ELS 2018

3RD ELS SCIENTIFIC MEETING

on leptospirosis and other rodent borne haemorrhagic fevers

24-26 May 2018
Alghero - Sardinia



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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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3rd ELS Scientific Meeting on leptospirosis and other rodent borne haemorrhagic fevers

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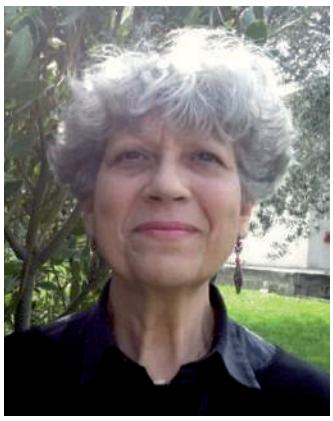
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WELCOME





Dear colleagues and friends

On behalf of the Organizing Committee and of the ELS President, we are pleased to welcome all of you to the 3rd European Leptospirosis Society Meeting which will be held in Alghero.

The long journey started in 2012, when the European Leptospirosis Society (ELS) was formed. The goals of our scientific society were to promote knowledge on leptospirosis and related haemorrhagic fevers and encourage scientific exchanges, especially between European researchers as well as to point out specificity of leptospirosis in Europe.

The ELS congress has become every 3 years a special meeting gathering the leading scientists and breaking news about the latest discoveries in the field.

The ELS meeting is also the place where friends and colleagues can find an opportunity for exchange information and create collaborative networks.

The Scientific Committee hopes to have prepared an interesting program including about 37 Oral, and 27 poster presentations and 6 lectures. Without neglecting other very important aspects of Leptospirosis in humans and animals, the Scientific Committee has put together a program that highlights an update of what we know - and still ignore -, about clinical and diagnostic aspects as well as host response and immunology. The program also pays close attention to the One Health concept that is the topic of a specific session of the Congress. Each session starts with a lecture by an invited speaker and ends with a general discussion.

The venue of the conference is the Hotel Carlos V that provides first class facilities. We are sure that this event represents an unmissable opportunity to visit Alghero, a truly remarkable and attractive place, providing a fantastic back-drop to the congress.

In the last decades Alghero has become a touristic main point not only because of its coast and natural beauties but also because of a fairly well preserved archeological patrimony. Indeed back from the Neolithic period up to more recent centuries many settlements remain in Alghero and in the close areas, including the Anghelu Ruiu necropolis, the Santu Pedru hill, the Villa Romana of Santa Imbenia or even the Purissima. Many *nuraghi,* ancient megalithic edifices, in some other points as Palmavera are also well preserved and open to visit.

We know that your participation and support is a key for a successful meeting and we hope that the program and reception will not betray your expectations.

We would like to thank all of you for your scientific contributions and a number of industrial sponsors for their generous funding of this scientific event. Finally, a special thanks to all colleagues who have collaborated to the realization of this event.

We wish you all a pleasure and inspiring time in Alghero

Nicoletta Ponti
Chair

Nenad Turk
ELS President

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KEYNOTE SPEAKERS

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Eric C.M. van Gorp



Prof. dr. Eric C.M. van Gorp studied medicine at the University of Amsterdam. In 2001 he finished his thesis entitled: "Studies on the pathophysiology of dengue haemorrhagic fever and dengue shock syndrome", granted by the Royal Netherlands Academy of Arts and Sciences (KNAW). He is currently working at the Erasmus MC Rotterdam, the Netherlands, appointed as Professor in Medicine, at the department of Viroscience and the department of Infectious diseases. He is visiting professor at the University of Airlanga in Surabaya, Indonesia. He is involved in the Rotterdam Global Health Initiative (RGHI\www.bmg.eur.nl/english/globalhealth). His field of interest is the social and scientific impact of infectious diseases; epidemiology, clinical management and pathophysiology. He is founder of the Cirion foundation [www.cirion.net], initiating and facilitating initiatives and projects to promote prevention of infectious diseases. One of the initiatives is the EU supported international education and prevention project Viruskenner for scholars [www.viruskenner.nl] in countries on four continents. The research line, with several PhD's, he is supervising is focussing on exotic viral infections, among which the mosquito and rodent borne hemorrhagic fevers, HIV and Fever studies, with an extensive (inter) national network in the Netherlands, the Caribbean, Suriname, China and Indonesia. He is supervisor of the vaccination outpatient clinic and the clinical trial unit from the Viroscience department.

Mario D'Incau



Mario D'Incau is a veterinary officer, head of the Laboratory of Bacteriology at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER) in Brescia (Italy). His main interest is the diagnosis of bacterial infectious diseases, with particular reference to leptospirosis, mycobacteriosis and salmonellosis, and his main duty is the coordination of lab activities and the control on the correct application of quality system.
He is in charge of the Italian Reference Centre for Animal Leptospirosis which main mission is the surveillance of leptospirosis in Italy. He is also the IZSLER Biobank specialist for bacteria.

Marga Goris



Marga Goris earned a Master of Science degree in Applied parasitology and medical entomology from the Liverpool School of Tropical Medicine. In 2000 she began working on leptospirosis. Her PhD thesis was titled: "Leptospirosis: Epidemiology, clinical aspects and diagnosis". She is currently head of the OIE and National Collaborating Centre for Reference and Research on Leptospirosis at the Department of Medical Microbiology at the Academic Medical Center/University of Amsterdam. Her research interests are improving diagnostics, typing methods, epidemiology and pathogenesis of leptospirosis.

Josipa Habus



Josipa Habus earned a Doctor of Veterinary Medicine degree at a Faculty of Veterinary Medicine University of Zagreb. She completed a PhD in Veterinary science at the same Institution with Dissertation on Genetic affinities of pathogenic *Leptospira* spp. within a natural focus of leptospirosis. As a clinician and infectious disease researcher she is interested in understanding and controlling the human-animal interface and zoonotic threats. She is leading and collaborating on multiple research projects concerned with different zoonotic agents but with particular interest for *Leptospira* spp. and leptospirosis. Josipa is working in National Reference Laboratory for leptospirosis and is a part of the multidisciplinary team that is collaborating on research of small rodents as carriers of different zoonotic diseases. She currently holds a position as an Associate Professor at Department of Microbiology and Infectious diseases with Clinic at Faculty of Veterinary Medicine. She is a founding member and General Secretary of European Leptospirosis Society since 2012.

Jarlath Nally



Jarlath Nally completed his Ph.D. in Veterinary Microbiology in 2001 at the University of Kentucky. From 2001-2005, he completed his postdoctoral research in the Division of Infectious Diseases, Department of Medicine at the University of California, Los Angeles. His postdoctoral research focused on understanding pathogenic mechanisms of the zoonotic disease Leptospirosis (Weil's disease) during acute and chronic infection and in particular, the severe pulmonary form of this disease. As a postdoctoral fellow, he was awarded a Ruth L. Kirschstein National Research Service Award from the National Institutes of Health. In 2005, Dr. Nally joined the School of Veterinary Medicine, University College Dublin, where he was awarded The President of Ireland Young Researcher Award from Science Foundation Ireland to further elucidate pathogenic mechanisms of leptospirosis. As a Veterinary Microbiologist, his research interests include zoonotic pathogens, proteomics and animal models of infection. In 2014, he returned to the United States to join the Infectious Bacterial Diseases of Livestock Research Unit, at the National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture where he continues to work on bovine leptospirosis and treponemes associated with digital dermatitis.

Mathieu Picardeau



Dr Mathieu Picardeau is Head of the Biology of Spirochetes unit, Dpt. Microbiology at the Institute Pasteur in Paris/France (research.pasteur.fr/en/team/biology-of-spirochetes/), and Director of the French Reference Centre for Leptospirosis which main mission is the surveillance of leptospirosis in mainland France and french overseas territories. He also leads the Taxonomic group of the International leptospirosis Society (ILS). He has an interest in genetics and genomics of Leptospira. His current research involves the study of different aspects of the biology of Leptospira such as the components of the endoflagellum and mechanisms of adaptation and resistance to stress conditions. Dr Picardeau published over 80 scientific articles about spirochetes, especially Leptospira.

KEYNOTE LECTURES

EMERGING INFECTIONS IN THE GLOBAL VILLAGE; THE RODENT BORNE HEMORRHAGIC INFECTIONS

Eric C.M. van Gorp
Erasmus MC Rotterdam, the Netherlands

Introduction

Infectious diseases are still - and even increasingly - an important health risk, not only in resource limited and/or less developed countries, but also increasingly in developed countries. The illusion that infectious diseases are virtually out of the world we have left behind more than 30 years ago. This illusion could arise from an underestimation of the complex dynamics between infectious diseases, people and their environment and yet also an overestimation of knowledge, knowledge obtained by science.

Emerging virus infections; clinical aspects and epidemiology

Exotic viral infections are virus infections that traditionally occur mainly in the tropics and subtropics. Characteristic of this group of infections is that they circulate permanently, endemic, or present themselves in smaller or larger outbreaks, with an epidemic or even pandemic character. On the basis of a few examples, we already saw that the distribution area, the epidemiology of these virus infections is changing rapidly. And thus we cannot exclude the possibility that a number of these infectious diseases will occur also in middle and northern European countries in the [near] future.
From the clinical perspective the hemorrhagic infections and the infections causing meningo-encefalitis are among the main clinical pictures we see in the [sub]tropical areas.
In the meantime, there is a growing likelihood that mosquito populations that have always been established in the tropics will also establish themselves in middle and north European countries. Beside the mosquito borne infections the rodent borne infections is a relevant group, containing pathogens with a major clinical impact in different areas. There is a need for sufficient rodent control programs, knowing that the rodent populations in many European countries is growing, which means an increased risk for rodent borne infections e.g. leptospirosis and hanta virus infection.

Haemorrhagic infections; pathogenesis

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

SURVEILLANCE OF ANIMAL LEPTOSPIROSIS IN ITALY

Mario D'Incau
Istituto Zooprofilattico Sperimentale della Lombardia/Emilia Romagna, Brescia, Italy

Leptospirosis is a worldwide zoonosis that infects a large spectrum of domestic and wild animals. Due to its prevalence on the Italian territory, and according to the national legislation, leptospirosis has always been included among the infections for which a mutual collaboration between human and veterinary medicine is required and, in particular, through sharing information of clinical cases and outbreaks in humans and animals.
Currently, human infection has very low annual prevalence and incidence (rates of less than 1%), while infection in animal species is present in livestock (cattle, pigs), pet animals (dogs) and wild animals.
Despite several seroprevalence studies have evidenced the continuous circulation of *Leptospira* in Italy, there are no systematic surveillance plans at national level; in the past, voluntary control plans were organized at regional level. However, in case of infection, it is mandatory to report to the Veterinary Authority with adoption of restriction measures.
Even if official surveillance plans are not in place, the control of infection in animals is ensured by the network of *Istituti Zooprofilattici Sperimentali* (IIZZSS) coordinated by the National Reference Center. Monitoring is mainly guaranteed by serological checks, performed by agglutination microscopy (MAT), with the use of the same panel of antigens by all laboratories, and collection of the data in a national database. Multi locus sequence typing (MLST) is also performed to monitor genotypes circulation.
During the period 2012 - 2017, IIZZSS laboratories examined, on average, over 18,000 sera per year; more than 80% of these sera came from cattle (over 40%), pigs (around 30%) and dogs (around 12%). Sera collected from wild animals (over 5%) and horses (over 2%) were also tested.
On a yearly average, 5% of the examined cattle were positive: Sejroe serogroup recorded the highest prevalence, followed by Pomona and Icterohaemorrhagiae serogroups.
In swine, more than 14% of the samples were positive: Australis serogroup was significantly the most prevalent serogroup followed by Pomona. MLST revealed two different Pomona sequence types referable to L. interrogans Pomona Pomona and L. kirschneri Pomona Mozdok. The Tarassovi serogroup has apparently disappeared (prevalence of less than 1%).
The sera collected from dogs revealed a high percentage of positivity (over 30%), due to the presence of vaccine antibodies; in this case, if we consider only sera with a titre greater than 1:400, we observed a higher prevalence of the Icterohaemorrhagiae serogroup followed by the Australis serogroup.
Among wild animals, mostly sera from wild boar were examined (over 80% of sera). In this species the Australis serogroup was the most prevalent, followed by Pomona and Grippotyphosa.
The data obtained confirm the presence of *Leptospira* both in domestic and wild animals and the prevalence of Sejroe serogroup among cattle, of Australis serogroup between pigs and Icterohaemorrhagiae among dogs. In addition, the seroprevalence data in cattle and pigs, now well established over the years, suggest the possibility of implementing control or eradication programs focused on specific serogroups/ serovars.

LEPTOSPIROSIS DIAGNOSIS IN HUMANS

Marga Goris
Academisch Medisch Centrum, Amsterdam, the Netherlands

Leptospirosis is one of the most wide spread zoonotic diseases in the world. It occurs at every continent except the polar regions where the disease has not been reported. The epidemiology of leptospirosis is complex and dynamic and there is a wide variety of (a)specific clinical manifestations with a broad differential diagnosis that makes diagnosis difficult. This explains that the disease is vastly underdiagnosed, underreported and hence ignored.
Leptospirosis can be treated with antibiotics but efficacy depends on the initiation of the treatment at an as early possible stage of the disease. Therefore early diagnosis is important. However, the variety of clinical manifestations makes the diagnosis challenging and therefore a laboratory is essential to confirm the diagnosis. However, the available diagnostic tests have several drawbacks such as being complicated and requiring expertise and experience, laborious, unreliable, slow and/or expensive. Diagnostic tests can be divided into conventional tests, that all provide confirmation at a relatively late stage of the disease, and novel molecular tests. Conventional tests are culturing leptospires from clinical samples, the microscopic agglutination test (MAT), which is the reference test and the enzyme-linked immunosorbent assay (ELISA). Both MAT and ELISA detect antibodies against leptospires. Molecular tests, mainly the polymerase chain reaction (PCR) provide a tool for diagnosis at the early acute stage since antigen is detected. Nowadays, rapid diagnostic tests have been developed based on detecting antibodies and giving a result within 15 minutes.
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.

THE ROLE OF SMALL RODENTS IN THE EPIDEMIOLOGY OF LEPTOSPIROSIS AND HEMORRHAGIC FEVER WITH RENAL SYNDROME

Josipa Habus
Faculty of Veterinary Medicine University of Zagreb, Croatia

Rodents are the most abundant and diversified mammalian order that can maintain and transmit more than 80 unique zoonotic pathogens. The increased incidence of rodent-borne diseases recorded over the last decade resulted in numerous studies aimed to clarify the role of different rodent species in harbouring distinct zoonotic pathogens. Leptospirosis and hantavirus infections are globally present, (re) emerging rodent-borne zoonosis that share the same rodent reservoirs and exhibit very similar clinical features. In Europe several hantaviruses are harboured by different rodent and insectivora species but most of the human infections are caused by widely distributed Puumala virus and Dobrava virus, which are present in the Balkans and central and eastern Europe. With few exceptions, each hantavirus is primarily associated with a single rodent host of the family *Muridae.* Consequently, the epidemiological pattern of hantaviruses will be heavily influenced by population dynamics of that rodent species. Due to heterogeneity of the *Leptospira* genus and high degree of serovar adaptation to variety of different animal species, epidemiology of leptospirosis is more complexed. Certain animal species may host distinct *Leptospira* serovars in different geographic areas, meaning that the knowledge of the prevalent serovars and their hosts in a particular region is essential to understand the epidemiology of the disease. Despite the wide host range, rats (*Rattus norvegicus*) are considered to be the major source of the *Leptospira* infection for humans. However, some recent studies suggest a change in epidemiological patterns of the disease with increasing incidence of human leptospirosis caused by serovars maintained by small rodents (murine and vole species).
High infection rates of small rodents with *Leptospira* spp. and hantaviruses with numerous co-infections have been documented in several studies. Peaks in rodent population abundance were correlated with outbreaks of both of the diseases while fluctuation in incidence appears to be driven by favourable climate that lead to increased food availability for rodents and prolonged pathogen survival. Certain differences in factors influencing rodent abundance have been found in different climate areas of Europe and should be further investigated.
In most of the European countries active surveillance of rodent ecology, population dynamics and circulation of zoonotic pathogens among rodents is seldom or not maintained on a regular level. Surveillance supported by public-health authorities is often initiated only as a response to a past or ongoing epidemic and even then investigations are of limited scale. Long term investigations and multidisciplinary approach involving lots of fieldwork followed by laboratory testing is complex, expensive and time-consuming. Yet, that work is necessary if we would like to identify different biotic and abiotic factors that could influence the rodent population dynamics and transmission patterns of both diseases.

THE INS AND OUTS OF PERSISTENT RENAL LEPTOSPIROSIS

Jarlath Nally
Infectious Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, U.S.A.

Pathogenic leptospires colonize the renal tubules of reservoir hosts of infection, including cattle and rats, and are excreted via urine. Cattle act as a reservoir host for L. borgpetersenii while rats act as a reservoir host of L. interrogans. A survey of urine samples (N=600) collected from a beef cattle slaughter facility identified 43 (7.2%) that were positive by culture or fluorescent antibody testing for leptospires. Twenty-three urine samples were culture positive. Sequence analysis of 16S ribosomal DNA and secY indicated that all isolates were Leptospira borgpetersenii. Typing by serology indicated that all isolates were serogroup Sejroe. There was poor correlation between seroprevalence and excretion of leptospires since 18/43 (41.9%) cattle, which were positive by culture or FAT, were seronegative. These findings highlight beef cattle as a reservoir host for the zoonotic transmission of leptospires. Rats are regarded as one of the most significant reservoir hosts of L. interrogans, and excrete large numbers of organisms in their urine. In order to investigate the unique biological equilibrium that exists between pathogenic leptospires and reservoir hosts of infection, an immunocompetent inbred rat model of persistent renal colonization was developed; leptospires were detected in urine of experimentally infected rats by three weeks post-infection and remained positive until 8 weeks post-infection. However, there was little, if any, evidence of inflammation in colonized renal tubules. At eight weeks post-infection, a robust antibody response was detected against lipopolysaccharide and protein outer membrane (OM) components. Purified B and T cells derived from the spleen of infected and non-infected rats proliferated in response to stimulation with 0.5 µg of OM fractions of Leptospira, including CD4+ T cells, which comprised 40% of proliferating cells, compared to 25% in non-infected controls. Lymphocytes purified from the lymph node draining the site of colonization, the renal lymph node, also showed an increase in percentage of proliferating B and T cells. However, in contrast to a phenotype of 40% CD4+ T cells in the spleen, the phenotype of proliferating T cells in the renal lymph node comprised 65% CD4+ T cells. These results confirm that the renal lymph node, the local lymphoid organ, is a dominant site containing Leptospira reactive CD4+ T cells and highlight the need to consider the local, versus systemic, immune responses during renal colonization infection. Whilst little is known about how Leptospira adapt to and 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. The rat model of persistent renal colonization provides a source of urinary derived leptospires for their characterization by transcriptomics, proteomics and their respective protein post-translational modifications. Collectively, the inbred rat model of persistent leptospirosis can be used to understand and define the unique equilibrium that exists between pathogenic leptospires and their reservoir host of infection.

TYPING OF LEPTOSPIRA IN THE GENOMIC ERA

Mathieu Picardeau
Institut Pasteur, France

Leptospirosis is an emerging zoonotic disease caused by pathogenic *Leptospira* strains. Each year, there is an estimated 1 million severe cases of leptospirosis and nearly 60,000 deaths worldwide. The genus *Leptospira* is highly heterogeneous and currently consists of 23 species and more than 300 serovars that can be isolated from diverse ecological niches and animal reservoirs. According to their phylogeny, *Leptospira* species are distributed in 3 groups: the pathogens, the intermediate species -which cause a milder disease- and the saprophytes, which do not cause disease in human nor animals. Recent study of environmental isolates from New Caledonia, a tropical island endemic for leptospirosis, revealed a previously unexplored diversity within the genus *Leptospira*, with the description of 12 novel species, including species from the pathogenic cluster that failed to produce an infection in animal models (Thibeaux *et al.* 2018).
Different serological and molecular typing methods have been used to study the epidemiology of *Leptospira*, but they are performed by few reference laboratories and usually designed for the most commonly found pathogens. Since the first complete *Leptospira* genome sequence was published in 2003, it is now possible to sequence bacterial genomes in a few hours at reduced cost. Whole-genome sequencing (WGS) has emerged today as an ultimate tool for both the identification of relevant genetic variations linked to virulence and for bacterial strain typing. The availability of whole-genome sequences of *Leptospira* allowed us to develop, for example, a core genome MLST (cgMLST) scheme targeting the entire genus of *Leptospira*. Our cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation among *Leptospira* isolates, allowing for comparison of and sharing typing results among laboratories worldwide. This scheme will enable to propose new standards for the classification of *Leptospira* strains to replace the current nomenclature, which is complex and obsolete. Genome analysis will also help to understand global and local phylogeographic trends, but also genetic factors that are associated with disease severity and reservoir specificity.
In conclusion, the last decade has seen major advances in our understanding of *Leptospira* diversity and genome evolution. In the near future, we should be able to generate metagenomic data and draft genomes from environmental and biological samples without the need for culture isolation. This will advance many aspects of the leptospirosis field: diagnostic, epidemiology, and basic knowledge including species diversity, ecology evolution, and virulence.

PROGRAM

	SCIENTIFIC PROGRAM
	Day 1 – Thursday 24th May 2018
09.00	Registration, poster session
12.00	Board Meeting ELS
14.00	Opening and Welcome address
	Alberto Laddomada, General Manager of Istituto Zooprofilattico Sperimentale della Sardegna Eraldo Sanna Passino, Director of Department of Veterinary Medicine, University of Sassari Nenad Turk, ELS President, Faculty of Veterinary Medicine University of Zagreb, Croatia M. Nicoletta Ponti, Chair of ELS Organising Committee, Istituto Zooprofilattico Sperimentale della Sardegna
15.00	Key note address – ONE HEALTH Key note lecture: ERIC C.M. VAN GORP – EMERGING INFECTIONS IN THE GLOBAL VILLAGE: THE RODENT BORNE HEMORRHAGIC INFECTIONS Erasmus MC Rotterdam, the Netherlands
CHAIR	Scilla Mastrandrea, Azienda Ospedaliero Universitaria di Sassari (Italy) Marga Goris, Academisch Medisch Centrum, Amsterdam, the Netherlands
15.30	THE HIDDEN RISK IN SNAKE BREEDING: AN ANECDOTE OF HUMAN LEPTOSPIROSIS Schuller Simone, Picardeau Mathieu, Brodard Isabelle, Bassi Cécile, Tataw Ashu James, Lienhard Reto, Rodriguez-Campos Sabrina
15.45	ENVIRONMENTAL, SOCIOECONOMIC DATA, AND MAPPING RISK OF LEPTOSPIROSIS IN ATLÁNTICO CARIBBEAN AREA, COLOMBIA Lagares Guzman Alfredo, Varela Prieto Lourdes, Mattar Salim, Fang José, Escaf Mayra
16.00	OF MICE, CATTLE AND MEN: USING CONCEPTUAL FRAMEWORKS TO UNDERSTAND THE ECO-EPIDEMIOLOGY OF LEPTOSPIRA BORGPETERSENII SEROVAR BALLUM IN NEW ZEALAND Moinet Marie, Yupiana Yuni, Nisa Shahista, Haack Neville, Wilson Peter, Vallee Emilie, Wilkinson David, Russell James C, Heuer Cord, Aberdein Danielle, Collins-Emerson Julie, Benschop Jackie
16.15	CAN HUMAN CASES REVEAL AN ANIMAL LEPTOSPIROSIS OUTBREAK? Piredda Ivana, Palmas Bruna, Noworol Malgorzata, Pintore Pierangela, Pedditzi Aureliana, Ruiu Angelo, Cubadda Antonio, Cancedda Maria Giovanna, Marongiu Antonio, Rebechesu Lucio, Ponti Maria Nicoletta
16.30	Coffee Break
17.00	DOGS AS SENTINELS FOR HUMAN EXPOSURE TO LEPTOSPIRES: IS THERE ANY SEASONALITY IN CLINICAL CASES? Natale Alda, Toson Marica, Gagliazzo Laura, Ceglie Letizia, Guerrini Eulalia, Mazzucato Matteo, Furnari Carmelo, Zuliani Federica, Mulatti Paolo, Lucchese Laura
17.15	FIRST REPORT ON HUMAN LEPTOSPIROSIS CAUSING STRAINS IN MALAYSIA SUGGEST RODENTS AS THE MAIN RESERVOIR Vasantha Kumari Neela, Noraini Philip Mimie, Ramli Siti Nuraliya, Joseph Narcisse, Azhari Natasya
17.30	LEPTOSPIRA INFECTION: WHAT HAVE WE LEARNED FROM ANIMALS INHABITING AN INSULAR ENDEMIC REGION? Rajeev Sreekumari, Shiokawa Kanae
17.45	LEPTOSPIRA IN RATS: A LITERATURE REVIEW TO UNDERSTAND THE GLOBAL PREVALENCE Boey Kenneth, Shiokawa Kanae, Rajeev Sreekumari

18.00	EMERGING SOURCES AND PATHWAYS FOR LEPTOSPIROSIS - A PARADIGM SHIFT Benschop Jackie
19.30	Welcome ceremony
	Day 2 – Friday 25th May 2018
09.00	Key note address – ONE HEALTH
	Key note lecture: MARIO D'INCAU – SURVEILLANCE OF ANIMAL LEPTOSPIROSIS IN ITALY Istituto Zooprofilattico Sperimentale della Lombardia/Emilia Romagna, Brescia, Italy
CHAIR	Nenad Turk, Faculty of Veterinary Medicine University of Zagreb, Croatia M. Nicoletta Ponti, Istituto Zooprofilattico Sperimentale della Sardegna, Italy
09.30	BOVINE LEPTOSPIROSIS IN ABATTOIRS IN UGANDA: MOLECULAR DETECTION AND LEPTOSPIRA EXPOSURE RISK TO WORKERS Alinaitwe Lordrick, Allan Kathryn, Rodriguez-Campos Sabrina, Torgerson Paul, Clovice Kankya, Dreyfus Anou, Dreyfus Anou
09.45	LEPTOSPIRA SEROPREVALENCE IN ANIMALS IN THE CARIBBEAN REGION: A SYSTEMATIC REVIEW Pratt Nicola, Rajeev Sreekumari
10.00	EPIDEMIOLOGY OF LEPTOSPIROSIS IN CENTRAL ITALY: FIFTEEN YEARS OF SEROLOGICAL DATA (2002-2016) Cilia Giovanni, Bertelloni Fabrizio, Turchi Barbara, Pinzauti Paolo, Cerri Domenico, Fratini Filippo
10.15	FLUCTUATION OF SEROPREVALENCE IN EQUINE POPULATION IN CROATIA Stritof Zrinka, Habus Josipa, Mojcec Perko Vesna, Milas Zoran, Hadina Suzana, Perharic Matko, Staresina Vilim, Turk Nenad
10.30	Coffee Break – Poster sessions
11.30	Key note address – CLINICAL AND DIAGNOSTIC ASPECTS IN HUMANS AND ANIMALS
	Key note lecture: MARGA GORIS – LEPTOSPIROSIS DIAGNOSIS IN HUMANS Academisch Medisch Centrum, Amsterdam, the Netherlands
CHAIR	Ciriaco Ligios, Istituto Zooprofilattico Sperimentale della Sardegna, Italy Marcella Mori, Coda Cerva Belgium
12.00	CHALLENGE MODEL DEVELOPMENT OF ACUTE LEPTOSPIROSIS IN DOGS Bouvet Jérôme, Cariou Carine, Cupillard Lionel, Guigal Pierre-Michel
12.15	PASSIVE SURVEILLANCE OF LEPTOSPIRA INFECTION IN SWINE IN GERMANY Strutzberg-Minder Katrin, Tschentscher Astrid, Beyerbach Martin, Homuth Matthias, Kreienbrock Lothar
12.30	ISOLATION AND IDENTIFICATION OF PATHOGENIC LEPTOSPIRA FROM RODENTS ON THE ISLAND OF SAINT KITTS Shiokawa Kanae, Rajeev Sreekumari
12.45	LEPTOSPIRA SEROVARS IN HORSES SUFFERING FROM ERU LOOKING AT INTRAOCULAR AND SERUM SAMPLES IN GERMANY AND NEIGHBOURING COUNTRIES Wollanke Bettina, Gerhards Hartmut, Brem Siegfried, Geiger Tobias, Wiehen Lina
13.00	Lunch

14.30	Key note lecture: JOSIPA HABUS – THE ROLE OF SMALL RODENTS IN THE EPIDEMIOLOGY OF LEPTOSPIROSIS AND HEMORRHAGIC FEVER WITH RENAL SYNDROME Faculty of Veterinary Medicine University of Zagreb, Croatia
15.00	SCREENING OF ANTI-LEPTOSPIRAL ANTIBODIES IN SANITATION WORKERS FROM LISBON AND TAGUS VALLEY REGION (PORTUGAL) Fernandes Maria, Carreira Teresa, Teodósio Rosa, Vieira Maria Luísa
15.15	LEPTOSPIRES AND LEPTOSPIROSIS IN SOME SUB-SAHARAN AFRICAN COUNTRIES: A PORTUGUESE COOPERATION EXPERIENCE Carreira Teresa, Nunes Mónica, Garcia Joana, Estanislau Elsa, Cá-Cá Baltazar, Fortes-Gabriel Elsa, Comia Isac, Vieira Maria Luísa
15.30	PREVALENCE AND BIOLOGICAL DISTRIBUTION OF LEPTOSPIRAL SEROVARS IN HORSES IN CENTRAL FRANCE Benamou-Smith Agnes, Djelouadji Zoree
15.45	EPIDEMIOLOGICAL AND PATHOLOGICAL ASPECTS OF LEPTOSPIROSIS IN WILD BOAR IN A DEFINED GEOGRAPHICAL STUDY AREA Pintore Antonio, Piredda Ivana, Palmas Bruna, Noworol Malgorzata, Cancedda Maria Giovanna, Desini Pietro, Manca Angelo, Bianco Giuseppe, Carta Antonio, Sgarangella Francesco, Tilocca Pasqua Maria, Rebechesu Lucio, Ponti Maria Nicoletta
16.00	OUTBREAKS OF BOVINE LEPTOSPIROSIS INFECTION IN TWO SICILIAN LIVESTOCKS (SOUTHERN ITALY) Grippi Francesca, Barreca Santino, Sciacca Carmela, Arnone Maria, D'Incau Mario, Galluzzo Paola, Guercio Annalisa
16.15	LEPTOSPIROSIS, A DIAGNOSIS NOT TO "UNDER-RATE-E" Paridaens Henry, Lambert Deborah, Van der Beken Yolien, Mori Marcella
16.30	Coffee Break – Poster sessions
17.30	LEPTOSPIRA CHRONIC CARRIAGE: A STUDY ON ELEVEN NATURALLY INFECTED PET RATS Mori Marcella, Djaroun M., Van der Heyden S., Vannoorenberghe P., Van Dosselaer I., Bakinahe R., Roels S., Serreyn P.-J., Fretin D., Truyens M.
17.45	EVALUATION OF TWO RAPID TESTS FOR THE IN-CLINIC DIAGNOSIS OF ACUTE CANINE LEPTOSPIROSIS Zamagni Silvia, Troìa Roberta, Balboni Andrea, Frigo Serena, Magna Luca, Perissinotto Linda, Battilani Mara, Billi Monica, Dondi Francesco
19.00	Gala Dinner

	Day 3 – Saturday 26th May 2018
09.00	Key note address – HOST RESPONSE AND IMMUNOLOGY
	Key note lecture: JARLATH NALLY – THE INS AND OUTS OF PERSISTENT RENAL LEPTOSPIROSIS Infectious Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, U.S.A.
CHAIR	Mathieu Picardeau, Institut Pasteur, France Rajeev Sreekumari, Ross University School of Veterinary Medicine Basseterre, St. Kitts, West Indies

09.30	PULMONARY ENDOTHELIAL EXPRESSION OF BETA-CATENIN, CD31 AND VON WILLEBRAND FACTOR IN DOGS WITH LEPTOSPIRAL PULMONARY HAEMORRHAGE SYNDROME Lettry Sophie, Geinoz Thibault, Posthaus Horst, Schweighauser Ariane, Francey Thierry, Wyder Marianne, Rodriguez-Campos Sabrina, Lyck Ruth, Schuller Simone
09.45	CHARACTERISATION STUDIES OF OUTER MEMBRANE PROTEINS FROM PATHOGENIC BOVINE LEPTOSPIRA SPP. Intan N. A. Kamaruzaman, Gareth J. Staton, Stuart R. Ainsworth, Stuart D. Carter, Nicholas J. Evans
10.00	ENDOTHELIAL CELL PROTEOMIC RESPONSES TO INFECTION WITH PATHOGENIC LEPTOSPIRA REVEALS ACTIVATION OF PATHWAYS LINKED TO CYTOSKELETAL REMODELING Lettry Sophie, Posthaus Horst, Wyder Marianne, Lyck Ruth, Rodriguez-Campos Sabrina, Schuller Simone
10.15	DEMONSTRATION OF CROSS-PROTECTION OF EURICAN$^{\Diamond}$ LMULTI AGAINST LEPTOSPIRA INTERROGANS SEROVAR COPENHAGENI IN AN ACUTE LEPTOSPIROSIS DOG CHALLENGE MODEL J. Bouvet, C. Cariou, F. Oberli, J. Reyes, P. Dhoms, L. Cupillard, P.M. Guigal
10.30	VACCINES AGAINST LEPTOSPIROSIS: A LIVE ATTENUATED MUTANT ELICITS CROSS-PROTECTION IN HAMSTER BUT NOT IN DOG Fontana Celia, Bouvet Jerome, Le Guyader Marine, Gasparini David, Ripart Prescillia, Guigal Pierre-Michel, Blain Cedric, Cariou Carine, Kodjo Angeli, Cupillard Lionel, Bomchil Natalia
10.45	REDUCTION IN INCIDENCE OF CANINE LEPTOSPIROSIS IN SWITZERLAND CORRELATES WITH THE INTRODUCTION OF A NEW QUADRIVALENT ANTI-LEPTOSPIRAL VACCINE Schuller Simone, Schweighauser Ariane, Reber Antonella, Francey Thierry
11.00	MEMBRANE TRANSLOCATING HUSCFV (HUSCFV-FC) ANTIBODY THAT TRANSVERSES Maneewatchararangsri Santi, Luangwattananun Piriya, Treeratanapaiboon Lertyot, Songprakhon Patcharee, Junking Muthita, Chieochansin Thaweesak, Kunwittaya Sarun
11.15	Coffee Break – Poster Sessions
12.00	Key note address – NEW FRONTIERS IN MOLECULAR DIAGNOSIS Key note lecture: MATHIEU PICARDEAU – TYPING OF LEPTOSPIRA IN THE GENOMIC ERA Institut Pasteur, France
CHAIR	Jarlath Nally, Infectious Bacterial Diseases Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, U.S.A. Ben Adler, Department of Microbiology, Monash University, Clayton, Australia
12.30	FIRST ISOLATION OF LEPTOSPIRA SPP. FROM A DOLPHIN (TURSIOPS TRUNCATUS) IN THE MEDITERRANEAN SEA. ASSESSMENT OF HALOTOLERANCE IN SEAWATER Piredda Ivana, Palmas Bruna, Noworol Malgorzata, Tola Sebastiana, Longheu Carla, Boniotti Maria Beatrice, Bertasio Cristina, Denurra Daniele, Cherchi Marcella, Pintore Antonio, Ponti M. Nicoletta
12.45	DETECTION OF BROWN RAT (RATTUS NORVEGICUS) IN DUTCH SURFACE WATERS USING EDNA TO DETERMINE A LINK WITH PATHOGENIC LEPTOSPIRA Becker Elmar, Ahmed Ahmed , Goris Marga, Ruijter de Hans, Wullings Bart, Kardinaal Edwin
13.00	Lunch

14.30	QUANTITATIVE REAL-TIME LEPTOSPIRA PCR ASSAYS: A QUICKER ALTERNATIVE TO THE CULTURE METHOD FOR THE DETECTION OF PATHOGENIC LEPTOSPIRA SPECIES IN DOG URINE, BLOOD AND KIDNEY DURING CLINICAL FOLLOW-UP Blanchard Stéphanie, Cariou Carine, Bouvet Jérôme, Valfort William, Cupillard Lionel, Poulet Hervé, Oberli Frantz, de Saint-Vis Blandine
14.45	DETECTION OF LEPTOSPIRA SP. BY PCR IN SWINE ABORTION - A CASE REPORT Steinrigl Adi, Liehl Beate, Steinparzer Romana, Bagó Zoltán, Schmoll Friedrich
15.00	TARGETING ATP SYNTHESIS IN LEPTOSPIRA THROUGH BIOREDUCED AGNPS: A POTENTIAL THERAPEUTIC ALTERNATIVE IN TREATMENT OF LEPTOSPIROSIS Govindaraju Shruthi, Vinayagmurthy Balamurugan, Kollur Shiva Prasad, Cletus J M D'Souza, Shivananju Nanjunda Swamy, Shivamallu Chandan
15.15	Closing Ceremony

ORAL PRESENTATIONS

ONE HEALTH

EMERGING SOURCES AND PATHWAYS FOR LEPTOSPIROSIS - A PARADIGM SHIFT

Benschop Jackie
Massey University ~ Palmerston North ~ New Zealand

Leptospirosis is considered to have a sporadic pattern in New Zealand and despite extensive nationwide interventions; it remains an unacceptable burden particularly on those living in rural communities and on Māori. Of critical concern is a current increase in incidence with 91 cases in the first half of 2017 (2016: 33 cases).We review public and animal health data and our research at the human-animal interface to develop hypotheses about sources and transmission pathways for leptospirosis in New Zealand - a disease seen as primarily occupational and controlled by animal vaccination.In addition to the tripling of incidence there are increased proportions of females (18%) and increased number of cases in those employed outside of the traditional high risk occupations (36%). The increase in incidence is nationwide but most noticeable in Waikato, a region with a large farming population, with 9 cases in 2015, 21 cases in 2016 and 39 cases to date in 2017. The recent national trend (2015-2016) of increased hospitalisation amongst notified cases, continues into 2017 (now approximately 70%), suggesting the 2017 increase in incidence is genuine. Prior to 2015, 50-60% of notified cases were hospitalised and the increase to 70%, although subtle, suggests an increase in the severity of disease over the past two and a half years and/or a later recognition and thus treatment of disease. We note increasing use of diagnostic polymerase chain reaction in many District Health Boards (DHB), but this change does not explain the large increase in notifications; increases in numbers have been seen in DHBs where PCR is not in use e.g. Northland. We have also received reports in 2017 of increased cases in dogs, cattle and a recent multi-animal species outbreak linked to an abortion storm in alpacas. NZ is progressively suffering more severe flooding due to extreme weather events and National Institute of Water and Atmospheric Research projections suggest a more tropical climate is set to continue.We hypothesise that the triad of changing epidemiology, increased flooding and emergence of new strains signals a shift towards a more tropical, epidemic-type transmission pattern in NZ, with rodent and environmental pathways becoming increasingly important.Communication of that shift requires careful consideration in consultation with all stakeholders, particularly those at risk of disease.

ENVIRONMENTAL, SOCIOECONOMIC DATA, AND MAPPING RISK OF LEPTOSPIROSIS IN ATLÁNTICO CARIBBEAN AREA, COLOMBIA

[1]Lagares Guzman Alfredo, **[1]Varela Prieto Lourdes, [2]Mattar Salim, [1]Fang José, [3]Escaf Mayra**

[1]Universidad del Atlantico ~ Barranquilla ~ Colombia

[2]Universidad de Córdoba ~ Monteria ~ Colombia

[3]Laboratorio de Salud Departamento del Atlántico, Barranquilla Colombia ~ Barranquilla ~ Colombia

Leptospirosis is an emerging infectious disease. Leptospirosis is a multi-factorial disease highly associated with both environmental and socioeconomic factors. It is most prevalent in tropical and subtropical areas with high rainfall and flood events. Here we present an epidemiological risk map for the Atlantic department in the northern Caribbean Coast region of Colombia, using environmental and socioeconomic data and predictive models to predict potential risk area of leptospirosis.

To estimate the geographic distribution of leptospirosis in humans an ecological niche model was used. Reports of confirmed notified cases between 2009 and 2015 were coded using addresses and global positioning system positions of houses in the Atlantic department (QGIS 2.18.4). A maximum entropy algorithm model using six environmental and socioeconomic variables was created in the Maxent v3.3.1 program.

Population density was the best predictor of spatial case occurrence with a contribution of 85.5% to the total variability. The places with the highest population density per unit area were those with the highest risk of contracting leptospirosis. It was found that the highest risk of leptospirosis in humans was located in five municipalities: Barranquilla, Soledad, Malambo, Sabanalarga and Baranoa.

The areas with the highest case occurrence in the Atlantic department were mainly urban areas associated with urban slums and extreme poverty and poor sanitation facilities. The Geographic information systems (GIS) and ecological niche modeling used in the present study were capable of revealing socioeconomic and environmental risk factors which inform strategic interventions by public health services in this coastal area of Columbia.

References

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Sorichetta, A., Hornby, G. M., Stevens, F. R., Gaughan, A. E., Linard, C. & Tatem, A. J. Americas Datasets, V1. Harvard Dataverse http://dx.doi.org/10.7910/DVN/PUGPVR (2015)

OF MICE, CATTLE AND MEN: USING CONCEPTUAL FRAMEWORKS TO UNDERSTAND THE ECO-EPIDEMIOLOGY OF LEPTOSPIRA BORGPETERSENII SEROVAR BALLUM IN NEW ZEALAND

[1]Moinet Marie, [1]Yupiana Yuni, [1]Nisa Shahista, [1]Haack Neville, [1]Wilson Peter, [1]Vallee Emilie, [1]Wilkinson David, [2]Russell James C, [1]Heuer Cord, [1]Aberdein Danielle, [1]Collins-Emerson Julie, [1]Benschop Jackie

[1]Massey University ~ Palmerston North ~ New Zealand

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Rodents and wildlife, unlike in numerous countries worldwide, were previously identified as a minor public health concern for leptospirosis in New Zealand (NZ). Here, this zoonosis is clearly related to occupation, with >66% of cases being farm or abattoir workers. Livestock species independently maintain serovars Hardjo and Pomona, both included in livestock vaccines. Livestock vaccination is thus a mainstay of Leptospira control in NZ. However, the increasing importance of L borgpetersenii serovar Ballum, that now causes 30% of notified human cases, suggests wildlife may be an overlooked source of infection. The role of livestock in the epidemiology of Ballum is unclear but recent findings indicate livestock could be bridge hosts for humans or part of the maintenance community. We drew from two disease ecology frameworks [1, 2] to study the determinants of Ballum transmission and spill-over. We collated published studies and preliminary findings of ongoing studies pertaining to (a) the infection dynamics of Ballum in potential maintenance hosts, including their distribution, densities, prevalence (MAT or culture) and pathogenesis, (b) Leptospira shedding and survival in the environment, (c) the competency of cattle as a potential bridge species, and (d) exposure (risk) factors for two potential spill-over hosts, namely cattle (bridge host) and humans (target hosts). Although genetic, physiological and immunological attributes of the spill-over hosts are also important determinants of transmission, these were beyond the scope of this work. Our literature review identified all known and suspected wild maintenance hosts of Ballum in NZ, and narrowed the focus on introduced rodents (Mus musculus, Rattus rattus, R. norvegicus), and hedgehogs (Erinaceus europaeus). These species are distributed throughout NZ, and are present on pastures. On Manawatu farms we estimated mouse densities of up to 53 [CI 34-82]/Ha and captured 0.3-3.2 ship rats and 0.7-1.3 hedgehogs /100 trap-nights. Ballum seroprevalence (MAT, cut-off 1:48) of these species was 35% [CI 27-43], 6% [0-29] and 56% [CI 31-78], and culture prevalence was 40% [CI 32-49], 6% [CI 0-29] and 33% [CI 13-59] (typing pending), respectively, both higher for mice than previous estimates. For cattle, experimental and natural infections suggest a low pathogenicity with seroprevalence lower in dairy (3% [CI 3-4]) than beef cattle [3] (14% [CI 12-16]). We found 13/4000 (0.3% [CI 0.2-0.6]) of adult dairy cows, all seronegative, shedding Ballum.Our approach highlights the potential for Ballum spill-over to cattle and identifies gaps in the knowledge of this serovar. Further studies will be important to understand its survival in the environment, to ascertain the role that cattle may play in its transmission to humans and to inform more effective control strategies.

1 Caron et al, Vet Res 2015. 46. 11
2 Plowright et al, Nat Rev Microbiol 2017. 15. 502-510
3 Mannewald et al, ILS 2017 Proceedings, p 219

CAN HUMAN CASES REVEAL AN ANIMAL LEPTOSPIROSIS OUTBREAK?

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Leptospirosis is a widespread zoonotic disease which can occur as asymptomatic, subclinical or fatal infection. Different categories of workers, involving farmers have higher risk of being affected by leptospirosis as a result of direct or indirect exposure to infected animal reservoir. Here we report an outbreak of clinical leptospirosis in 2 farmers and different species of livestock in a rural holding.
In August 2016, two farmers of Sardinia (Italy) were hospitalized with clinical aspects indicative of acute febrile infection. Based on clinical signs and serological findings diagnosis of acute leptospirosis was suspected. To investigate the possible source of infection in the rural holding of the 2 farmers, blood samples from pigs (n = 11), dogs (n = 3) and donkeys (n = 3) were submitted to Microscopic Agglutination Test (MAT). In addition pigs were euthanized and, at necroscopy, samples of kidneys and urine were collected for being examined by 16S rRNA gene PCR, bacteriological assay in semisolid EMJH medium as well as by immunohistopathological means. With the same methods we also examined samples of urine from the 3 donkeys. Finally, the isolates of Leptospira spp. were categorized with Multi-Locus Sequence Typing (MLST) and Multi-Locus Variable-Number Tandem Repeats Assay (MLVA) by amplification a portion of the 16S rRNA gene.
The 2 farmers showed acute febrile illness, jaundice, oliguria, elevated bilirubin level and MOF. Urine cultural assay resulted negative in both farmers, while antibody titer (1:20) against serovar Pomona was detected in one. In the same farmer urine sample was positive by real- time PCR for L. interrogans serovar Pomona 16S rRNA gene. All the animals were clinically healthy, interestingly, all pigs and donkeys showed MAT titers ranging 1:200 to 1:3200 for serovar Pomona. The 3 dogs were serologically negative for Lepstospira spp. Real time PCR analysis revealed positive reaction in kidneys or urine in 8 pigs and 3 donkeys while, by bacteriological examination, Leptospira spp. isolates were obtained from 4 swine and one donkey. MLST and MLVA genotyping showed that all the Leptospira spp. isolates matched the serovar Pomona. Severe focal interstitial-nephritis was found in 7 kidneys, among which 4 showed immunoistochemical intratubular reactivity for Leptospira spp.
These results indicate that *L. interrogans* serovar Pomona is an allarming zoonotic agent with a large number of animal species which could act as reservoirs, thus increasing the risk of disease transmission to humans. In addition our findings stimulate the practice to investigate the source of human infection more careful for gaining a better understanding of the prevalence and biological behavior of Leptospira spp.

DOGS AS SENTINELS FOR HUMAN EXPOSURE TO LEPTOSPIRES: IS THERE ANY SEASONALITY IN CLINICAL CASES?

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Leptospirosis is a re-emerging zoonosis with a worldwide distribution. In north-eastern Italy the illness is often notified in dogs, whereas the infection is probably underreported in humans. The epidemiological knowledge about the infection in dogs may be useful in a One Health approach, especially considering them as sentinels for the risk assessment in humans.

From April 2013 to March 2016 a total of 1412 suspected clinical cases in dogs were collected. The samples (sera and urines) were submitted to the IZSVe laboratories for the diagnosis of Leptospirosis, and they were respectively analysed with microagglutination test (MAT) and real-time PCR. The cases were considered as “confirmed cases” if MAT showed a titre ≥1:400 (cut-off 1:100) and/or the PCR gave positive results. After tested, 527 confirmed cases of Leptospirosis have been reported. Seasons were analysed to obtain cases’ distribution during the four seasons in three years. Data on location of suspected and clinical cases were organized based on three altimetry areas (mountain, hill, flat), allowing to consider the effect of the altitude on climate determination.

On the basis of the location, the percentage of confirmed on suspect cases was significantly different (Chi-square test’s p-value=0.001), being the percentage reported in mountain (21.3%) lower compared to those found in other altimetry areas (hill and flat area: 34.1% and 39.9% respectively).

Seasonality was observed (Chi-square test’s p-value=0.008): overall, the proportion of cases was higher in autumn (41.8%) than in other seasons (36.5% in summer, 36.0% in winter, 29.4% in spring), with some differences in percentage among years.

The percentages of cases were also compared among three years (Chi-square test’s p-value<0.001): in particular the percentage was higher in 2014 (45.6%) than in other years (32.0% in 2013, 31.9% in 2015).

Autumn seemed to be the highest risk season, but other factors, such as altimetry area and year showed some influence on Leptospirosis distribution in dogs.

Further analysis will be performed to determine whether the data suggest effect modification by potential confounders and to evaluate if the annual fluctuations were due to other climate factors (humidity, mm of rain, ground temperature).

FIRST REPORT ON HUMAN LEPTOSPIROSIS CAUSING STRAINS IN MALAYSIA SUGGEST RODENTS AS THE MAIN RESERVOIR

[1]Vasantha Kumari Neela, [1]Noraini Philip Mimie, [1]Ramli Siti Nuraliya, [1]Joseph Narcisse, [1]Azhari Natasya

[1]Universiti Putra Malaysia ~ Serdang ~ Malaysia

Leptospirosis is endemic in Malaysia and gazetted as the notifiable disease since 2010. The first human case associated with leptospirosis was reported in 1925. Since then several studies have been reported on seroprevalence, serovars from patients and animals, animal reservoirs, risk factors, risk groups, case, incidence and mortality etc. However, despite the skyrocketing number of cases from 2,268 in 2011 to more than 7000 with increased mortality rates during recent years, leptospiral species causing human leptospirosis in Malaysia is not yet known. It is utmost important to identify the human infecting species not only for treatment and public health management but also to be included in the MAT panel to improve diagnostic sensitivity. On the other hand, it is equally important to identify the reservoirs for the efficient control and prevention of the zoonotic pathogen leptospira.

In the present investigation, blood samples were collected from patients clinically suspected for leptospirosis warded in two tertiary care hospitals in the state of Selangor, Malaysia. A total of 75 samples were collected over a period of 18 months from June 2016 to December 2017. All samples were subjected to lipL32 PCR, ELISA, and MAT. PCR positive samples were further characterized 16SrDNA typing.

Among the 75 samples, 14 showed positive amplification for the lipL32 fragment. These 14 samples when subjected to 16S rDNA typing elucidated Leptospira interrogans and L. kirschneri as the infecting species. Phylogenetic analysis with 16S rDNA of L. interrogans and L. kirschneri isolated from rodents trapped from human settlements and recreational areas in Selangor showed 100% similarity with human- infecting species.

The data from the present study for the first time identifies the human-infecting leptospiral strains in Malaysia and also suggests rodents as the main reservoir. A one health approach is important to effectively control and prevent leptospirosis incidences and mortalities.

LEPTOSPIRA INFECTION: WHAT HAVE WE LEARNED FROM ANIMALS INHABITING AN INSULAR ENDEMIC REGION?

[1]Rajeev Sreekumari, [1]Shiokawa Kanae

[1]Ross University School of Veterinary Medicine ~ Basseterre, ~ Saint Kitts and Nevis

Leptospirosis is endemic in humans and animals in the Caribbean region. The insular nature of the islands, limited surface area, high endemism and restricted host species may facilitate exploration of the natural history and host parasite relationship of pathogens such as Leptospira. As a first step to achieve this, we initiated a study to understand Leptospira prevalence in animals on the Caribbean island of Saint Kitts.

Seroprevalence studies were conducted in multiple animal species inhabiting the island using microscopic agglutination test against 21 Leptospira serovar panel. Presence of Leptospira renal infection was tested by bacterial culture and PCR, when kidney or urine samples were available. H&E stained kidney sections of various animal species were examined for lesions and its correlation to Leptospira infection and exposure.

Differences in overall seroprevalence and serovar distribution was observed between animal species. The overall seroprevalence was highest in rats (82.8), followed by cattle (81.4%), monkeys (49.4%), pigs (35.3%), mongoose (8.1%), sheep (11.3%), goats (15.4%), dogs 72.3%), and cats (4%). Renal carrier stage was observed in 6.2% of mongoose, 18.2 % of cattle, 12.9% of pigs, 58.6% of rats, and 44.4% of mice tested by PCR. Twenty Leptospira isolates were obtained from cultures performed on kidneys from multiple animal species. Lesions were observed in the kidneys of multiple animal species examined. Presence of brown pigments within the renal epithelium (11/28) and interstitial nephritis were common in rat kidneys (12/28). Interstitial nephritis and Lymphoplasmacytic interstitial nephritis was present in cattle kidney (16/25) sections. Mild to severe histopathological abnormalities were detected in 25 out of 75 (33.3%) of mongoose kidney samples and severe lesions were comparable to a chronic nephropathy.

Exposure to 16 Leptospira serovars was observed in multiple animal animal species examined on the island. There were differences in seroprevalence and serovars exposed, and renal prevalence among animal species. Variations in renal pathology were observed in the animals examined in this study. Although no clear associations were observed between renal lesions and exposure to Leptospira, a potential effect of a complex host pathogen relationship should not be disregarded. As infectious agents are implicated in the initiation of chronic kidney conditions such as “Mesoamerican nephropathy”, studies related to the natural history of Leptospira infection and associated potential chronic pathologic consequences in animals in the endemic regions is urgently needed.

LEPTOSPIRA IN RATS: A LITERATURE REVIEW TO UNDERSTAND THE GLOBAL PREVALENCE

[1]Boey Kenneth, [1]Shiokawa Kanae, [1]Rajeev Sreekumari
[1]Ross University School of Veterinary Medicine ~ Basseterre, ~ Saint Kitts and Nevis

Rodents play an important role in Leptospira epidemiology worldwide. Wild and peridomestic rats (Rattus spp.), especially the Norway/brown rat (R. norvegicus) and the black rat (R. rattus), are main reservoirs of Leptospira. This study aimed to collate the global prevalence of Leptospira infection in rats.
We conducted a literature review to collate the global published data on rats and their role in Leptospira transmission. A thorough search on PubMed was conducted using the terms, but not limited to, "(Leptospira) AND (Prevalence) AND ((Rats) OR (Rattus))" without restrictions on publication date, and retrieved 303 titles. After screening the articles for duplicates and inclusion/exclusion criteria, we had a total of 120 articles.
Leptospira prevalence in rats varied considerably based on geographic region, with some reporting 0% prevalence in countries such as Madagascar and the Faroe Islands, and others reporting as high as >80% prevalence in studies done in Brazil, India, and Philippines. The top five countries that were reported based on number of articles include India (13), Brazil (9), Malaysia (6), France (5), and Thailand (5). Methods of detecting or isolating Leptospira also varied among studies. Leptospira prevalence among different Rattus species showed no patterns as to which species are more likely to be infected by the bacterium. However, it can be agreed upon that L. interrogans serovar Icterohaemorrhagiae is the most prevalent serovar found in Rattus spp.
Our review indicates that global distribution of Leptospira in rats varies widely, and there are many different considerations to be discussed, including variations in detection methods used and sample size across studies.

BOVINE LEPTOSPIROSIS IN ABATTOIRS IN UGANDA: MOLECULAR DETECTION AND LEPTOSPIRA EXPOSURE RISK TO WORKERS

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In Uganda, the burden of leptospirosis and the importance of specific hosts in transmitting and maintaining leptospires are not clearly understood. Leptospira seropositivity has been demonstrated in hospital patients (Dreyfus et al., 2016) and cattle (Dreyfus et al., 2017) by Microscopic Agglutination Test. Little is known about the role of cattle as a source of human Leptospira infection in Uganda. The aim of our study was to determine the prevalence of Leptospira renal infection and urinary shedding in cattle and to estimate the risk of exposure to abattoir workers in Uganda.

Two purposively selected cattle abattoirs in Kampala were enrolled in this cross-sectional study. In total, kidney, urine and blood samples were collected from 500 cattle from across the country between June and July 2017. Leptospira infection status in cattle was determined by real-time PCR (qPCR) targeting lipL32 on kidney and urine samples. The daily risk of a worker being exposed to ≥1 (kidney and/urine) positive carcass (%) for different work positions was modelled stochastically using a binomial distribution. The input data were the number of Leptospira positive carcasses found in the sample, the total number of sampled animals, and the number of handled carcasses per year and work position. To account for sampling uncertainty a Beta distribution was applied.

Of 500 cattle, 36 (7.2%) had Leptospira positive kidneys (“carriers”) and 29 (5.8%) positive urine (“shedders”), hence 44 (8.8)% were either kidney and/ urine positive. Urinary shedding was detected in 21 (58.3%) of carriers. The daily exposure risk to ≥1 (kidney and/urine) positive carcass ranged from 27% (95% credible interval 18.6-52.3) to 100% (91.0-100.0), with halal butchers and meat inspectors being highly exposed.

Confirmation of renal infection and urinary shedding of pathogenic leptospires in cattle demonstrates that cattle have the potential to act as a source of Leptospira infection for people in Uganda. In particular, abattoir workers and other persons handling cattle (i.e. milking, obstetrics) have a great potential risk of exposure. Monitoring abattoir workers for clinical disease and increased awareness of workers is recommended, especially for halal butchers and meat inspectors. Appropriate managerial and behavioral changes to reduce the risk of infection may be necessary.

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THE HIDDEN RISK IN SNAKE BREEDING: AN ANECDOTE OF HUMAN LEPTOSPIROSIS

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A case of human leptospirosis was diagnosed in Switzerland by real-time PCR targeting LipL32 from a DNA sample extracted from blood of the patient and by seroconversion to serogroup Hardjo by microscopic agglutination test (MAT). The patient held mice and rats for feeding purposes for snake breeding. Pet rodents are known to be a source of infection for humans (Mori et al. 2017). An epidemiological investigation based on real-time PCR, Multilocus Sequence Typing (MLST), culture and MAT was initiated.

First, litter samples from the mouse and rat cages were collected, and 3 mice and 2 rats randomly selected, euthanized and DNA extracted from the kidneys examined by real-time PCR targeting LipL32. Second, all remaining rodents (mice, n=39; rats, n=12) were euthanized and DNA isolated from the kidneys examined by real-time PCR. Multilocus Sequence Typing (MLST) was performed on the real-time PCR positive samples with a Ct<30 using the 7L scheme (Boonsilp et al. 2013). Additionally, from 10 mice and 10 rats the kidneys were cultured in semi-solid EMJH medium supplemented with 6% rabbit serum and 2% 5-fluorouracil. MAT was carried out according to availability of serum as previously described (Delaude et al. 2017).

From the total of 43 samples from mice and 15 samples from rats, 34 and 7 were PCR positive, respectively. One mouse and one rat were pregnant and the fetuses also tested positive. MLST was carried out on 21 samples with Ct<30 (human, n=1; mouse litter, n=1; rat litter, n=1; mice, n=17, rats, n=1): 17 samples identified as ST155 and 2 samples as ST149. The DNA isolated from the patient was typed as ST155, for which two matching strains were found in the PubMLST database (https://pubmlst.org/), both belonging to serogroup Sejroe. For ST149 three matching strains were retrieved from the PubMLST database, all belonging to serogroup Ballum. To date, 4 Leptospira spp. strains were isolated (3 strains ST155, 1 strain ST149). Serological investigations of rodent sera were limited due to the little serum volume obtained from the mice. From 11 mouse sera examined by MAT, 7 showed antibodies against serovar (sv) Hardjo strain Hardjoprajitno (titers ranging from 1:40 to 1:400), 4 sera to sv Sejroe strain M84 (titers ranging from 1:40 to 1:400), 7 sera to sv Australis (titers ranging from 1:20 to 1:200), and 4 sera to sv Copenhageni strain M20 (titers ranging from 1:20 to 1:40). From 12 rat sera examined by MAT 6 sera showed antibodies against sv Hardjo strain Hardjoprajitno (titers ranging from 1:20 to 1:100), 7 sera to sv Australis (titers ranging from 1:100 to 1:200) and 2 sera to sv Copenhageni (titers 1:400 and 1:800).

Rodent breeding may pose a risk to humans through shedding of pathogenic Leptospira spp. in urine as indicated by positive kidney and litter samples matching the ST from the patient. Different strains may persist in the same breeding; however, the likely more virulent strain, in this case ST155, caused the infection in the owner.

Errata Corrige

LEPTOSPIRA SEROPREVALENCE IN ANIMALS IN THE CARIBBEAN REGION: A SYSTEMATIC REVIEW

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Leptospirosis accounts for significant mortality and morbidity in humans in the Caribbean region and small tropical islands generally have the highest incidence of infection, with Leptospira infection being one of the major zoonotic cause of morbidity in these regions. This systematic review was conducted using Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines and is solely focused on Leptospira seroprevalence studies on the islands of the Caribbean region to obtain a detailed overview and summarises the data published on the Leptospira seroprevalence, serovar diversity and distribution among animal species in the Caribbean region.
Following Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, and checklist, relevant articles were identified and data were extracted and recorded.
The review provided Leptospira seroprevalence data from 16 Caribbean islands in a variety of animal species. Much of the seroprevalence information came from a single study published by Levett et al., in 1996 in livestock animals in the Lesser Antilles. This study encompassed 12 islands and used 22 Leptospira serovars for MAT testing. Variations in exposure to serovars were observed between islands in this study ranging from exposure to 0-9 serovars. Other relevant studies we identified are from Barbados (n=9), Trinidad (n=7) Grenada (n=3), Jamaica (n=2) Puerto Rico (n=1), Saint Croix (n=1). The number of serovars used in the MAT panel in these studies ranged from 2 to 23. Reviewing the literature highlighted the limited amount of data available from limited number of islands. Many of the studies conducted have recorded seroprevalences based on variable and small samples sizes. Besides, serovar panels used for MAT were not consistent between studies.
The review indicates that the Leptospira exposure in a given geographic location may change with time and climatic and environmental conditions, and highlights the need to conduct continual surveillance in tropical countries where the climate supports the survival of Leptospira in the environment.Specific attention must be given to standardization of MAT panels and protocols and providing training across laboratories involved in testing. Further, animal and environment testing to isolate and identify circulating Leptospira spp. in geographic region must actively be pursued. This knowledge is important to implement geographically specific control programs, as risk factors of Leptospira transmission is favoured by various factors such by change in climatic conditions, urbanization, encroachment of wildlife inhabitation, import/export of animals, increase in adventure travel, and water related recreational activities.

EPIDEMIOLOGY OF LEPTOSPIROSIS IN CENTRAL ITALY: FIFTEEN YEARS OF SEROLOGICAL DATA (2002-2016)

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Leptospirosis represents an important widespread re-emerging zoonosis. Climatic changes, modifications of ecological niches and new potential maintenance-hosts could represent the most important factors involved in leptospirosis epidemiology. This study aim was to evaluate the diffusion of Leptospira in Central Italy.

From January 2002 to December 2016, 8488 sera belonging to cow (1328), sheep (2682), swine (1332), dog (1144), wild boar (479), goat (327), hare (162), fox (94), coypu (70), horse (74), roe deer (74), fallow deer (65), red deer (56), wolf (43), rat (34), mouflon (8), mouse (8), cat (4), donkey (2), guinea pig (2), ferret (1), bear (1), alpaca (1) and man (329) were analysed for Leptospira. They were sampled in Central Italy from healthy and ill subjects. Serological investigation was carried out by Micro Agglutination Test using as live antigens Icterohaemorrhagiae (strain Bianchi), Canicola (strain Alarik), Pomona (strain Mezzano), Tarassovi (strain Jhonson), Grippotyphosa (strain Moscow V), Bratislava (strain Riccio 2), Ballum (strain Castellonis 3) and Hardjo (strain Hardjoprajitno). Titers of 1:100 were considered positive; 2-fold serial dilutions were tested to determine the endpoint titer.

Positive sera for Leptospira were 8.35% (709/8488) at 1:100 titer and 2.57% (218/8488) for titer ≥1:400. Table 1 shows the positive sera distribution in relation to serovars and animal species. The highest positive sera percentages were recorded for coypu, swine and cow at low (22.86%, 19.67% and 13.10%, respectively) and high titer (5.61%, 6.23% and 3.16%, respectively). Wild boar, fox and hare sera resulted positive at titer ≥1:100 (8.56%, 3.04% and 1.85%, respectively). Positive human sera were 4.26% (10/329), while 3 (1.06%) showed a titer ≥1:400. Serovars Pomona and Bratislava resulted more often detected, with 267 and 224 at titer 1:100, and with 68 and 67 at titer ≥1:400 positive reactions, respectively. Low number of positive sera was detected for serovars Ballum, Canicola and Tarassovi (13, 28 and 32, respectively). Hare and goat sera were the only samples negative to serovar Icterohaemorrhagiae and Bratislava, respectively. Serovars Icterohaemorrhagiae and Canicola were found mainly in dog sera, though Pomona and Bratislava in swine sera. Serovar Tarassovi was detected mainly in wild boar and serovars Grippotyphosa and Hardjo especially in cow sera. Icterohaemorrhagiae was the most detected serovar in human sera (Table 1). Percentage of positive sera for each year decreased from 2002 to 2008 and increased from 2009. In 2008 only 0.17% (1/597) sera was positive and in 2014, 2015 and 2016 high positive percentages were recorded (17.23%, 19.61% and 38.05%, respectively). Although Leptospira prevalence seems to be decreasing, some serovars has been increased during last years. Moreover, some wild species seems to play a key role in leptospirosis epidemiology.

Table 1: *Leptospira* serovars positive sera distribution regarding to animal species.

Animal species (examined)	Titer	Serovar								Total	Positive to more serovars
		Ic	Ca	Po	Gri	Tar	Br	Har	Ba		
Cow (n:1328)	>100	21	4	55	48	3	3	94	4	232	44
	>400	4	1	3	5	1	1	30	2	47	3
Dog (n.1144)	>100	58	14	16	4	1	48	0	0	141	33
	>400	16	1	2	0	1	19	0	0	39	11
Goat (n: 327)	>100	9	0	0	0	0	0	0	0	9	0
	>400	6	0	0	0	0	0	0	0	6	0
Horse (n:242)	>100	3	0	1	0	0	2	1	0	7	0
	>400	0	0	0	0	0	0	0	0	0	0
Wild boar (n:479)	>100	3	1	4	1	18	12	14	1	54	11
	>400	1	0	1	0	3	1	7	1	14	1
Hare (n:162)	>100	0	0	0	0	0	1	0	2	3	0
	>400	0	0	0	0	0	0	0	0	0	0
Coypus (n:70)	>100	1	0	0	14	0	8	0	0	23	7
	>400	0	0	0	2	0	3	0	0	5	7
Sheep (n:2682)	>100	20	1	30	20	8	14	19	5	117	21
	>400	2	0	17	6	3	1	12	1	42	3
Swine (n:1332)	>100	2	6	160	0	2	131	1	0	302	40
	>400	0	0	45	0	1	41	0	0	87	3
Fox (n:94)	>100	1	0	0	0	0	3	0	0	4	0
	>400	0	0	0	0	0	1	0	0	1	0
Man (n:329)	>100	7	2	1	2	0	2	0	1	15	2
	>400	2	0	0	1	0	0	0	0	3	0
Total	>100	125	28	267	89	32	224	129	13	907	158
	>400	31	2	68	14	9	67	49	4	244	28

Legend. Ic: Icterohaemorrhagiae; Ca: Canicola; Po: Pomona; Gri: Grippotyphosa; Ta: Tarassovi; Br: Bratislava; Har: Hardjo; Ba: Ballum.

FLUCTUATION OF SEROPREVALENCE IN EQUINE POPULATION IN CROATIA

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Leptospirosis is a worldwide zoonosis affecting many animal species and humans. In Croatia it is endemic, with human leptospirosis rates among highest in Europe. Testing of horses is a part of multidisciplinary approach being conducted with purpose of surveillance and control of leptospirosis in Croatia.
In the period of six years (2011-2016) a total of 42965 horse sera were tested, with number of sera ranging from 5000 in 2014 to 10735 in 2016. All sera were tested by microscopic agglutination test (MAT) which was performed following standard procedure (Dikken and Kmety, 1978), using a panel of eight Leptospira serovars: Grippothyphosa, Sejroe, Bratislava, Pomona, Canicola, Icterohaemorrhagiae, Saxkoebing and Bataviae. In accordance with regulations ordered by Veterinary Directory of the Ministry of Agriculture, Republic of Croatia, cut-off value was 1:200 for serovar Bratislava and 1:400 for other serovars. Presumptive infective serogroups were determined according to the highest titres to one or more serovars within certain serogroup.
Overall seroprevalence during six years was 8.8% Continuous decline of seroprevalence was observed from 2011 (14.8%), through 2012 (13.3%) to 2013 (6.2%), with the incidence rising in 2014 (10.8%), and again declining in 2015 (9.4%) and 2016 (8.5%). Decline in seroprevalence during the first three years might be explained by introduction of legally regulated measures for identification and treatment of positive animals. Extreme weather conditions contributed to the rise of seroprevalence in 2014, although even then seroprevalence wasn't as high as before introduction of control measures. In 2015 and 2016 decline in seroprevalence was observed again. Analysis of data by counties revealed that in spite of extreme climate conditions present in the whole country, with precipitation amounts higher than average in the last 30 years, in most Croatian counties rise in seroprevalence in 2014 hasn't occured. Overall rise in seroprevalence in 2014 was a consequence of very significant rise detected in seven counties situated in proximity of big rivers, that were affected by severe floodings occuring as a result of high precipitation amounts and saturation of soil with water. Analysis of presumptive infective serogroups revealed that in 2010 and 2011 the most prevalent presumptive infective serogroup was Australis, in 2012 and 2013 Pomona, in 2014 Grippotyphosa and in 2015 and 2016 Pomona.
This work once again confirmes endemicity of leptospirosis in Croatia and indicates that flooding, above all other climate conditions, had major impact on leptospirosis incidence in horses.

CLINICAL AND DIAGNOSTIC ASPECTS IN HUMANS AND ANIMALS

CHALLENGE MODEL DEVELOPMENT OF ACUTE LEPTOSPIROSIS IN DOGS

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Leptospirosis, the most widespread zoonosis, can cause severe and potentially lethal disease in dogs, if not treated. Current licensed bacterin vaccines elicit serogroup restricted protection which requires development of specific and reliable animal models for evaluation of efficacy. Despite the need to develop a model for each strain, we defined a common process for acute challenge model development (CMD) from strain selection to clinical, biological and bacteriological monitoring.

First step is to obtain several well-characterized isolates of the strain of interest and test their virulence in hamsters (screening step). A frozen stock material is then produced for one to three highly virulent strains (production step with serial passages in hamsters) and stored in liquid nitrogen.

After verification of identity, a selection step is carried out in puppies to selected one or two strains which gave satisfactory results by intraperitoneal challenge performed as required by European Pharmacopeia monograph 0447. Afterwards, an optimization step (dose/puppy, parameters of in vitro culture) is performed to maximize severity of disease. Finally, as adult dogs are often less sensitive to a given strain than puppies, the relevance of each challenge model developed in puppies is confirmed in adult dogs and specific adjustments made, if needed.

This common process of CMD provides robust means to evaluate the beneficial effects on six different criteria (mortality, clinical signs, leptospiremia, leptospiruria, renal carriage and renal lesions) of Boehringer Ingelheim Animal Health canine leptospirosis vaccines.

PASSIVE SURVEILLANCE OF LEPTOSPIRA INFECTION IN SWINE IN GERMANY

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Leptospirosis is presumed to be the most widespread zoonosis worldwide. It is a cause of reproductive loss in swine breeding herds and has been reported in swine from all parts of the world. Unfortunately, current data about the prevalence of leptospiral infection in swine are rare.

Laboratory data were analysed from diagnostic examinations carried out on samples from swine all over Germany from January 2011 to September 2016. A total of 29829 swine sera were tested by microscopic agglutination test (MAT) for antibodies against strains of eleven Leptospira serovars (Australis, Bratislava, Autumnalis, Canicola, Grippotyphosa, Copenhageni, Icterohaemorrhagiae, Pomona, Hardjo, Sejroe, and Tarassovi).

Overall, 20.2 % (6025) of the total sample collection tested positive for leptospiral infection. Seropositivity ranged between 16.3 % (964) in 2011 and 30.9 % (941) in 2016 (January to September only). Of all samples, 11.6 % (57.3 % of the positives) reacted with only one Leptospira serovar, and only 8.6 % (42.7 % of the positives) reacted simultaneously with two or more serovars. The most frequently detected serovar was Bratislava, which was found in 11.6 % (3448) of all samples, followed by the serovars Australis in 7.3 % (2185), Icterohaemorrhagiae in 4.0 % (1191), Copenhageni in 4.0 % (1182), Autumnalis in 3.7 % (1054), Canicola in 2.0 % (585), and Pomona in 1.2% (368). Modelling (Table 1) shows that both the year and the reason for testing at the laboratory had statistically strong effects on the test results; however, no interactions were determined between those factors. The results support the suggestion that these seropositivities may be considered to indicate the state of leptospiral infections in the German swine population.

Although data from passive surveillance are prone to selection bias, stratified analysis by initial reason for examination and analyses by model approaches may correct for such biases. A prevalence of about 20 % for a leptospiral infection is most probable for sows with reproductive problems in Germany, and with an increasing trend. Swine in Germany are probably a reservoir host for serovar Bratislava, but were not for Pomona and Tarassovi in the period of this study, in contrast to findings of other studies.

Table 1: Two-factor logistic regression analysis on MAT outcome

General model type III tests of fixed effects			
	DF[1]	F[2]	p[3]
Year	5	10.79	< 0.0001
Reason for sampling	2	7.66	0.0005
Interaction	10	1.58	0.1049

Effect	n	Positives %	OR[4]	CI[5] lower	CI[5] upper	p[6]
Year						
2011 (ref)	5908	16.3	1	-	-	-
2012	5472	19.0	1.490	1.204	1.843	0.0023
2013	5568	19.3	1.251	0.996	1.572	0.1292
2014	5106	19.5	1.296	1.026	1.638	0.1935
2015	4733	21.3	1.951	1.549	2.457	0.0014
2016 / 01 to 09	3042	30.9	2.565	1.862	3.533	0.0240
Reason for sampling						
Monitoring (ref)	1813	16.9	1	-	-	-
Reproductive problems	12017	22.1	1.478	1.189	1.838	0.0017
Unknown reasons	15999	19.1	1.326	1.068	1.646	0.0093

[1]Degrees of freedom; [2]F-test statistics for model parameter; [3]p-value for F-Test; [4]odds ratios adjusted for year, reason for sampling, and interaction; [5]lower/upper bound 95 % confidence interval for the odds ratio; [6]p-value for Wald test

ISOLATION AND IDENTIFICATION OF PATHOGENIC LEPTOSPIRA FROM RODENTS ON THE ISLAND OF SAINT KITTS

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Leptospirosis is endemic in the Caribbean region and is estimated to result in high human morbidity and mortality. Contact with infected animals and contaminated environment is a major risk factor for Leptospira infection in humans and animals. Rodents are well known reservoirs of Leptospira and is a key player in epidemiology and transmission of Leptospira. Leptospira infection in rodents has not been documented on Saint Kitts. This study describes isolation and identification of Leptospira from rodents. Kidneys from rats (n=29), mice (n=9) were tested for Leptospira infection, by real time PCR (RT-PCR), direct immunofluorescence assay (DFA) and Leptospira culture. Serum samples were tested by microscopic agglutination test (MAT) when available. Phylogenetic tree was constructed with adk gene sequences amplified from kidneys and from isolates. Whole genome sequencing was conducted from selected isolates. Mitochondrial cytochrome b sequencing was performed to identify the rodents species.

Eleven Rattus rattus (R. rattus), 18 Rattus norvegicus (R. norvegicus) and nine Mus musculus (M.musculus) were captured. Leptospira DNA was amplified from kidneys of 17/29 rats (58.6%) and 4/9 mice (44.4%). Kidneys from 12 rats and two mice were DFA positive. Eighteen Leptospira isolates were obtained from 14 rats and one mouse. Whole genome sequencing suggested that three rodents might be co- infected with L. borgpetersenii and L. interrogans. Phylogenic analysis of adk sequences indicated the presence of L. interrogans and L. borgpetersenii in rats and L. borgpetersenii in mice, respectively. A preliminary attempt to classify isolates identified that isolates belonged to Icterohaemorrhagiae, and Ballum. Twenty-five out of 29 (86.2%) rats had antibodies to at least one Leptospira serovar tested in the 21 serovar MAT panel. Predominant serovars detected by MAT included Icterohaemorrhagiae (82.6%), Copenhageni (13.7%), Grippotyphosa (3.4%), and Wolffii (3.4%) in rats.

Heavy Leptospira renal colonization was observed in rats and mice in Saint Kitts. Our study suggests potential evidence of coinfection with at least two Leptospira species. It is intriguing that a high proportion of rats had antibodies against serovar Icterohaemorrhagiae, but none of the rats had antibodies against serovar Ballum, although both serovars were infecting the rat population.

LEPTOSPIRA SEROVARS IN HORSES SUFFERING FROM ERU LOOKING AT INTRAOCULAR AND SERUM SAMPLES IN GERMANY AND NEIGHBOURING COUNTRIES

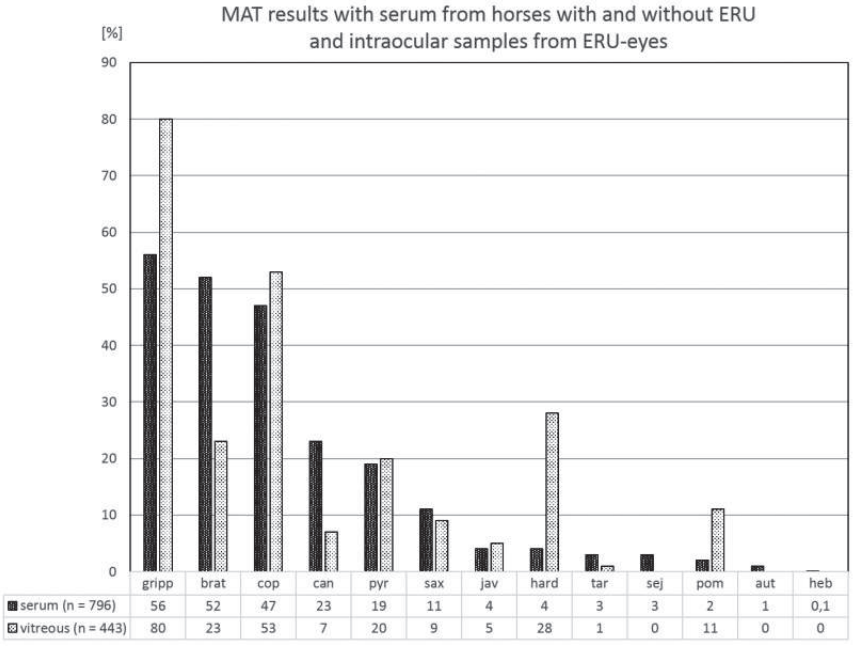
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Recurrent leptospiral uveitis is an important disease in horses, often leading to blindness. Collecting serum samples is easy but not indicative for leptospiral uveitis which is a late sequela of systemic leptospirosis in horses. Uveitis recurrences are caused by persistent leptospiral infection within the equine eyes. Vitrectomy had been proven as an effective treatment in respect of preventing further uveitis attacks and preserving vison if performed early in the course of the disease. In the beginning of this surgery, it is possible to collect undiluted vitreous material for microbiological investigations. It is assumed, that small rodents and especially field mice are the most important transmitters of leptospires. Serum samples have been taken from 664 horses suffering from recurrent uveitis (equine recurrent uveitis, ERU) as well as from 132 controls and were tested for antibodies using O.I.E. guidelines conform MAT for serovars Grippotyphosa, Bratislava, Copenhageni, Canicola, Pyrogenes, Saxcoebing, Javanica, Hardjo, Tarassovi, Sejroe, Pomona, Autumnalis and Hebdomadis. Vitreous samples were collected from 443 ERU eyes as well as from 54 control eyes and tested for antibodies identically. Antibody titres of 1:100 or higher were considered positive. Furthermore, 358 intraocular samples from ERU-eyes were prepared for leptospiral culture.

Looking at MAT results of serum samples, there was no difference between ERU horses and controls, whereas there was a highly significant difference between MAT results of intraocular samples from ERU horses and controls. Vitreous samples from control horses were negative in 95 %, 5 % had an MAT titre of 1:100, none was higher than 1:100. In vitreous samples from ERU eyes, 10 % were MAT negative, 6 % showed a MAT titre of 1:100 and 84 % had higher titres than 1:100. Many serum and vitreous samples showed antibodies against several serovars. The most frequently detected antibodies were directed against serovar Grippotyphosa as well in serum as in intraocular samples (see figure 1). Culture was positive in 189/358 vitreous samples (53 %). Cultures were specified using specific antibodies. In 144/189 cultures (76 %), serovar Grippotyphosa was detected. In 21 samples there was serovar Bratislava, in 8 cases serovar Pomona, in 4 cases serovar Pomona, and in 2 cases serovar Javanica. Thus, serovar Grippotyphosa is the most common one among horses in Germany and neighbouring countries. As field mice are known to shed serovar Grippotyphosa, they seem to be the most important source of infection for horses, but there are other ways of infection as well. Furthermore, it could be shown that different serovars cause chronic intraocular infection with accompanying recurrent uveitis in horses.



	gripp	brat	cop	can	pyr	sax	jav	hard	tar	sej	pom	aut	heb
serum (n = 796)	56	52	47	23	19	11	4	4	3	3	2	1	0,1
vitreous (n = 443)	80	23	53	7	20	9	5	28	1	0	11	0	0

SCREENING OF ANTI-LEPTOSPIRAL ANTIBODIES IN SANITATION WORKERS FROM LISBON AND TAGUS VALLEY REGION (PORTUGAL)

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Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of Leptospira genus, whose importance worldwide is increasing. The clinical manifestations are broad-spectrum and may be characterised by an apparent flu-like syndrome or even severe situations. Contact with the infectious agent can be detected by the presence of anti-Leptospira spp antibodies in the serum. Rodents, mainly rats and mice, are the most common reservoirs of these bacteria. Transmission to humans occurs mainly through direct contact with urine, soil or water containing leptospires. There are occupational groups considered at risk for this disease (veterinarians, farmers, sanitation workers, among others). Thus, in this study we aimed to evaluate the presence of anti-leptospiral antibodies in sanitation workers from Lisbon and Tagus Valley Region (Portugal).

Blood samples were collected from 347 sanitation workers (WWTP's, City Hall and privatized companies) and a clinical-epidemiogical questionnaire was applied to each one. The study participants also attended to a lecture about the disease and how it is transmitted. The samples were tested in the Leptospirosis and Lyme Borreliosis laboratory at the IHMT/UNL, initially by a screening test (MACROLepto - an agglutination test on the slide), to search the presence/absence of anti-leptospiral antibodies in the serum. Samples with "Positive" and "Non- Conclusive" results were evaluated with the confirmatory test for leptospirosis - Microscopic Agglutination Test (MAT).

It was not observed anti-L. interrogans s.l. antibodies in the worker's samples. However, the survey results showed that 83% were unaware of the disease, 77% had previously seen rodents in the workplace, 94% always used PPE (Personal Protective Equipment) and 79% always carried out the general hygiene measures "Daily Bath" and "Handwashing".

Despite the majority of workers that didn't know any information about the disease and the regular presence of rodents in the workplaces, the use of PPE and the general hygiene measures practiced by them seemed to be useful in the infection prevention by Leptospira spp. It is now intended to study a sampling of the rodent population where this survey was performed, aiming the determination of the bacterial rate in order to prevent Leptospira infection in this professional group.

LEPTOSPIRES AND LEPTOSPIROSIS IN SOME SUB-SAHARAN AFRICAN COUNTRIES: A PORTUGUESE COOPERATION EXPERIENCE

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Leptospirosis is the most worldwide distributed zoonosis caused by pathogenic bacteria of genus Leptospira in tropical regions. Its diagnosis can be complicated, where similar febrile illnesses are common and can be confused with other disorders such as malaria, viral hepatitis, dengue, among others. In Angola, Guinea-Bissau and Mozambique, the diagnosis is difficult due to lack of availability and implementation of specific tests, and the prevalence of disease remains unknown. Portugal, as a member of the Community of Portuguese Speaking Countries, maintains in these countries active cooperation, namely in promoting Health. Therefore, is crucial to help the research in order to include leptospirosis in the differential diagnosis of fever diseases, improving the population's health.This work reports the collaboration and technical support to those countries through the Portuguese Laboratory for Leptospirosis, contributing to determine the leptospirosis seroprevalence in patients/users with undetermined febrile syndrome who use the health services in Angolan Provinces of Lubango, Lobito, Huambo and Luanda, and detect/identify Leptospira spp in rodents captured in Huambo and Luanda (Angola), Bissau (Guinea-Bissau) and on the Island of Mozambique (Mozambique).

A total of 1091 serum samples was collected from patients/users of the referred health services. Fever was the primary symptom in all patients. Serological and molecular tests were performed by Microscopic Agglutination Test (MAT) to detect antibodies (abs) against Leptospira, and to detect leptospiral DNA by Polymerase Chain Reaction (PCR), targeting the hap1 gene, respectively.

Regarding the rodents, a total of 238 animals was captured (Angola n=77; Guinea-Bissau n=104 and Island of Mozambique n=57). DNA from kidney tissue was extracted for further analysis by PCR, targeting the same hap1 gene (rodents from Angola and Guinea-Bissau), and the 16S rRNA and lipL32 genes (rodents from Mozambique). Leptospiral DNA of some samples (serum and kidney tissue) were sequenced to identify Leptospira spp.

The serological study revealed 175+/950 (18%) positive samples for abs against pathogenic Leptospira and 130/950 (14%) with borderline reactivity. Icterohaemorrhagiae, Ballum and Australis serogroups were the most prevalent. In parallel, leptospiral DNA was detected in 43+/274 (17.4%) of sera. Concerning the rodent's, specific DNA was present in 52+/238 (21.8%) of kidney samples. Of the sequenced samples the results showed 99% of similarity to L. interrogans and L. borgpetersenii genospecies.

The results show the essential role of a Reference Laboratory in the support to the studies carried out in countries with weak laboratory facilities for leptospirosis diagnosis. These findings, the first so far in these countries, can contribute to the future development of the diagnostic ability of an emerging and often neglected disease.

PREVALENCE AND BIOLOGICAL DISTRIBUTION OF LEPTOSPIRAL SEROVARS IN HORSES IN CENTRAL FRANCE

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The aim of this prospective study is to assess prevalence of leptospirosis in clinically healthy horses in Central France, based on detection of Leptospira interrogans by MAT and PCR from relevant biological fluids such as blood, urine and vitreous humour.
8 healthy horses euthanized for independant reasons were studied and sampled. Blood, urine and vitreous humour were all analysed using classic quantitative MAT technique for serology, as well as PCR extraction and amplification to detect gene 16S, and also by BLAST sequencing.
Serological results showed that 36/48 horses were positive at 1/100 dilution indicating contact with Leptospira,, 31/48 were weakly seropositive at 1/200, 20/48 moderately positive at 1/400, and 5/48 were strongly positive at a 1/800 dilution. Dominant identified serovars were Bratislava (89%), Bim (50%), Copenhageni (47%), Pyrogenes (39%), Panama (33%) and Saxkoebing (22%).
PCR and sequencing results indicate presence or Leptospiral DNA in 11/48 (23%) of all samples, with a prevalence of 10% in blood, 10% in vitreous humour, and 2% in urine.
Looking at correlations between tests: 18% horses were positive for MAT and PCR, whilst 17% horses were negative for both. 58% were MAT positive & PCR negative, and 6% were PCR + and MAT -
64% of our horses were seropositive which is higher than in previous studies by other authors and studies arising from other countries performed in horses. 23% of our horses were positive based on PCR which is lower than in the Miere et al. study (2000). The MAT+/ PCR - horses could reveal cases of old infections that have been cleared.
The most frequently isolated serovar in this study is Bratislava, which seems to concur with a previous study by Wayne et al., 1994 in horses.
This study provides quantitative data of exposure of horses to Leptospirosis in Central France as no other similar study had been conducted in that area beforehand, and very few data are available otherwise. Using several biological media provides an interesting array of relevant samples to equine leptospiral manifestations.

EPIDEMIOLOGICAL AND PATHOLOGICAL ASPECTS OF LEPTOSPIROSIS IN WILD BOAR IN A DEFINED GEOGRAPHICAL STUDY AREA

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Leptospira spp. commonly affect domestic and wild species, including swine which may shed this bacteria in the environment. In wild boars *Leptospira* Pomona, *Leptospira* Bratislava and *Leptospira* Grippothyphosa have been considered to be the most frequent serovars. In this study we investigated the potential role of wild boars as reservoirs of *Leptospira* spp. within a defined geographical area of Sardinia (Italy).

In our study, we firstly tested by Microscopic Agglutination Test (MAT) the sera of 13,870 wild boars, which were mostly hunted in the Northern part of Sardinia. In 430 out of these animals, all belonging to a small geographical area of 960 km^2, we examined the kidney by REAL TIME PCR targeting lipL 32 gene, specific bacteriological assay and, in a number of them (n. 214), by immuno-histopathological means. In addition, in the same area, 60 slaughtered rural pigs from no-professional holding were also included in the study and submitted to the same diagnostic procedures.

Serological examinations showed that 744 (5.4%) out of the 13,870 wild boars serum samples were MAT positive for serovars Pomona (73%), Bratislava (35%) and Grippothyphosa (12.4%). In 215 (29%) blood samples we detected agglutinating antibodies against both serovar Pomona and serovar Bratislava. Among the 430 wild boar kidney samples 37 (8.6%) were real-time-PCR positive for pathogenic Leptospira, while from 9 (2%) we isolated serovar Pomona. Immuno-histopathologically, we observed in the examined kidneys focal chronic interstitial nephritis (9.3%) and intratubular colonization of *Leptospira* spp (1.4%). All the 60 rural pigs resulted negative for Leptospira infection by serological test, PCR and bacteriological means, while only 3 (1.4%) showed histological lesions in the kidneys.

Our study shows that in Sardinia wild boars are highly exposed to *Leptospira interrogans* serovar Pomona and *Leptospira interrogans* serovar Bratislava and can potentially act as a maintenance host for these serovars. Differently, pigs in the rural non-professional farms, although they often share the same environmental niches are less exposed to *Leptospira* spp. infection. From a public health viewpoint, other studies need to estimate the significance of this potential exposure route for human Leptospirosis.

OUTBREAKS OF BOVINE LEPTOSPIROSIS INFECTION IN TWO SICILIAN LIVESTOCKS (SOUTHERN ITALY)

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Until now, there are no data about Leptospirosis cases in bovines in Sicily. In this work, we first report leptospirosis infection outbreaks in bovines farmed in two different livestocks located in mountainous area in the heart of Madonie Natural Park (Sicily). After some cases of abortion and fertility problems, the freelance and public veterinary, supposed a probable leptospirosis infection.

The bovines object of our study belonged to two different livestocks. The first one was composed of only bovine of *Charalaise* race. The second was composed of *Limousine* bulls and *Cinisare* and *Modicane* cows. The outbreaks emerged in October 2017. Number 23 samples of bovine sera were taken in the first livestock and n. 75 in the other one. Sampling was carried out before and after the antibiotic treatment with oxytetracycline (10mg/kg of body weight with two injections at four days interval). Two urine samples were taken from positive bovines (2/2) belonging to the first livestock and four (4/29) in the second one; in addition, a sample of brain and placenta were taken from a single abortion. The diagnosis of leptospirosis infection was based on the antibodies detection through serological Microagglutination Test (MAT) conducted as prescribed in the O.I.E. Manual (Chapt. 2.1.12. Par. B2.1. 2014). The analysis concerned eight common serogroups circulating in Italy (*L. interrogans* serogroup Australis serovar Bratislava, *L. interrogans* serogroup Ballum serovar Ballum, *L. interrogans* serogroup Canicola serovar Canicola, *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni, *L. interrogans* serogroup Pomona serovar Pomona, *L. borgpetersenii* serogroup Sejroe serovar Hardjo, *L. borgpetersenii* serogroup Tarassovi serovar Tarassovi). "Real-Time PCR method" was performed for the gene *lipL*32 detection of pathogenic *Leptospira* spp. in the brain, placenta and urine samples. The "isolation method" was performed only urine sample. Afterwards a differential diagnosis, no presence of bacterial or viral abortion agents was detected.

In the first livestock, 2 serological positive bovines (2/23) to antibodies against *L. borgpetersenii* serogroup Sejroe serovar Hardjo were found. While in the other livestock, there were 29 serological positive bovines (29/75), with different antibody titres against *L. borgpetersenii* serogroup Sejroe serovar Hardjo (1:100 – 1:3200). The Real Time PCR test detected *Leptospira* DNA in one of the two samples of urine of the first livestock. But the same Real Time PCR test on both placenta and brain was negative. The isolation is still in progress. After 40 days from antibiotic treatment, the antibody titres to *L. borgpetersenii* serogroup Sejroe serovar Hardjo decreased in the first livestock. Instead, in the second livestock, the titres of 6 bovines increased, the titres of 4 diminished and 2 new positives emerged. The titres of the other positive 19 remained at the same level as before. In both livestocks the symptoms disappeared.

The good results obtained with antibiotic treatment in the bovines of the first livestock, let us to believe that outbreak will be over soon. Instead, the situation of the second livestock seems to be more complicated and makes the leptospirosis eradication more difficult. Infact, a second antibiotic treatment is still on going. In order to control the progression of the *Leptospira* spp. infection, it is our intent to check the bovine antibody titres in the next future.

LEPTOSPIROSIS, A DIAGNOSIS NOT TO *"UNDER-RATE-E"*

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Leptospirosis is increasingly recognized as a clinically important and potentially life-threatening illness. We hereby report the case of a 43- year-old male gardener who was admitted to our hospital with fever, myalgia, cough, dyspnea and asthenia. He evoked an indirect contact with rats a month ago. During his initial evaluation he was noted to be febrile (38.8°C), hypotensive and profoundly jaundiced.

A Leptospira IgM rapid immunochromatographic test was performed on a serum specimen collected 5 days post-symptom onset. In addition, a Microscopic Agglutination Test (MAT) was carried out. Serum was brought into contact with 10 cultures of different Leptospira serotypes. Specific antibodies, present in the sample, will agglutinate. One drop of suspension was brought onto a slide and interpretation was done by dark field microscopy. DNA was extracted from the same sample and challenged to a TaqMan PCR targeting the Hap1/LipL32 gene (diagnostic PCR). Positive PCR samples were further tested by lfb1 mono-gene targeted PCR for genotyping.

Laboratory findings on admission showed a mild anemia (Hb 10.9 g/dl) and leukocytosis (WBC 11 300/µl) with a deep thrombocytopenia (32 000/µl) without morphological abnormalities. The high CPK level (1857 U/L) indicated a rhabdomyolysis and the kidney- and liver-function tests were strongly pathological. Initial serology testing ruled out infections with Chlamydia pneumoniae, Treponema pallidum, Hepatitis B and C, HIV and Hantavirus. Further laboratory testing demonstrated a decreasing hematocrit (24%) and myelocytosis. A serum specimen collected 5 days post-symptom onset resulted in a positive Leptospira IgM rapid test. A MAT was also performed on this serum sample (with negative result) and on another convalescent specimen collected 12 days post-symptom onset and revealed elevated titers for multiple Leptospira serovars. Cross-reactions between serovars or serogroups are common and well described with MAT. To identify the infecting serovar, the serum collected on day 5 of illness was sent to Coda-Cerva to perform a diagnostic PCR which resulted positive (Ct 37). High resolution melting typing of the lfb1 gene indicated the species to be of L.interrogans while an analysis of the sequence type is ongoing. Later the patient developed an acute respiratory distress syndrome (ARDS) requiring mechanical ventilation for a duration of 3 days. Despite our patient presented the five prognostic factors for a severe outcome (hemoptysis, thrombocytopenia, hyperbilirubinemia, hyperleucocytosis and hematocrit < 30%), he was discharged thanks to his good clinical evolution after a treatment of 7 days with intravenous penicillin G.

In Belgium, in contrast to other European countries, there is no preventive vaccination policy against leptospirosis. Because early recognition is mandatory to rapidly initiate an accurate antibiotic therapy, clinicians should familiarize themselves with the clinical presentation of leptospirosis.

LEPTOSPIRA CHRONIC CARRIAGE: A STUDY ON ELEVEN NATURALLY INFECTED PET RATS

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We previously described a case of human leptospirosis associated with direct contact with eleven pet rats (1). Epidemiological investigation led to identify key risk factors such as working in a petshop and pet rat exchanges within a pet-rat daycare community. The eleven rats were collected alive at the CODA/CERVA animal facility and maintained for three months to study the natural *Leptospira* chronic carriage in reservoir animals. Serological analyses were performed with MAT by using the following serogroups' panel: Australis, Autumnalis, Bataviae, Canicola, Ballum, Grippotyphosa, Javanica, Sejroe, Icterohaemorrhagiae, Pomona, Pyrogenes, and Tarassovi. Strains were maintained/isolated in liquid EMJH medium supplemented with 0.2% w/v yeast extract and 10% fetal calf serum and cultured at 29°C. Diagnostic PCR (RT-PCR), obtained by amplification of the Hap1/LipL32 locus, was applied on blood, urine and organ samples. Strains isolated from positive PCR samples were further characterized by mean of sero- and a 3-loci MLVA typing. Whole genome sequencing (WGS) was performed using a MiSeq reagent kit with 2x 300-bp paired-end sequencing on a MiSeq system (Illumina). Urine, blood bacteremia and serology were followed over a 3-month period in the eleven rats. One rat was sacrificed few days after the beginning of the study to limit pain associated with an already weak health status. Urinary shedding was detected in 63% (7/11) and 45% (5/11) of cases by RT-PCR and direct isolation, respectively. Bacteremia in blood was not observed all along the study. Serology and MLVA indicated the infection being clonal at both serological and genotyping levels and highlighted the strains belonging to the serogroup *Icterohaemorrhagiae*. Considering serology and MLVA approaches of a low resolution for outbreak investigation, we applied WGS to three strains. Bioinformatics analyses are ongoing. Times before sacrifice, two of the rats were treated with doxycycline 10 mg/kg for 5 days and shedding analyzed by RT-PCR till 60 days post- treatment. Bacterial shedding, which was high before treatment for these two rats, decreased steadily to be undetectable at the end of the study. Histology examination of the euthanized rats identified interstitial nefritis as the common lesion in positive rats. Diagnosis of Leptospira chronic carriage in reservoir animal can be cumbersome. However, the combination of several approaches might lead to increase sensitivity in diagnosis. Antibiotic treatment of infected pet rodents decreases shedding. Further studies are required to fully evaluate pros versus cons (i.e. selection of resistant strains) to the application of this approach and whether it could be suggested in extremely sensitive contexts.

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EVALUATION OF TWO RAPID TESTS FOR THE IN-CLINIC DIAGNOSIS OF ACUTE CANINE LEPTOSPIROSIS

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The early diagnosis of canine leptospirosis is still considered challenging in the daily veterinary practice. Recently, point of care tests have been proposed with promising results in retrospective studies. The aim of this study was to prospectively evaluate the accuracy of two rapid in-clinic tests detecting anti-leptospiral antibodies for the diagnosis of acute canine leptospirosis.

Dogs with history, clinical and clinicopathological signs suggestive of leptospirosis (acute kidney injury and/or systemic inflammatory response syndrome with multiorgan damage) were prospectively enrolled in the study and tested with IgM-detecting (RTD-A; WITNESS Lepto, Zoetis) and IgG-detecting (RTD-B; SNAP Lepto, IDEXX Laboratories) rapid tests. The confirmation of leptospiral infection (confirmed leptospirosis cases, CLC) was obtained by a combination of microagglutination test (MAT) performed upon admission (titre ≥1:800), convalescent MAT (four-fold increase in titre) and qPCR on blood and/or urine samples (gold standard criteria to achieve a definitive diagnosis). Negative leptospirosis cases (NLC) were also included in the study for comparative purpose. Performances of the two in-clinic tests and MAT were compared to the gold standard adopted in this study and statistically analysed using ROC curve analyses. A P value <0.05 was considered significant. Furthermore, a Bayesian latent class model was performed, assuming that all diagnostic tests are imperfect, as previously described in human medicine.

A total of 42 CLC and 36 NLC were included. MAT upon admission and RDT-A had a sensitivity of 76.2% and 71.4%, respectively, while the specificity was 100% for both. RDT-B had a sensitivity of 78.6% and a specificity of 72.2%. Areas under the ROC curve (AUC) were 0.88 (MAT), 0.85 (RDT-A), and 0.75 (RDT-B). No statistical difference was documented between the diagnostic performances of the MAT upon admission and the RDT-A (P=0.56), nor was between the RDT-A and the RDT-B (P=0.06). A significant difference was observed between the MAT upon admission and the RDT-B (P=0.01). According to the Bayesian latent class model, RDT-A and RDT-B had a sensitivity of 78.9% and 86.5%, with a specificity of 97.6% and 75.0% to diagnose leptospirosis in this study population.

Our results demonstrate the utility of the two in-clinic diagnostic tests as a first screening tool in presence of a clinical suspicion of leptospirosis in dogs. Statistical analyses documented that RDT-A had slightly better diagnostic performances than RDT-B; nevertheless, a positive result in dogs with clinical and laboratory signs indicative of acute leptospirosis highly supports the diagnosis for both the rapid tests. A negative result requires additional diagnostic investigation to rule out the infection.

HOST RESPONSE AND IMMUNOLOGY

PULMONARY ENDOTHELIAL EXPRESSION OF BETA-CATENIN, CD31 AND VON WILLEBRAND FACTOR IN DOGS WITH LEPTOSPIRAL PULMONARY HAEMORRHAGE SYNDROME

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Leptospiral pulmonary haemorrhage syndrome (LPHS) is a particularly severe form of leptospirosis associated with high mortality. The pathogenic mechanisms of LPHS are poorly understood but a role of the pulmonary microvascular endothelium in the breakdown of the alveolar barrier function has been suggested. Aims of this study were to describe the in vivo expression of endothelial cell markers including ß-catenin, CD31 and van Willebrand Factor (vWF) in lung tissues of dogs with LPHS due to natural infection with Leptospira. Differences in the abundance and intracellular distribution of ß-catenin in canine LPHS tissues were then further investigated using canine and murine endothelial cell culture models.

Lung tissues of 8 dogs without pulmonary pathologies (A), 12 dogs with LPHS (B) and 9 dogs with pulmonary haemorrhage due to other causes (C) were immunhistochemically labeled for ß-catenin, CD31 and vWF. Staining patterns for capillaries, small and bigger pulmonary blood vessels were semi-quantitatively scored and compared between groups. Confluent primary canine aortic (CAEC) and VE-cadherin- GFP murine pulmonary endothelial cells (MPEC) were incubated with either pathogenic L. interrogans serovar Copenhageni or L. biflexa serovar Patoc I for 3, 6 and 24 hours. Cells were labelled for total and non-phosphorylated (NP) ß-catenin and differences in staining patterns and intensities assessed.

Immunohistochemical staining patterns and intensities for CD31 were not different between groups, whereas staining intensities for vWF and ß-catenin were significantly reduced in lungs with haemorrhage (A+B) compared to healthy control lungs. Internalization of ß-catenin away from the cell-cell junction (linear cytoplasmic pattern) was observed almost exclusively in LPHS lungs (C).

In in vitro infected endothelial cells, staining intensity of total and NP ß-catenin at cell borders was reduced, and focal spotting was increased in cells infected with pathogenic Leptospira, most noticeably after 24h. VE-cadherin expression was reduced in MPEC.

We conclude that loss of ß-catenin and VE-cadherin could be important factors in the development of LPHS as these proteins are constituents of the endothelial adherens junction complex, which is crucial for the maintenance of the endothelial barrier function.

CHARACTERISATION STUDIES OF OUTER MEMBRANE PROTEINS FROM PATHOGENIC BOVINE LEPTOSPIRA SPP.

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Leptospirosis is a substantial cause of milk drop syndrome and reproductive failures in cattle[1]. Cattle are known to maintain serovar Hardjo which is pathogenic to both cattle and humans and poses a public health risk. Commercial bovine vaccines are considered limited due to serovar-restriction, short-term immunity and persistent renal colonisation.[2] Outer membrane proteins (OMPs) are considered worthy vaccine candidates due to their ability to express during natural infection. Furthermore, OMPs are surface exposed which enables substantial immunogenicity. Using a reverse vaccinology approach, we have identified several leptospiral OMP genes, produced recombinant proteins, characterised their function and investigated their interaction with the host immune system.

In silico identification of target genes: OMP loci from two reference bovine leptospiral strains were identified by using various bioinformatic programs to predict the β-barrel structures.

Cloning, expression and purification of target OMPs: Candidate OMP genes were amplified and cloned into pDEST17 expression vector containing a N-terminal 6X His-tag. The purified plasmids were then transformed into Escherichia coli BL21-AI for expression.

Recombinant genes were grown overnight and expressed with L-arabinose, refolded and purified by Ni^{2+}-NTA affinity chromatography.

Functional and immunological assays of recombinant OMPs: Successful rOMPs were subjected to functional assays to measure binding affinity for host molecules. Host antibody responses within milk were also surveyed for the rOMPs.

Seven target genes with predominant β-sheet were selected (from ~4000 genes) and were expressed and purified. Circular dichroism (CD) spectrums demonstrated predominance of β-sheets within rOMPs (example shown in Figure 1). From the panel of rOMPs only cattle rOmpL1 showed response towards bovine IgG1 in positive milk samples when investigating host antibody responses to rOMPs (Figure 2). Our finding demonstrates that the rOMPs from bovine leptospiral strains are capable of binding to several host molecules. This study helps elucidate how these microbes are capable of adhering to the host. Interestingly bovine Leptospira rOmpL1 was identified as an antigen that reacts with host immunity in cattle milk and this could be used as a diagnostic development in the future[3]. Further studies are planned to survey attachment of these recombinant proteins against kidney cells and kidney damage markers given that the renal tubules are considered prone to Leptospira colonisation.

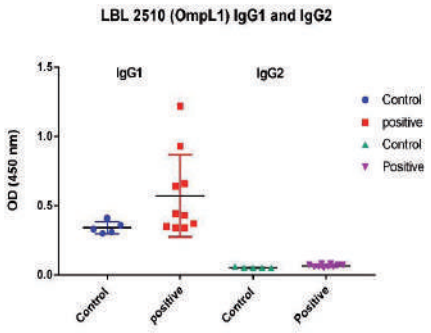


Figure 2: Antibodies response of rOmpL1 in milk samples

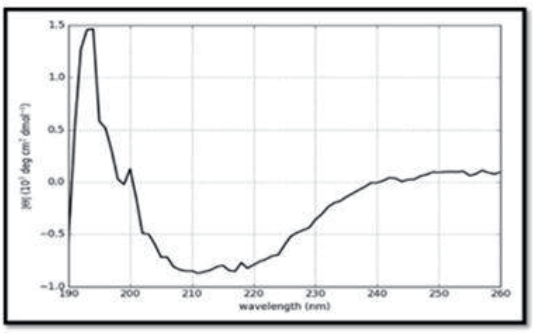


Figure 1:CD spectra of rOMPL1

Kd value (μM)/ ECM component	LBL2510	LBL0972	LBL2618
Fibrinogen	0.367	0.107	0.024
Fibronectin	1.004	0.235	0.085
Laminin	-	0.769	0.183
Elastin	0.563	-	-
BSA (control)	Not significant	Not significant	Not significant

Table 1: Binding to selected host molecules as a function of recombinant proteins

Reference

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2. Zimmerman AD, Springer EW, Barling KS, et al. J Am Vet Med Assoc 2013; 242: 1573–7.
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ENDOTHELIAL CELL PROTEOMIC RESPONSES TO INFECTION WITH PATHOGENIC LEPTOSPIRA REVEALS ACTIVATION OF PATHWAYS LINKED TO CYTOSKELETAL REMODELING

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Leptospiral pulmonary hemorrhage syndrome (LPHS) is a severe form of leptospirosis. Due to a limited understanding of the pathomechanisms of LPHS, there are currently no efficient treatments and mortality is high. Endothelial dysfunction, contributing to a decreased pulmonary alveolar barrier function in LPHS, has been suggested in previous studies. Aim of this study was to investigate the effect of direct contact of pathogenic leptospires with primary endothelial cells on cell morphology and proteome.
Cultured endothelial cells (EC) from porcine, murine and canine origins were infected with either pathogenic L.interrogans serovar Copenhageni (EC-Cop), or saprophytic L.biflexa serovar Patoc I (EC-Pat). Fixed cells were labelled with phalloidin and Hoechst stain. Morphological criteria, such as cell elongation, rounding, gap formation, cortical and central actin, and stress fibers were assessed semi- quantitatively at 3h, 6h and 24h. In parallel, changes in the proteome of EC at 3h and 6h were studied using mass spectrometry (LC-MS) of infected and TNF-α-stimulated (EC-TNF-α) murine primary pulmonary EC.
Increased scores for cell rounding, gap formation and central actin condensation, were found in EC-Cop compared to Control and EC-Pat. Findings were consistent across all three species. LC-MS identified 3036 proteins, 16 of which were leptospiral proteins. At 3h, 86 proteins were found in different abundance uniquely in EC-Cop, 63 uniquely in EC-Pat, 84 were common to both, of which 57 were also common to EC-TNF-α. At 6h, 68 proteins were different in abundance uniquely in EC-Cop, 75 uniquely in EC-Pat, 105 were common to both, of which 66 were also common to EC-TNF-α.
Proteins found in different abundance included VCAM-1, PAI-1, P-selectin and GBP5. Pathway analysis suggested remodeling of the actin cytoskeleton through signaling by Rho Family GTPases at 3h as the most relevant pathway in EC-Cop. Analysis of the biological functions suggested that cell morphology, quantity of tight junctions and synthesis of pro-inflammatory mediators were altered in EC-Cop, and that HMOX1 was a highly significant upstream regulator. In comparison, after 6h, coagulation, tight junction and calcium signaling were pathways specific for EC-Cop, and nitric oxide quantity and EC viability were prominent biological functions.
These results indicate that direct interaction of pathogenic Leptospira with EC leads to actin cytoskeleton, and cell junction remodeling, and, consequently, a change of EC morphology. This could affect the integrity of the endothelium and contribute to the development of LPHS.

DEMONSTRATION OF CROSS-PROTECTION OF EURICAN® LMULTI AGAINST LEPTOSPIRA INTERROGANS SEROVAR COPENHAGENI IN AN ACUTE LEPTOSPIROSIS DOG CHALLENGE MODEL

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Efficacy of the *Leptospira* components of EURICAN[1] DAPPi-Lmulti, a dog multivalent vaccine, was demonstrated after a primary course of two subcutaneous injections of one dose given 4 weeks apart to 7 to 9 weeks old puppies. Challenges with three different pathogenic serovars of *Leptospira spp*. (Canicola, Icterohaemorrhagiae and Grippotyphosa) were carried out 14 days (onset of immunity studies - OOIs) or up to15 months (duration of immunity studies - DOIs) after primary vaccination using heterologous strains of the same serovar than vaccine strains. Beneficial effect of the vaccine was clearly demonstrated based on six criteria of protection with sterile immunity for all three serovars in OOIs and at different levels in DOIs (Bouvet et al., 2016). Here, we report cross-protection within the Icterohaemorrhagiae serogroup conducting an OOI challenge study against *L. interrogans* serovar Copenhageni.

Fourteen conventional Beagle puppies, aged between 8 and 9 weeks, were randomly allocated to two groups of 7 vaccinates and 7 controls. Animals were vaccinated with one dose of DAPPi-Lmulti (vaccinates) or DAPPi (controls) vaccine on Day0 and Day28. On Day41, all animals were challenged by intraperitoneal route with a virulent suspension of *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni. Following challenge, all animals were examined for clinical signs of leptospirosis on a daily basis and weight regularly. Blood and urine were regularly sampled for blood biochemistry and platelets count (blood), or detection of Leptospira by qPCR (blood and urine). At the time of death or euthanasia, animals were necropsied, and kidneys and liver were sampled for detection of Leptospira by qPCR (kidneys) or histological analysis (kidneys and liver).

Throughout the post-challenge monitoring period, all vaccinates remained healthy, without clinical sign and alteration of biologal parameters, no Leptospira DNA was detected in blood, urine or kidney, and no renal lesions were recorded; while all of the control animals presented *Leptospira* DNA in blood, urine and kidney and/or typical signs of the disease, and renal lesions.

Conclusion: similarly to the *L. interrogans* serovar Icterohaemorrhagiae OOI challenge study, dogs were completely protected against mortality, clinical signs of the disease, renal infection, bacterial excretion, renal lesions and renal carriage.

These results show that a primary course of two doses of EURICAN DAPPi-Lmulti provides cross-protective immunity against *L. interrogans* serovar Copenhageni with a quick onset of immunity (14 days following V2) and with a high level of protection demonstrated in a challenge model reproducing acute leptospirosis.

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VACCINES AGAINST LEPTOSPIROSIS: A LIVE ATTENUATED MUTANT ELICITS CROSS-PROTECTION IN HAMSTER BUT NOT IN DOG

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Leptospirosis is the most widespread zoonosis worldwide. Current licensed bacterin vaccines for human or animal applications elicit serogroup restricted protection while epidemiology is complex and regionally fluctuating. This imposes a long and costly vaccine development process with multiple strains assemblies and recurrent updates by species and by geographic location. A cross-protective solution is therefore a major unmet need for human and veterinary medicine. Live vaccines have already proved to be an interesting alternative for a long term and broad spectrum protection for other bacterial or viral diseases. We previously described a non-motile L. interrogans Australis isolate carrying a spontaneous mutation in its flagellar motor fliM gene. It was shown to be non-virulent in hamster model.

In the present work, we evaluated this strain as a live attenuated vaccine candidate against homologous and heterologous virulent challenges.

Hamsters were efficiently protected against homologous Australis and heterologous Icterohaemorrhagiae challenges. In dog, the live vaccine was safe upon subcutaneous administration and elicited sterilizing immunity against a severe homologous challenge. However, no significant protection was obtained against an heterologous Icterohaemorrhagiae challenge.

These results demonstrate that the nonmotile fliM mutated strain, although a promising candidate based on the hamster model, is unable to cross-protect dogs, one of the main veterinary vaccine target species. This study suggests that further work is still necessary to reach the ambitious goal of a universal vaccine against leptospirosis.

REDUCTION IN INCIDENCE OF CANINE LEPTOSPIROSIS IN SWITZERLAND CORRELATES WITH THE INTRODUCTION OF A NEW QUADRIVALENT ANTI-LEPTOSPIRAL VACCINE

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A marked increase in incidence of acute canine leptospirosis was documented in Switzerland between 2003 and 2012 with 70% of infected dogs showing serologic evidence of infection with serogroup Australis. In 2013, a new tetravalent killed whole cell anti-leptospiral vaccine (Nobivac® L4 – MSD Animal Health) was introduced onto the Swiss market. This vaccine includes serogroups Australis and Grippotyphosa in addition to serogroups Canicola and Icterohaemorrhagiae present in the previously available bivalent vaccines (L2). It had shown excellent protection against experimental challenge of dogs with heterologous strains from the same serogroups. Aim of this retrospective longitudinal case control vaccine efficiency study was to examine whether the introduction of L4 is associated with a reduction in hospital incidence of canine leptospirosis.
Dogs with leptospirosis (n=211) or acute kidney injury not due to leptospirosis (AKI-nL, n=119) treated at the Vetsuisse Faculty Bern between 2011 and 2017 were included if they had an accurate vaccination history. A diagnosis of leptospirosis was confirmed based on compatible clinical findings and the presence of at least one of the following: positive single MAT titre of ≥1:800 (n=66), MAT seroconversion (n=102), single positive IgM lateral flow assay (n=16), positive urine RT-PCR (n=4), or strong clinical suspicion not confirmed serologically due to early death (n=23). Control dogs were diagnosed with AKI-nL based on a convincing alternative diagnosis and at least a negative single serology. Vaccination status, vaccine type, and correctness of vaccination schedule were recorded. Annual incidences and vaccine effectiveness were compared between groups, the latter being expressed as odds ratio (OR) for being diagnosed with leptospirosis.
The control AKI-nL group showed a marked decrease in dogs vaccinated with L2 (from 100% in 2011 to 19% in 2016), and a steep increase in dogs vaccinated with L4 (from 0% to 81%), with stable numbers of dogs current on their vaccines (63 ± 13%) or unvaccinated (2 ± 3%). Simultaneously, the number of cases of canine leptospirosis significantly decreased from 63 (2011-2012) to 14 cases/y (2016-2017), while the number of control cases with AKI increased from 17 to 39 cases/y. The OR for a dog with AKI to be diagnosed with leptospirosis while being vaccinated vs not being vaccinated was 1.12 (95% CI, 0.89-1.42, P 0.33) for any anti-leptospirosis vaccine; 2.66 (95% CI, 1.87-3.79, P<0.001) for a L2-vaccine; and 0.28 (95% CI, 0.17-0.47, P <0.001) for L4.
These results indicate that the introduction of L4 was associated with a marked reduction in incidence of severe canine leptospirosis in Switzerland.

MEMBRANE TRANSLOCATING HUSCFV (HUSCFV-FC) ANTIBODY THAT TRANSVERSES MEMBRANE BARRIER AND FUNCTIONALLY EXHIBITS ANTI-LEPTOSPIRA COLONIZATION

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Leptospirosis is a global zoonosis that causes a significant human and veterinary health burden, and also has a large socio-economic impact. Pathogen-host interaction mediated through *Leptospira* adhesins leads to host inflammation and pathology, bacterial invasion, and dissemination to renal-tropic homing at immune privilege of animal reservoir. Passive immunotherapy by using therapeutic antibodies such as immunoglobulin G (IgG) and small molecules such as single-chain variable fragment (ScFv) against *Leptospira* adhesin might be optional therapeutic approach for antibiotic-allergic patients and for severe leptospirosis.

Human single-chain variable fragment antibodies (huScFv; VH-linker-VL) with anti-bacterial colonization were screened from a naïve 2.6×10^8 huScFv-displayed phages library. Membrane translocating huScFv (huScFv-Fc) antibodies were also developed to deliver HuScFv-Fc across membrane barrier. The huScFv-Fc antibody was tested for apical-to-basolateral transport through human FcRn receptor in antibody transcytosis assay by using HuLEC-5a endothelial membrane barrier model under in *in-vivo* (pH 7.5-8.0) and *in-vivo* acidosis (pH 7.0) conditions (50% serum, 37°C, 5%CO_2). Antibodies exocytosis was measured by ELISA assay.

Human ScFv antibody clones with anti-*Leptospira* colonization activity were screened from 500 selected huScFv-displayed phages. Pooled *Leptospira* specific human ScFv (cocktail of 20 clones) demonstrated adhesion inhibitory activity of 44.44% against 3.125×10^6 leptospires adhering to HuLEC-5a endothelial cell monolayer while IgM- or IgG-positive human anti-sera (dilution of 1:100) demonstrated 88.89% and 66.67% inhibition, respectively. Doxycycline (50 µg/ml) demonstrated anti-bacteriostatic to *Leptospira* and did not inhibit bacterial adhesion to fixed HuLEC-5a monolayer cells. In *ex vivo* antibody transcytosis assay, standard IgGs and huScFv-Fc antibodies were intracellular detected in acidic organelles at 5 hours incubation. The huScFv-Fc antibodies translocate across endothelial and epithelial membranes of both *in-vivo* (pH= 7.5-8.0) and *in-vivo* acidosis (pH= 7.0) conditions. Paracellular flux of albumin (65 kDa) was also observed.

Human ScFv antibodies specific to pathogenic *Leptospira* adhesins with adhesion inhibitory activity were produced from a well-established naive huScFv-displayed phages library. Membrane translocating huScFv (huScFv-Fc) antibody format can traverse across membrane barrier. Cocktail HuScFv-Fc antibodies are awaiting to test their therapeutic efficacy in experimental leptospirosis model.

Acknowledgements

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NEW FRONTIERS IN MOLECULAR DIAGNOSIS

FIRST ISOLATION OF LEPTOSPIRA SPP FROM A DOLPHIN (TURSIOPS TRUNCATUS) IN THE MEDITERRANEAN SEA. ASSESSMENT OF HALOTOLERANCE IN SEAWATER

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The pathogens Leptospira species are very widespread in nature, persisting and multiplying in the kidney tubes of many domestic and wild animals (reservoir). Infected animals shed the pathogen in their urines. Many mammals, including humans, are subject to infection, but information on leptospirosis associated with cetacean species is currently very limited. This paper describes the first isolation of a pathogenic *Leptospira interrogans* from a dolphin stranded along the coasts of Sardinia, showing that bottlenose dolphin represents a potential source for human leptospirosis in marine environments.

The bacteriological survey is conducted sowing 25 mg of renal tissue homogenate in three tube which contained 3 ml of Ellinghausen- McCullough-Johnson-Harris (EMJH) semi-solid medium (Difco) supplemented with 200 µg/mL 5-fluorouracil and incubated at 28-30°C for 60 days and regularly observed under dark-field microscopy every 7 days. Three panels designated as A, B, and C were used in this study to test halotolerance of isolated strain. Real-time PCR targeting highly conserved region of the LipL32 gene was used in the study. Three target genes rrs, secY and rpoB were used for sequencing. For typing, Multilocus Sequence Typing (MLST), Multilocus Variable-Number Tandem- Repeat Analysis (MLVA), and Pulse Field Gel Electrophoresis (PFGE) were performed on dolphin isolate.

On June 2016, an adult female bottlenose dolphin (Tursiops truncates species) was found dead and stranded near to the gulf of Orosei Although bad conditions of kidneys were observed, a positive culture of Leptospira spp. was obtained after about three weeks. Real time PCR reported positivity for pathogenic Leptospira. Genetic characterization classified the strain as *Leptospira interrogans*. Dolphin isolate survived different seawater concentrations (up to 20%). Residual viability thus infectivity after exposure to pure seawater for 3 days was demonstrated by subculturing surviving bacteria in fresh EMJH.

Our study provides the first evidence of pathogenic Leptospira classified as belonging to the genus *Leptospira interrogans* in a dolphin stranded in the Mediterranean Sea. PFGE and MLVA, applied to the dolphin isolate and 2 other wild boar isolates analyzed in parallel, showed the same genetic profile demonstrating that the same strain can be transmitted from terrestrial to marine animals and can contaminate the coastal environment. These findings suggest some questions about the route of infection and the potential role of cetaceans in the epidemiology of leptospirosis. The zoonotic nature of this strain and its diffusion, between both wild and domestic terrestrial animals as well as in marine mammals, does pose a significant risk to human health. These results highlight the need for conducting deeper longitudinal surveys including other animal species living in this area.

DETECTION OF BROWN RAT (RATTUS NORVEGICUS) IN DUTCH SURFACE WATERS USING EDNA TO DETERMINE A LINK WITH PATHOGENIC LEPTOSPIRA

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Higher winter survival of rodents and, specifically, the brown rat (Rattus norvegicus), has been suggested as a cause for the recent rise in Dutch leptospirosis cases. Two-thirds of the Dutch autochthonous cases could be attributed to contact with surface waters, indicating its role as infection pathway. Brown rats are the carrier of serogroup Icterohaemorrhagiae. This was the main infecting serogroup during 1925-2008, to which about 70% of the cases of autochthonous leptospirosis could be attributed. This serogroup causes the most severe symptoms necessitating the most hospital care. As environmental (e)DNA has been employed for the rapid and non-invasive detection and monitoring of (semi)-aquatic animals that have recently been in contact with water, this study describes the development of a qPCR-based method for detecting and monitoring brown rats to elucidate the relation between pathogenic Leptospira and their hosts in Dutch surface waters.

Screening survey of 13 bathing sites 86 surface water samples from 13 bathing sites in the Netherlands were analysed.

Intensive sampling. Two locations were chosen, from each 10 and 16 water samples were taken. eDNA extraction Real-time PCR

DNA extraction from all water samples, negative controls and PCR's internal control was performed using filtration and PowerBiofilm® DNA Isolation kit (MO BIO inc., Carlsbad, USA) according to the manufacturer's protocol. Primers and probe were designed specifically targeting Cytochrome-b (Cyt-b) sequence of R. norvegicus. The assay was optimized, and specificity and sensitivity were tested according to the standard procedure. Detection of pathogenic Leptospira was carried out using in house Real-time PCR targeting lipL32.

Screening survey of 13 bathing sites.Presence of brown rat DNA was detected in 15% of all screened samples. In 35% of all samples pathogenic Leptospira were detected. In three sites no pathogenic Leptospira were detected throughout the period studied. At 2 locations leptospires were present in 56 and 60% of the samples.

Co-occurrence of pathogenic Leptospira and brown rat eDNA

In 6 locations both pathogenic Leptospira and brown rat eDNA were detected. In 8% of the samples both DNA's were detected simultaneously.

Local distribution of pathogenic Leptospira and brown rat eDNA

Two locations were sampled more intensively. In 1 location, 3/10 samples contained pathogenic Leptospira. Two samples harboured brown rat eDNA. Of the 16 samples taken at another location, 4 contained pathogenic Leptospira and no brown rat eDNA was detected.

Using a novel method for detecting brown rat eDNA from environmental surface water samples, this study showed that the brown rat is a likely source of pathogenic Leptospira in a number of bathing sites in the Netherlands. An intensive sampling strategy employed at two locations showed that the method is not likely to be susceptible for genetic material that did not originate close to the sampling site.

Moreover, taking multiple samples on one site allows for the inference of the exact location of a source which can be used to inform management policies. Optimizing sampling strategy as well as sample filtration and DNA isolation will likely result in less false negatives. However, a lack of fundamental knowledge regarding the human infection with environmental pathogenic Leptospira hinders advancement of guidelines that demark an acceptable limit of pathogenic Leptospira numbers.

QUANTITATIVE REAL-TIME LEPTOSPIRA PCR ASSAYS: A QUICKER ALTERNATIVE TO THE CULTURE METHOD FOR THE DETECTION OF PATHOGENIC LEPTOSPIRA SPECIES IN DOG URINE, BLOOD AND KIDNEY DURING CLINICAL FOLLOW-UP

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Leptospirosis is a vaccine-preventable bacterial zoonotic disease caused by pathogenic Leptospira species. As part of the assessment of the efficacy of the multivalent canine vaccine EURICAN®DAPPi-Lmulti, groups of vaccinated and unvaccinated dogs were challenged with virulent serovars of Leptospira and the presence of Leptospira in blood, urine and kidney tissue after challenge was compared between groups. The reference method for detection of Leptospira involves microscopic observation of leptospires after up to six weeks of culture.

This method is laborious and time-consuming. Recently-developed quantitative real-time qPCR assays could offer advantages in terms of rapidity and sensitivity, as alternative diagnostic tool for Leptospira detection.

In this study, we compared the sensitivities of species-specific qPCR (targeting the lipL32 gene) and the reference culture method for pathogenic Leptospira spp. detection in vaccinated and control dogs from onset and duration of immunity (OOI, DOI) laboratory studies. An internal control of the qPCR was simultaneously amplified in the samples to verify the absence of inhibitors. A statistical analysis (McNemar test, Kappa coefficient, percentage of agreements) was performed to compare methods.

The detection of three pathogenic Leptospira serovars (Canicola, Icterohaemorrhagiae, Grippotyphosa) in urine, blood and kidney tissue samples from six OOI and two DOI challenge studies was performed with both methods. An internal control in the qPCR assay showed no inhibitory effects for amplification in qPCR-negative samples. The statistical analyses (McNemar test, Kappa coefficient, percentage of positive/negative agreements) showed a good correlation between the methods in urine, blood and kidney tissue for all 3 challenge strain serovars.

These results demonstrate that qPCR can be used for the rapid and specific detection of pathogenic Leptospira spp. in urine, blood and kidney tissue during clinical protocols, without loss of sensitivity, compared to the reference culture method.

DETECTION OF LEPTOSPIRA SP. BY PCR IN SWINE ABORTION - A CASE REPORT

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Leptospirosis is commonly diagnosed in cases of swine abortion by serological methods, most often by microagglutination test (MAT). In contrast, direct detection of the infectious agent by PCR, immunofluorescence or culture is seldom reported. Even high MAT titers are often associated with negative PCR results, having caused uncertainty about the validity of both serology and PCR to correctly diagnose the disease. Here, we present a case of abortion in swine, where Leptospira was demonstrated by real-time PCR in numerous fetal tissues, while MAT of the sow was only transiently positive and no Leptospira sp. genome was detectable in sow tissues or urine.
A case of reproductive failure in a sow was submitted by a private practitioner for etiological diagnosis. Samples included three fetuses, placental tissue and blood from the sow. Tissues from two fetuses were tested by real-time PCR for African and Classical swine fever virus, Brucella sp., Aujeszky's disease virus, Chlamydia, Porcine Parvovirus (PPV), Porcine Circovirus 2, Porcine reproductive and respiratory syndrome virus (PRRSV) and Leptospira. Tissues were also subjected to bacterial culture. Blood serum was tested for antibodies against PPV, PRRSV and Leptospira. Following the initial submission, blood and urine were collected from the sow for MAT and real-time PCR, respectively. Finally, the sow was euthanized and numerous tissues and blood were collected for analysis.
Leptospira sp. genome was detected by real-time PCR in several tissues collected from one of the two sampled fetuses. All other real-time PCR tests as well as bacterial culture were negative in both tested fetuses. By serology, the blood from the sow was MAT-positive for L. serovar Icterohaemorrhagiae (1:400), but negative for all other Leptospira serovars tested. No PRRSV antibodies were detectable, but the sow tested positive for antibodies against PPV. Blood collected from the sow at two time-points following the initial submission tested MAT- negative against all Leptospira serovars in the panel, including Icterohaemorrhagiae. Neither urine nor any of the numerous tissues collected from the sow following euthanasia were positive for Leptospira sp. by real-time PCR.
Leptospirosis is an important differential diagnosis in the context of reproductive failure in swine. While direct detection of the infectious agent by PCR or other techniques is rare, demonstration of Leptospira antibodies in sows is common. In the presented case of abortion in a sow, Leptospira sp. genome was readily detectable in fetal tissues by real-time PCR. Interestingly, no Leptospira sp. genome was found in sow tissues or urine despite the collection of an extensive tissue panel. Furthermore, Leptospira antibodies were undetectable in the sow as early as three weeks following abortion. This case report emphasizes the necessity of more research to understand this complex disease and to increase the validity of laboratory results.

TARGETING ATP SYNTHESIS IN LEPTOSPIRA THROUGH BIOREDUCED AGNPS: A POTENTIAL THERAPEUTIC ALTERNATIVE IN TREATMENT OF LEPTOSPIROSIS

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Leptospirosis is a zoonosis, occurring in a wide range of animals including humans. Rodents are found to be the primary carriers of the organism, where the infection is asymptomatic. In humans the disease is highly symptomatic eliciting severe damage to the liver and kidneys, also causing meningitis. Limited effective treatment options, benzyl penicillin and doxycycline, are available upon infection. Due to increasing resistance strains and the threat posed by benzyl-penicillin to cause an anaphylactic reaction, there is an urgent requirement for an alternative therapeutic option to treat Leptospirosis. Hence, in this study bioreduced silver nanoparticles (AgNPs) are used as an antileptospiral agent. The AgNPs are used to treat Leptospirosis by specifically targeting and inhibiting the metabolic functions of the electron transport chain and ATP synthesis in Leptospira. The differential action of AgNPs on Leptospira interrogans is analysed in comparison with mitochondria and yeast models.

Antihemolytic activity of AgNPs analysed against RBC infected with Leptospira interrogans

Inhibition of Electron transport chain by AgNPs in Leptospira in comparison with Mitochondria and Yeast studied through DCPIP and Ferricyanide assay

Inhibition of ATP synthesis by AgNPs in Leptospira in comparison with Mitochondria and Yeast studied using Luciferase assay Scanning electron Microscopy of Leptospira Before and After treatment with AgNPs

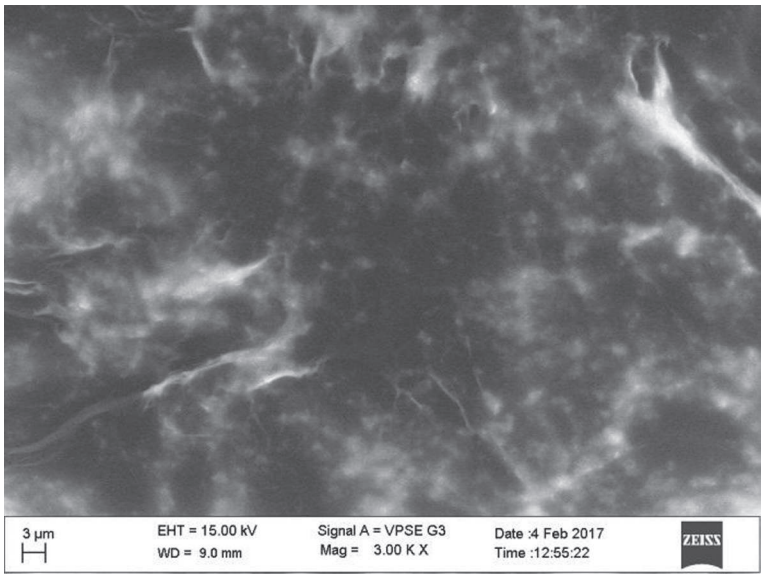
AgNPs were found to have absolute bactericidal activity against Leptospira interogans strains: pomona, hardjo, javanica, pyrogens, australis AgNPs were found to have 99% hemeprotective activity

AgNPs were found to inhibit Electron transport chain In Leptospira within 2 minutes of treatment against the above mentioned strains, whereas it partially inhibited electron transport chain in Mitochondria and Yeast

AgNPs were found to inhibit ATP synthesis completely in Leptospira, whereas reduction in ATP synthesis was seen in Mitochondria and Yeast

Scanning electron Microscopy of Leptospira showed total disintegration of intact Leptospiral cells after treatment with AgNPs

Bioreduced AgNPs prove to be novel therapeutic alternatives to treat Leptospirosis with high specificity towards Leptospira interrogans with low toxicity to the host. Metabolic inhibition of Leptospira interrogans proves to be a novel alternative therapeutic approach to treat Leptospirosis, and does not provide the organism the opportunity to develop antimicrobial resistance, providing a solution for treatment of drug resistance strains. This approach could prove to be a solution for impending threat of superbug.



POSTER PRESENTATIONS

LEPTOSPIROSIS IN FREE-RANGING CARNIVORES IN SOUTHERN ITALY

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Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. The disease has a worldwide distribution and is known to affect humans, domestic animals, and wildlife. Wildlife is relevant in the epidemiology of leptospirosis because some wild species, markedly small mammals, act as reservoir of some serovars. Free-ranging carnivores being at the top of the feeding chain may be exposed to pathogens through their preys. Most of them are predators and/or scavengers, get in touch with a wide range of wild species, thus representing good sentinels in order to determine the presence and relative importance of Leptospira spp. in the natural environment (Millán et al. 2009, Vector-Borne Zoonotic Dis 9: 549-554). In this study, we investigated using standard and real-time PCR and sequencing, the occurrence and molecular characterization of Leptospira spp. species in free-ranging carnivores from the southern Italy. We used carcasses of 111 free-ranging carnivores including 92 red fox (Vulpes vulpes), 9 gray wolf (Canis lupus), 4 Eurasian badger (Meles meles), 4 Eurasian otter (Lutra lutra),1 European polecat (Mustela putorius), and 1 beech marten (Martes foina) obtained from the southern Italy from January 2015 to December 2017. Tissue samples of kidneys were used for Leptospira DNA detection.DNA was extracted using the QIAamp DNA mini kit, (QIAGEN). Leptospira spp. detection was performed by a real-time PCR targeting the lipL32 gene, encoding an outer membrane lipoprotein, virulence factor non present in nonpathogenic species. Reaction was performed in a total volume of 25 µL containing Quantifast Pathogen MasterMix (Qiagen) 0.7 µM of each primer and 5 µL of DNA. The primers set of L32-45F (5′- AAGCATTACCGCTTGTGGTG -3′) and LipL32-286R (5′- GAACTCCCATTTCAGCGATT -3′), were selected to amplify a fragment of 242 bp, which was detected by probe, LipL32-189P (FAM-AAAGCCAGGACAAGCGCCG-MGB). A positive control containing genomic DNA for L. interrogans serovar Pomona strain L222, and a negative and non-template control were included in each PCR reaction. The Leptospira- positive samples were amplified by classical PCR assay, using the rrs (16S) gene as a target sequence (Merien et al. 1992 J Clin Microbiol 30: 2219-2224). PCR products were resolved by the QiaXcel automated electrophoresis system (QIAGEN). Amplicons were purified and bi- directionally sequenced (Big Dye Terminator Cycle Sequencing Kit v1.1,Thermo Fischer Scientific, USA) in an automated sequencer. Sequences were aligned using ClustalW program and compared with those available in GenBank (BLAST– http://blast.ncbi.nlm.nih.gov/Blast.cgi). A total of 10 carcasses (including 9 red foxes and one beech marten) were positive to Leptospira spp. by rt PCR. and this presence of was confirmed by classical PCR assay (rrs 16S). Three of the sequenced PCR products from 3 red foxes were 100 per cent identical to the corresponding portion of 16S rRNA gene sequence of L. interrogans, one amplicon from a red fox showed a low similarity (92%), the remaining six products showed sequence heterogenity, suggesting the simultaneous presence of different species.

Infections with Leptospira spp. occurr in red foxes of southern Italy. Red foxes may be considered as valid sentinels for leptospirosis due to its assorted diet composition, its wide distribution, and its susceptibility to the infection.

SURVIVAL OF LEPTOPIRA SPP. IN SOIL AND WATER: A LITERATURE REVIEW

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The survival time of Leptospira spp. in surface or soil water is a major factor influencing the exposure and risk of infection of both humans and animals. The viability of the bacteria is affected by various environmental conditions such as temperature, pH value and humidity.
Several studies showed the ability of the bacteria to survive in fresh water and soil under experimental conditions; however it is a challenge to estimate the survival time of Leptospira spp. in varying natural matrices in combination with different parameters. The aim of this review is to extract and summarize data on the tenacity of Leptospira spp. from published studies.
A comprehensive literature search was performed using 5 databases (PubMed, Scopus, ScienceDirect, Google Scholar, Web of science core collection). In order to gather information concerning the survival time in water and soil, the research was comprised with the terms “leptospira AND survival AND time”, “leptospira AND tenacity”,”leptospira AND viability”, “leptospira AND survival AND water”, “leptospira AND survival AND soil”,”leptospira AND tenacity”, “leptospira AND survival AND environment” or “leptospira AND growth conditions”.
The search generated a total of 29 publications covering the period from 1918-2017. After excluding 3 abstracts with irrelevant information, 26 studies were identified that described the survival of Leptospira spp. under different conditions. After comparing and studying the full-text articles of the available studies, 7 were excluded because of incomplete, irrelevant or low quality data. In the end 19 reports were identified which met the inclusion criteria. The observed survival times of leptospires in different studies were very variable. Leptospires remained viable for a very short time (<1 hour) up to several months depending on the surrounding conditions. It was shown that several factors like a neutral or alkaline pH, high humidity and no direct exposure to UV-light were positively associated with the survival rate. Also the accompanying bacterial flora, the temperatures and the salinity had an influence on the survival time. Few data are available to allow a comparison of Leptospira spp. growth and survival in the environment under different conditions. Nevertheless, the data demonstrate the important role of factors including pH, humidity and temperature influencing the duration of survival of leptospires.
Future work should focus on studying different Leptospira spp. under various environmental conditions and biotic and abiotic factors, which affect the survival of pathogenic Leptospira spp. outside the host. This would be helpful to estimate the survival time of leptospires in the environment which determines the risk of human exposure.

CRYOPRESERVATION OF STRAINS OF LEPTOSPIRA SPP, A SOLUTION TO MAINTAIN OUR COLLECTION

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The stable supply of strains for the production of vaccines is a determining process for the quality of the final product. The choice of an adequate and effective conservation method will ensure that the strains maintain properties such as viability, purity and genetic stability. Leptospires, traditionally, are maintained by subculture in media such as Fletcher, a process with high risk of loss of strains.
Cryopreservation using DMSO or glycerol as cryoprotectants has been reported, but stability over time is not always guaranteed. In the IFV the strains are maintained by subcultures and it is necessary to have constant resources to maintain the virulence of the vaccine strains. We have studied the effect of different cryoprotective additives with good results, however, undesirable effects; it forced to consider other variants such as the use of Greaves additive, reported for other bacteria.Ojetive:To evaluate the protective capacity of Greaves in the conservation of Leptospira sp. at -70oC as an alternative to the method traditionally used.
The experiment occurred in two stages. In the first, the effect of four cryoprotectants (DMSO 2.5%, Glycerol 10%, skim milk 10% and Greaves) on the vaccine strains (Canicola, Copenhageni and Mozdok serovar) was evaluated and compared with the values of the conserved Fletcher. The best results criprotector for the second stage was chosen, in which the Greaves additive is used to prepare banks of vaccine strains and in addition to the 23 reference serovars of the collection (avirulent). In both stages the vials were thawed and planted in EMJH medium and checked by the Quality Specifications. Controls: Purity, Feasibility and cell concentration in Petroff-Hausser chamber, Identity by MAT and Degree of virulence by 50% lethal dose assay). The lots were checked at 0, 4, 10 days, 3, 6, 12 months, 3, 5, 8 and 10 years of storage.
First stage: it was demonstrated that the strains conserved by subculture lost virulence after 6 months of preservation. The effect of glycerol and DMSO on the frozen strains limited the recovery of the cultures due to their toxicity. The skim milk was effective; however it caused interference in the observation of dark field crops. Greaves additive proved to be the best and did not interfere with microscopic observation.
Second stage: it was reaffirmed that the cryoprotective Greaves, does not affect the strains, kept their properties during the period evaluated. It was observed that after 6 years the viability of the crops decreases after freezing.
The cryopreservation method can be applied at -70 ° C, using the Greave as a cryoprotectant to make a sowing lot of Leptospiras strains, and store them for a period of 5 years without affecting their properties. The method is also economical.

EVALUATION OF VIRULENCE FACTORS BY PATHOGENIC LEPTOSPIRA SPP. ISOLATED FROM ICTERIC AND ANICTERIC HUMAN CASES OF LEPTOSPIROSIS

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Leptospirosis is a worldwide distribution zoonosis caused by pathogenic serovars of Leptospira species. The infection is considered a public health concern in many countries, including Brazil. Although the mechanisms of virulence of this pathogen are not completely understood, biofilm formation and proteins involved in pathogenesis are considered to be important factors in the establishment of the disease. The aim of this study was to evaluate the biofilm production and the expression of whole proteins of different strains of Leptospira spp. isolated from human clinical cases.

Initially, 18 strains were selected (one saprophytic and 17 pathogenic), all belonging to the Leptospira Collection (CLEP/IOC-Fiocruz). The pathogenic strains were obtained from different clinical cases of patients with leptospirosis presenting anicteric, icteric or severe pulmonary (SPFL) forms of the disease. Previously, a growth curve was stablished for all strains during ten days in order to define the phase of biofilm production. Subsequently, a crystal violet assay to quantify the biofilm was evaluated after seven days of incubation by using 24-wells polystyrene plates. Statistical analysis were made by the GraphPad Prism software by using the One-way ANOVA test. The scanning electron microscopy (SEM) was used for observing the production of biofilm by selected strains. A comparative protein profile will be evaluated by SDS-PAGE by using the whole protein extract from planktonic and biofilm adherent cells. The characterization and identification of the proteins will be performed by a Western blotting assay, using sera from human patients previously diagnosed with leptospirosis, followed by mass spectrometry (MALDI TOF-TOF MS).

Until now, our results show that three clinical isolates, two obtained from anicteric cases and one involved in SPFL, were strong biofilm producers, compared to other clinical strains, and to the saprophytic one. Among them, the strain isolated from a fulminant case of leptospirosis associated with massive pulmonary hemorrhage and renal failure presented a higher level of biofilm formation. The biofilm production seems to be initiated in the beginning of the Log phase until the stationary phase. SEM analysis revealed a dense biofilm production corroborating with the quantification assay, although no differences were observed in the patterns of cell aggregation among saprophyte and pathogenic strains.

The elucidation of the virulence potential by pathogenic leptospires might reveal mechanisms of survival, colonization and virulence associated with clinical isolates and a better understanding of the infectious disease.

IMPROVEMENT OF LABORATORY DIAGNOSTIC OF LEPTOSPIROSIS AND INVESTIGATION OF PREDOMINANT SEROVARS CIRCULATING IN THE COUNTRY OF GEORGIA

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Leptospirosis is a zoonotic disease caused by spirochetes of the genus Leptospira and affects humans and a wide range of domestic and wild animals. The formal registration of leptospirosis in the country of Georgia began in the 1950s. The epidemiological characteristics of leptospirosis identifies that the infection is widely distributed in the country. It has an increasing tendency and duration and the course of the disease is frequently severe. Water is mainly implicated as a risk factor in the infection transmission. Since 2006 significant an increase of leptospirosis in Georgia has been observed. The highest incidence was reported in 2016 with 2,83 (105 cases) per 100 000 population.

Young and middle age people mainly expose to leptospirosis in Georgia. The disease is spread with equal intensity both in urban and rural areas.

Current laboratory diagnosis of leptospirosis in humans in Georgia is limited and performed only by screening with a commercially available leptospira IgM and IgG ELISA. So far only little is known about circulating serovars in the country.

Within the framework of the German-Georgian Biosecurity Programme we initiate a study to investigate key questions about leptospirosis diagnostics in animals and humans in Georgia. The research activity includes identification of prevailing serovars of leptospira in the country and the evaluation of reservoir-human serotype relationship.

Blood or urine human and animal sample will be taken in acute phase of infection and cultured for leptospira isolation. Additionally aseptically collected kidney tissue from rats will be collected for culture isolation of leptospira. For antibody demonstration against specific serovars, human or animals (preferably ELISA positive) sera will be tested with microscopic agglutination test (MAT). The panel used for MAT testing will consist of 24 Serovars. Obtained serovars would be confirmed by Polymerase chain reaction (PCR) testing. Testing will be performed at

R. Lugar Center for Public Health Research at NCDC, Georgia.

Implementation of a new or improving existing technics for diagnosis will significantly improve the capabilities for detecting leptospirosis infections. It will provide medical professionals with more valuable diagnostic information about emerging serogroups of Leptospira and public health professionals with improved epidemiological information.

VNTR AND MST LEPTOSPIRAL GENOGROUPING OF DNA FROM DOG AFFECTED WITH ACUTE LEPTOSPIROSIS

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The genus Leptospira is subdivided into 23 genomic species and, 25 serogroups including more than 300 serovars. Humans as well as animals, in particular dogs, are exposed to multiple strains, with strong temporal and regional variations in the epidemiology. Precise knowledge of the prevalence of a given serogroup is necessary in order to best understand epidemiological fluctuations and associated reservoirs. Moreover, it is key to the development of vaccines relevant of regional and target species specificities.

Diagnosis of leptospirosis through the Microscopic agglutination test (MAT) or PCR-based detection of pathogenic leptospiral nucleid acids in blood and urines allow confirming infection. But these methods provide no reliable, specific indication on the infecting serovar/serogroup.

Molecular approaches such as Variable Number Tandem Repeat analysis (VNTR) or Multi Sequence Typing (MST) have been developed to characterize serovars of cultured strains.

In the present study, we have investigated the feasibility of applying these tools directly to DNA extracts from biological fluids, without culturing.

88 DNA samples isolated from canine urine or blood collected across 11 European countries were positive under leptospiral real-time PCR. We showed that successful typing was overall achieved in 60% of the case and that this rate increased with the amount of DNA contained in the samples. 97% of DNAs samples with cycle threshold values below 34 were fully typed. Our data further indicate that L. interrogans, L. kirschneri and L. borgpetersenii are circulating across Europe, with Icterohaemorrhagiae as the most frequently encountered serogroup.

Overall our study demonstrates that data on seroprevalence and geographic circulation of leptopires could and should be followed more extensively by usage of rapid, reliable molecular techniques in addition to serology.

IDENTIFICATION OF LEPTOSPIRA STRAINS ISOLATED FROM PATIENTS IN KOPRIVNICA - KRIŽEVCI COUNTY, CROATIA

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Introduction. Leptospirosis is the most frequent zoonotic disease all over the world. It is present also in north - western part of Croatia. During the period 2000 to 2004 we observed 68 patients with clinical signs of leptospirosis presenting in Koprivnica – Križevci County. From 10 of these patients we were able to isolate Leptospira spp. The aim of the study was to determine leptospira strains isolated from patients in our region and to establish the frequency of particular strain involved in infection.

Material and methods. All leptospira strains were isolated from the blood of patients the first day of their hospitalization. Samples were inoculated in in Korthof's medium, incubated at 28 oC and weekly checked for the presence of spirochets. For isolated strains, we determined Leptospira species, serovar and serogroup. The Leptospira species was identified by melting temperature (Tm) determination of the PCR product of the gene LAO322, serovars by determination of NotI restriction fragment length polymorphism (NotI-RFLP) while serogroup by hyperimmune sera obtained from the reference centre.

Results. Most frequently isolated species from patients of Koprivnica – Križevci County was L. interrogans sensu stricto (8/10), one isolate was identified as L. kirschneri while one strain was not able to grow after storage at liquid nitrogen. Regarding leptospira serogroup and serovar, 8/10 (80%) patients were infected with serogroup Icterohaemorrhagiae, one patient (10%) with serogroup Grippotyphosa. For one strain we failed to determine the serogroup because the strain agglutinated with hyper-immune serum from the Bataviae (1: 6400) and Tarassovi (1: 3200) serogroup, and we also failed to delineate serovar because the isolated strain had its own NotI-RFLP profile that we did not have among our reference leptospira strains; strain belonged to previously reported L. kirschneri species.

Conclusions. Study confirmed L. interrogans sensu stricto serovar Icterohaemorrhagiae as the most frequent leptospira strain isolated in humans in Koprivnica – Križevci County. This is the first report about human Leptospira species in Koprivnica – Križevci County.

CHARACTERIZATION OF CLINICAL ISOLATES OF LEPTOSPIRA FOR VETERINARY VACCINES

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The leptospirosis, is the zoonosis more diffused in the world, suffer it equally men and animals, so much domestic as wild. Due to the increment of the incidence of this illness, in advanced and in underdeveloped countries, It is important their study for the veterinary and human medicine. The classification of isolations, although did not have a lot of clinical valor, has a great impact in the epizootiológic studies, since it allows us a strict control of the serogrupos and serovares circulating in animals in any geographical region. One of the most important approaches for the development of Leptospira immunogens is to have very well characterized strains by their virulence and representative of the circulating serogroups from the clinical and veterinarian point of view. All of this allows guaranteeing immunogens capable to protect against the serovars present in the formulation and that affect animals at risk.
This paper was carried out from isolations of the causal agent of five autochthonous clinical cases of Leptospira at Holguín. These isolations were characterized in pathogens and no pathogens by means of phenotypic conventional methods: growth at 13ºC, in supplemented media with 8-azaguanina and in the presence of sodium chloride. The isolations were also classified until serogroup by the microaglutination technique with the employment of reference policlonal antiserums specific for the 11 circulating serogrupos in Cuba: Ballum, Pomona, Canicola, Icterohaemorrhagiae, Pyrogenes, Tarassovi, Australis, Hebdomadis, Bataviae, Automnalis and Sejroe. A strain of Ballum serogroup was selected and characterized from these isolations as a vaccinal candidate. A qualitative and quantitative evaluation of its virulence was carried out and its serologic classification until the level of serovar was made, using a panel of four monoclonal antibodies (AcM F74C1-6, AcM F74C4-4, AcM F74C7-3 and AcM F74C12-1). The isolations did not show growth at 13ºC, not even when 8-azaguanina (2.25 mg/mL) was added to the culture, while all the strains showed conversion to spherical form in the presence of sodium chloride (1M). The classification of the isolations allowed having two strains (245-12 and 192-12) belonging to the serogroup Ballum and two to Pomona with de title between 1:512 and 1:2048. The strain 245-12 showed a high virulence and in the biomodel animal employee, since it produced the death of all animals inoculated with smaller dose or similar to 0,2 mL of the bacterial suspension. While the strain 192-12 showed a moderate virulence and consequently it was not selected as vaccine candidate. The classification until the serovar level by means monoclonal antibodies determined that the strain 245-12 belonged to Ballum serovar, with a title of 1:1028.
The present paper sets for the first-time the bases for novel vaccinal formulations in animals, containing the serogroup Ballum.

MOLECULAR IDENTIFICATION OF PATHOGENIC LEPTOSPIRA SPP. IN CULTURES FROM ABORTED BOVINE FETUSES

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Leptospirosis is a zoonotic disease that affects several species and produces economic losses due to deaths of weak newborns, abortions, or infertility (Ellis et a., 1977; Anderson, 2007; BonDurant, 2007). 71 fetal fluids cultured in enriched medium for Leptospira EMJH from 23 bovine fetuses aborted from April 2006 to May 2014 in Venezuela, were used to identify pathogenic Leptospira spp. using molecular tools. After DNA extraction, PCR with primers G1-G2 (Gravekamp et a.,1993) and Internal 1-Internal 2 (Haake et a., 2000) was performed. Leptospira bacterins along with reference strains (Leptospira Hardjopratjino) were used as positive controls.
Pathogenic Leptospira spp were identified in 8 out of 23 aborted bovine fetuses (34.8%) with at least one primers. 10 out of 71 cultures of fluids were positive (14.1%) as for G1/G2, and 2 out of those 10 were positive for Internal 1-Internal 2 (2.8%), as well. Fluids with the highest sensitivity using G1-G2, after culture in EMJH medium, were: renal and maternofetal fluids (16.7% and 16.7%, respectively). As for Internal 1-Internal 2, abdominal and ocular fluids showed the highest sensitivities (6.7% and 4.3%, respectively). The use of DNA extraction Wizard® kit resulted in higher sensitivity, when compared to phenol/chloroform/isoamyl alcohol, with both markers: 55.6% and 44.4% were positive to pathogenic Leptospira spp using G1/G2 and Internal 1/Internal 2, respectively. Species with the highest occurrences were Bos taurus (6/7;85.71 %) and Bubalus bubalis (2/9; 22.2%). There were no amplifications in Bos indicus fetuses. These results are similar to what was found by Marianelli et al. (2007) who detected Leptospira DNA in 22 of 80 aborted Bubalus bubalis fetuses, that is, in 27.5%. On the other hand, Subharat et al. (2010) only obtained a positive doe fetus of 27 analyzed by real-time PCR, the culture in EMJH medium of kidney of that fetus was positive and the mother had a serological titre by MAT of 192 to the harrobovis serovar.The I1-I2 marker that amplifies the gene encoding the LipL32 protein (Haake et al 2000, Haake and Matsunaga, 2002), a protein that is expressed during infection in cattle (Hauk et al., 2009), is therefore a a very important determinant of pathogenicity in vivo (Haake et al 2000, Haake and Matsunaga, 2002).
PCR with primers G1-G2 and Internal 1-Internal 2 could be used as a diagnostic tool for pathogenic Leptospira spp. in aborted fetuses.

Fetal fluids



Own source

EVALUATION OF TWO IGM IMMUNOASSAYS FOR THE DIAGNOSIS OF EARLY HUMAN LEPTOSPIROSIS

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Diagnosis of leptospirosis frequently relies on specific and rapid detection of IgM, we propose to compare the performance of two kits available in Europe.

Sera from swiss and cuban patients are categorized to challenge sensitivity, specificity and correlation between the two EIA. Group I is composed of confirmed cases having either PCR positive samples or MAT titer >160 and follow-up sera from confirmed patients. Group II includes patients with clinical suspicion of leptospirosis and group III sera of patients positive in IgM for another etiologic agent (Toxoplasma gondii, cytomegalovirus, herpes simplex, Epstein-Barr virus, rubella virus, malaria, dengue, Bartonella henselae, Borrelia burgdorferi s.l and Coxiella burnetti. The kits tested are Panbio Leptospira IgM (Panbio) and Virion/Serion Leptospira IgM (VS)

Hundred and eighty sera from 173 patients are tested with both kits. Including positive and equivocal results, sensitivity of Panbio and VS is 67.5% (27/40) and 50% (20/40), respectively. A higher sensitivity is observed for Panbio showing 9 reactive sera that are non-reactive on VS. Specificity measured on group III is very similar for both tests with 86.5 % (32/37) and 83.8 % (31/37) respectively. In group II (suspected cases) reactive results are 2 times higher with Panbio, 28.1 % (29/103), than VS, 14.6 % (15/103). However this indefinite group cannot be used to attest higher sensitivity for Panbio or higher specificity for VS, but only test the correlation between their results.

Agreement in group III (83.8%) is acceptable for specificity but low for sensitivity in group I (72.5 %) and intermediate for group II (78.6%). Global correlation is moderate considering the kappa test of 0.531 (95%: 0.416 - 0.646).

Serology offers a simple and rapid diagnostic of leptospirosis. However the uncertainty of the results appeals to the use of a much more specific and sensitive molecular method on serum or plasma and urine, depending on the time of infection.

These EIA for IgM do not correlate well and underline the need for a more liable test to rapidly confirm a leptospirosis diagnostic.

LEPTOSPIRA SPP RESEARCH IN FRESHWATER COLLECTIONS IN THE REGION OF LEIRIA (PORTUGAL)

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Leptospirosis is a disease caused by pathogenic spirochetes of the genus Leptospira that occurs worldwide, and it is endemic in many countries. Rodents are the primary reservoirs, but also other mammals such as pigs, cattle, dogs and many wildlife species might be infected. When these infected animals deposited or drain urine in the freshwater or soil, the bacteria can survive for months or years. Thus, these environments can be an important vehicle of transmission to humans and animals exposed to them. This study aimed to investigate the presence of Leptospira spp in environmental water samples collected in the district of Leiria (Portugal) and evaluate the risk of the potential human leptospirosis cases, due to the direct contact with these water collections.

A total of 100 samples of freshwater was collected from several locations previously selected, in the Leiria district. Leptospira DNA detection was performed by three different PCR protocols: I) a nested-PCR with universal primers for Leptospira genus (A/B) targeting rrs (16S) gene;

II) a PCR protocol using primers G1/G2 targeting secY gene present in some pathogenic Leptospira species, and III) another nested-PCR protocol targeting lipL32 gene, that encoding the surface protein with the same name, common to all pathogenic leptospires. Some of amplified products were sequenced and analyzed by BLAST, to identify the Leptospira species.

Of the samples analysed, 41% were positive for the presence of leptospiral DNA, and 28% of these were identified as pathogenic Leptospira species, and the majority revealed a high similarity (98-99%) with pathogenic Leptospira species, namely L. interrogans and L. borgepetersenii.

This was a pioneering study in Portugal regarding freshwater collections, being crucial for Public Health since there are already some reported cases of human leptospirosis in this region. Therefore, this environmental water is believing to act as a potential vehicle of Leptospira infection to the population. Moreover, it is important to alert the Health Authorities, to perform a tight control of the waters collections of free public access, in order to decrease the leptospirosis cases among the population in this region of the country.

MOLECULAR IDENTIFICATION OF PATHOGENIC LEPTOSPIRA SPP IN URINE SAMPLE OF HUMAN PATIENT WITH CLINICAL SUSPICION OF LEPTOSPIROSIS IN VENEZUELA

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Leptospirosis is a zoonotic disease of worldwide distribution, caused by a spirochaete of the genus Leptospira spp. In humans, it produces a febrile disease, headaches, myalgias severe complications can occur at pulmonary level with hemorrhagic syndrome and renal failure (Cerqueira, G and Picardeau M., 2009; Vieira, M. et al., 2009; McBride, A. et al., 2007;). Mortality is significant due to delays in diagnosis, lack of adequate infrastructure and faults in clinical suspicion (Bharty, A. et al, 2003).
In Venezuela, leptospirosis is an important public health problem, although the true prevalence of this pathology is unknown (Cardona M.et al., 2008).
A 69-year-old female patient presented malaise, 39°C fever, chills, asthenia, adynamia, muscle pain in the legs, headache, cough, drowsiness, CF 110ppm, BP 92/44 mmHg, FR 36rpm, oxygen saturation 95%. Moderate anemia (Hb 10,10 gr / dl; Htto 32%) thrombocytopenia, electrolyte disorder (hypokalemia), increased CPK (CPK Total 1,529 U / L, CPKMB 17 U / L), serum iron 37 ug / dl, TIBC 412 Ug / ml. The epidemiology refers coexistence with a dog that died with signs compatible with leptospirosis, unconfirmed diagnosis and 18 cats; 4/4 cats had abortions, 6/18 jaundice 18/18 polydipsia, polyuria, low body condition, sialorrhea, food packaging showed evidence of rodent bites, this is handled by the unprotected patient, three times a day.
The objective of this work was to molecularly identify the presence of Leptospira spp. pathogens in a human patient, with clinical suspicion of leptospirosis in Venezuela, in pet food and cat fetus, using the polymerase chain reaction technique, PCR.
Samples were taken of: pet food, aborted fetus of a cat and the patient's urine were inoculated into tubes with 5 ml of EMJH culture medium, plus 10% BSA. Frozen 1.5 ml aliquot was stored for molecular identification.
The DNA extraction was done with the Wizard® Genomic DNA Purification kit (Promega). A PCR was performed to identify pathogenic Leptospira spp.; a pair of primers was used for the markers, Internal 1 and Internal 2 described by Haake et al. (2000). 2% agarose gel electrophoresis, generated amplicons with a band pattern of 497 bp.
Pathogenic Leptospira spp was identified by the PCR technique in pet food sample culture, cat fetus culture, urine sample and patient urine culture confirming the clinical suspicion of leptospirosis. Similarly (Cardona M., et al. 2008); (Quaresma and Cota, 2006) demonstrated the usefulness of urine samples in the early diagnosis of the disease by PCR. For their part (Pagès F. et al, 2014) they report that approximately 41% of leptospirosis infections occur in the home.
PCR with primers Internal 1-Internal 2 can be used for the diagnosis of pathogenic Leptospira spp in urine samples of patients with clinical suspicion of leptospirosis, in turn it is useful to identify the bacteria in contaminated foods, infected animals that can be a source of infection for humans.

SEROLOGICAL EVIDENCE OF LEPTOSPIROSIS IN STRAY DOGS OF PALERMO AND PANTELLERIA (SICILY, ITALY)

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In this work, we report the results of a research carried out from 2016 to 2017 about Leptospirosis in stray dogs of Palermo and Pantelleria. During this research, some dogs were resulted serologically positive to *Leptospira* spp.

Sera samples have been taken from 269 stray dogs, temporarily hosted into the Municipal Dog Pound of Palermo, and from 47 stray dogs hosted into the Municipal Dog Pound of Pantelleria. All the dogs were healthy and did not show any leptospirosis clinical sign. The positive dogs to *Leptospira* spp. hosted into the Municipal Dog Pound of Palermo were treated with 10.000I/U of benzylpenicillin and 15.000 I/U of dihydrostreptomycin for 15 days. The positive dogs to *Leptospira* spp., hosted into the Municipal Dog Pound of Pantelleria were treated with doxycycline 5mg / kg every 12 hours for a period of 15 days. The dog sera were examined to *Leptospira* spp. through Micro Agglutination Test (MAT). The test was performed as it is prescribed in the O.I.E. Manual (Chapt. 2.1.12. Par. B2.1. 2014). The analysis were conducted on eight common serogroups circulating in Italy (*L. interrogans* serogroup Australis serovar Bratislava, *L. interrogans* serogroup Ballum serovar Ballum, *L. interrogans* serogroup Canicola serovar Canicola, *L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni, *L. interrogans* serogroup Pomona serovar Pomona, *L. borgpetersenii* serogroup Sejroe serovar Hardjo, *L. borgpetersenii* serogroup Tarassovi serovar Tarassovi). After the antibiotic treatment, a second blood sample was taken and a second MAT test was carried out, to evaluate the antibody titres trend. It was impossible to trace all the positive dogs, because the sampling was been performed months before.

Among the 269 dog sera samples of Palermo, four were positive (1.5%) to antibodies to *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni with titres of 1:100, 1:400, 1:800 and 1:1600. After 30 days from the antibiotic treatment, the 1:100 titre became negative and the 1:400 titre remained so. It was not possible to track the dogs with the highest titres (between 1:800 and 1:1600). Among the dog sera samples of Pantelleria only one sample was positive (2.1%) with 1:1600 antibody-titre to *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni. Thirty days after antibiotic treatment, a second check was made and the 1:1600 titre decreased to 1:800. After 60 days, another serological test was made and the antibody-titre returned to 1:1600. It was impossible to carry out further checks on this dog because it was adopted.

Our results revealed that leptospirosis asymptomatic dogs might be reservoires of *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni or more in general of *L. interrogans* serogroup Icterohaemorrhagiae. For this reason, the serological control for *Leptospira* spp. would be appropriate to become mandatory, for example in case of adoption.

IMPACT OF THE CHANGES MADE IN THE CELLULAR MULTIPLICATION STAGE DURING THE OBTAINING OF THE ERYTHROCYTE SENSITIZING SUBSTANCE USED IN THE SEROLOGICAL DIAGNOSIS OF LEPTOSPIROSIS

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Introduction: In Cuba since 1981 has implemented the indirect hemagglutination as an alternative technique of Leptospirosis diagnosed, in the absence of others that may be more costly but effective at different stages of the disease. For the development of the technique requires the Erythrocytes Sensitize Substance (ESS), assuming the production Finlay Institute in 2009, from obsolete technology in the process of cell multiplication. Makes changes in the stage of cell culture (biomass) to obtain the SSE, using the available culture media in the production plant, for the optimal yield of the product, without affecting the biological activity as a fundamental parameter of quality. To obtain the cell biomass, two strains of Leptospirawere used: L. biflexaPatoc I from Finlay Institute (FINLAY strain) and L. biflexaPatoc I from LABIOFAM (LABIOFAM strain). The general conditions for growth were at a temperature of 28-30 °C, in Kortof medium (MK) or medium MEJH for both strains and using static or agitated growth at 130 r.p.m in sieve, for eight variants. Each variant was determined the doubling time and the growth rate (μ), by measuring the optical density at 400 nm every 24 hours for seven days (three replicates for each combination). From the eight biomasses, the protein extracts were obtained by detergent solubilization of the membrane and subsequent extraction with ethanol. Then, the titration of each protein extracts was carried out with 12 positive reference sera from IPK, thus obtaining by dilution the SSE. For the comparison between variants, the ANOVA test with a level of significance $p < 0.05$ was used, using Statsoft for Windows.

The best variant was the combination (LABIOFAM strain, growth in MEJH and agitated), with a considerable decrease in the time of the ESS obtaining by means of this new technology, with a consistent title with the demands of quality.

This work allowed using the best variant to begin the different designs of stability studies of the product.

SEROLOGICAL ASPECTS OF LEPTOSPIROSIS OF ANIMALS IN THE RUSSIAN FEDERATION

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Leptospirosis comprises a group of naturofocal infections believed to be the most widespread zoonoses associated with the greatest number of anthropurgic foci. In Russian Federation, leptospirosis is relatively common among both domestic and wild animals of various species.

Statistical and analytical methods of analysis were used in the present study. Between 2007 and 2016 our team performed serological tests on more than 10 million animals. 55.7% of the studies were performed on cattle, whilst 29.9% were performed on pigs, 6.4% on horses, 6.9% on sheep and goats and 1.1% on dogs. The infection rate was estimated at 15.1% in dogs, 14% in horses, 10.1% in cattle, 3.3% in pigs and 1.8% in sheep and goats.

During this period a trend towards a decrease of infection rates among animals of various species could be observed.

The etiological structure of the infection is as follows: serological groups *Sejroe (hardjo)*, *Hebdomadis*, *Pomona, Tarassovi* and *Grippotyphosa* are found in cattle; *Icterohaemorrhagiae*, *Pomona* and, to a lesser extent, *Tarassovi* found in pigs; *Icterohaemorrhagiae*, *Pomona*, *Tarassovi*, *Grippotyphosa* and *Canicola* found in horses; *Icterohaemorrhagiae*, *Canicola* and, in fewer cases, *Grippotyphosa* found in dogs. An increasing number of cases when a positive reaction with *Leptospira icterohaemorrhagiae* in animals of different species has been recorded in Russian Federation over the past years. Over the past 46 years the percentage of positive results with *Leptospira icterohaemorrhagiae* has grown from 3.7% to 57.1% in pigs and from 12% to 40.4% in dogs.

Changes in the etiological structure of leptospirosis have been observed in humans as well. City dwellers tend to get infected with leptospirae belonging to the *Canicola* and *Icterohaemorrhagiae* serogroups, of which the main source and reservoir are gray rats and dogs. In the countryside, on the other hand, more prominent are the *Grippotyphosa* and *Sejroe* serogroups, which might be coming from murine rodents and cattle.

Animals normally tend not to display clinical signs of leptospirosis, but nonetheless tens and hundreds of thousands of animals carry antibodies against leptospirae in their blood and have leptospirae in their urine. It is therefore crucial to be able to identify infected and sick animals, and a framework of tools should be delineated to eliminate the disease, including an array of diagnostic tools, general sanitary and veterinary measures, implementation of specific prophylaxis of leptospirosis and spreading the awareness among the human population.

Complex diagnosing of leptospirosis should be made possible, to be followed and confirmed by laboratory results of analysis, adopted by WHO.

LEPTOSPIROSIS IN PORTUGAL: A RETROSPECTIVE STUDY FROM 2012 TO 2017

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Leptospirosis is a zoonotic disease caused by spirochete bacteria of the genus Leptospira. There are 10 pathogenic species, and more than 250 pathogenic serovars. Humans and animals are infected with Leptospira through direct contact with the urine of an infected animal or indirectly by environmental contamination such as soil and water. Transmission occurs through mucous membranes, conjunctiva and skin cuts or abrasions. Leptospirosis ranges in severity from a mild, self-limited febrile illness to a fulminant life-threatening illness. The disease is widely spread all over the world, with a higher incidence in the tropical areas of the globe. Leptospirosis is a nationally notified disease, and 176 cases were declared during the 2012-2015 period, with a higher incidence on adult men. The aim of this study is to present an overview of cases observed during the past 6 years (2012-2017) in a convenience sample of the Portuguese population.
Microscopic Agglutination Test (MAT) was performed on 640 serum samples sent to INSA by Portuguese hospitals, during 2012-2017. The tested serogroups were: Australis, Autumnalis, Canicola, Celledoni, Cynopteri, Grippotyphosa, Icterohaemorrhagiae, Louisiana, Mini, Pomona, Rachmati, Sejroe, Shermani. Samples with titre ≥ 100 were defined as positive.
Forty five patients presented MAT titers ≥ 100 and were considered Leptospirosis cases. The majority of positive cases (n= 34; 75,6%) were found on men. The average age was 55 years (min=25; max=80), with similar distribution for men and women. The most frequent specific serogroup agglutination was found with serogroup Ballum (n=9; 20%). In 40% of the cases no specific serogroup agglutination was observed. A considerable number of cases occurred during the autumn, a rainy season in Portugal, as expected.
There is not much information on the circulating strain serovars in Portugal. This study presents an overview on leptospirosis, with a considerable number of cases per year, and provides information on probable infecting serogroups. Leptospirosis is still an important health problem, with a considerable number of cases per year in Portugal, and, in the eminence of climate changes, it may become a very important problem in Mediterranean countries that needs a greater awareness and surveillance.

EVALUATION OF THREE SEROLOGICAL TESTS FOR DETECTION OF ACUTE LEPTOSPIROSIS IN EXPERIMENTALLY INFECTED DOGS

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Canine leptospirosis is an acute zoonotic infection of dogs with worldwide distribution, caused by pathogenic spirochetes belonging to the genus Leptospira. Early detection and appropriate therapeutic intervention are required to resolve infection and prevent zoonotic transmission; however diagnosis is complicated by ambiguous clinical signs and a lack of rapid diagnostic tests capable of reliably detecting indicators of acute disease. In the last several years, two rapid point-of-care tests (Zoetis WITNESS® Lepto and Idexx SNAP® Lepto) for detection of Leptospira-specific antibodies in canine sera became commercially available. WITNESS Lepto detects IgM antibodies specific to multiple Leptospira antigens derived from whole cell extracts of serovars Grippotyphosa and Bratislava, whereas SNAP Lepto detects antibodies to the membrane lipoprotein LipL32. This study tested the hypothesis that IgM-based WITNESS Lepto can detect Leptospira- specific antibodies to four common leptospiral serovars earlier in the course of infection as compared to SNAP Lepto and the reference method microscopic agglutination test (MAT).

Thirty-two healthy, seronegative male beagles 6-8 months old were inoculated experimentally with Leptospira serovars Canicola, Grippotyphosa, Icterohaemorrhagiae and Pomona (n=8 dogs/serovar). Acute-phase sera were collected on days 0, 4, 7, 10, and 14 post- inoculation and monitored for Leptospira-specific antibodies using WITNESS Lepto, SNAP Lepto, and MAT. A minimum four-fold increase of antibody titer (≥1:400) for any serovar of a 7-serovar MAT panel indicated seroconversion. Some dogs were removed from the study on Day 10 to allow for the collection of larger quantities of serum for future use.

Seroconversion was detected in all dogs by day 10 by WITNESS Lepto and in 30 of 32 (93.8%) dogs by day 14 by MAT. Seroconversion was detected as early as day 7 in 28/32 (87.5%) dogs by WITNESS Lepto and 21/32 (65.6%) by MAT during the same period. The serovar with the highest MAT titer was often different from that of the infecting serovar, and strong cross-agglutination was observed across serovars. In contrast, SNAP Lepto detected seroconversion in only one dog (1/32; 3.1%) at day 7 and in two additional dogs by day 10 (3/31; 9.7%).

None of the dogs inoculated with serovar Canicola were detected by SNAP Lepto.

WITNESS Lepto detected immune responses specific to four common leptospiral serovars early in the course of infection and identified seroconversion in all animals earlier than SNAP Lepto and MAT. Additionally, cross-agglutination was observed across multiple serovars reinforcing the paradigm that the infecting serovar should not be determined by titer alone.

POSSIBLE ROLE OF WILD BOARS AS RESERVOIR FOR LEPTOSPIROSIS

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The opportunistic natural etiology of the animal, the ability to adapt to various habitats including urban, and the natural fecundity of suids have all led to expansion of feral swine population in Belgium. This increase poses problems particularly within the agricultural sector. In July 2014, the cattle breeding has faced an emerging phenomenon associated with an increased incidence of icteric abortions associated with infection with Leptospira of the serogroups Grippotyphosa (the majority of cases) and Australis (1). In search of natural reservoirs to relate with this phenomenon, we challenged feral swine samples derived from the Brucellosis surveillance programme to leptospirosis diagnosis.
A total of 116 samples, 56 sera and 60 kidneys, were retrospectively tested with a 12 serogroups microscopic agglutination test (MAT12) panel or challenged to a diagnostic PCR test, respectively. The tests were performed in accordance to the OIE manual and they undergo a strict quality-based control. The serological prevalence was evaluated by MAT12 in 56 animals that were sampled between 2015 and 2018. The sampling comprised all animals submitted at the national reference laboratory during the years 2016 and 2017. The prevalence was estimated at 25.0% (95% CI: 15.5–37.7) for cut-off titre of 1:100. Represented serogroups were Australis (11), Grippotyphosa (2), and Javanica (1) (none with mixed serogroups). DNA was extracted from kidney sample and tested by a TaqMan PCR targeting the Hap1/LipL32 gene. All kidneys, which were derived from another 60 animals, resulted negative at molecular diagnosis.
In Belgium, wild boars are susceptible to be infected with at least Leptospira of the serogroups Australis, Grippotyphosa and Javanica, albeit with very low titers. Indication for chronical infection and role in shedding was lacking. The deemed serogroups responsible for the majority of icteric abortion cases in cattle, Grippotyphosa and Australis, were seldom found in feral swine making this animal a possible natural reservoir for these serogroups in Belgium.
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REAL TIME PCR DETECTION OF LEPTOSPIRA SPP. IN KIDNEYS OF DOGS AND CATS IN SICILY

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In order to valuate the presence of *Leptospira* spp. in dogs and cats from Sicily, we sampled kidneys and tested with a specific PCR Real Time.
Kidney samples were collected from October 2016 to December 2017. We collected nr. 114 kidney of dogs and nr. 29 of cats. Both dogs and cats were conferred to our laboratory in order to know the cause-mortis assessment. Most of their were suspected of poisoning. DNA extraction from tissue was performed by PureLink Genomic DNA (Invitrogen). The target of Real Time PCR was *lip*L32 gene, absent in non-pathogenic *Leptospira* spp. Primers were: forward 3'-GGTCTTTACACAATTTCTTTCACT-5' and reverse 3'-TGGGAAAAGCAGACCAACAGA-5'. The probe was: 3'-FAM-AAGTGAAAGGATCTTTCGTTGTTGC-BHQ-5'. As a positive control, DNA extracted from a *L. interrogans* serogroup Australis serovar Bratislava reference strain was used, provided by National Reference Center for Leptospirosis (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy) and maintained in culture at our laboratory. The positive samples were sequenced by BMR Genomics (University of Padova, Italy).
No *Leptospira* spp. DNA was found in cat kidneys. Three dog kidneys were positive for presence of *Leptospira* spp. DNA and one of these was identified as *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae by sequencing. Two of the positive kidneys belonged to stray dogs and the third to a domestic dog. The death of the three positive dogs was probably imputed to poisoning.
Autopsy examinations did not show any clinical sign of leptospirosis. It was observed heart megalic and lungs congested and edematous. Moreover, the stomach appeared relaxed and full of semi-digested food. Nothing relevant was observed in reproductive system, urinary system, skin, lymphatic system, skeletal and muscular system.
Real Time PCR analysis is the most sensitive technique for detection of leptospiral DNA in tissue samples. Through Real Time PCR was confirmed the presence of *Leptospira* spp. only in 3 of 114 dogs. No presence of *Leptospira* spp. was observed in cats. This preliminary report lets to make some observations: i. only few dogs were found positive for *Leptospira* spp.; ii. in these cases their death is not attributable to this infection; iii. since the dogs are prone to Leptospirosis, it always necessary to exclude *Leptospira* spp. infection. Moreover, in accordance with other serological data obtained by our laboratory, *L. interrogans* serogroup Icterohaemorrhagiae seems to be the most common serogroup in Sicilian dogs.

PREVALENCE OF *LEPTOSPIRA* INFECTION IN RAT POPULATIONS IN LEPTOSIROSIS OUTBREAK AREAS IN CENTRAL JAVA AND YOGYAKARTA PROVINCE, INDONESIA

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Leptospirosis is a zoonotic disease, which is endemic in Indonesia, although the clinical cases is rarely reported. Investigations of *Leptospires* infection in rats population caught in rural areas were conducted during the rainy season (October) (n = 215) and those caught during the dry season (February-March) (n = 135) in some Central Java and Yogyakarta. Detection of Leptospira infection in rats was performed by two methods, which were PCR for the pathogenic *Leptospira* using LipL32 gene segments and by microscopic agglutination test (MAT) for the gold standard. In addition, the examination of human samples was done only by MAT. The results between infections occurring in rats were compared with the distribution of human case serovars from hospitals in the study areas (Semarang, Kebumen, Banyumas, Central Java and Kulon Progo Distric, D.I. Yogyakarta). Leptospirosis seroprevalence by MAT method in rats was 16.6%. The highest seroprevalence occurred in: *Rattus norvegicus* (45.0%), *Rattus tanezumi* (34.8%), *Bandicota indica* (6.5%), *Rattus exulans* (2.3%), and *Rattus tiomanicus* (2.1%). Serovar Djasiman (3.7%) was recognized as the most commonly infecting serovar in the rats, followed by bangkinang (2.9%), and bataviae (2.5%). Those serovars are also common in humans. In conclusion, the peridomestic rats tend to have a higher prevalence than the domestic rats. This study shows that human serovars and rats serovars are relatively similar.

IMMUNE STATUS EVALUATION BY MICROSCOPIC AGGLUTINATION TEST PERFORMED ON BOVINE FROM A LEPTOSPIROSIS OUTBREAK

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Leptospirosis are infectious diseases common to humans and many domestic and wild species of animals that can become infected, bearing or eliminating pathogens and infection carriers, respectively. The purpose of this study was to evaluate the evolution of immune status of a cattle herd in a leptospirosis outbreak.

The study was conducted on 32 cattle, part of which was from the beginning in the farm and the rest were introduced into the herd from other farmers from different areas. Following the first test, the result in MAT was negative for 14 cattle and positive for 18 cattle. The microscopic agglutination test (MAT) is the standard serological test recommended for use in the leptospirosis diagnosis, based on the detection of the relative concentration of antibodies in the serum of animals that react with the specific antigen represented by different serotypes of Leptospira spp. MAT was performed with live antigens represented by 9 serovars of Leptospira spp., from the Royal Tropical Institute, KIT Biomedical Research WHO / FAO / OIE Collaborating Center for Reference and Research on Leptospirosis, Amsterdam, Netherlands (Pomona, Icterohaemorrhagiae, Canicola, Wolffi, Hardjo, Grippothyphosa, Australis, Autumnalis and Bataviae), with 4 trials performed during a 3-year period.

After the analysis of the results were identified 6 different groups of immunological profile: 2 positive cattle for Pomona, 4 for Australis, 2 for Australis and Grippothyphosa, 4 for Grippothyphosa, 5 for Wolffi and Hardjo and a positive bovine for Wolffi serotype, Hardjo, Australis and Grippothyphosa. At the last test, the result in MAT was negative only for 5 cattle and positive for 27 cattle. For 9 of initially 14 negative bovines, 1 was positive for the Pomona, 2 for Pomona and Australis, 1 for Canicola, 3 for Australis, 1 for Canicola and Australis, and one cattle positive for the Grippothyphosa serovar.

Analyzing the immune profile of the tested animals during 3 year period: for positive cattle on the first test, no major increase of level antibody was detected from the first test to the last, the antibody titer was kept on the same level. In some positive bovine, positive reactions against the other serovars have also occurred, so that, the immunity developed against a serovar can not be considered protective for subsequent infection with another serovars. During this period, the positive cattle cohabited with the negatives ones, these have not been isolated. Although repeated antibiotic treatments have been performed, these did not significantly affect seroconversion and prevented the infection or reinfection of cohabiting animals. Following the epidemiological investigation of the leptospirosis outbreak, a direct correlation between the immune profile of the animals and their origin was identified, so each immune profile was come from different location/area.

LEPTOSPIROSIS IMMUNOGENICITY INDUCED AFTER EURICAN DAPPI LMULTI® VACCINATION IN DOG

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Leptospirosis is the most widespread zoonotic disease in the world affecting a broad range of mammals. It is caused by infection with spirochaetes belonging to several pathogenic species. EURICAN DAPPi Lmulti® is a whole cell, non-adjuvanted vaccine prepared from inactivated cultures of three serovars of *Leptospira interrogans* Grippotyphosa, Canicola and Icterohaemorrhagiae. Previous studies demonstrated that the tested vaccine provides a quick onset of immunity as early as two weeks post-vaccination and a long-term immunity of 13-15 months with a very good protection against fatal leptospirosis for the three serovars. Several studies have shown that vaccine-associated Leptospira MAT titers are generally low, short-lived or ununderectable in most of protected dogs. Thus, agglutinins are unlikely to be the only protective immune mechanism reinforcing the role of antibodies after vaccination and against Leptospira infections. So, we decided to develop and evaluate other analytical techniques such as antibody ELISA in order to increase the level of sensitivity and to detect other types of antibodies. In addition, as three serovars of *Leptospira* are contained in the vaccine, each ELISA technique was developed to titrate the specific antibodies against one serovar among three. The data presented here show the performance of the technique to detect a raise of mean antibody titer following primovaccination, boost at one year, or challenge Sensitivity of the technique has been compared to the MAT technique. Briefly, ELISA allows detecting a systematic increase amount of antibody following primovaccination, one year boost and challenge. Sensitivity and specificity is clearly superior to MAT technique and more easy to use in routine for vaccine development.

PREVALENCE OF ANTILEPTOSPIRAL SERUM ANTIBODIES IN CLINICALLY HEALTHY HORSES IN CZECH REPUBLIC

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Leptospirosis is a zoonotic disease of global importance, and has a worldwide distribution. Problems of this disease can be solved by using the effective vaccine with suitable composition of Leptospira serovars.

The aim of this study was to determine the seroprevalence of Leptospira spp. in horses in Czech Republic. At present, the examination is performed on 573 samples of blood serum of horses from all over the Czech Republic.

The sera were collected in period 2008 – 2016 and were tested by microagglutination test (MAT) to detect antibodies to Leptospira species, employing the following strains as antigens:

Serovar Grippotyphosa – strain P125; serovar Icterohaemorrhagiae – strain Fryšava; serovar Sejroe – strain M 84; serovar Canicola – strain C7; serovar Pomona – strain Šimon; serovar Bratislava – strain Jež Bratislava; serovar Sorexjalna – strain Sorex Jalna; serovar Tarassovi – strain Mitis Johnson; serovar Hebdomadis – strain Hebdomadis; serovar Autumnalis – strain Akiyami A.

Composition of selected serovars corresponds approximately to occurrence and prevalence of leptospiral serovars in Europe.

Of the tested samples, 43.28% were found to be positive. Responses against only one Leptospira serovar were detected in 49.6%, responses with two or more Leptospira serovars were detected in 50.4%. The most prevalent was serovar Grippotyphosa (47.32%) followed by Sejroe serogroup (18.65%) and serovar Bratislava (17.72%). Antibodies against other leptospiral serovars used in diagnosis were detected only sporadically.

It can be concluded that the current interim results suggest, that horses are in the course of their live exposed to infection by different Leptospira serovars. The results so far confirmed already known fact that the antibodies against serovar Grippotyphosa has dominant incidence.

How this serovar contributes to influence of health status of affected animals is a matter of follow-up.

This work was supported by Project FV 10125 from Ministry of Industry and Trade, CR.

EVALUATION OF IMMUNOGENICITY AND PROTECTIVE HOMOLOGOUS CAPACITY OF A TETRAVALENT LEPTOSPIRA VACCINE CANDIDATE, FOR VETERINARY USE, INCLUDING SEROGROUP BALLUM

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Immunogenicity is an important parameter for studies of immunogens. The humoral immune response against leptospirosis is vital for resistance to infection; hence this work is proposed to evaluate the immunogenicity and protective homologous capacity of a tetravalent candidate that includes serogroup Ballum in its formulation.

In this work, it used the strain 245-12 classified as L. borgpetersenii serovar Ballum, isolated from a confirmed case of leptospirosis. With this strain previously characterized from their virulence were formulated 5 batches of a tetravalent

vaccine preparation, which also contains strains Canicola, Icterohaemorrhagiae and Pomona contained in the Polivalente-Leptospira vaccine for veterinary use. The immunogenicity of these preparations were assessed by agglutination technique and protective homologous capacity in hamster was evaluated by challenge against 100 LD50. Animals were immunized with two doses of vaccine (0.1mL) with an interval of 15 days; the challenge was carried out 14 days after immunization schedule was ended.

All lots of tetravalent vaccine formulated were satisfactorily and showed a significant immunogenicity and protective homologous capacity against serogroup Ballum, as well as eliminate the carrier state in the immunized animals.

A significant response was observed against Ballum and Canicola even with the first dose, for Icterohaemorrhagiae and Pomona the response was higher only after the second immunization, reaching in the case of Icterohaemorrhagiae values close to those of Canicola and Ballum at the end of the study, for Pomona the levels remain lower. In the non-immunized control group, no antibody response was observed in any of the cases.The evaluation by MAT of the response of specific agglutinins induced by both preparations against the homologous antigens revealed a significant seroconversion (T9 / T0 ≥ 1: 1024). All the tetravalent lots induced a significant increase of specific agglutinin titres against the Ballum antigen after the first and second doses ($p < 0.05$). During this study of the evaluation of the homologous protective capacity and elimination of the carrier state induced by the tetravalent preparations, 100% protection was observed in the animals immunized against the lethal infection after 15 days of completion of the immunization scheme. This work, for the first time set the base for novel vaccine formulations, in animals, containing the serogroup Ballum.

The tetravalent vaccine preparations obtained from the strain L. borgpetersenii serovar Ballum, showed a significant immunogenicity and homologous protection capacity in the animal biomodel, as well as managed to eliminate the carrier state. It was evidenced that the homologous protection against the Mozdok serovar in the tetravalent preparation, although satisfactory, is smaller, probably due to the interference of serovar Ballum.

IDENTIFICATION OF NOVEL IMMUNOGENIC PROTEINS FROM LEPTOSPIRA SPP. BY CELL-SURFACE IMMUNOPRECIPITATION (CSIP)

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Leptospirosis is a re-emerging zoonosis caused by pathogenic *Leptospira* spp. that is difficult to diagnose and that lacks an effective universal vaccine. Several approaches have been used to identify leptospiral vaccine candidates and targets for diagnostics, yet most of these studies were based on *in vitro*-grown bacteria. It is well-established that *Leptospira* spp. adapt to the host environment, expressing proteins that are absent *in vitro*. These host-adapted proteins represent an unexplored pool of antigens for the development of novel vaccines and diagnostics for leptospirosis.

Leptospira interrogans strain Fiocruz L1-130 was cultured under *in vivo*-like conditions within dialysis membrane chambers. Pooled serum samples from human convalescent patients were used to immunoprecipitate surface-exposed leptospiral proteins. The leptospiral cell membrane was lysed and the antibodies, together with bound proteins, were recovered by using Protein A/G columns. Bound proteins were eluted from the antibodies and resolved by 1D SDS-PAGE. The proteins underwent tryptic digestion and the fragments were identified by LC-MS/MS. Proteins identified by CSIP-MS were further analysed by bioinformatics analysis to select proteins that were likely to be surface exposed. SignalP was used to predict the presence of a signal peptide; LipoP, SpLip predicted the presence of a lipobox; PSORTb, Gneg-Ploc predicted subcellular location (outer membrane or secreted) and Bomp and HHomp were used to predict outer membrane beta-barrel structures. Using host-adapted *L. interrogans* strain Fiocruz L1-130 and sera collected from leptospirosis patients, we immunoprecipitated and identified seroreactive proteins by mass spectrometry. A total of 157 immunoreactive proteins were initially identified. *In silico* analysis suggested that 24 of these proteins are surface exposed and represent potential vaccine candidates.

CSIP using host-adapted leptospires allowed the identification of novel targets for vaccine and diagnostic development and will provide novel insights into host-pathogen interaction.

COMPARISON OF EFFICACY OF TWO LICENSED TETRAVALENT LEPTOSPIRA VACCINES FOR DOGS USING VIRULENT CHALLENGE STRAINS OF SEROGROUPS GRIPPOTYPHOSA AND ICTEROHAEMORRHAGIAE

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Tetravalent leptospirosis vaccines for dogs were not available on the European market until 2013, when Nobivac® L4 (MSD Animal Health) was introduced, followed in 2015 by Versican® Plus L4 (Zoetis). In the present studies the efficacy of the two vaccines has been compared in two challenge studies using pathogenic strains of serogroup Grippotyphosa# (Grippo) and of serogroup Icterohaemorrhagiae## (Ictero). [#Serovar Bananal/Liangguang; ##Serovar Copenhageni]

Two similar studies in beagle dogs were performed, one with a Grippo challenge strain, and one with an Ictero challenge strain. Each study comprised the following treatment groups (7 or 8 dogs/group): group 1, Nobivac L4; group 2, Versican Plus L4; group 3, non-vaccinated control group. Each L4 vaccine was administered according to manufacturer's recommendations (1 ml subcutaneously). In both cases two vaccinations were administered, at 6 and 10 weeks of age. Three weeks after the second vaccination all dogs were challenged by conjunctival, intranasal and intraperitoneal administration of the respective challenge strain. Necropsy was done on any dogs which had to be euthanised during the study (according to humane end point criteria), and at four weeks after challenge on the surviving dogs.

Efficacy was assessed based on the following criteria: titres of agglutinating serum antibodies (microscopic agglutination test, MAT) three weeks after second vaccination, rectal temperature, clinical signs, culturing of challenge organisms from blood, urine, kidney and liver, macroscopic and histopathological examination at necropsy.

See Table.

Both vaccines were able to provide a high level of protection against clinical signs caused by a pathogenic strain of serogroup Grippo. However, only Nobivac L4 was able to prevent clinical signs following a severe Ictero (serovar Copenhageni) challenge.

Table: Results cultures, clinical signs, nephritis and number euthanised dogs

Study no. (challenge serogroup)	Group (vaccine)	Group Size	Days of positive blood cultures (mean per dog)	Days of positive urine& kidney cultures (mean per dog)	Number dogs with clinical signs	Number dogs with interstitial nephritis	Number dogs euthanised
1 (Grippo)	1 (Nobivac L4)	8	0.3 S*	0.0 S	0/8 S	0/8 S	0/8 NS
	2 (Versican Plus L4)	8	0.3 S	0.0 S	1/8** 0/7 S	0/8 S	1/8* 0/7 NS
	3 (--)	7	4.9	2.3	7/7	7/7	0/8
2 (Ictero)	1 (Nobivac L4)	8	2.6 S	1.5 S	0/8 S	2/8 S	0/8 S
	2 (Versican Plus L4)	7	5.6 NS	4.3 NS	4/7 NS	5/7 NS	2/7 NS
	3 (--)	8	7.0	5.8	8/8	8/8	6/8

* Difference with control group: S and NS, statistically significant ($P<0.05$) and non-significant, respectively.
**One dog had very low body weight (BW) before challenge (1.5 kg; mean BW group 2 was 3.3 kg); it became ill on day 3 post-challenge and was euthanised on day 14 post-challenge but there was no evidence of leptospirosis from laboratory tests.

TRAPPING AND IDENTIFICATION OF POTENTIAL SUBSTRATES OF THE MOLECULAR CHAPERONE CLPB(HSP100) FROM THE PATHOGENIC BACTERIUM LEPTOSPIRA INTERROGANS

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Bacterial ClpB is a molecular chaperone belonging to the Hsp100 subfamily of the AAA+ ATPases that reactivates aggregated proteins in cooperation with the DnaK chaperone system. It is well known that ClpB acting as an ATP-dependent protein disaggregase is essential for survival of bacteria under stress conditions. A number of publications also indicate that ClpB supports bacterial virulence. However, the exact role of ClpB in bacterial pathogenicity has not been well characterized. It can be assumed that ClpB, due to its role as the molecular chaperone, mediates refolding of some essential bacterial proteins, including the known virulence factors, which may be more prone to aggregation under infection-induced stresses.

We are especially interested in ClpB from the pathogenic spirochaete Leptospira interrogans (ClpBLi), responsible for Leptospirosis, a zoonotic disease of global importance. In this study, we constructed a “substrate trap” variant of ClpBLi (ClpBLi-Trap) with mutations of the Walker B motif in both ATP-binding domains (E281A/E683A) to identify potential ClpBLi substrates by using the protein-protein-interactio-based pull down strategy coupled with LC-MS-MS/MS and bioinformatics analyses.

We identified over 60 potential protein substrates for the chaperone ClpB in cell lysates prepared from L. interrogans serovar Copenhageni strain B42 cultures submitted to mild or serve heat stress. The captured proteins were grouped into various functional categories, but most of them were related to major metabolic pathways like the tricarboxylic acid (TCA) cycle, glycolysis-gluconeogenesis or amino acid and fatty acid metabolism. It is likely that these proteins have an important impact on the growth and pathogenicity of Leptospira. Other identified proteins were associated with the transcriptional process, protein synthesis, membrane biogenesis or cell signaling.

Based on our proteomic investigations we suggest that ClpB can support the virulence of L. interrogans through protecting the conformational integrity and catalytic activity of multiple metabolic enzymes and thus maintaining the energy homeostasis in the bacterial cells.

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Signs of renal or hepatic failure, acute febrile illness, pulmonary hemorrhage, anorexia, lethargy, jaundice, vomitus...

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