keywords: Buffaloes, Cattle, LAMP, Leptospirosis, Uroprevalence, Thailand

*Corresponding author: D. Suwancharoen, National Institute of Animal Health, Department of Livestock Development, Bangkok, 10900 Thailand. (Email: dj90705@hotmail.com)

A LEPTOSPIROSIS OVERVIEW ON WILD fauna IN SARDINIA, ITALY

[1]Ponti M.N., [1]Palmas B., [1]Noworol M., [1]Falchi A., [1]Pintore A., [1]Ruiu A., [1]Vannini A. [2]Boniotti M.B., [1]Piras A., [3] Picardeau M., [1]Piredda I.

[1]Istituto Zooprofilattico Sperimentale della Sardegna, Italy

[2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy

[3]Institut Pasteur Unitè de Biologie des Spirochetès, Paris

Keywords: Leptospirosis, infection, wild

INTRODUCTION: Leptospires are usually transmitted through contact with the urine of infected animals, either directly or through exposure to contaminated water or soil. Among wildlife species, rodents are the most important maintenance hosts for *Leptospira* spp. and may transfer infection to livestock, pets and humans. This study reports the prevalence of leptospirosis in wild animals such as deer, wild boar, rodent, rabbit, hare, marten, mouflon, shrew, nutria, fox and hedgehog circulating in Sardinia.

MATERIALS AND METHODS: Between 2012-2014 were examined: 7608 sera by MAT using a reference panel of 9 serovars, and 466 organ, blood and urine samples used to inoculate four tubes of liquid EMJH medium. Cultures were incubated at 29°C and evaluated weekly by darkfield microscopy for up to 3 months. 451 of 466 were examined by PCR using the LigA/LigB primer pair amplifies an expected product of 331 bp. The PCR products were analyzed by agarose gel electrophoresis and visualized by staining with SYBR-green. The isolated *Leptospira* species were identified by Multi Locus Variable-number tandem repeat (VNTR) analysis (MLVA) and rrs gene sequencing.

RESULTS: 563 (7,5%) of 7512 wild boar serum samples evaluated in our study presented anti-*Leptospira* spp. antibodies. 3,4% had agglutinating antibodies against *L. interrogans* serovar Pomona, 2,6% against Bratislava, and 1,S% against Grippotyphosa. 141out of 563 (25%) have reported titres ≥ 1:800. 466 (99%) kidneys were tested by culture. *Leptospira* spp was isolated from 26 (5,6%) samples. Out of these, using molecular tools, such MLVA and MLST, 11 wild boar isolates were identified as *L. interrogans* serovar Pomona, 2 out of 9 from rodents as *L. borgpetersenii* and *L. interrogans*, 1 out of 4 from hedgehogs as *L. borgpetersenii*. A single isolate from foxes was typed as *L. interragans* serovar Bratislava. 62 (54 kidneys and 8 livers of 451 organ samples) tested positive by PCR. Emerging technologies like MLVA and MLST facilitate the identification of infective leptospire species, thereby contributing to the knowledge of the serovars circulating in our region.

CONCLUSIONS: Our study indicates that pathogenic leptospires are widely distributed in Sardinia. The excretion of highly pathogenic serovars in the environment could represents an increased risk for humans and animals, especially those living in rural areas. Our data suggest a high level of environmental contamination and indicate that wild animals could become good sentinels for the monitoring of leptospirosis.

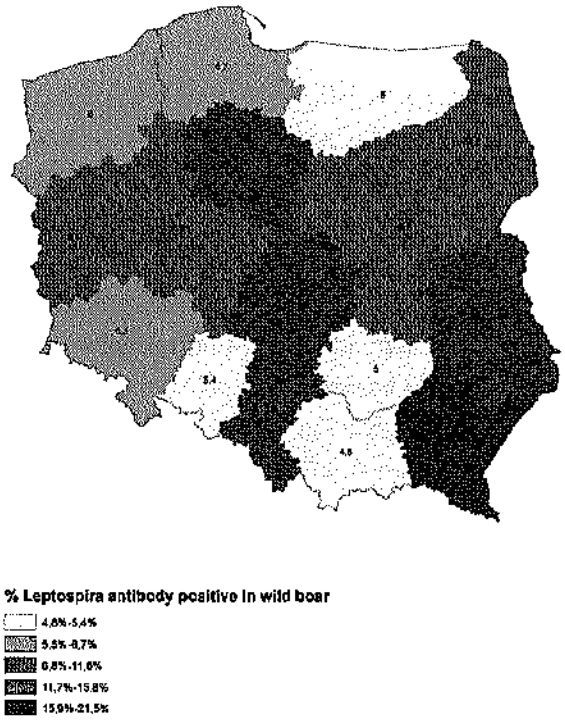
A serologic survey of selected *Leptospira* serovars in wild boar in Poland

J. Zmudzki[1], A. Jablonski[1], S. Zebek[1], A. Nowak[1], Z. Arent[3], A. Stolarek[2], L. Bocian[2], Z Pejsak[1]

National Veterinary Research Institute, Swine Diseases Department[1] and Department of Epidemiology and Risk Assessment[2], Pulawy, Poland, Leptospirosis Reference Laboratory, Agri-Food and Biosciences Institute, Belfast[3]

Introduction

Leptospirosis is widely distributed zoonotic disease caused by pathogenic serovars of the genus *Leptospira*. Animals are an important reservoir of leptospirosis for humans. Leptospirosis can be transmitted by rodents, domestic animals such as cattle and swine and companion animals. Previous survey on leptospirosis in in polish wild boar reported overall antibody prevalence on the level of 24% in one region (northern Poland) but based on limited number of serum samples (3). The aim of the study was to estimate the scale of *Leptospira* sp. infections in polish population of wild boar.



Materials and Methods

A total of 3621 serum samples were collected from wild boars during hunting season (2013-2014) and from serological monitoring of wild boars to classical swine fever in 2012-2014. Serum was tested by MAT with the following *Leptospira* serovars: Icterohaemorrhagiae, Grippotyphosa, Sejroe, Tarassovi, Pomona, Canicola, Bratislava, Autumnalis, Hardjo and Ballum. The minimum sera dilution was 1:100.

Results From a total of 3621 serum samples antibodies against particular *Leptospira* serovars, were found in 377 samples, which gave overall prevalence on the level 10.4% from all polish provinces (voivodeships). The highest percentages of seropositive samples in wild boars to tested *Leptospira* serovars were found in the following voivodeships: Podkarpackie (Subcarpathia) – 21.5%, Lubelskie – 15.8%, Śląskie (Silesia) – 15.5%, Łódzkie – 14.3% and Kujawsko-pomorskie (Kuyavian-Pomerania) – 14.1%, while the lowest were found in Małopolskie (Leser Poland) – 4.8%, Warmińsko-mazurskie (Warmia-Masuria) – 5.0%, Świętokrzyskie – 5.0% and Opolskie – 5.4%. The most common serovars of *Leptospira* include: Pomona, Hardjo, Grippotyphosa and Bratislava.

Conclusions and Discussion The results of our study show that the prevalence of seropositive samples tested for serovars of *Leptospira* in wild boars in Poland are quite similar to the results presented by other European countries especially in Spain – 12% (5) and in Germany – 18% (2). In contrast low prevalence antibody titers were reported in Italy – 2.6% (4), and Sweden – 3.1% (1). Presented data confirm that wild boars in Poland can be an important vector in the spread of leptospirosis in humans and especially in people working in the forest environment.

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Past, current and future surveillance of leptospirosis in animals in the Netherlands

Jaap A. Wagenaar1, 2, Els Broens1, Marc Engelsma2, Hendrik-Jan Roest2 and Jet Mars3

1. Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
2. Central Veterinary Institute, Lelystad, the Netherlands
3. Animal Health Service, Deventer, the Netherlands

Surveillance of leptospirosis in domesticated animals in the Netherlands is limited to the presence of *Leptospira hardjo* in cattle. This surveillance program is driven by the dairy industry and carried out by the Animal Health Service. Since the introduction and spread of *L. hardjo* in the Dutch cattle population in the eighties of the last century a successful control program has been implemented primarily based on screening for antibodies in bulk tank milk. For other animal species either only clinical cases are investigated (e.g. dogs) or samples from animal based on export requirements (e.g. pigs and horses). Although in pigs leptospirosis is associated with fertility problems, the number of samples from pigs investigated for clinical reasons is very limited, although with the increase of free range pigs, the exposure to leptospira might increase. For dogs only samples from suspected cases are submitted to the diagnostic lab. Remarkably, the increase in human cases in 2014 paralleled an increase in positive fraction of canine samples (see abstract Els Broens). For wild life rats have been investigated (see abstract Joke van der Giessen). Altogether, the information on leptospirosis in animals in the Netherlands is limited and fragmented. The pro's and contra's of a systematic monitoring in other animals species than cattle will be discussed with regard to clinical disease in animals and zoonotic transmission to humans.

Large epidemiological investigation about leptospirosis within the non-rodent wildlife in the French landscapes

B.COMBES; Z.DJELOUADJI; V.RATON; F.BAURIER; E.FAURE; A.KODJO

Leptospirosis is the most widespread zoonosis all around the world. This zoonosis is caused by serovars of pathogenic *leptospira* species. *Leptospira* multiply in the kidneys of mammals and is excreted in their urine. The infection then occurs by direct contact with infected animal or indirect contamination by contact with water previously contaminated by the urine of a carrier animal. The understanding of geographical *leptospira*'s contamination process from one area to another one needs to acquire epidemiological data from all mammal species. The role of rodent as a reservoir has been extensively investigated, however despite their sensitivity a very few non-rodent species has previously been studied as potential carriers of *leptospira*.

From 2012 to 2014, we collected around 3456 kidneys from 24 different wild French mammals (from weasel to red deer excluding rodents and bats) to determine which of these species could be of some importance both in the carrying and excreting pathogenic *leptospira* and to detect the most prevalent circulating *leptospira* species and/or genovars.

A specific pathogenic *leptospira* real time PCR was performed on kidneys. All positive samples were then genotyped together by VNTR and MST methods. A total of 170 samples off 2893 yet analysed kidneys (prevalence = 5.9 %) were detected positives on PCR. The main prevalent host is the hedgehog with 43.8 % prevalence (n=50). Mustelids group occurs also as important carrier with a mean prevalence of 12.5 % (n=689). The *rrs* gene sequencing identified *Leptospira interrogans* as the prevalent infected species (74%) followed by *L. kirschneri* (16%) and *L. borgpetersenii*. All the VNTR and MST profiles were related to serovars Icterohaemorrhagiae, Australis and Grippotyphosa's genotype in addition to new original genotypes identified.

The use of adequate epidemiological tools to assess our data will help in understanding the eco-epidemiological role of the investigated hosts.

Corresponding author: benoit.combes@e-l-i-z.com

CARACTERIZATION OF THE GENUS *LEPTOSPIRA* ISOLATES FROM RODENTS CAPTURED IN ANGOLA

Elsa V. Fortes-Gabriel, Teresa Carreira and M. Luísa Vieira
Group of Leptospirosis and Lyme Borreliosis, Unit of Medical Microbiology, Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT) Universidade Nova de Lisboa (UNL), Lisbon, Portugal

The role of rodents in carriage and transmission of pathogenic *Leptospira* spp., is well known. However, in Angola, neither the natural reservoirs of these spirochetes nor leptospirosis diagnosis in human population have been considered. To address this gap, we carried out a study to identify circulating *Leptospira* spp. Rodents were caught in Luanda and Huambo provinces, and kidney tissue was cultured in selective medium and analyzed by integrated approaches, such as serological methods [microscopic
agglutination test (MAT) with monoclonal Antibodies and rabbit anti-sera] and molecular methods such as, Polymerase Chain Reaction (PCR) targeting the *hap1* gene and sequencing, and an irep1 protocol to compare the leptospiral DNA of new isolates with the reference serovars profiles. A total of 37 captured rodents were classified as: *Rattus rattus* (nRr = 15; 40.5%), *R. norvegicus* (nRn = 9; 24.3%) and *Mus musculus* (nMm = 13; 35.1%). From kidney tissue, we obtained four (10.8%) *Leptospira* spp., isolates.

The serological approaches showed that the isolates belonged to Icterohaemorrhagiae and Ballum serogroups and Icterohaemorrhagiae and Arborea serovars, respectively. PCR results reveled the presence of *Leptospira* DNA with the evidence of simple amplicon of 262 base pairs in eight (21.6%) kidney samples. The comparison of the irep1-PCR product from each isolate showed similarity of the profiles obtained with Icterohaemorrhagiae and Arborea reference serovars. Finally, sequencing confirmed that these isolates belong to *L. interrogans* and *L. borgpetersenii* genospecies. All results showed consistence in *Leptospira* isolates characterization. This study, restricted yet to the Luanda and Huambo provinces, provides for the first time, important information about the circulating pathogenic leptospires disseminated by rats and mice in the Luanda and Huambo provinces. Despite the small sample of the present study, the results are promising because the serovars and genospecies of *Leptospira* isolated now can be useful in the future to support the serological and molecular diagnosis of leptospirosis in Angola.

Elsa V. Fortes-Gabriel, elsa.gabriel@ihmt.unl.pt

ORAL PRESENTATIONS Saturday 18 April
Immunology and vaccine development

The host response to hantaviruses: a delicate balance between resolution and pathology

Byron E. Martina and Marco Goeijenbier
Erasmus MC, Rotterdam, the Netherlands, Department of Viroscience

Hantaviruses can cause two severe human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Hantaviruses are spread to humans mainly through inhalation of infectious virions, secreted in the excreta from infected rodents. The human diseases are characterized by an increased capillary leakage syndrome. Although hantaviruses are known to infect endothelial cells, infection is not cytopathogenic. The mechanism behind human disease is not well understood, but an overactive immune response is implicated in the pathogenesis. In humans, high levels of inflammatory cytokines are present in the lungs of patients that succumb to infection. This, along with other observations, suggests that disease has an immunopathogenic component. An increasing amount of evidence suggests the existence of an extensive correlation between inflammation and coagulation, whereby inflammation not only leads to coagulation, but coagulation also affects inflammatory activity. One of the most important functions of the endothelial cell layer is the maintenance of haemostasis, a physiologic mechanism that maintains blood in a fluid state within the vasculature and responds with coagulation at time of injury to prevent exsanguination. Inflammation impacts the initiation, propagation and inhibitory phases of blood coagulation. Clinical studies focusing on alterations in primary and secondary haemostasis during hantavirus disease showed thrombocytopenia in both HFRS and HCPS, a decreased plasma activity of coagulation factors in acute HFRS patients infected with puumula virus. Bleeding complications are associated with disease severity and most often arise following consumptive coagulopathy or even disseminated intravascular coagulation.

Mannose-binding lectin inhibits motility of *Leptospira*

Jun Xu
Labatory of Animal Microbiology, Graduate School of Agricultural Science, Tohoku University, Japan
E-mail: xu.jun.r4@dc.tohoku.ac.jp

Introduction

Bacterial motility is one of the essential pathogenic factor that enhances the invading ability and eventually leads bacteria to reach the target tissue. In this study, various kinematic parameters of the *Leptospira* motility were measured under the affection of Mannose-binding lectin (MBL), to identify the interrelation between MBL and motility. The results are expected to give a new insight into complement pathways in the response to leptospiral infection.

Materials and Methods

Leptospira biflexa serovar patoc strain Patoc I, and *Leptospira interrogans* serovar pomona strain Ponoma were used in the experiment. It has been confirmed that mannose as one of the sugar component in the lipopolysaccharide of Patoc I but not in Pomona. Cells were grown in EMJH liquid medium at 30ºC for 4 days (Patoc I) and 6 days (Pomona) respectively, and then maintained in 10% normal rabbit serum. The cells were then suspended in fresh EMJH medium, which was used as motility medium. The methods which are used to measure the motile fraction, swimming speed and rotation rate, briefly, 4 days cultured *Leptospira* cells in the density of 1.0×10^8 cells/ml, were treated with human MBL in 2.5 µg/mL and otherwise the combination with 20 mM EGTA buffer, commonly used chelator to bivalent calcium ions. Then observed under dark-field microscope, the locomotion of bacteria was captured by a charge-coupled device camera and recorded by video recorder. Appropriately captured DVD videos were converted to AVI movies. Swimming trajectory and speed of were analyzed by using software ImageJ and Macros of Excel. Rotation rate of *Leptospira* cell was analyzed by using high speed CCD camera.

Results and Conclusion

The results showed that number of tumbled rotating cells was increased by adding with MBL to the culture, and further the reduction of rotation rate of these rotating cells, but no substantial change on swimming speed compared to control groups. These indicate that the motility of *Leptospira* was impaired by the presence of MBL. However, such affection was only transient, bacteria regained their original motility after several hours, which may be explained by the fluidity of *leptospira* cell membrane.

Efficacy of EURICAN® DAPPi2-L3 against leptospirosis

J. Bouvet, C. Cariou, F. Oberli, J. Minke, P.M. Guigal, L. Cupillard
MERIAL S.A.S., 254 rue Marcel Mérieux, 69007 Lyon, France

Protection against clinical disease and prevention of the renal carrier state remain the key objectives of vaccination against leptospirosis in the dog. In the current study, we demonstrate the efficacy against leptospirosis (serovars Canicola, Icterohaemorrhagiae, and Grippotyphosa), using a canine combined vaccine (EURICAN® DAPPi2-L3). Puppies received a first injection of vaccine at the age of 7 to 9 weeks, then a second injection at the age of 11 to 13 weeks. Dogs were challenged by intraperitoneal and ocular route with virulent suspension of heterologous strains representatives of serovars, 2 weeks (onset of immunity) or 12-14 months (duration of immunity) after the second vaccination. Control dogs were not vaccinated and kept with the vaccinated dogs. Clinical observations were recorded during 28 days post-challenge. Dogs displaying clinical signs were humanely euthanized according to pre-set endpoints. Blood (culture, biochemistry, and haematology), urine (culture) and liver and kidney (culture and/or histopathology) samples were collected during the study or at necropsy. Challenges in the control puppies (onset of immunity studies) reliably produced clinical signs consistent with *Leptospira* infection, leading to the euthanasia of at least 29% of the animals for each serovar, while no vaccinated puppies in any study displayed any clinical signs. As a confirmation of clinical protection, all control puppies and none of the vaccinated puppies, were infected, had urine or kidney samples positive for *Leptospira* isolation, demonstrating a complete protection against renal carriage, with no histological lesions of kidney in the vaccinated animals. Challenges in control adults (duration of immunity studies) can be less severe, depending on challenge strain : 100%, 70% or 10% of the dogs had clinical signs with Icterohaemorrhagiae (leading to the euthanasia of 27% of them), Canicola, or Grippotyphosa, respectively. None of the vaccinated dogs in any study displayed any major clinical signs, with significant differences between groups for Icterohaemorrhagiae and Canicola challenges. Analysis of blood cultures showed significant differences between groups in duration of infection for Icterohaemorrhagiae and Canicola, and close to significance for Grippotyphosa. As for the onset of immunity against Icterohaemorrhagiae, adult dogs were also completely protected against renal carriage. For Canicola and Grippotyphosa, at least 70% of the adult controls became renal carriers. Despite the heavy challenges, 18 out of the 23 vaccinated adult dogs did not developed a renal carrier state. These results show that a primary course of two doses of EURICAN® DAPPi2-L3 provided quick onset and a long-lasting protection of at least 12 months against serovars Icterohaemorrhagiae, Canicola, and Grippotyphosa.

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Leptospira regulate protein expression during persistent infection of reservoir hosts

Jarlath E. Nally.

Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture.

Reservoir hosts of leptospirosis, including rodents, dogs and cattle, exhibit little to no signs of disease but shed large numbers of organisms in their urine. Transmission occurs when mucosal surfaces or abraded skin come into contact with infected urine or urine-contaminated water or soil. Whilst little is known about how *Leptospira* persist within a reservoir host, *in vitro* studies suggest that leptospires alter their transcriptomic and proteomic profiles in response to environmental signals encountered during mammalian infection. When *L. interrogans* is cultivated within dialysis membrane chambers (DMCs) implanted into the peritoneal cavities of rats, 166 genes are differentially-expressed in response to mammalian host signals, as well as candidate small regulatory RNAs. Whilst the proteome of *L. interrogans* is also modulated in response to mammalian host signals, results indicate that differential expression of specific post-translational modifications (PTM) also occurs. Proteomic analysis of leptospires excreted in urine from experimentally infected rats confirms differential protein expression during infection. Whilst the major outer membrane protein, LipL32 was not differentially expressed, specific PTM of LipL32 were detected. LipL32 derived from rat urine-derived leptospires contained either acetylation or tri-methylation of lysine residues within multiple LipL32 peptides, including peptides corresponding to regions of LipL32 previously identified as epitopes. A synthetic peptide containing an identified tri-methylated lysine, which corresponds to a previously identified LipL32 epitope, demonstrated significantly reduced immunoreactivity with serum collected from leptospirosis patients compared to the peptide version lacking the trimethylation. Collectively, these results highlight the importance of detecting and understanding the role of proteins of leptospires, and their respective PTM, that are expressed during active disease processes to understand mechanisms of persistent infection.

E-mail: Jarlath.Nally@ars.usda.gov

Safety and efficacy of a new octavalent combined Erysipelas, Parvo and *Leptospira* vaccine in gilts against *Leptospira* serovar Pomona associated disease and foetal death.

A. Jacobs*, F. Harks, M. Hoeijmakers, M. Collell and R. Segers
MSD Animal health, PO Box 31, 5830 AA Boxmeer, The Netherlands

*corresponding author, ton.jacobs@merck.com

The safety and protective efficacy of a new octavalent combination vaccine containing inactivated *Erysipelothrix rhusiopathiae*, Parvo virus, and *Leptospira* serogroups Canicola, Icterohaemorrhagiae, Australis (Bratislava), Grippotyphosa, Pomona and Tarassovi - Porcilis® Ery+Parvo+Lepto - was evaluated in laboratory studies and under field conditions.
The safety (2x overdose and repeated dose) was tested in 26 gilts. In this study, neither vaccine related temperature increase nor other systemic reactions were observed after the intramuscular vaccinations. No local reactions were observed except for one animal with a small local reaction (2cm diameter) lasting for 5 days after third vaccination.
The efficacy was tested in 40 gilts. A group of 20 gilts was vaccinated at 20w and 24w of age with Porcilis® Ery+Parvo+Lepto and a group of 20 age and source matched animals served as control. The gilts were inseminated at 41w or 66w of age and were challenged with serovar Pomona 10w after insemination corresponding to 6 months (n=2x10) and 12 month (n=2x10) after last vaccination. After both the 6 and 12 months challenge the control animals developed clinical signs (fever, lethargy and anorexia) and leptospiraemia as determined by positive blood culture. In addition, both the 6 and 12 months challenge resulted in foetal death of 21% and 27% of the total number foetuses in the control groups, respectively. The vaccinates showed a statistically significant reduction in clinical signs and leptospiraemia after both the 6 and 12 months challenge. In addition the vaccinates showed a significant reduction in foetal death to 3% and 2% of the total number of foetuses, respectively.
The vaccine was further tested under field conditions on a Portuguese farm with a history of increasing abortion rate associated with a *Leptospira* serovar Pomona infection (confirmed by PCR and serology). This study was designed as an observational-longitudinal field study. At the start of the study, all breeding sows and replacement gilts on the farm were vaccinated twice with Porcilis® Ery+Parvo+Lepto with an interval of 4 weeks. The animals were re-vaccinated in the second week of every following lactation. New replacement gilts were vaccinated using the same schedule. After vaccination a rapid and statistically significant reduction in abortion rate was manifested, from 12.6% in winter months Dec 2011- March 2012 down to 0.5% in winter months Dec 2012-March 2013, a decrease of 96% compared to the previous year. The total number of abortions on the farm decreased from 55 in 2012 to 6 in 2013. The trend of having a low number of abortions at the farm was stable after the vaccination program started and in the period Dec 2013-April 2014 the frequency of abortions was still low (0.6%).
In conclusion, the present studies demonstrate that the octavalent Porcilis® Ery+Parvo+Lepto vaccine can be safely used in gilts and sows and induces statistically significant protection, for the duration of at least one year, against serovar Pomona induced clinical signs, leptospiraemia and foetal death. Protection against Pomona associated reproductive failure was confirmed under field conditions where a significant reduction in abortion rate was observed.

Leptospirosis severity association with the humoral immune response

Roshan Niloofa[1], Narmada Fernando[1], Lilani Karunanayake[2], H Janaka de Silva[3], Sunil Premawansa[4], Senaka Rajapakse[5], Shiroma Handunnetti[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka
[2]Department of Microbiology, Medical Research Institute, Sri Lanka
[3]Faculty of Medicine, University of Kelaniya, Sri Lanka
[4]Faculty of Science, University of Colombo, Sri Lanka
[5]Tropical Medicine Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka

Leptospirosis patients present with a vast varying clinical manifestations ranging from mild flu like illness to complicated presentations involving multiple organs. Severe leptospirosis is characterized by jaundice, renal and pulmonary failure. Signs and symptoms of renal, hepatic and pulmonary involvement in leptospirosis appear in the immune phase of infection and specific agglutinins are detected by then. Here we studied the intensity of humoral immune response in severe leptospirosis patients (n=30) compared to mild patients (n=30). Clinically diagnosed leptospirosis patients were confirmed by microscopic agglutination test. MAT titer of ≥400 in single sample, four-fold rise or sero-conversion ≥200 in paired samples were considered as confirmed leptospirosis. Patients with any of the following were categorized as having severe leptospirosis; jaundice (bilirubin > 51.3 µmol/L), renal insufficiency (oliguria with a urine output less than 400ml per day or creatinine>133 µmol/L or blood urea >25.5 mmol/L) and other indicators of poor outcome (intensive care unit stay, initiation of dialysis, hospital stay greater than 10 days and multi-organ dysfunction). Thirty age and gender matched healthy controls were also used for this study. Acute serum from each patient was tested for Immunoglobulin M and G (IgM & IgG), using an in-house enzyme linked immune-sorbent assay (ELISA) which detects antibodies against the antigens prepared with *Leptospira biflexa* serovar Patoc strain Patoc I. The magnitude of anti-leptospiral IgM and IgG antibodies were evaluated by measuring the optical density (OD_{450}) values of 1/100 serum dilution. Cut-off OD_{450} for IgM and IgG were 0.388 and OD_{450} 0.523 respectively. Total anti-leptospiral IgM antibody prevalence in control, mild leptospirosis patints (ML) and severe leptospirosis patients (SL) were 90% and 100% respectively, whereas anti-leptospiral IgG prevalence were 30% and 63.3% respectively. ML patients showed significantly lower anti-leptospiral IgM and IgG antibody prevalence (chi-square test, $p < 0.001$) than that of SL patients. The magnitude of anti-leptospiral IgM of ML and SL patients (Mean OD_{450} 1.391 & 1.539 respectively) were significantly higher than that of control group (Mean OD_{450} 0.206; ANOVA, $P < 0.001$), but there was no significant difference between ML and SL. However, the anti-leptospiral IgG antibody levels of SL patients were significantly higher (Mean OD_{450} 0.719; ANOVA, $P < 0.001$) than that of ML patients and control group (Mean OD_{450} 0.409 & 0.257 respectively), but there was no significant difference between ML patients and control group. Anti-leptospiral IgM and IgG antibodies were not dependent on age, gender, number of days between on-set of symptoms and sampling and past infection. Patients with severe leptospirosis showed higher anti-leptospiral IgG antibodies. These results suggest that there may be an association with IgG antibody response and disease severity.

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Corresponding author: Miss Roshan Niloofa, e-mail: rosh86niloo@gmail.com

A mouse model of Leptospire infection designed for the development of anti-shedding drugs or vaccines

Ana Vieira, Rita Melo, Luciana Richer and Maria Gomes-Solecki*
University of Tennessee Health Sciences Center

Development of transmission blocking vaccines to prevent bacterial shedding is currently hindered due to the lack of a good rodent model to assess vaccine efficacy within Leptospires' enzootic cycle paradigm. We pursued the C3H-HeJ mouse model given that it is susceptible to Leptospiral infection and it is deficient in TLR4 making it an attractive tool to further dissect immunological mechanisms of protection of putative candidates. In the study reported here we infected C3H-HeJ mice intraperitoneally with *Leptospira interrogans* serovar Copenhageni strain L1-130 and evaluated parameters such as physical symptoms of disease, kidney function, weight loss, and leptospiral burden in different target tissues (blood, kidney and urine) over a period of 15 days post infection (dpi) by dark field microscopy and by quantitative PCR. Infection of mice at 12 weeks with 10^7 Leptospires resulted in impairment of physical capability as well as kidney function as measured by observation of ruffled coats, lethargy and a low output of urine from dpi2 to dpi4; detection of ~50 Leptospires in blood on dpi2 and dpi4; weight differences ranging from 4% to 8% loss of body weight from dpi2 to dpi13; shedding of ~1500 Leptospires from dpi8 to dpi15; and presence of ~3000 Leptospires in kidney at termination on dpi15. Mice infected with 10^7 Leptospires at 10 weeks of age shed about 1000-7000 Leptospires in urine from dpi5 to dpi15; and mice infected at 14 weeks of age shed negligible amounts of Leptospires within the same time frame. Our data suggests that an initial acute phase of infection (between dpi2 and dpi4) which is characterized by a decline of overall health quantified by the presence of Leptospires in blood and by impairment of kidney function, is subsequently followed by recovery of physical symptoms around dpi5 to dpi7 and by colonization of the kidneys and shedding between dpi8 to dpi15. Our mouse model of infection produced a solid read out against which to test vaccine candidates that prevent shedding of pathogenic Leptospires in urine.

Superoxide production bydifferentiated HL-60 cells interacted with *Leptospira*

Narmada Fernando[1], RoshanNiloofa[1], LilaniKarunanayake[2], H Janaka De Silva[3], Senaka Rajapakse[4], Sunil Premawansa[5], ShiromaHandunnetti[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka

[2]Department of Microbiology, Medical Research Institute, Sri Lanka

[3]Faculty of Medicine, University of Kelaniya, Sri Lanka

[4]Tropical Medicine Research Unit, Faculty of Medicine, University of Colombo, Sri Lanka

[5]Faculty of Science, University of Colombo, Sri Lanka

NADPH oxidase in professional phagocytes catalyses the respiratory burst and produces superoxide free radicals which generates microbicidal reactive oxygen species (OH^-, H_2O_2 etc.). Pathogenic *Leptospira* bacteria are known to be capable of withstanding high ROS concentrations in macrophages over saprophytic serovars. Knowledge about superoxide production of human neutrophils in response to *Leptospira* pathogens is extremely limited. Therefore, the aim of this study was to evaluate the superoxide production of neutrophils exposed to saprophytic and pathogenic *Leptospira* in the presence of serum of leptospirosis patients. HL-60 cells were treated with 1.25 % dimethyl sulfoxide for 6 days under standard conditions to differentiate into neutrophils in Iscove's Modified Dulbecco's Medium with 20 % FBS. *Leptospira biflexa* serovar Patoc 1 and *L. interragans* serovar Pyrogenes were grown in Ellinghausen-McCullough-Johnson-Harris media containing 10 % rabbit sera. For the quantitative nitrobluetetrazolium (NBT) assay, neutrophils were seeded at 5 x 10^6/ mL concentration in antibiotic free medium. NBT at 2 mg/ mL, bacteria 5 x 10^6/ mL and 10 % test sera were added and incubated. Sera from laboratory confirmed (via microscopic agglutination test) leptospirosis patients clinically categorized as severe and mild (SL and ML; n=10/group) and age and gender matched healthy individuals (HC; n=10) were tested. Neutrophils were washed, fixed with methanol and intracellular formazan was dissolved using KOH and DMSO and OD_{620} was measured. Neutrophils incubated with *L. interrogans* had produced a significantly high level of superoxide compared to *L. biflexa* (402.94 and 291.83 pM respectively; p = 0.001). Incubation with patient and HC sera had no significant effect on superoxide production. The quantitative NBT assay was again carried out using *L. biflexa* were separately opsonized with acute patient serum samples which contained only IgM antibodies against *L. biflexa* (SL and ML, n=6/ group) and their convalescent sera containing both IgM and IgG. Results revealed that superoxide production was similar in neutrophils incubated with pre-opsonized *L. biflexa* using both acute and convalescent sera. These results indicate that IgM and IgG antibodies that cross react with saprophytic *Leptospira* have no effect on ROS production by neutrophils. However, interaction with the *L. interrogans* Serovar Pyrogenes induces a higher level of innate immune response by human neutrophils compared that by the saprophytic, *L. biflexa* Serovar Patoc 1.

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Corresponding Author's name : Narmada Fernando

Email address : narfernando@gmail.com

POSTER PRESENTATIONS Thursday 16 April
Pathophysiology and clinical aspects

Intracranial hemorrhage during leptospirosis: a case report

Zhenisa Hysenaj, Edmond Puca, Arben Pilaca, Gentian Stroni, Elda Qyra, Dhimiter Kraja
Service of Infection Diseases, University Hospital Center, Tirana, Albania

Leptospirosis is a zoonosis with a broad spectrum of clinical manifestations. Intracranial hemorrhage is a rare complication of it.

Aim: To present a rare case of leptospirosis complicated with intracranial hemorrhage.

Material and methods: A female diabetic patient, 65 years old presented in hospital with: fever, myalgia, arthralgia and echimosis in the upper limbs. On admission, patient was conscious (Glasgow coma scale 14/15), pale and icteric. He had a heart rate of 120 beats/min, blood pressure of 90/50 mmHg, and respiratory rate of 24 breaths/min. In laboratory examinations she resulted with: WBC 12400/mm^3, erythrocytes count 3.25x10^6/mm^3, platelet counts 27000/mm^3, glicemia 355 mg/dl, urea 191.5 (range 10-43 mg/dl), creatinemia 3.13 (range 0.6-1.2 mg/dl), total bilirubinemia 36.9 (range 0.3-1.2 mg/dl), ALT 84 (range 0-45IU), AST 108 (range 0-35IU), CPK 983 (range 0-171), INR 1.3 and protrombine time 75%. Based on clinical and laboratory examinations, epidemiologic data we suspected her for leptospirosis. Serological examination resulted positive IgM for leptospirosis. During hospitalization, her hemodynamics, liver and renal functions started improving, but she complicated with left hemiparesis.

Results: On MRI examination resulted with frontoparietal dextral hematoma with mild perilesional edema with no midline. Both neurological and neurosurgical opinion was in favor of conservative management.

Conclusions: Leptospirosis has protean manifestations and rare and unusual presentations should be kept in mind in relevant epidemiological scenario. Severe leptospirosis can cause generalized endothelial dysfunction. Endothelial dysfunction initiates bleeding manifestation especially with thrombocytopenia or platelet functional abnormalities.

Corresponding and presenting author
Edmond Puca
Email address: Edmond_puca@yahoo.com

An overview of leptospirosis in Albania

Edmond Puca, Arben Pilaca, Gentian Stroni, Elda Qyra, Pellumb Pipero, Arjan Harxhi, Najada Como, Tritan Kalo, Entela Kolovani, Ergys Ramosaco, Klodiana Shkurti, Shkelqim Kurti, Arben Ndreu, Zhenisa Hysenaj, Gjoke Tosku, Dhimiter Kraja.
Service of Infection Diseases, University Hospital Center "Mother Teresa", Tirana, Albania.

Leptospirosis is a zoonotic disease caused by organisms of the genus *Leptospira*. A broad spectrum of clinical manifestations may occur in humans.

Aim: To give same data of leptospirosis in Albania during a six period time of investigation from January 2009- December 2014.
Methods: We study cases with leptospirosis in our clinic UHC "Mother Teresa" which is the only tertiary center in Albania and mostly of cases are treated in our hospital. All cases were confirmed serologically with ELISA (enzyme-linked immunoassay) positive for IgM antibodies.
Results: This study investigated retrospectively 107 consecutive patients with serologically confirmed leptospirosis admitted to our hospital during the period January 2009- December 2014. They were 89.7% males (96) and 10.2% females (11). Mean age at the time of diagnoses were 43.7 years old with 17,8±DS range 17-78. The time from the first symptoms until they presented to hospital were 6.8 days. Sign and symptoms were: myalgia 88.7%, fever with average 38.2°C in 86% of cases; fatigue 84.1%, headache 71%, jaundice in 67.2%, vomiting and abdominal disturbations in 56%, respiratory signs in 29.9%, renal insufficiency in 31.7% of cases. Laboratory results included: hyperleukocytosis in 66.3% of cases, thrombocytopenia in 42.9%. Hyperbilirubinaemia were present in 71.9% of cases with an average level 7.48 mg/dl, hepatic abnormalities (85.9%): AST (aspartate aminotrasferase) were elevated in 75.7% of cases, ALT(alanine aminotransferase) were elevated in 79.4% of cases and renal abnormalities: elevated serum creatinine 38.3%, proteinuria in 73.8% of cases, haematuria in 30.8% of cases, leukocyturia in 42% of cases. All of patients received antibiotics.

Conclusions: There was a predominance of the males in ratio 8.7:1.The disease was more frequent during summer-autumn. Leptospirosis is a multiorgans disease. The study confirmed the variable clinical and biological symptoms of leptospirosis, and indicated that hepatic and renal abnormalities are common feature of leptospirosis.
Keywords: leptospirosis, symptoms, hepatorenal.

Correspondending and presenting author
Edmond Puca,
Email edmond_puca@yahoo.com

Mortality during leptospirosis

Elda Qyra, Edmond Puca, Arben Pilaca, Ermira Muca, Gentian Stroni, Zhenisa Hysenaj, Arjan Harxhi, Dhimiter Kraja,
Service of Infection Diseases, University Hospital Center "Mother Teresa", Tirana, Albania.

The severe form of leptospirosis (M. Weill) is quite e life-threatening disease. It can cause multiorgan damage and hepato-renal failure is the most common clinical feature. Case-fatality rates in different parts of the world have been reported to range from < 5% to 70%.

Aim: To determine the risk factors of death in severe leptospirosis.

Material and methods. During a six years period time from January 2009 to December 2014, 107 patients with clinically leptospirosis and serologically confirmed were recruited in this study,

Results: In our study we had 107 patients, they almost presented with clinical features like jaundice, oligo-anuri, fever, myalgia, dyspnea, or presence of hemorrhage. Most of them were male. The mortality resulted 6.54%. All of them were males. In all these patients we noticed the impairment of hepatobiliar, pulmonary, renal and hematologic system.
The risk factor that led to death were: acute renal failure in 5 patients, pulmonary hemorrhage and respiratory distress in 6 patients, multiorgan failure in 7 patients, hemorrhage 2 patients.

Conclusions: Many patients with severe leptospirosis will have complications on admission. The presence of oliguria, hypotension, hemorrhage and pulmonary involvement are risk factors. Involvement of pulmonary system is prognostic factor for case fatality especially when it's companied from renal failure and anuria.

Corresponding author Elda Qyra,
eldaqyra@yahoo.com

A DOMINANT OF LEPTOSPIRA NAAM SEROVAR ASSOCIATED WITH AN OUTBREAK OF HUMAN LEPTOSPIRA IN EAST JAVA INDONESIA

Purwati

Tropical and Infectious Diseases Division - Department of Internal Medicine – Fac. of Med.- Airlangga University – Dr. Soetomo Teaching Hospital. Surabaya – East Java – Indonesia

Leptospirosis is the world's most widely distributed zoonosis, yet it is also one of the most neglected zoonotic infectious diseases (Adler & de la Pena Moctezuma, 2010). The incidence of leptospirosis in tropical countries wet 5-20 / 100.000 population per year the World Health Organization (World Health Oraganization / WHO) noted, cases of Leptospirosis in subtropical climates estimated at 0.1-1 per 100.000 persons per year, whereas in the tropics case this increases to more than 10 per 100.000 people each year. At the time of the outbreak, a total of more than 100 people from high-risk groups among the 100.000 people may be infected. In Indonesia, Leptospirosis is spread among others in the province of West Java, Central Java, Yogyakarta, Lampung, South Sumatra, Bengkulu, Riau, West Sumatra, North Sumatra, Bali, West Nusa Tenggara, South Sulawesi, North Sulawesi, East Kalimantan and West Kalimantan Figures Leptospirosis death in Indonesia is high, reaching 2.5 to 16.45 percent over

In our studies when outbreak in Sampang, East Java Indonesia, based on the MAT examination showed that most leptospira infecting serovar is Naam 11/36, hereinafter Copenhageni serovar much as 9/36, then 6/36 serovar shermani, sejroe, Brasilava, Tarrasovi and each serovar Lai 2/36 and Australis and Bataviae 1/36 respectively. The distribution of the most affected age group is 34-43 years of age, followed by 44-53 age group, and is at least 64-73 years age group. Outcome of leptospirosis infection is passed and the survival. Among the causes of outbreaks of leptospirosis was obtained that died most often caused by Leptospira serovar Naam with 5 mortality of 11 patients were infected, whereas the mortality shermani serovar 2 of 6 patients infected with serovar Lai and mortality of 2 from 2 infected patients. Leptospira serovar Brasilava setovar and Tarrasovi not obtained mortality

In an outbreak of leptospirosis with PH on the Andaman Islands, notably flanking the Malaysian coast near Langkawi, a "Lai-like" strain was isolated, but from a patient without PH. The antigenic profile of this strain, determined by mAbs, is closest to the strain from our patient. Consistent with this finding, DNA analysis revealed that the strain from our patient belongs to *L. interrogans* sensu stricto and represents a newly recognized type of serovar Lai. Therefore, we designate this strain as *L. interrogans*, serogroup Icterohaemorrhagiae, serovar Lai, type Langkawi, strain "Langkawi". We suspect that this variant of Lai is common in Southeast Asia (Jiri, et.al., 2005)

POSTER PRESENTATIONS Friday 17 April
Epidemiology and ecology

Epidemiological overview of human leptospirosis in Albania

Luljeta Alla,[1] Edmond Puca[2], Artan Mesi[2], Silva Bino [1].

1) Institute of Public Health, Tirana, Albania
2) Service of Infection Diseases, University Hospital Centre, "Mother Tereza", Tirana, Albania

Introduction: Leptospirosis is a zoonotic disease caused by bacteria of the genus *Leptospira*. It is most commonly spread via water contaminated with urine from infected animals, but contaminated food or soil can also act as vehicles for the disease. Leptospirosis is an endemic diseases in Albania.
Aim: To give an epidemiological overview of human Leptospirosis in Albania during the period 2010-2013.
Material and methods: Epidemiological data according to person-place-time were collected from individual forms reported through passive surveillance and active surveillance including occupation exposure to animals and laboratory investigation.
Results: A total of 75 cases to leptospirosis were reported to Institute of Public Health during the study period. The incidence of Leptospirosis varied from 0.6 to 0.4 cases/100.000 population. The most affected agegroup were 45-59 years and 60+ years old. According to profession most affected were farmers and other with 61.8% of the reported cases. The most used serological diagnostic test is ELISA IgM and IgG.
Men were more affected than females, due to occupational character of the disease.
The disease is more frequent in spring and summer and in middle and north part of the country. 79% (95%CI 61.1-89.8%) of the cases were laboratory confirmed.
Conclusions: Despite the existence of the vaccination program in animals, vaccination of heads is sporadic though the prevalence of infection in animals shows a decreasing trend. The use of protective measures during occupational exposure and prevention of flooding is essential in reducing the spread of infection of leptospirosis in humans.

Keywords: leptospirosis, epidemiology, zoonosis, flood

Correspondendin and presentin author
Edmond Puca
Email edmond_puca@yahoo.com

From typing to genomic epidemiology in *Leptospira*

P. Ruybal, V. Varni, K.Caimi*

Leptospirosis is one of the most common and widespread zoonotic disease worldwide with nearly 900,000 people infected annually. The disease is caused by more than 250 different serovars (sv) of the genus *Leptospira*. Earlier phylogenetic analyses have classified the genus into 3 distinct lineages that include pathogenic, intermediate and saprophytic species. The introduction of Multilocus Sequence typing (MLST) for strain identification provided the first sequence-based approach to strain resolution, and established the promise of unraveling the phylogeny of *Leptospira*, although in studies of limited strain panels or strains with restricted geographic prevalence. Regarding this, a pan-*Leptospira* genome project has provided data of very fine-scale resolution enabling the the study of the evolutionary history of this genus. For these reasons, the main objective of this study was to apply a 7 loci MLST scheme previously reassessed by our group to an extended group of strains of worldwide distribution which genome sequences are now available. A total of 403 strains were analyzed in this work. The collection includes strains from the *Leptospira* genome project and strains used in our previous work. All the sequences were analyzed by MLST 1.7 web application selecting *Leptospira* spp. #2 configuration which correspond to *Leptospira* R7L MLST scheme database. Allelic profiles and sequence types (STs) of genome sequences were assigned by MLSTest software. GoeBURST algorithm was applied to determine the relationships between STs. Intermediate species (*L. licerasiae* and *L. fainei*) that showed a complete hit in the genome sequences for all loci, were included in the subsequent analysis. The application of R7L scheme to the whole collection produced 199 different STs. GoeBURST analysis showed that the six pathogenic species are confined within 14 different clonal complexes (CC) and that there is no coexistence of different species within the same CC. We have also observed that the correlation among this larger set of isolates fits our previous observation, where founder genotypes contained isolates belonging to a single (or at most two) serogroup but not inversely.

This work constitutes a starting point in the attempt to generate a deeper phylogenetic and epidemiological scenario of *Leptospira* genus.

*corresponding author; e-mail: caimi.karina@inta.gob.ar

Human Leptospirosis in Portugal: a retrospective study of ten years (2004-2013)

Teresa Carreira, Mónica Nunes, Maria Luísa Vieira

Group of Leptospirosis and Lyme Borreliosis, Unit of Medical Microbiology, Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT) Universidade Nova de Lisboa (UNL), Lisbon, Portugal

Leptospirosis is a zoonotic and recognized neglected disease that represents a public health problem affecting both industrialized and developing countries. About 500 000/year severe cases occur worldwide with a mortality rate of 5-20%, being 588 cases reported in 25 European countries with an overall incidence of 0.13/100 000 inhabitants, in 2010. Although the disease does not have a great expression in Europe, in Portugal (Azores Archipelago) between 1993 and 2003, the incidence rate was 11.1/100 000 inhabitants. This value is ten times higher than the incidence verified, for the same period, in mainland Portugal. In the country, leptospirosis is a notifiable disease since 1950. However, the under diagnosis persists due to the marked clinical polymorphism, the difficulties of the serological diagnosis and the lack appropriate reporting systems. The purpose of this retrospective study was to summarize an update of leptospirosis in Portugal [mainland and Azorean islands (São Miguel and Terceira)] and compare the incidence, clinical symptoms, prevalent serovars, and occupational risk of infection, with the study done in 2003 by our laboratory. As one of the two reference leptospirosis laboratories at Portugal, between 2004-2013, we describe a series of 2,314 symptomatic patients for whom a routine laboratory diagnosis by microscopic agglutination test (MAT) was performed, along with other approaches, representing a unique picture of human leptospirosis in Portugal (mainland and Azorean islands). From the 2,314 patients (1,642 men and 672 women) with clinical suspicion of leptospirosis, a total of 3,054 serum samples were obtained, being 64% from the mainland and 36% from Azorean islands. A positive MAT result was obtained for 398 patients (average of 40 cases/year), being middle-aged men adults (45-64 years) the major affected group. Icterohaemorrhagiae and Ballum presumptive serogroups were the most prevalent with 19% (76/398) and 13% (53/398) respectively. However, other serogroups were also observed such as Pomona and Sejroe. Leptospirosis has been considered a public health problem in Portugal, particularly in Azores islands. This retrospective study reveals a marked decrease of cases; although, the annual incidence rate in Azores remains still high (9.97/100 000 population) when compared to the previous study. Nevertheless, it is important to emphasize that no fatalities have occurred during the period of this study. Concerning the mainland, the incidence rate is 0.5 cases, value slightly lower than the rate obtained in 2003, (0.7/100 000 population). The present data show that in Portugal, particularly in Azores, leptospirosis should continue to have the attention of the health authorities, despite the surveillance program underway in the region. It would be also useful, to increase the access to more sensitive diagnostic tools, such as the molecular approaches.

Corresponding Author's name: Teresa Carreira
E-mail: tcarreira@ihmt.unl.pt

Research of anti-hantavirus antibodies in suspected cases, but discarded, leptospirosis in a Collaborating Center of the World Health Organization for Leptospirosis (2008-2011) in Rio de Janeiro.

Angelica Tapia de Lima Barbosa3; Renata Carvalho de Oliveira1; Ilana Teruszkin Balassiano2 ; Juliana Magalhães Vital-Brazil2; Katia Eliane Santos Avelar2 ;Andrea Maria Assis Cabral3; Paulo Vieira Damasco3; Martha Maria Pereira2; Elba Regina Sampaio de Lemos1

1Laboratório de Hantaviroses e Rickettsioses, Fundação Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, Brazil. 2Centro Colaborador da OMS/OPS para Leptospirose e Laboratório de Referência Nacional para Leptospirose, Instituto Oswaldo Cruz / Fundação Oswaldo Cruz, Rio de Janeiro/RJ, Brasil Coleção de Leptospira, Instituto Oswaldo Cruz / Fundação Oswaldo Cruz, Rio de Janeiro/RJ, Brasil. 3Hospital Universitário Pedro Ernesto- UERJ

Introduction: Leptospirosis is an endemic zoonosis in Brazil and in the State of Rio de Janeiro (RJ) that is characterized as an acute, potentially fatal febrile infectious disease, especially in the pulmonary form. The differential diagnosis of this zoonosis with several endemic infectious diseases is required, due to its distribution in the same geographical areas, a fact which makes its diagnosis difficult and complex, especially in periods of epidemics. Among these infectious diseases, there is the hantavirus cardiopulmonary syndrome (HCPS) an emerging zoonosis restricted to the Americas, acquired from wild rodents, whose clinical spectrum is more severe and fulminant pulmonary and cardiovascular frames characterized by respiratory insufficiency with mortality 40-60 %. Whereas both zoonosis have rodents as reservoirs and present similar clinical features, mainly between the pulmonary form of leptospirosis (PFL) and HCPS, differential diagnosis is necessary in patients with acute febrile infection with respiratory manifestation coming from areas where there are species of wild rodents. Although no cases of HCPS have been reported in the state of RJ, wild rodents infected with Juquitiba virus genotype have been identified in RJ territory, whose border with the states of Minas Gerais and São Paulo, where there are cases of HCPS, reinforces the need for studies.

Material and methods: The purpose of this retrospective study was to (i) analyze the clinical and epidemiological data of samples of suspected leptospirosis forwarded to the WHO Collaborating Centre of the World Health Organization for Leptospirosis Institute Oswaldo Cruz - RJ between 2008-2011 and (ii) investigate the presence of hantavirus infection in samples from suspected cases – patients with fever and respiratory manifestation during the clinical course, but laboratory negative for leptospirosis , coming from the municipalities of the state of RJ where rodents infected with hantaviruses have been identified (iii) Contribute to the reasoning of differential diagnosis of both zoonosis and propose an epidemiological and syndromic surveillance flow in acute febrile illness with respiratory manifestation in the state of RJ.

Results: 108 suspected cases were serologically negative agglutination for leptospirosis. Males comprised the majority with 77% of cases, a predominant age group of 20-29 years and 30-39 years, positive for the presence of rodents in 28.7% of cases and in the period of greatest rainfall epidemiological history, respecting the seasonality of leptospirosis. In relation to clinical analysis, the frequency of events is: myalgia (87%), jaundice (47.2%), anuria (31.5%), oliguria (31.5%), headache (18.5%), conjunctival injection (18.5%), signs bleeding (12%) and pulmonary hemorrhage (6.5%).

Conclusion: The 108 samples tested were anti-hantavirus antibodies negative and the viral RNA was not detected. Although the results point to the absence of hantavirus infection in human specimens in this population, more studies are needed due the presence of the rodent reservoir *Oligoryzomys nigripes* infected with Juquitiba virus recently identified in regions of the RJ state.

Extremely high *Leptospira* carriage rate influenced by climate conditions

Stritof Majetic, Zrinka; Habus, Josipa; Mojcec Perko, Vesna; Vucelja, Marko; Margaletic, Josip; Staresina, Vilim; Milas, Zoran; Perharic, Matko; Turk, Nenad

Leptospirosis is classified as a typical disease of the natural foci, meaning that leptospires are maintained in nature by a large variety of animal hosts with rodents as the most important reservoirs. It is well known that a combination of various climatic, hydrologic and edaphic factors have a major impact on incidence of leptospirosis in humans and animals. However, the exact mechanisms and the contribution of individual factors are not fully understood. In order to dissect the impact of various factors, we undertook a systematic study of mouse like rodents in a limited area known as endemic focus of leptospirosis. Investigated site, Lipovljani area, is located in the close proximity of river Sava and has been systematically investigated for the last four years. Average relative abundance of rodents was calculated based on the number of animals trapped in trap snaps positioned along transect lines. Rodents were aseptically dissected and kidney tissue from each animal was cultured in Korthoff's medium and sampled for DNA extraction. Average relative abundance for the period from 2011 to 2013 ranged from 0.1 % to 16.5% with renal carriage rates ranging from 8% to 28%. However, in September 2014, during the regular annual survey, an extremely high relative abundance of rodent population (55.5%) as well as *Leptospira* isolation rate of 55.1% (38/69) were detected. Due to relatively high percentage of contaminated kidney cultures, additional PCR testing was performed on kidneys of related animals and additional 11 positive animals were detected. In total, extremely high carriage rate of 71% (49/69) was determined. To our knowledge, this is the highest rodent infection rate ever recorded in Europe.
According to percentile ranges and classification ratings, climate conditions in 2014 in the whole Croatia, including Lipovljani area, have been described as extremely warm and extremely humid with precipitation amounts higher than average in the last 30 years. High precipitation amounts and saturation of soil with water as a consequence, resulted in severe flooding in some parts of Croatia, especially in surroundings of big rivers.
Climate specificities in 2014 that were recorded throughout the year obviously had a major influence on relative abundance of rodents by increasing food availability and lowering mortality during winter period. High abundance of rodents inevitably resulted in more direct and indirect contacts between animals, which, together with prolonged survival of *Leptospira* in environment due to favorable climate conditions, increased possibility of infection within rodent population.
We can conclude that extremely high *Leptospira* carriage rate recorded in this study was a consequence of climate conditions affecting many important ecological factors that synergistically lead to increase in *Leptospira* carriage rate in reservoirs.

Prevalence of anti-*Leptospira* IgM antibodies in Sudanese febrile and non-febrile patients

Nada A. Abdelrahim[1] and Wisal M. Ibrahim [2]

1\ Research Methodology & Biostatistics Unit, Sudan Medical and Scientific Research Institute, University of Medical Sciences and Technology, Khartoum, Sudan

2\ Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology, Khartoum, Sudan

Background

Leptospirosis is the most widespread zoonosis in the world. The incidence is significantly higher in warm climate conditions. In Sudan, leptospirosis remains an unknown health problem. Further, leptospirosis, as a febrile illness, might be mistaken for other infectious diseases that are well known and most common in Sudan. Undifferentiated human febrile illness is a major public health problem, and awareness is growing by demonstrating the extent of over-diagnosis of malaria in parts of Africa as well as revealing the potential importance of other previously neglected causes of febrile illness.

Objectives

The current project aims at detecting the existence and prevalence of leptospirosis among febrile and non-febrile patients attending several hospitals in Khartoum by the use of a new lateral flow immunochromatographic assay.

Methodology

This is a laboratory-based descriptive study; it included febrile patients with and without a definitive diagnosis and patients under renal dialysis attending several hospitals in Khartoum city during the period of April to September 2013. Serum samples were screened qualitatively for the existence of anti-leptospiral IgM antibodies using a rapid lateral flow immunosorbent assay. The ethical clearance was obtained from the Ethical Committee Board of UMST. Data were analyzed by the statistical package program SPSS version 20.

Results

A total of 119 serum samples were collected. The overall prevalence of anti-*Leptospira* IgM antibodies among all screened samples was 7%. The prevalence among the 57 with end stage renal disease was 9%, and among the 47 with fever of unknown origin was 6%. The prevalence among the 15 febrile patients with a definitive diagnosis of malaria or typhoid was 0%. Almost all positive patients had recurring episodes of fever, are in close contact with livestock, farmers and have a natural untreated source of drinking water.

Conclusion

Leptospirosis is probably a common febrile condition in Sudan. It may be the cause of renal abnormalities in a wide group of patients. Combined infections may occur. The lateral flow immunochromatographic assay is a reliable, easy to perform and rapid serological test. It is easily available and is cost effective.

Marked increase in autochthonous leptospirosis in humans and dogs in the Netherlands, 2014

Roan Pijnacker[1], Marga G.A. Goris[2], Kitty Maassen[1], Daan W. Notermans[1], Rudy A. Hartskeerl[2], Jaap A. Wagenaar[34], Els M. Broens[3], Joke W.B. van der Giessen[1,4], Margreet J.M. te Wierik[1], Barbara Schimmer[1]

[1] Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[2] Leptospirosis Reference Centre, KIT Biomedical Research, Amsterdam, the Netherlands

[3] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands

[4] Central Veterinary Institute, Wageningen University (WUR), the Netherlands

Introduction: Leptospirosis is a zoonosis caused by pathogenic *Leptospira* and may range from a subclinical to a life-threatening infection in humans. In the Netherlands, leptospirosis is a notifiable disease in humans and is caused by two main serogroups; Icterohaemorrhagiae in rats and Grippotyphosa in voles. About 30 human cases are notified annually. Since 2005, more than half of the leptospirosis cases acquired the disease outside the Netherlands, mainly in South-East Asia. Leptospirosis has a seasonal distribution with most cases occurring in late summer and fall. In animals, only leptospirosis caused by serovar Hardjo is a notifiable disease. Leptospirosis can cause severe infections in dogs.

Materials and methods: We report national surveillance data of human leptospirosis cases in the Netherlands that are diagnostically confirmed by the National Reference Lab for Leptospirosis (NRL) with culture and/or PCR and/or serology (IgM ELISA or MAT). Dogs clinically suspected for leptospirosis are serologically confirmed by ELISA at the Veterinary Microbiological Diagnostic Center (VMDC). We describe the notified human cases in 2014 and case characteristics such as age, sex, country of infection (autochthonous/import) and type of activity (occupational/non-occupational) at point of infection compared with notified cases during 2010-2013.

Results: In 2014, 97 cases were notified. The majority (60/97; 62%) were autochthonous, representing a 4.6-fold increase compared to 2010-2013. A 1.6 fold increase (33/97; 34%) was observed in imported cases, which mainly contracted leptospirosis in Thailand (18/33; 55%). The median age of autochthonous cases was 48 years (range: 10-75 years) and 42 years (range: 13-64 years) in imported cases. Most autochthonous (82%) and imported cases (85%) were male. Autochthonous cases mainly acquired leptospirosis during recreational activities (40/60; 67%) such as swimming and fishing. The median diagnostic delay was 15 days (range 3-50) in autochthonous cases and 12 days (range 1-49) in imported cases. The VMDC reported 13 dogs diagnosed with leptospirosis in 2014 compared to 2 to 6 dogs annually during 2010-2013.

Conclusion: A marked increase in autochthonous leptospirosis was observed in the Netherlands in 2014. A possible explanation for this increase is the preceding mild winter of 2013-2014 followed by the warmest year in three centuries, enabling rodents and possibly *Leptospira* to better survive. More awareness and early recognition of this rodent-borne zoonosis by medical and veterinary specialists is warranted.

Presenting author: Roan Pijnacker
Email address: Roan.Pijnacker@rivm.nl

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Diagnostics and molecular aspects

Reduced MLST scheme optimized for direct application on *Leptospira* spp. human clinical samples

V. Varni, Y. Chiani, A. Nagel, P. Ruybal, N. B.Vanasco, K. Caimi*

Leptospirosis is an infectious disease caused by different species of the genus *Leptospira* and is a zoonosis worldwide distributed. The traditional classification of strains of *Leptospira* spp. is based on serological methods having high complexity in implementation and outcome. Currently, alternative molecular methods for typing isolates have arisen. Among them, multilocus sequence typing (MLST) allows genotyping pathogenic *Leptospira* being one of its main advantages the overall comparison of isolates through their allelic profile.

Recently, our group conducted a reassessment of such methods by which an optimized scheme of 7 loci was described. Because obtaining isolates is extremely difficult, particularly for human samples, the aim of this study was to adapt this scheme for direct application to clinical samples, increasing the number of samples analyzed and enable better epidemiological surveillance of this zoonosis.

Serum samples and DNA extracted from sera from patients with positive serology or positive LipL32-qPCR were used; the samples were characterized to species level by 16S-rRNA PCR. From our reassessed 7 loci scheme (R7L), the 3 more discriminating loci (MLST reduced) were select for direct amplification on clinical samples. The typing scheme proficiency was studied from sequences previously used in generating the complete scheme. Three loci were selected and its concatenated sequences were applied for phylogenetic analysis (Maximum Likelihood, MEGA6).

Phylogenetic analysis with previous sequences confirmed that the reduced scheme matches the whole scheme in the clustering of isolates of the same species; it also allows to recognize sub-groupings of isolates within each specific cluster, validating its potential for typing.

A total of 61 clinical samples were studied. Thirty-six could be amplified by 16S rRNA PCR. It was found that 94% belonged to the major pathogenic species: *L. interrogans*, *L. kirschneri* and *L. borgpetersenii*. Positive amplification was obtained for the MSLT reduced scheme in 4 of them. Additionally, 5 samples could be amplified with only 1 or 2 of the 3 loci. Positive samples for the three loci belong to the species *L. interrogans*. Phylogenetic analysis allowed to correlate this samples with isolates belonging to serogroups Canicola and Icterohaemorraghiae.

The results obtained here constitute an important precedent for the molecular epidemiology of *Leptospira* spp., as the development of tools for typing directly on clinical samples will significantly increase the information on circulating genotypes, and will simplify the diagnosis and epidemiological control of this disease.

*corresponding author; e-mail: caimi.karina@inta.gob.ar

Comparison of PCR and classic bacteriological methods in monitoring of leptospirosis in natural foci.

Jarekova J.[1], Bakoss S.[1], Racka K.[2], Simek B.[2], Stanko M.[3]

[1] Comenius University, Medical Faculty, Department of Epidemiology, Bratislava, Slovakia

[2] State Veterinary Institute Jihlava, Czech Republic

[3] Institute of Zoology, Slovak Academy of Sciences, Košice, Slovakia

Wild and commensal rodents are considered to be the most important reservoirs for leptospires which persist in the proximal kidney tubules of the infected reservoir hosts. The aim of this study was to compare the use of PCR with bacteriological and serological methods for detection of leptospires in animal tissues.

90 rodents including 6 species (*Microtus orvalis, Apodemus agrarius, Apodemus flavicollis, Myodes glareolus, Micromys minutes, Pitymys subterraneus*) were trapped in 9 localities in all regions of the Slovak Republic. Their serum samples were tested by a microscopic agglutination test (MAT) with a reference panel of *Leptospira* strains belonging to 18 pathogenic serovars (Copenhageni, Icterohaemorrhagiae, Grippotyphosa, Sejroe, Istrica, Saxkoebing, Polonica, Sorexjalna, Poi, Pomona, Canicola, Bratislava, Jalna, Tarassovi, Bataviae, Castellonis, Zanoni and Pyrogenes). Kidney samples were submitted for direct microscopy and culture isolation on Korthof's or EMJH medium. The same kidney samples were stored at -20^{0}C and subsequently tested by real-time PCR using SYBR Green with *sec Y* gene region targeting. This test is designed for the specific detection of pathogenic leptospires.

Serological positivity was found in 54 (60%) of the total 90 sera examined. Leptospires were detected by direct microscopy in 36 (40%) kidney samples whereas kidney cultures were positive in 40 (44.4%) cases. 52 (57.8%) samples were PCR positive.

A total concordance between positive results of PCR and at least one of the classical methods (serological and bacteriological) was obtained from samples of 52 (57.8%) animals, and negative results using all methods in 20 (22.2%). A total agreement considering all diagnostic methods was found in samples of 72 (80%) animals. It is noteworthy that 14 (15.6%) animals gave only MAT positive results while PCR was negative, presumably due to the persisting antibodies after a previous infection. On the other hand, kidney samples of 11 (12.2%) animals with MAT positive results were also PCR positive but direct microscopy and culture isolation was negative.

The latter result demonstrates that real-time PCR should be preferentially used for the direct detection of animal leptospirosis due to higher sensitivity and early diagnosis. Our results also indicate the usefulness of employment of the PCR as an additional method for detection of leptospires in natural foci of these agents, whereas cultivation remains as the "gold standard" for obtaining live strains for the further identification of *Leptospira* serovars.

Corresponding author: Karol Racka, racka@svujihlava.cz

New Public Health England national diagnostic and reference leptospirosis service

Chalker V, Harrison I, Menezes A, Opoku-Boateng A, Bailey D, Simpson A, Jameson L, Chand MA

Indigenous and imported human leptospirosis is detected in England and Wales, with 40-100 laboratory confirmed cases per year. It is likely that there are further cases which are undiagnosed, particularly those with milder manifestations. Small outbreaks are detected intermittently, the most recent being a cluster of cases associated with a triathlon in 2014. Surveillance of human cases of leptospirosis forms part of the health protection activities around major sporting events, such as the London 2012 Olympics, and recent flooding in the UK. Leptospirosis is not a statutorily notifiable disease in the UK and surveillance depends exclusively on laboratory data. In 2013, there were 47 laboratory confirmed cases, compared to 71 cases in 2012, including 2 deaths. 23 cases were UK-acquired and 24 were acquired overseas including in South East Asia, South America, South Africa and Europe. Where an exposure was identified, the majority were recreational water exposures such kayaking, fishing or swimming. The infecting serogroup was not determined in the majority of cases, but where it was identified, *L. interrogans* serogroup icterohaemorrhagiae was the most frequent. We present a **new national leptospirosis diagnostic and reference laboratory service for England and Wales**, replacing the previous Leptospirosis Reference Unit in Hereford from April 2015. The new service, provided by Public Health England at Colindale and Porton sites, will include primary serological and molecular testing, confirmatory testing and typing, culture from human specimens where possible, maintenance of a strain collection, clinical advice, input into health protection, applied research, education for health professionals and data collation for epidemiological purposes. Selection and validation of the screening ELISA has been undertaken in house, as has development of a PCR assay for use in early diagnosis of infection. We present the new service and related developmental work, including our case and specimen algorithm and our approach to assay development and evaluation.

Corresponding author
Meera Chand (meera.chand@phe.gov.uk)

Enumeration of *Leptospira* using flow cytometry

Célia FONTANA[1,2], Steve CRUSSARD[1], Nathalie SIMON-DUFAY[1], Natalia BOMCHIL[1] and Jean REYES[1]
1 MERIAL S.A.S., R&D, 254 rue Marcel Mérieux, 69007 Lyon, France, **2** INSTITUT PASTEUR, Unité de Biologie des Spirochètes, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.

Enumeration of *Leptospira*, the causative agent of leptospirosis, is arduous mainly because of its slow growth rate. Rapid and reliable tools for numbering leptospires are still lacking. The current standard is the count on Petroff-Hausser chamber under dark-field microscopy, but this method remains time-consuming, requires a well-trained operator and lacks reproducibility. Here we present the development of a fast, sensitive and reproducible flow-cytometry based counting technique for leptospires. We show that it is well correlated to Petroff-Hausser counting and allows to assay bacterial viability. Flow cytometry is therefore a promising technology to replace current enumeration techniques of *Leptospira*.

Corresponding author : Jean REYES, jean.reyes@merial.com

Development and validation of an IgM ELISA for early diagnosis of human leptospirosis

Vanasco NB; Jacob P; Landolt N; Chiani Y; Schmeling MF; Cudos C; Tarabla, H, Lottersberger J.

ELISA tests may play a key role for early case detection and treatment in developing countries. The aim of this study was to develop and validate an IgM ELISA under field conditions. Six hundred and nineteen patients (n=1339 serum and blood samples) were selected by simple random sampling from N=5773 patients referred to the leptospirosis national reference laboratory in Santa Fe, Argentina from 2004 to 2014. Confirmed leptospirosis cases (MAT ≥1/400, ≥four-fold MAT titer rise or positive culture) were compared to discarded cases of leptospirosis (MAT <1/50), confirmed cases of diseases other than leptospirosis (Argentinean Haemorrhagic Fever, Hantavirus, dengue, flu or psittacosis, n=148) and healthy blood donors from endemic areas (n=154). Samples were collected at acute (<10 days post onset -DPO- of symptoms) and convalescence phases of illness (≥10 DPO). IgM ELISA was performed using a sonicated antigen prepared from cultures of the serovar Hardjo (strain Hardjoprajitno) and coated on polystyrene plates. The MAT was performed with a panel of live leptospires that included of 12 live reference strains used as antigens which represented the following 9 serogroups: Ballum (strain Castellòn 3), Canicola (Hond Utrecht IV), Pomona (Pomona), Grippotyphosa (Moska V), Icterohaemorrhagiae (RGA and M20), Pyrogenes (Salinem), Tarassovi (Perepeletsin), Sejroe (3705 and Hardjoprajitno), Bataviae (Swart) and Patoc (Patoc 1). Both tests were performed using a blind design. Data was de-identified and were no attributable to individual patients. Data analysis included ROC curve and Younden's index (J). The area under the curve (AUC) for IgM ELISA was 0.940 and J= 0.849. Overall specificity (Sp) was 97.0% (95.6; 98.1). Sp increased from 95.1% (92.8; 96.8) in suspected cases without leptospirosis to 99.4% (96.4; 100) in healthy blood donors and 100% in patients with diseases other than leptospirosis. While sensitivity (Se) was higher in convalescence (98.4%) than in the acute phase (76.6 %), Sp remained high in both phases (96.9 and 94.9%, respectively). This rise in Se is consistent with the highest peak of IgM antibodies that is reached at approximately 15 DPO, and may explain its increase from 0.892 (0.859; 0.919) during the acute phase to 0.986 (0.972; 0.994) during convalescence phase. Leptospirosis is frequently not recognized, underreported, and hence neglected. MAT can confirm the disease at a late acute phase when antibiotic treatment is already less effective. Therefore, there is a strong global need for methods that can confirm leptospirosis at an early acute phase. Moreover, the excellent Sp and high Se may support the view for using IgM ELISA as confirmatory test.

Norma Bibiana Vanasco
E-mail: nbvanasco@gmail.com

Comparison of two PCR, IgM and IgG ELISA for early diagnosis of human leptospirosis

Vanasco NB; Jacob P; Landolt N; Chiani Y; Schmeling MF; Cudos C; Tarabla, H, Lottersberger J.

Real time PCR (rtPCR), conventional PCR (cPCR), IgM and IgG ELISA have been proposed for diagnosing leptospirosis in its early stages, in that order. The aim of this study was to compare the diagnostic accuracy among IgG, IgM ELISAs, cPCR and rtPCR during the acute phase of the disease. *Leptospira* DNA was extracted with QIAamp DNA Blood mini extraction kit, rtPCR and cPCR were performed using LipL32-45F and LipL32-286R primers. All techniques were performed using a blind design. Data was de-identified and were no attributable to individual patients. Case definitions were as the following four groups: confirmed cases (MAT ≥1/400, ≥four-fold MAT titer rise or positive culture) were compared to discarded cases of leptospirosis (MAT <1/50), confirmed cases of other diseases (Argentinean Hemorrhagic Fever, Hantavirus, dengue, flu or psittacosis) and healthy blood donors from endemic areas. Acute phase samples (n=232) were collected and stratified into three groups according to the number of days post onset (DPO) of symptoms (0-3, 4-6 and 7-9 days). IgG and IgM ELISA were performed as presented in a companion communication. MAT was performed with a panel of 12 strains (nine serogroups) of live leptospires. Data analysis included diagnostic sensitivity (Se), specificity (Sp) and Younden´s index (J). Specificity (Sp) was high: rtPCR (97.6%, 93.2; 99.5), IgM ELISA (96.1%, 91.0; 98.7) and rtPCR (91.3%, 85.0; 95.6), with the only exception of IgG ELISA (76.0%, 68.0; 83.5). IgM ELISA showed the highest overall Se (63.8%), followed by rtPCR (43.8%), IgG ELISA (30.5%) and cPCR (23.8%). As DPO increased Se diminished on both PCRs and increased on both serological methods (particularly IgM ELISA). During the first 0-3 DPO, rtPCR (48.6%) and IgM ELISA (45.7%) showed the highest Se. IgM ELISA showed the greatest diagnostic accuracy at 1-9 DPO (J=0,6) followed by rtPCR (J=0,4), cPCR (J=0,2) and IgG ELISA (J=0,1). Our recent studies had already shown that IgM ELISA was seven times more efficient than IgG ELISA. Low Se on PCRs may be attributed to low/short leptospiremia, the administration of antibiotics, long sample storage period or the presence of PCR inhibitors. Memory bias of patients could mislead DPO estimates. Our results support the use of IgM ELISA and rtPCR for early diagnoses of the disease. However, tests have to be validated on the population that will be applied, and final diagnosis compiles not only leptospirosis test results, but also other laboratory findings and clinical and epidemiological data.

Norma Bibiana Vanasco
E-mail: nbvanasco@gmail.com

Evaluation of LipL32 real-time PCR for diagnosis of leptospirosis

Podgoršek Daša[1], Ružić-Sabljić Eva[1], Cerar Tjaša [1],

[1]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana

Introduction: Leptospirosis is a febrile worldwide zoonosis caused by pathogenic strains of *Leptospiro*. Clinical presentation in humans varies considerably, consequently making the diagnosis difficult. Performance of the reference serological test is the microscopic agglutination test (MAT), which performance and interpretation requires significant expertise. Additionally, specific antibodies can only be detectable 1 or more weeks after the onset of symptoms. Molecular methods can aid to faster diagnosis detecting leptospiral DNA in first days of infection. The aim of this study was to evaluate real-time PCR targeting *lipL32* gene which encodes an outer membrane lipoprotein and is specific for pathogenic *Leptospira* spp.

Materials and methods: In the present study 147 blood samples, 7 cerebrospinal fluids and 97 urine samples from 251 patients with clinical manifestations suggestive for leptospirosis were tested with three classical PCR (one step LeptoA/LeptoB and L3/L4 and nested L4/Lpato2) from which single result was combined and real-time PCR (RT-PCR) targeting *lipL32* gene. Patients were observed in different hospitals from Slovenia. All samples were collected at the first patient's visit to the hospital and referred to the Institute of microbiology and immunology in Ljubljana from 2011-2014.

Results: Three classical PCR gave 10/251 (4 %) samples were positive while PCR targeting *lipL32* gene gave 11/251 (4.4 %) positive results. Tests gave concordant results in 96,4 %. Four positive samples with classical PCRs that were negative with RT-PCR originated from urine samples and were subjected to sequence analysis that showed identity to *Atopobium vaginae* suggesting false positive results with classical PCR. Using RT-PCR targeting *lipL32* gene leptospiral DNA was detected in additional 5 samples, which were negative using classical PCRs.

Conclusion: Comparing two PCR methods, RT-PCR targeting *lipL32* gene was faster, more sensitive and specific for determination of leptospiral DNA in clinical samples.

DIAGNOSTIC PERFORMANCE OF TWO RAPID DIAGNOSTIC TESTS FOR LEPTOSPIROSIS

M. Hussein Gasem, Setyo G Pramudo, Nur Farhanah, A. Dainuri, B. Isbandrio[*)]
Department of Medicine, Division of Infectious Diseases, Tropical Medicine and Immunology, and
[*)] Department of Microbiology, Faculty of Medicine, Diponegoro Univwerity, Dr Kariadi Hospital, Semarang, Indonesia

Introduction

Clinical manifestations of leptospirosis vary from mild, non-specific to severe and fatal illness. Early diagnosis is very important to manage the patient. MAT (Microscopic Agglutination Test) is the gold standard for diagnosis of leptospirosis requires complicated procedures and skilled personnel, need a paired sera samples, and the tests is available in limited number of laboratories. Two serologic rapid diagnostic tests (RDTs) for leptospirosis based on immunochromatographic method (also known as lateral flow) have recently developed. The first RDT is SD Leptospira IgM (SD), and the second is PB Leptospirosis Test (PB).

Materials and Methods

A cross-sectional study for diagnostic test evaluation was conducted from January 2010 - September 2012 in Dr Kariadi Hospital, Semarang, Indonesia. Subjects were patients fulfiled the criteria of suspect and probable leptospirosis (WHO - SEARO 2009) and confirmed by MAT using 31 strains. The positivity of the RDTs was read by 2 independent examiners to know the kappa index of agreement.

Results

From 145 samples, 114 (78.6%) were tested positive for MAT . Of these samples, 79 were positive to SD test, and 82 samples to PB test. The diagnostic performance of the two RDTs were as follows: the sensitivity, specificity, positive and negative predictive values of SD test were 69.3%, 80.6%, 92.2%, and 41.7%, respectively. The sensitivity, specificity, positive and negative predictive values of PB test were 71.3%, 66.7%, 89.1%, and 37.7%, respectively.

The kappa index of agreement of SD was 0.82 and PB was 0.80.

Conclusions

Both RDTs have moderate diagnostic performance. When applying the test, we should consider the clinical stage of the patients and the level of endemicity of the setting.

Influence of the serovar on the limit of detection of pathogenic *Leptospira* spp. by Real time PCR

Stettler M., Brodard I., Rodriguez-Campos S.
Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Leptospirosis is caused by pathogenic serovars of the spirochaetes *Leptospira* spp. A wide range of mammal species, amphibians and reptiles can be infected. Maintenance hosts infected with host adapted serovars are usually clinically inapparent, but act as carriers playing an important role in the epidemiology of the disease. Despite being one of the world's most widespread zoonoses, leptospirosis is fairly neglected and, moreover, incidence rates are most probably underestimated due to the lack of rapid and reliable diagnostic tools. Serological analysis by microscopic agglutination test (MAT) remains the gold standard for diagnosis. However, for the MAT the maintenance of live *Leptospira* spp. cultures is required, it is time-intensive and cannot differentiate infection from vaccination. Hence, simple and rapid molecular analysis to screen for the presence of pathogenic *Leptospira* spp. in blood, urine and organs is useful, but the performance of existing real-time (RT) PCR assays remains controversial. In this study we assessed the limit of detection (LOD) of a TaqMan® RT PCR targeting the *lipL32* gene for 10 reference strains: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo type Prajitno, Icterohaemorrhagiae, Pomona and Pyrogenes. DNA from the cultures was extracted and the content of genome equivalents (GE) estimated assuming an average genome size of 4.6Mb. In order to generate standard curves we performed ten-fold as well as two-fold serial dilutions with resulting DNA concentrations ranging from $2.01x10^6$ GE/µL to $2.01x10^2$ GE/µL and from 10 GE/µL to 0.625 GE/µL. We found the highest LOD with serovars Bataviae and Australis ($5x10^2$ GE/reaction) and the lowest LOD with Icterohaemorrhagiae and Hardjo (0.625 GE/reaction). Comparing Ct (threshold cycle) values all over the serovars, we found a standard deviation (SD) of 3.303 [95% confidence interval (CI) (18.89-22.89)] for the lowest dilution ($5x10^6$ GE/reaction) and for the highest dilution (0.625 GE/reaction) an SD of 8.707 [95% CI (27.10-45.37)]. Our results indicate that, although the RT PCR can be a useful tool for prompt diagnosis of acute infection and identification of carriers, its performance may be largely influenced by the causative serovar. Further studies are needed to better understand the differences between the serovars of pathogenic *Leptospira* spp. and to improve the performance of diagnostic tests such as the RT PCR.

Corresponding author:
Sabrina Rodriguez-Campos sabrina.rodriguez@vetsuisse.unibe.ch

Genotyping of *Leptospira* sp. strains, circulating in Saint-Petersburg, Russia using multilocus VNTR analysis

Yu. Panferova, T. Lukyanova, N. Stoyanova, N. Tokarevich
Pasteur Institute in Saint-Petersburg

Introduction. Leptospirosis is one of the most widespread zoonosis in the world and has grat importance for North-Western Russia. Serogroups Icterohaemorrhagiae and Canicola prevail in St-Petersburg as a source of human infection last two decades, but little is known about epidemiological relations and genetic structure of local *Leptospira* sp. strains. The aim of this work is to apply VNTR analysis for molecular characterization of *Leptospira* strains isolated from leptospirosis patients in St.-Petersburg. Material and method. 33 strains of pathogenic *Leptospira* isolated from human during 1990-2013 were serologically characterized using standart microagglutination test two reference strains (Icterohaemorrhagiae - RGA680 and Canicola - Hond Utrecht IV). Genotyping was performed using five tandem repeats loci (vntr 4, 10, 11, 19 and 23) as described by Majed et al. (2005) with some modifications. Generated MLVA-types were used to construct a dendrogram. Results. All investigated *Leptospira* strains from human source and reference strains revealed two clades with minimal genetic similarity. One clade was formed by unique strain (serogroup Canicola, isolated in 2008). 32 clinical strains and reference strains formed second clade. Two MLVA-types joint strains from both serogroups. One MLVA-type (type 1) was predominant during 1990-1999 (frequency 86%), but was represented by only three strains from twelve isolated during 2000-2013 (frequency 25%). Moreover, only 4 different types revealed from strains isolated in 1990th and 8 types in 2000-2013. Discussion. VNTR-5 typing scheme showed significant genetic diversity among pathogenic *Leptospira* strains, circulating in St.-Petersburg during last two decades. The prevalence of certain types change during different periods and the frequency of rare and unique types increases during 2000th. Considering epidemiological anamnesis, this difference may arise from different infection sources for human (dogs in 1990th and principally rats in 2000th). MLVA scheme performed by 5 VNTR loci is a rapid method for *Leptospira* isolates molecular typing and can be performed if sequencing is inaccessible but in some cases it's unable to discriminate strains from different serogroup (Icterohaemorrhagiae and Canicola) and should be extended with additional loci for precision analysis.

Corresponding author: Yulia Panferova, zoonoses@mail.ru

Differentiation of *Leptospira* strains by MALDI-TOF mass spectrometry

E.V. Zueva., N.A. Stoyanova., N. K.,Tokarevich., A.A. Totolian
St. Petersburg Pasteur Institute, Saint Petersburg, Russia

The identification of bacteria by a rapid and reliable method of MALDI-TOF mass spectrometry (MS) is based on the comparison of the protein mass spectra of unknown microorganism and the spectra of known, reference bacteria from the taxonomic libraries. However, the identification of *Leptospira* strains is limited by the absence of a database containing the validated reference spectra. The study's aim was to build up a reference *Leptospira* classification model and to evaluate the use of MALDI-TOF MS for the differentiation of *Leptospira* strains within *Leptospira* genus. In the research we used ten reference strains, according to the microscopic agglutination test, that belong to *L.interrogans* (sv. Canicola, Copenhageni, Icterohaemorrhagiae, Pomona), *L.borgpetersenii* (sv. Sejroe, Ballum, Tarassovi), *L.kirschneri* (sv. Mozdoc, Grippotyphosa), *L.biflexa* (sv. Patoc) genomospecies, and ten well characterized leptospiral isolates. All strains were taken from the St. Petersburg Pasteur Institute collection. The leptospiral strains were cultured for 10 days in Terskikh medium at 28°C. The cell's extracts were prepared by ethanol/formic acid method. The solution of α-cyano-4-hydroxycinnamic acid was used as matrix. The mass spectra were acquired by "Microflex" mass spectrometer (Bruker Daltonics, Germany). The spectra from the reference strains specimens were imported into "ClinProTools 3.0" (Bruker Daltonics, Germany) software to create the classification models and the statistical analysis. The external validation of the constructed models was made by using new spectra from each reference strains. Then test spectra from *leptospira* isolates specimens were classified by the constructed model to evaluate its performance. The external validation results showed 100% correctly classified values within Sejroe, Ballum, Tarassovi, Copenhageni, Mozdoc, Grippotyphosa and Patoc classes, indicating a high predictive capability of the model in these classes. However, the entries of validation matrix indicated that 50% of spectra from Canicola and Pomona serovars were classified to Patoc class. Some spectra from Icterohaemorrhagiae serovar were classified to Copenhageni class. The test spectra of two isolates belonging to sv.Tarassovi and spectra of two isolates belonging to sv.Sejroe matched with the correct *L.borgpetersenii* genomospecies. Test spectra of two isolates of the sv.Grippotyphosa were also correctly identified to the species level, but four spectra of the isolates belonging to sv.Canicola and Pomona matched with the same score value with class Patoc (*L.biflexa*) and with classes belonging to *L. interrogans*. Our study showed that the created classification model makes it possible to differentiate strains belonging to *L.borgpetersenii* and *L.kirschneri* species.

corresponding author – Elena Zueva elenazueva9@gmail.com

A rapid test accurately detects acute canine leptospirosis

Josh Lizer, Christophe Callega
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA

Introduction: Leptospirosis is a serious global disease of dogs and humans, among other species. Current diagnostic methods are time consuming, difficult to interpret, or not immediately available at the point of care (POC). Because of the zoonotic potential and necessity to initiate treatment as early as possible in the dog, POC test methods are needed that give an immediate accurate answer. Zoetis has developed a rapid, POC lateral flow test that accurately detects canine IgM made in response to acute leptospirosis in the dog's whole blood, serum, or plasma.
Materials and Methods: Test performance was demonstrated by testing four sets of samples: 1) 48 dogs with a clinical suspicion of leptospirosis and eight dogs experimentally infected with *Leptospira* to determine sensitivity (Se) and specificity (Sp); 2) 150 samples collected from 71 dogs at different time points and vaccination conditions to test for interference from vaccination; 3) 92 laboratory dogs with no previous exposure to *Leptospira* to confirm Sp in a healthy dog; and 4) analytical specificity samples collected from dogs positive for Lyme disease [n=10], canine distemper virus [n=1], and etiologies other than leptospirosis [n=36]. The microscopic agglutination test (MAT) and a commercially available *Leptospira* IgG test (ImmunoComb®; Biogal) were used as paired reference tests. A clinical outcome was defined by the presence of clinical signs in conjunction with a MAT titer $\geq$400 and a negative IgG test or a MAT titer of $\geq$400 for multiple serovars including non-vaccine serovars and a positive IgG test. A negative clinical outcome was defined as a MAT titer <400 or a MAT titer of $\geq$400 for vaccination serovars, a positive IgG test, and vaccination within 6 months.
Results: Results are reported with Jeffrey's 95% CI included in brackets. Previously, in a separate study on different samples, the Se was 98% [0.887, 0.999]; [positive n=50] with Sp of 93.5% [0.874, 0.971]; [negative n=85], comparing only to MAT using a weighted prevalence model (data under review for publishing). With the paired reference methods (sample set one), the current preliminary study determined 91.7% Se (11/12 dogs) and 100% Sp [n=36] from the clinically suspect dogs. All eight experimentally infected dogs were positive. In sample set two, 58/58 samples [0.958, 1] were negative 21 days post-vaccination, 62/65 samples (95.4%) [0.619, 0.996] were negative 21 days post-booster vaccination, and 25/27 dogs (92.6%) [0.783, 0.984] were still negative after post-vaccination challenge. In the third and fourth sample sets, all healthy lab dogs [n=92]; [0.973, 1] and dogs tested for analytical Sp [n=47] were negative.
Conclusions: This test is highly sensitive and specific with no bias to serovar and minimal interference from vaccination. Given its accuracy, ease of use, and fast time-to-results, this test provides a solution to the POC veterinarian to quickly and confidently identify and treat cases of acute leptospirosis.

Corresponding author: Josh Lizer (Joshua.lizer@zoetis.com)

The accuracy investigation of the reported leptospirosis cases in high prevalence counties in Sichuan Province, China

Zeng Lin-zi
College of Basic and Forensic Medicine,SiChuan University,China

Abstract Objective SiChuan Province is one of the most high disease rate area of leptospirosis in China.we analysis experiment data of 2012 in three high prevalence counties in incident areas of Sichuan province, to assess the diagnosis accuracy of the reported cases and to provide a scientific basis for disease control and prevention .
Methods Sera of clinical diagnosis cases in three high incident areas in 2012 were collected and examed by MAT(antibody titer of single serum≥1:400 justfied positive, antibody titer of duble serum raise 4 times justfied positive).Pathogens were isolated from the samples and then cultured, and the strains were identified by MAT. **Results** 87 cases of leptospirosis were reported in three high prevalence counties in 2012. All cases sera were examined. Collected 64 cases of double serum, of which 54 cases were positive for antibodies against *Leptospira*, positive rate is 84.4%. A total of 23 cases single serum and 6 positive, positive rate is 26.1%. most highest rate is *Leptospira interrogans* serovar(*L*) Icterohaemorrhagiae that is 63.3%,the second rate is *L* Autumnalis that is 23.3%,the third rate is *L* Australics that is 3.3%.Cultivate 4 strains of *Leptospira*,3 of which is *L* Hebdomadis and 1 is L.Australis,Pathogens culture positive rate was 4.6%.
Conclusion The diagnosis accuracy of the reported cases in high prevalence counties in Sichuan province is high, but still need to strengthen case double sampling and detection of serum. Also continually strengthen surveillance to avoid outbreak of the rain type and flood type leptospirosis

Keywords: leptospirosis cases accuracy investigation

POSTER PRESENTATIONS Saturday 18 April

Veterinary aspects

Leptospira antibodies in wild boars (_Sus scrofa_) from Northern Portugal: risk factor analysis.

Vale-Gonçalves HM[1,2,3], Cabral JA[1,2], Faria MC[3], Nunes-Pereira M[3], Faria AS[2,3], Veloso O[3], Carreira T[4], Vieira ML[4], Paiva-Cardoso MdN[2,3,5]

1 Laboratory of Applied Ecology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

2 Centre for the Research and Technology of Agro-Environment and Biological Sciences (CITAB), Universityof Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

3 Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

4 Group of Leptospirosis and Lyme Borreliosis, Unit of Medical Microbiology, Institute of Hygiene and Tropical Medicine (IHMT), University Nova of Lisboa (UNL), Global Health and Tropical Medicine (GHTM), IHMT, UNL, Lisboa, Portugal

5 Centre for Veterinary and Animal Science (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

Leptospirosis is an emerging zoonosis of worldwide importance, caused by pathogenic spirochaetes of the *Leptospira* genus which are shed to the environment via urine of infected rodents, the main renal carriers of *Leptospira*. The wild boar (*Sus scrofa* Linnaeus, 1758), one of the most important hunting species both in Portugal as in the rest of Europe, appears to play a significant role in the epidemiological cycle of leptospirosis, as increasing evidence points it as a potential reservoir for numerous infectious diseases. One hundred and one serum samples from wild boars shot in the Trás-os-Montes region (Northern Portugal) were collected during the hunting seasons of 2011/12 and 2012/13 and tested by microscopic agglutination test (MAT) with seventeen different pathogenic serovars of *Leptospira*, in order to detect leptospiral antibodies. Sixty six serum samples (65.4%) presented antibodies against nine serovars, with Tarassovi and Altodouro showing the highest seroreactivity rates (23.8% and 16.8%, respectively), followed by Autumnalis (7.9%) and Bratislava (6.9%). Statistical analysis of the data showed that age and district of origin are risk factors for the presence of antibodies against *Leptospira*, while gender does not appear so. Considering these results, the wild boar seems to represent a potentially important source for the dissemination of leptospires for humans and animals (both wild and domestic) that share the same environments, as is the case of the Trás-os-Montes region where they continually intersect.

Corresponding author:
Maria das Neves Paiva Cardoso
maria_neves_cardoso@msn.com

LEPTOSPIROSIS IN SARDINIAN STRAY DOGS

[1]Piras A., [1]Piredda I. , [1]Palmas B. , [1]Noworol M. , [1]Falchi A. , [1]Ponti M.N.

[1]Istituto Zooprofilattico Sperimentale della Sardegna ~ Sassari ~ Italy

INTRODUCTION: Leptospirosis is a worldwide zoonosis caused by aerobic gram-negative spirochaetales belonging to the genus *Leptospira*. Classic canine leptospirosis is associated with infection induced by the serovars Canicola and Icterohaemorrhagiae, though recent researches show the importance of Bratislava, Grippotyphosa and Pomona serovars in canine infection. The aim of this report was to determine the prevalence of leptospirosis in dog population housed in Sardinia's kennels and to identify the prevalent serovars, as well as to determine the role dogs have as reservoirs of infection, which would be relevant information for efficient design of preventive measures to control human leptospirosis.

MATERIALS AND METHODS: A total of 1296 stray dogs (642 males and 654 females) randomly selected from 11 different kennels located in northern Sardinia, aged between 6 months and 18 years, were subjected to blood sampling from November 2012 to March 2014. 1114 dogs (88%) were vaccinated against leptospirosis less than a year before sampling.

Serum samples were tested for *leptospira* antibodies by Microscopic Agglutination test (MAT) with a panel of 8 *Leptospira* reference strains (sv Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Tarassovi). A titre ≥ 1:100 was considered positive. To evaluate hepatorenal parameters and seroconversion, a second blood sample of 26 seropositive dogs was drawn; 18 urine specimens were collected for bacteriological and molecular analysis.

RESULTS: The overall seroprevalence was 13 %. 154 of 1296 sera tested were found seropositive for one or more serovars. Positive cases were concentrated in dogs aged 2 to 8 years and in medium sized dogs, independent of gender. In our study, the most predominant titre was directed against serovars Icterohaemorrhagiae and Copenhageni (72%) followed by Bratislava (13%), Canicola (9%) and Grippotyphosa (6%). All urine samples were found to be bacteriologically negative, while PCR assay confirmed the presence of leptospiral DNA in one urine sample.

CONCLUSIONS: Our study confirms that this disease is widespread in dogs housed in kennels, which could be an important source of infection for humans. It must be consider that MAT titres ≤1:800 directed against serovars canicola and icterohaemorragiae probably can be attributed to recent vaccination. Appropriate control measures are therefore needed in order to prevent or reduce the introduction of this pathogen, such as serologic screening in dogs before admission to kennels and the use of a polyvalent commercial vaccine that includes the new serovars.

Implications of *Leptospira* spp. in bovine abortion

Vidal S.[1], Syring C.[2], Rodriguez-Campos S.[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

[2] Clinic for Ruminants, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

Leptospirosis in cattle is mainly characterized by reproductive problems like infertility, low conception rate, abortion, occurrence of stillbirths and weak offspring. It particularly occurs in regions with extensive pasture grazing and is most often associated with serovars Hardjo, Pomona and Grippotyphosa. Cattle are considered to be maintenance hosts of Hardjo resulting in endemically infected herds. The microscopic agglutination test (MAT) remains the gold standard for diagnosis, but real-time (RT) PCR has been also found suitable. We screened 415 organs of bovine abortion (placenta and/or fetal stomach corresponding to 353 cases) for the presence of pathogenic *Leptospira* spp. by RT PCR and 83 related serum samples from the cows by MAT. Additionally, we examined 14 cows of a farm with elevated abortion rate serologically and checked 4 organs from 2 abortions by RT PCR. After DNA extraction from the abortion material [placenta (n=293); abomasum (n=126)] with the QIAamp Mini Kit (Qiagen) RT PCR was carried out in a 7500 Real Time PCR System (Applied Biosystems) using primers and corresponding probe targeting the gene *lipL32*. MAT was carried out according to OIE guidelines using serum dilutions from 1:100 to 1:3200 for titration of 12 serovars [Australis (A), Autumnalis (Aut), Ballum (B), Bataviae (B), Bratislava (Br), Canicola (C), Grippotyphosa (G), Hardjo (H), Icterohaemorrhagiae (I), Pomona (P), Sejroe (S), Tarassovi (T)]. Of the 419 tissue samples analyzed by RT-PCR 11 (10 cases) were positive. Of the 83 sera analyzed by MAT 14 were seropositive (≥1:100) to at least one serovar [A (n=2); C (n=1); H (n=6); S (n=1); A/Br (n=2); H/S (n=1); H/S/T (n=1)]. In one case the positive RT PCR result correlated with seropositivity to Hardjo (1:800) in MAT indicative for a case of acute leptospirosis. Out of the 14 animals from the same farm, 6 were positive for serovars Hardjo and Sejroe; yet, the corresponding abortion material was negative by RT PCR. Since other bacterial, fungal, parasitic or viral agents could be ruled out, we concluded that a chronic infection with leptospires may be causative of the elevated abortion rate and vaccination was carried out to prevent further losses. The effect of *L. borgpetersenii* serovar Hardjo on fetal loss remains controversial as some studies failed to show an association between seropositivity to this pathogen and abortion, while others described Hardjo as a cause of abortions. Cattle appear to be the primary maintenance host of serovar Hardjo with abortions occurring only sporadically as opposed to abortion 'storms' which may occur due to infection with Pomona or Grippotyphosa. Our results highlight the controversy about the implication of leptospires in abortions. We consider thorough investigation combining clinical findings, serological and molecular analyses crucial to evaluate a possible implication of these pathogens in abortion scenarios.

Corresponding author:
Sabrina Rodriguez-Campos sabrina.rodriguez@vetsuisse.unibe.ch

First pulmonary observation of *Leptospira interrogans* in *Rattus norvegicus*

Anne-Laure Zilber, Patrick Belli, Marc Artois, Angeli Kodjo and Zoheira Djelouadji

Most mammalian can be to carry leptospires, which caused the leptospirosis in human and domestic animals. The main maintenance hosts of *Leptospira* are the wild rodents, specially the brown rat (*Rattus norvegicus*). In this animal, the leptospirosis is asymptomatic and the leptospires persist only by a colonization of renal tubules. Our study realized in the context of a preliminary investigation, in a trapping site with a high level of leptospirosis in rodents. We reported two first cases of pulmonary presence of the leptospires in apparently healthy rats, which carried *Leptospira* in kidney and captured in a City Park of Lyon (France). We performed the serology by the micro-agglutination test on the blood and the renal *Leptospira*'s isolation. On the kidney and lung samples, a specific pathogenic *Leptospira* TaqMan real-time PCR was performed and completed by a genotyping (including a species' identification by the *rrs* (16s) gene PCR and MST genotyping). We investigated the localization of leptospires in the kidney and the lung by histological analyses: warthin-starry staining and immunohistochemistry. The results showed a *Leptospira*'s isolation on the kidneys of two rats, which were confirmed by specific pathogenic *Leptospira* real-time PCR. Moreover, the *Leptospira* DNA was also detected in the lung tissues (in one of the two rats). In these two rats, the Warthin-Starry staining and the immunohistochemistry showed that the leptospires were localized both in the renal tubules and on the surface of the ciliated epithelium of the bronchi. Using the *rrs* (16s) gene PCR and MST genotyping, the DNA extracts of the kidney and the lung were identified as belonging to the *Leptospira interrogans* species and the Icterohaemorrhagiae serovar "CHU Réunion" strain. Thus, the renal and pulmonary leptospires could have originated from the same infectious source. This observation of the *Leptospira*'s pulmonary presence was different to the observations in the experimental infection (pulmonary clearance phase after some days). This presence raises the questions about other possibilities of *Leptospira*'s transmission via air-borne route, which would require an understanding by the future research, not only on the evaluation of the leptospires prevalence in the lung, but also on the air transmission of the infection.

Corresponding author: Anne-laure Zilber; e-mail: anne-laure.zilber@vetagro-sup.fr

Molecular characterisation of UK isolates of *Leptospira interrogans* serogroup Pomona

Zbigniew J. Arent, Colm Gilmore, William A. Ellis
OIE Leptospirosis Reference Laboratory
Veterinary Sciences Division, AFBI, Belfast, Northern Ireland, UK

Current typing of *Leptospira* strains requires a combination of both serological and molecular methods in order to allow the precise identification of field isolates. Study of the epidemiology of strains within the pomona serogroup has proved problematic due to the limitations of serological typing methods. This is particularly true of the two of the main complexes within this group – namely the Pomona/Monjakov/Kennewicki (PMK) cluster and the Mozdok/ Tsaratsova (MT) cluster.

Strains from the south of England have been shown previously to belong to the MT cluster. Such strains are maintained by small rodents in western Europe and while they may occasionally cause disease in domestic animals they are unlikely to be of economic importance.

In contrast, strains belonging to the PMK cluster, in particular serovar Kennewicki strains have been shown to be significant veterinary pathogens, especially in north America. The purpose of this study was to examine 10 UK PMK isolates by serological methods, restriction endonuclease analysis and VNTR analysis. All the isolates were identified as serovar Pomona and therefore unlikely to pose a significant threat to domestic animals. This is supported by the very low prevalances, or absence of, serovar Pomona antibodies in UK livestock.

Leptospiral infections in pigs from Northern Italy during the period 2002-2014

Boniotti MB, Papetti A., D'Incau M., Tagliabue S.

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, via Bianchi 9, 25124 Brescia, Italy

Swine are one of the most significant reservoir and source of *Leptospira* infections for man. Endemic infections in swine farms generally remain subclinical and they are mainly caused by serovars Pomona, Bratislava and Tarassovi while other serovars tend to occur only incidentally. The aim of this study was to investigate the leptospiral infections of the most important serovars in pig population and to understand the genetic characteristic of serogroup Pomona circulating in Northern Italy in the period 2002-2014. Microscopic agglutination test (MAT) with use of serovars Ballum, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo, Pomona, Tarassovi, was performed on a total of 117136 swine serum samples collected from 2002 to 2014. *Leptospira* DNA detection was carried out on 155 samples by a Real time PCR directed against the *lipL32* gene. Culture isolation was tempted on 272 kidney samples. The isolates were serotyped by MAT using a panel of reference hyperimmune sera. Moreover, 36 isolates collected from 2002 to 2014 from 36 different farms were identified and genotyped by molecular methods as Multi Locus Sequence Typing and Multi Locus Variable Number Tandem Repeat Analysis (MLVA) (loci: 4, 7, 10, Lb4, Lb5). Out of 117136 collected sera examined by MAT, 14685 (12,5%) samples were positive. Positive titers against more than one serovar were detected in 21,4% positive samples (3143/14685). Among MAT positive samples, 9,31% of sera demonstrated titers with serovar Bratislava, 4,23% with Pomona and less than 1% with Ballum, Canicola, Copenhageni, Grippotyphosa, Hardjo and Tarassovi. *Leptospira* detection by *lipL32* Real time PCR revealed 53 positive samples out of 155. During this study, 111 strains were isolated and all of them were serotyped as serogroup Pomona. Genotyping by MLST showed that 35 out of 36 isolates had the same profile as the reference strains serovar Pomona (ST 140) and one isolates showed a profile with 99,9% of nucleotide identity with serovar Mozdok (ST 117). By MLVA, the 35 Pomona isolates showed the same profile as the Italian reference strain (Mezzano I) but they differed from the international one (Pomona). Among the tested serovars, Bratislava remains the most prevalent while Tarassovi, unlike in the past, has became negligible. Culture isolation was successful only with Pomona infected kidneys showing interstitial nephritis. The genotype of Pomona isolates showed to be very stable over a long period of time (2002-2014). In fact, with the exception of one Mozdock isolate, a unique MLST and MLVA profile was detected in our study. These results are very important to design and develop new vaccines that could be use on the local pig population.

Corresponding author: Boniotti Maria Beatrice, email: mariabeatrice.boniotti@izsler.it

Can a serosurvey on animal Leptospirosis in Morocco be representative of the epidemiology of the infection in the Mediterranean region?

Natale A.[1], Benkirane A.[2], Lucchese L.[1], Zuliani F.[1], Hakimi I.[2], Essamkaoui S.[2], Nally J.E.[3]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

[2]Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco

[3]School of Veterinary Medicine, University College Dublin, Dublin, Ireland

Leptospirosis is a neglected zoonosis of global importance with a complex epidemiology. Due to the diversity of clinical signs and difficulties in establishing a confirmatory laboratory diagnosis, the disease remains poorly investigated, particularly in the developing world. The study focused on abortions reported in some areas of the country and used a selected panel of *Leptospira* serovars among the most representative in the Mediterranean region. The aim of the study was to attempt understanding if leptospirosis in ruminants has recurring and repeatable patterns, representative of a wider epidemiological situation, possibly linked to the farming system. In 2013 a total of 295 sera were collected from cattle (99), goats (98), and sheep (97) and analysed with the microscopic agglutination test (MAT).

The cut-off has been set at 1:20, 1:40 and 1:100 to highlight the circulation of subclinical infections and differentiate the most relevant serovars. With the cut-off 1:100, only one multiple positivity was found. The most represented serovars were Copenagheni (2,06%) in sheep, Tarassovi (4,08%) in goats and Pomona (6,06%) in cattle, with antibody titres up to 1:800. With lower cut-off values, a wide circulation of *Leptospira* could be demonstrated and many serovars showed prevalences higher than 20%. Sheep and goats shared a more similar distribution with respect to cattle. It must be noted that the study was conducted in a handful of dairy farms and in various extensively reared herds/flocks, respectively for cattle and for sheep and goats.

Serovar Hardjo, typical of cattle, has been rarely found. Only one cow showed a high titer (1:800), associated to abortion. Pomona, known to cause clinical forms of leptospirosis in cattle, was the most represented serovar in this species, with titres up to 1:400.

Serovars Copenagheni and Icterohaemorrhagiae, belonging to the same serogroup, showed an only partial cross-reactivity. This finding underlines the importance of using different serovars/strains respectively within the same serogroup/serovar for the MAT. The very rare finding of positivity against serovar Ballum with respect to a former study may be explained with the use of a different strain.

Considering animal species, geographic area and antigens used, the data show no repeatable patterns, especially when a low cut-off is used, with respect to studies carried out in other Mediterranean countries (Italy, Albania, Spain); nevertheless, few systematic data are available. An etiological investigation aimed at identifying the circulating strains and excluding cross-reactions or aspecific results should be recommended. Using the cut-off of 1:100 the infection appears to be quite rare and the results are more repeatable referring to other studies. The extensive farming system could be a main factor for maintaining a low infection frequency, nevertheless the few cases of high-titre response, limited to cattle, are relevant from a zoonotic point of view.

Corresponding Author:
Alda Natale anatale@izsvenezie.it

PET RODENTS AS RESERVOIRS OF HUMAN LEPTOSPIROSIS CASES: NEW FAVORITE DOMESTIC ANIMALS AND OLD VECTORS

Mori M*., Truyens M., Bakinahe R., Van der Heyden S., Van Dosselaer I., Roels S., Serreyn P.-J., Fretin D.

Bacterial Zoonoses of Livestock, Operational Directorate Bacterial Diseases, Veterinary and Agro-chemical Research Centre (VAR), Brussels, Belgium

Leptospirosis is a zoonotic disease in which the role of wild rodents as vector is well established. Within the leptospiral ecological cycle, rats function as asymptomatic reservoirs proving the perfect niche for spirochetal growth in the proximal renal tubules and contributing to its spreading through urine shedding. Transmission to humans occurs via direct or indirect contact with contaminated urines of infected animals. This report describes two cases of pet rodents as vector for human leptospirosis (involving three patients) possibly contracted from a domestic mouse (*Mus musculus*) and domestic rats (*Rattus norvegicus*). Clinical symptoms of patients at admission included sepsis and/or meningitis. Case 1: the patients' own household pets were a parakeet, a turtle, a dog, a guinea pig, a rat and a mouse. Among the rodents, the rat resulted negative, the guinea pig presented antibodies against *Leptospira* at 1:10 dilution for serovars (sv.) Ballum, Icterohaemorrhagiae, and Pyrogenes, and the mouse at 1:10 dilution for sv. Ballum and Pyrogenes. The kidneys, liver and lungs of the mouse were additionally positive at bacteriological examination (*lipL32* RT-PCR). Case 2. The patient held 11 rats, 2 cats and a dog. Five out of 11 rats presented antibodies against *Lesptospira* for at least one of the following sv: Ballum, Icterohaemorrhagiae, Javanica and Pyrogenes. We used these 11 naturally infected animals as model to investigate Leptospirosis in rats. Urine shedding and serology was followed over a 3-month period. Urine resulted positive by RT-PCR in 6 out of 11 animals examined on single collection and in 7 out of 11 during a 24 hours follow-up. Serological profile was stable and did not change over the study period. Histological examination of the euthanized rats showed interstitial nefritis in 3 out of 7 positive rats, which can be associated with Leptospirosis. The genetic profile of the isolated strains (5) showed the clonal origin of the infection. The dogs resulted negative at serology while 1 out of 2 cats presented antibodies against *Leptospira*, providing indication of a possible contamination from the rats.
As for other domestic animals, pet rodents need to be considered as thread for transmission of zoonotic diseases. Therefore, efforts need to be dedicated to implement cost-effective controls in those animals to increase public health measures.

*corresponding author: Marcella.Mori@coda-cerva.be

Serological investigation on *Leptospira* infection in Albanian cattle population

Lucchese Laura[1], Alla Luljeta [2], Nardelli Stefano[1], Marchione Silvia[1], Natale Alda[1], Morini Matteo[1], Koleci Xhelil[3]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

[2] Public Health Institute, Tirana, Albania

[3] Faculty of Veterinary Medicine, Tirana, Albania

In Albania, the cattle livestock sector represents an important part of the household economy. The cattle management is based on traditional system (small farms, 1-4 animals), where co-grazing with other species is a common practice. The prevalence and circulation of *Leptospira* serovar Hardjo, for which bovine represents the maintain host, as well as the occurrence of infections caused by other pathogenic serovars of *Leptospira* are unknown, although reproductive disorders are frequent. Furthermore, hundreds of *Leptospira* human cases were reported in Albania in the 1990s, involving cattle as recognised source of infection with rodents and pigs.

The study was aimed at evaluating the leptospirosis seroprevalence in Albanian cattle population. Samples were collected from cows aging >12 months*; sample size was defined assuming a prevalence of 20% (95% of confidence, 2.5% precision). A total of 1017 cows were randomly selected in 31 villages of 13 municipalities, mainly located on the coastline except for two mountain municipalities that had human cases' history of leptospirosis. Among the 506 sampled farms, one was under intensive management. The 1017 samples were tested by means of the microagglutination test (MAT) using a panel of 9 strains of pathogenic serovars of *Leptospira*: Icterohaemorrhagiae, Canicola, Copenagheni, Pomona, Hardjo, Bratislava, Tarassovi, Grippotyphosa and Ballum. The sera screening dilution was 1:100, then two-fold serial dilutions were prepared to obtain the titre.

The overall individual seroprevalence was 9,2%; 10 of 13 municipalities (77%) and 22 of 31 villages (71%) tested positive. All the coastline municipalities but one resulted positive (seroprevalence between 6,1% and 14%), while mountain districts tested negative. The most relevant serovars were Icterohaemorrhagiae and Hardjo with a percentage of positive samples of 3,4% and 2,8% respectively, followed by Grippotyphosa (1,7%) and Pomona (1,2%). The other serovars showed a seroprevalence lower than 1%. Among the 28 Hardjo-positive samples, 11 were located in the intensive farm. Considering the cut-off 1:400, suggestive of acute infection, only serovars Hardjo and Pomona were significantly represented. Mixed infections were scarce (11 samples), with no more than two serovars involved.

The serovar Hardjo was found sporadically in the study, probably because of the extensive cattle farming in Albania. Indeed, a higher number of positive results were found in the intensive farm, where the cattle management may favour Hardjo transmission. On the other hand, the extensive farming was found to facilitate the occasional animal infection with serovars for which cattle do not represent a reservoir, due to promiscuity with other species or environmental contamination. The study results suggested the possibility of a moderate exposure to *Leptospira* connected to direct contact with cattle.

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Corresponding author:
Lucchese Laura LLucchese@izsvenezie.it

Canine leptospirosis in the Netherlands

E.M. Broens[1], M.A.M. van Dijk[1], Y.J. Schaap[2], H.L.B.M. Klaasen[2], J.A. Wagenaar[1]

[1] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University [2] MSD Animal Health, Boxmeer

Introduction. Leptospirosis is an infectious disease of global importance. In humans and cattle, leptospirosis is a notifiable disease in the Netherlands, but in dogs it is not. Incidences of canine leptospirosis are likely to be underestimated, partially due to the fact that suspected patients are treated without a confirmed diagnosis and to diagnostic difficulties. At the Veterinary Microbiological Diagnostic Centre (VMDC) of Utrecht University a validated IgM/IgG ELISA for the detection of Leptospira-specific antibodies is used (Hartman, 1983). For this test a single sample is sufficient for diagnosing acute canine leptospirosis as specific IgM antibodies quickly rise during the early stage of infection; a limitation is that there is no indication of the infecting serogroup. Information on the epidemiology of serogroups is important, as vaccination is an important tool in the control of canine leptospirosis. Recently a new European tetravalent vaccine containing serogroups Icterohaemorrhagiae, Canicola, Grippotyphosa and Australis has been introduced (Nobivac® L4 – MSD Animal Health). The objectives of this study were (1) to study trends in the incidence of canine leptospirosis in the Netherlands and (2) to gain more insight in the epidemiology of serogroups. *Materials and Methods.* (1) Data from the VMDC from the year 2010 onwards were retrospectively analysed and (2) 43 non-paired serum samples (2000-2012) testing positive in the ELISA at the VMDC were tested in the Microscopic Agglutination Test (MAT); the panel included the 4 serogroups of Nobivac-L4. *Results.* (1) From 2010 – 2014, an average of 41 dogs showing clinical signs of leptospirosis were tested at the VMDC every year. Just a small proportion (15%) turned out to be positive. In 2014, an increase of (suspected) cases was seen. With 7 positive samples in the 3rd quarter of 2014, this number clearly exceeded the average number of 1.5 positive sample per quarter in the preceding years. (2) In the MAT, 16% of the serum samples tested positive for serogroup Australis, 12% for Icterohaemorrhagiae, 5% for Canicola and 2% for Grippotyphosa; the remaining 65% showed no or mixed reactivity allowing no conclusion on the serogroup. *Conclusions.* A marked increase in canine leptospirosis cases was observed in the Netherlands in 2014, particularly from July until October. In humans, a parallel increase was observed in the same period. A possible explanation for this increase is the preceding mild winter of 2013-2014 followed by a long summer period with high temperatures. Despite small numbers, the results of the MAT support the use of tetravalent vaccines instead of traditional bivalent vaccines (containing Icterohaemorrhagiae and Canicola only).

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Corresponding author: Els Broens e-mail: e.m.broens@uu.nl

Leptospirosis in bovine. Case reports of *Leptospira* Hardjo in Piedmont Region.

Gennero M.S.[1], Barbero R.[1], Canale G.[1], Bergagna S.[1], Dezzutto D.[1], Falzone R.[2], Tarello V.[3], Boniotti M.B.[4], Lombardi G.[4], Tagliabue S[4].

[1]Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta;[2]Servizi Veterinari Regione Piemonte ASL TO5;[3]Servizi Veterinari Regione Piemonte ASL Biella;[4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna.

Introduction

Leptospira borgpetersenii is a bacterial pathogen of cattle and it also causes zoonotic infections in humans. It is important because is a significant public health problem and because causes organ failure in the liver, lungs, kidney, and brain. (*Naiman, Brian M. et. al., 2002*). *L. borgpetersenii* serovar Hardjo type hardjo-bovis is the most common cause of leptospirosis in bovine, in addition, hardjo-bovis is one of the most common serovars because cattle are maintenance hosts.

Materials and Methods

We report a cluster of two cases of leptospirosis in Italian farms, with regard to clinical, laboratory, environmental findings and pharmacological therapy: a beef farm of Piedmontese cows with 100 cows and 38 sheep, outbreak on 2013; a dairy farm of Pezzata Rossa d'Oropa with 70 cows, outbreak on 2014. The two cases were investigated by case interview and review of clinical and laboratory information. A site visit was made to the farm to assess environmental risk. Different methods of laboratory diagnosis were used with each case: microagglutination test MAT to reveal antibody in serum, microbiological techniques and PCR Real Time LipL32 to detect leptospiral DNA in tissue samples including urine and milk. The isolates were serotyped by MAT using a panel of reference hyperimmune sera. Moreover, serovar identification was performed by Multilocus Sequence Typing (MLST). Auto-vaccines whole cell bacterins were manufactured from *Leptospira* isolates of the two outbreaks.

Results and Conclusions

The two outbreaks were confirmed as leptospirosis by MAT (increasing Ab titers against serogroup Sejroe) and in both cases the isolated causative agent was identified as *L. borgpetersenii* serovar Hardjo type hardjo-bovis by MLST (ST 152). By PCR all the urine samples (9) and 8 out 12 urine samples examined were positive respectively in the first and in the second case.

Laboratory tests showed the presence of *Leptospira spp.* antibodies in individual cow serum and *Leptospira spp.* organisms in urine. *L. borgpetersenii* serovar Hardjo type hardjo-bovis is one of the most important and costly contributors to reproductive loss in the beef and dairy industries. In Italy there are not vaccines for Leptospira in cattle, so auto-vaccines were prepared in five months with hardjo-bovis strains from urine samples from the two outbreaks. The antibiotic treatment and the vaccination allowed to close the outbreaks after several months. On the basis of these data, vaccination of farm livestock for leptospirosis is an integral factor in preventing new infection and human cases. Particularly, in bovine livestock, auto-vaccines are extremely useful in case of animal movements to pasture.

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Corresponding author: Maria Silvia Gennero, MariaSilvia.Gennero@izsto.it

SEROLOGICAL, MOLECULAR AND HISTOPATHOLOGICAL DETECTION OF *Leptospira* spp. IN RODENTS CAPTURED IN SWINE FARMS IN PERU

Chris E. Pinto, Juan Siuce M., Guillermo Salvatierra R., André Sedano S., Renato Palomino V., Gabriela Pacheco S., Sonia Calle E.

Leptospirosis is a worldwide zoonotic disease caused by the spirochete *Leptospira* spp. In recent years the disease has gained importance in Peru due to the increased number of human cases. According to the Peruvian National Institute of Health (INS), Lima has the fourth highest number of Leptospirosis confirmed cases (11.1%) in Peru. It is well known that rodents can act as reservoirs and vectors of the bacteria becoming an important factor in the persistent dissemination and endemic circulation of *Leptospira* spp. to domestic animals, livestock and humans. The objective of the present study was to determine the presence of *Leptospira* spp. in samples of blood, urine and kidneys of rodents captured in the surroundings of swine production systems in Lima city. This is the first step to elucidate the reservoir status of rodents in the veterinary and human epidemiology of Leptospirosis in Lima. A systematic trapping was performed with Sherman and Tomahawk traps to catch rodents living around swine farms. Forty rodents were caught, 31 of which were identified as wild brown rats (*Rattus norvegicus*), 6 were black rats (*Rattus rattus*) and 3 were mice (*Mus musculus*). They were evaluated serologically with the Microscopic Agglutination Test (MAT), molecularly with a TaqMan PCR assay and pathologically with haematoxylin–eosin and silver staining. The frequency of leptospiral infection was high, but there was variation in the results obtained with the different diagnostic tests. In total 52.5% (21/40) of rodents were seropositive by MAT being Autumnalis, the serogroup with the highest percentage of reactions 37% (15/40), followed by Tarassovi 10% (4/40), Abramis 7.5% (3/40) and Iquitos 5% (2/40). The antibodies titre ranged between 1/100 and 1/200. No reactions were detected against serogroups Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Panama, Pomona, Pyrogenes, Shermani, Australis, Ballum, Hurtsbridge, Manhao, Mini, Ranarum, Sejroe and Semaranga. In contrast, just 2.5% (1/40) of kidneys were positive by silver-staining, and 27.5% (11/40) were positive to the presence of pathogenic leptospires DNA by the TaqMan PCR assay. A broad range of morphological alterations were detected in the kidneys from captured rats by H-E staining, however all cases had a similar pattern of focal infiltration of lymphocytes compatible with leptospira infections. No black rats were positive to any of the performed tests. Fifty percent of brown rats and all of the mice were positive to at least one of the tests performed. The high percentage of rodents positive to *Leptospira* suggest that small rodents can have an important role in the transmission and persistence of *Leptospira* in the swine farm environment becoming a source of infection for farm animals and farm workers.

Corresponding author: Chris E. Pinto Jiménez (cpintoj@veterinaria-unmsm.edu.pe). Laboratory of Veterinary Microbiology and Parasitology, Section of Bacteriology and Mycology, Faculty of Veterinary Medicine – Universidad Nacional Mayor de San Marcos (UNMSM), Lima-Peru.

POSTER PRESENTATIONS Saturday 18 April Immunology and vaccine development

VacciPure™ filtration process for manufacturing leptospirosis vaccines with reduced levels of bovine serum albumin to minimise the risk of allergic reactions after vaccination

S.J. Slagman[1], H.L.B.M. Klaasen[1], D. Sutton[2]
[1]MSD Animal Health, Boxmeer, the Netherlands; [2]MSD Animal Health, Milton Keynes, UK

Inactivated bacterins against leptospirosis are widely used worldwide and are produced by a number of different vaccine manufacturers. In the production of these vaccines bovine serum albumin (BSA) is routinely used in the culture media for the *Leptospira* bacteria. However, BSA can cause hypersensitivity reactions in dogs and other animal species following vaccination. Consequently, reducing the concentration of BSA is desirable. MSD Animal Health (MAH) has developed a unique filtration process with the objective of consistently removing more than 90% of the BSA from its *Leptospira* antigens during vaccine production.

Detailed analyses were performed using laboratory studies and mass balance analyses. These showed that during the original antigen filtration process BSA was being retained on the filter with the antigen, which is undesirable. Subsequently, it was found that BSA retention could be significantly reduced through optimisation of various critical parameters of the filtration process. In addition, the washing step in the diafiltration scheme was improved using mathematical modeling and verified in laboratory runs. Finally, the optimised ultrafiltration process was scaled-up and transferred to the large-scale manufacturing of the *Leptospira* antigens. Upon validation it was found that ≥ 95% of the BSA was consistently removed. The resulting filtration process is referred to by the trademark: Vaccipure™.

In a subsequent comparative study the BSA content of three bivalent European canine leptospirosis vaccines (vaccines A, B and C) was compared with the BSA content of MAH's bivalent Nobivac Lepto® vaccine and new tetravalent Nobivac L4® vaccine. The vaccines were all assayed for BSA content using the Pierce® BCA protein assay (Thermo Scientific) and a commercial BSA ELISA, and were also tested on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The BSA content of all of five batches of vaccines A, B and C was much higher (2.4-6.3 mg/ml) than that of the three Nobivac Lepto® batches or the five Nobivac L4® batches (0.2-0.5 mg/ml). The BSA ELISA and SDS-PAGE results confirmed that the major protein that was quantified with the BCA protein assay was BSA and, therefore, confirmed this significant difference in BSA content. Assuming that all of these batches are representative of the respective licensed products, this implies that Nobivac Lepto® and Nobivac L4® contain the lowest amount of potentially allergenic BSA. It can be concluded that after vaccination of dogs with Nobivac Lepto® or Nobivac L4® there is likely to be a lower risk of allergic reactions.

In vitro cross-neutralisation of serovar Australis with sera from dogs immunised with live or killed bacteria from serovar Bratislava

H.L.B.M. Klaasen*, M. van der Veen, M.J. Francis
MSD Animal Health, PO Box 31, 5830 AA Boxmeer, The Netherlands

The objective of the present study was to assess *in vitro* cross-neutralisation of bacteria of *Leptospira interrogans* serogroup Australis serovar Australis with antibodies directed against *L. interrogans* serogroup Australis serovar Bratislava. For this purpose an *in vitro* test method was used, the growth inhibition test (GIT), which has been demonstrated elsewhere to detect neutralising (mainly IgG) antibodies in the serum of vaccinated animals. In contrast with the microscopic agglutination test, the GIT is thought to correlate well with *in vivo* protection.
Sera from two non-vaccinated (serologically naive) dogs taken 4 weeks after challenge with a strain of serovar Bratislava and sera from two dogs taken three weeks after the second vaccination of the basic vaccination schedule for Nobivac L4 were analysed for the presence of neutralising antibodies using the GIT. Titres of growth-inhibiting antibodies (GIT titres) in the four sera were determined against two strains of serovar Bratislava and two strains of serovar Australis. Sera from the same two non-vaccinated dogs taken pre-challenge and sera from the same two vaccinated dogs taken pre-vaccination were used as negative controls.
GIT titres of the post-challenge sera as well as the post-vaccination sera demonstrated that the growth inhibition of the serovar Australis strains was within the range of the growth inhibition of the two serovar Bratislava strains.
It is concluded that antibodies against serovar Bratislava induce complete *in vitro* cross-neutralisation of strains of serovar Australis. This was shown for antibodies produced after experimental serovar Bratislava infection as well as antibodies produced after two vaccinations with Nobivac L4. The latter suggests that Nobivac L4 will induce cross-protection in dogs against infection with serovar Australis.

Corresponding author: H.L.B.M. Klaasen, e-mail: eric.klaasen@merck.com

Versican™ Plus prevents infection, shedding and clinical signs following experimental challenge with four Leptospira serovars

Stephen Wilson, Catrina Stirling, Anne Thomas, Vickie King, Adrien Lefebvre, Bruno Besognet, Gordon Sture, Alicia Urniza
Zoetis

Although effective canine vaccines have been developed against the common *Leptospira* serovars, these pathogens are still reported in clinical cases, while others are increasingly prevalent.
The results from four challenge studies following vaccination of dogs with a new combination vaccine (DHPPi/L4R) containing inactivated *Leptospira* serovars Canicola, Icterohaemorrhagiae, Bratislava and Grippotyphosa are reported. These studies were conducted to satisfy the requirements of the European Pharmacopoeia monograph (01/2008:0447). Six week old dogs received two vaccinations as indicated by the product label, three weeks apart, and were challenged 25 days after the second vaccination with different isolates of the *Leptospira* serovars. Clinical observations were recorded. Blood, urine and tissue samples were collected for analysis.
Following challenge, non-vaccinated dogs demonstrated various clinical signs including apathy, anorexia, jaundice or conjunctivitis . No vaccinated dogs were affected; with significant differences in mean clinical scores observed. Measurable antibody titres to each *Leptospira* antigen were seen in vaccinated dogs 21 days following the first vaccination, with further increases in antibody titres observed following challenge with the respective *Leptospira* strain. Non-vaccinated dogs remained seronegative until challenge. *Leptospira* were re-isolated by culture from the blood, urine, kidney and liver of all non-vaccinated dogs following challenge. In contrast, no vaccinated dogs had *Leptospira* re-isolated from the same tissues. Significant differences were seen in number of days with positive isolation (blood and urine) and in number of dogs with positive samples (kidney and liver).
In conclusion, vaccination of dogs with the new vaccine induced protective immunity 25 days after second vaccination with protection against tissue infection, urinary shedding and clinical signs following challenge.

List of participants

Marian Aalberts	GD Animal Health	Netherlands
Nada Abdelghani Abdelrahim	Sudan Medical and Scientific Research Institute	Sudan
Theresia Abdoel	KIT Biomedical Research	Netherlands
Ben Adler	Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics	Australia
Ahmed A. Ahmed	Royal Tropical Institute, KIT Biomedical research	Netherlands
Kathryn Allan	Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow	United Kingdom
Yulia Ananyina	Gamaleya Institute for Epidemiology and Microbiology	Russia
Zbigniew Arent	Agri-Food and Biosciences Institute	United Kingdom
Bianca van Bavel	Trinity College Dublin	Ireland
Bruno Besognet	Zoetis	United Kingdom
Monica Billi	Zoetis	Italy
Natalia Bomchil	MERIAL	France
Maria Beatrice Boniotti	Istituto Zooprofilattico Sperimentale Lombardia e dell'Emilia Romagna	Italy
Jerome Bouvet	Merial	France
Karina Caimi	Biotechnology Institutte-Inta	Argentina
Christophe Calleja	Zoetis	United Kingdom
Giuseppina Canale	Istituto Zooprofilattico Sperimentale PLVA	Italy
Maria das Neves Paiva Cardoso	University of Trás-os-Montes e Alto Douro, Vila Real, Portugal	Portugal
Teresa Carreira	Instituto de Higiene en Medicina Tropical, Universidade Nova de Lisboa	Portugal
Maximiliano Cesio	Hipra Scientific	Spain
Vicki Chalker	Public Health England	United Kingdom
Meera Chand	Public Health England	United Kingdom
Benoit Chatrenet	Virbac	France
Pauline Chorier	Imaxio	France
Benoit Combes	Entente de Lutte Interdep Contre les Zoonoses	France
Lionel Cupillard	MERIAL	France
Zoree Djelouadji	VetAgro Sup	France
Laura Doornekamp	Erasmus MC	Netherlands
Anou Dreyfus	University of Zurich	Switzerland
Francoise Emily	Virbac	France
Marc Engelsma	Central Veterinary Institute	Netherlands
Marjan van Esbroeck	Instituut voor Tropische Geneeskunde	Belgium
Narmada Fernando	Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo	Sri Lanka
Genevieve André-Fontaine	Oniris	France
Celia Fontana	Merial	France

Elsa Fortes Gabriel	Instituto de Higiene e Medicina Tropical - Universidade Nova de Lisboa	Portugal
Muhammad Hussein Gasem	Dr Kariadi Hospital, Diponogoro University	Indonesia
Maria Silvia Gennero	Istituto Zooprofilattico Sperimentale piemonte, Liguria e Valle d'Aosta	Italy
Joke van der Giessen	RIVM	Netherlands
Corinne Gloor	University of Bern, Vetsuisse Faculty	Switzerland
Marco Goeijenbier	Erasmus MC	Netherlands
Maria Gomes-Solecki	University of Tennessee Health Science Center	USA
Marga Goris	KIT Biomedical Research	Netherlands
Eric van Gorp	Erasmus MC	Netherlands
Saba Goytom	Virion\Serion GmbH	Germany
J.B. Grapiglia	Universidade Federal Fliminense	Brazil
Josipa Habuš	Faculty of Veterinary Medicine, University of Zagreb	Croatia
Usman Hadi	Dr Soetomo Hospital - Airlangga University	Indonesia
Jo Halliday	University of Glasgow	United Kingdom
Ian Harrison	Public Health England	United Kingdom
Katrin Hartmann	Ludwig-Maximilians-Universitat	Germany
Rudy Hartskeerl	KIT Biomedical Research	Netherlands
Heikki Henttonen	Metla	Finland
Sandra Hermelijn		Suriname
Cord Heuer	Institute of Veterinary, Animal and Biomedical Sciences	New Zealand
Marine Hugonnard	VetAgro Sup - Campus Veterinaire de Lyon	France
Mario D'Incau	IZSLER	Italy
Bambang Isbandrio	Diponegoro University Semarang	Indonesia
Artur Jabłoński	National Veterinary Institute	Poland
Ton Jacobs	MSD Animal Health	Netherlands
Tomer Keren	Biogal	Israel
Eric Klaasen	MSD Animal Health	Netherlands
Maarten Limper	Erasmus MC	Netherlands
Hans van der Linden	Koninklijk Instituut voor de Tropen	Netherlands
Javier Lottersberger	Universidad Nacional del Litoral	Argentina
Laura Lucchese	Instituto Zooprofilattico Sperimentale delle Venezie	Italy
Ake Lundkvist	Uppsala University	Sweden
Byron Martina	Erasmus MC	Netherlands
Rita Matos Fonseca	Instituto Nacional de Saude Dr Ricardo Jorge	Portugal
Mariko Matsui	Institut Pasteur de Nouvelle-Caledonie	France
Anne Mayer-Scholl	Federal Institute for Risk Assessment, Berlin, Germany	Germany
Dorothy McColl		Canada
Alain Medawar	Virbac	France
Angela Menezes	Public Health England	United Kingdom
Georgies Mgode	Sokoine University of Agriculture	Tanzania
Zoran Milas	Faculty of Veterinary Medicine, University of Zagreb	Croatia

Juan Jose Siuce Moreno	Universidad Nacional Mayor de San Marcos	Peru
Marcella Mori	CODA-CERVA	Belgium
Mark Moseley	University of Aberdeen	United Kingdom
Jarlath Nally	National Animal Disease Center	USA
Alda Natale	Istituto Zooprofilattico Sperimentale della Venezie	Italy
Roshan Niloofa	Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo	Sri Lanka
Malgorzata Noworol	Instituto Zooprofilattico Sperimentale della Sardegna	Italy
Davor Ojkic	Animal Health Labatory, University of Guelph	Canada
Marleen van Oosten	UMC Groningen	Netherlands
Agatha Opoku-Boateng	PHE Colindale	United Kingdom
Bruna Palmas	Instituto Zooprofilattico Sperimentale della Sardegna	Italy
Yulia Panferova		Russia
Mathieu Picardeau	Institut Pasteur, Unité de Biologie des Spirochètes, Paris, France	France
Roan Pijnacker	RIVM	Netherlands
Chris Evelyn Pinto Jimenez	Faculty of Veterinary Medicine - UNMSM	Peru
Maria Nicoletta Ponti	Istituto Zooprofilattico Sperimentale della Sardegna	Italy
Edmond Puca	University Hospital Center 'Mother Theresa' Tirana	Albania
Elda Qyra	University Hospital Center 'Mother Theresa' Tirana	Albania
Karol Racka	State Veterinary Institute Jihlava	Czech
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Zrinka Štritof	Faculty of Veterinary Medicine, University of Zagreb	Croatia
Purwati Prawito Sumorejo	Airlangga University	Indonesia
David Sutton	MSD Animal Health	United Kingdom
Duangjai Suwancharoen	National Institute of Animal Health, Bangkok, Thailand	Thailand
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Jacek Zmudzki	National Veterinary Research Institute	Poland
Elena Zueva	St Petersburg Pasteur Institute	Russia

Design & Lay out
Sickmann Congres Organisatie
Erik Sickmann
www.sickco.nl
info@sickco.nl
+31 6 4569 3847

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1. Ellis, W.A. (2010). Control of canine leptospirosis in Europe: time for a change?, Vet. Rec, **167**, 602-605.

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Errata Program 2nd ELS meeting on leptospirosis and other rodent born haemorrhagic fevers

Page 5

09.45 title will be:
Leptospirosis Incidence and Risk Factors in Meat Workers in New Zealand - *Comparison between Standard Generalized Linear Modelling and Additive Bayesian Network*

10.45 Key note Yulia Ananyina, Russian Academy of Medical Science, Russia
Ecology of Leptospires: pregenomic and postgenomic era
CANCELLED

New

10.15 Workshop on Micro Agglutination Test for the diagnosis of animal leptospirosis
A need for consensus?
Marcella Mori (CODA-CERVA) and Zorée DJELOUADJI (VetAgro group).

Page 6

15.00 Pathogenic *Leptospira* spp. causing aseptic meningitis in Sudanese children
Nada Abdelghani Abdelrahim, Sudan Medical and Scientific Research Institute, Sudan
CANCELLED

Page 7

12.15 Speaker will be **Artur Jablonski** instead of Jacek Zmudki

Page 8

14.45 Characterization of the genus *Leptospira* isolates from rodents captured in Angola
Elsa Fortes Gabriel, Universidade Nova de Lisboa, Portugal
CANCELLED