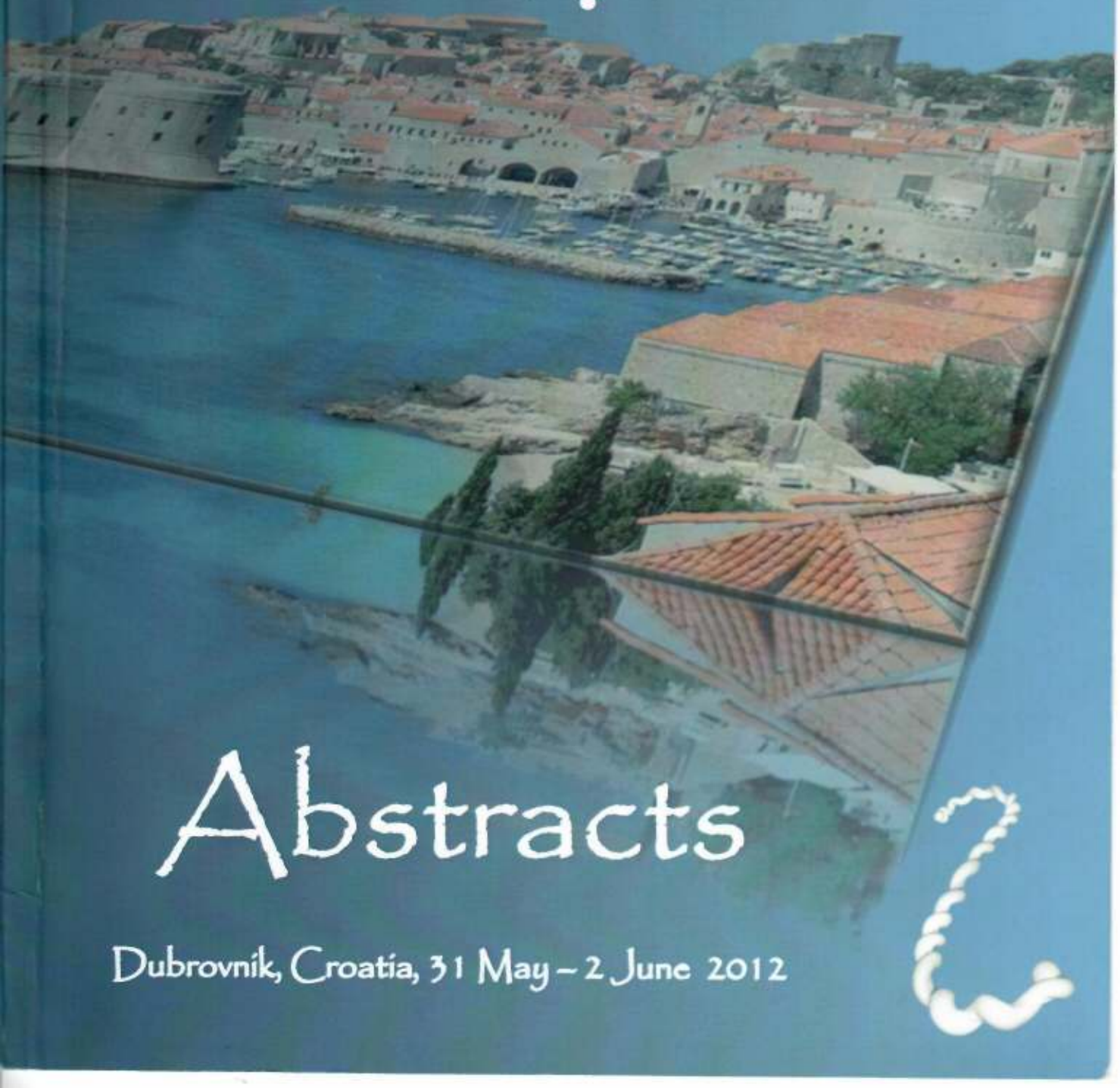
European meeting of leptospirosis

Eurolept 2012



Abstracts

Dubrovnik, Croatia, 31 May – 2 June 2012

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Hosted by
Faculty of Veterinary Medicine
University of Zagreb

Venue
Hotel Excelsior, Dubrovnik

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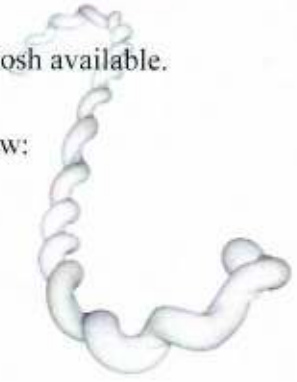


Instructions for speakers & poster presenters

Oral presentation

Important: If you are not able to present your talk, it is essential that you contact the Congress secretariat immediately to notify them of any change of presenter or withdrawal.

1. Sessions will be held at conference room "Lavandula" at Excelsior Hotel, Dubrovnik.
2. The language of all presentations is English, no translators will be provided.
3. Oral presentation should contain: background, objectives, methods, results, conclusions/implications. Objective should be clearly stated. Avoid unnecessary details in methods unless the methodology is the central topic of your talk.
4. The duration of presentation is 15 minutes, approximately 10 minutes for the presentation itself and 5 minutes for questions from audiences.
5. The chairperson of your session will be strict in allowing no more than the time allotted for your presentation and will signal 10th and 12th minute of presentation.
6. For keynote lectures, the duration for the presentation itself is 20-25 minutes and 5-10 minutes for discussion.
7. Presentation must start on time with no exceptions, in order to comply with the Congress schedule.
8. Please bring your presentation on the following media: USB-removable drive or CD-ROM, and give it to Organizing Committee day before your presentation or latest early morning on the day of presentation.
9. The meeting rooms are equipped with Windows-PC, no Apple/Macintosh available.
10. Please note that all media should be formatted for Windows-PC.
11. Please create and save your file name in accordance with the rule below:
    Ref. number/last name.ppt (e.g.) OP1 Smith.pp
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12. Laser pointers will be available.



Poster presentation

Important: If you are not able to present your poster for any reason, you are kindly requested to contact the meeting secretariat immediately and to notify them of any change of presenter or withdrawal.

1. The language of all posters is English only.
2. Poster panel size: 100 cm (height) x 80 cm (width)
3. One poster board will be allocated for each poster presentation.
4. Suggested poster format: title, authors and affiliations, abstract, methods, results, discussion, references.
5. Posters will be presented in the Poster area in front of the Conference room "Lavandula".
6. Posters will be posted in the Poster area by organizing committee members, so please mark them and hand them over at registration or at the latest on the day before your poster presentation is scheduled.
7. In order to enable discussion and interaction with other participants, we request you or one of your group to be at your poster board. Poster presenters should stand by their posters to explain and discuss posters during the discussion time on the presentation day (see Programme).
8. Display and removal of your poster should be conducted according to the Programme.



Programme

Wednesday, 30 May 2012

	Arrival		
17:00 – 20:00	Registration		
20:00	Welcome drink		

Thursday, 31 May 2012

08:00 – 10:30	Registration		
10:30 – 11:00	Opening ceremony		
11:00 – 11:30	KL 1	**CHALLENGES IN ANIMAL LEPTOSPIROSIS** Ellis, W. A.	Clinical leptospirosis -animal
11:30 – 11:45	OP 1	**ANIMAL LEPTOSPIROSIS IN THE RUSSIAN FEDERATION** Panin A. N., Viktorova E. V.	Epizootiology
11:45 – 12:00	Coffee break		
12:00 – 12:15	OP 2	**INFECTION RATES AND ETIOLOGICAL STRUCTURE OF LEPTOSPIROSIS IN RUSSIAN FEDERATION** Soboleva G. L., Malakhov Y. A., Aliper T. I.	Epizootiology
12:15 – 12:30	OP 3	**CURRENT SITUATION OF LEPTOSPIROSIS IN SARDINIA** Ponti M. N., Palmas B., Noworol M., Canu M., Fiori E., Picardeau M., Carboni G. A., Pintore A., Ruiu A., Piredda I.	Epizootiology
12:30 – 12:45	OP 4	**PASSIVE SURVEILLANCE OF SWINE IN GERMANY FOR INFECTION WITH LEPTOSPIRES** Strutzberg-Minder K., Tschentscher A., Hartmann M., Beyerbach M., Kreienbrock L.	Epizootiology

13: 00	**Lunch**		
15:00 – 15:15	**OP 5**	**DETERMINATION OF SPECIFIC ANTIBODIES TO *Leptospira* spp. IN POPULATION OF STRAY DOGS IN THE REPUBLIC OF SERBIA** Vojinović D., Samokovlija A., Elezović M., Rogožarski D., Đuričić B.	Epizootiology
15:15 – 15:30	**OP 6**	**SEROLOGIC EVIDENCE OF *Leptospira* spp. SEROVARS IN CATTLE IN CROATIA** Zdelar-Tuk M., Račić I., Cvetnić Ž., Vujnović A., Duvnjak S., Špičić S.	Epizootiology
15:30 – 15:45	**OP 7**	**CATTLE INCIDENTAL INFECTIONS DUE TO *Leptospira* STRAINS CARRIED BY SMALL MAMMALS IN TRÁS-OS-MONTES E ALTO DOURO REGION, NORTHERN PORTUGAL** Paiva-Cardoso M. N., Collares-Pereira M., Gilmore C., Santos-Reis M., Rodrigues J., Ellis W. A.	Epizootiology
15:45 – 16:00	**OP 8**	**OBJECTIVISATION OF ANTIBIOTIC THERAPY PRACTICE FOR LEPTOSPIROSIS SEROPOSITIVE DAIRY COWS** Bajrović T., Eterović T., Zvizdić Š., Sarajlić R.	Clinical leptospirosis -animal
16:00 – 16:15	**OP 9**	**LEPTOSPIROSIS IN SHEEP: ISOLATION OF LEPTOSPIRES FROM THE GENITAL TRACT** Arent Z., Gilmore C., Mackie D., Ellis W.	Clinical leptospirosis -animal
16:15 – 16:30	**OP 10**	**DIAGNOSIS LEPTOSPIROSIS OF ANIMAL BY POLYMERASE CHAIN REACTION** Panin A. N., Viktorova E. V.	Diagnostics
16:30 – 18:00	**Coffee break and Poster presentation (categories; Epizootiology)**		

FRIDAY, 1 JUNE 2012

9:00 – 9:30	**KL 2**	**GLOBAL LEPTOSPIROSIS AND TOOLS FOR THE DETECTION AND CHARACTERIZATION OF LEPTOSPIRES** Hartskeerl R. A.	Diagnostics
9:30 – 9:45	**OP 11**	**HUMAN LEPTOSPIROSIS IN CROATIA** Balen-Topić M.	Epidemiology
9:45 – 10:00	**OP 12**	**HUMAN LEPTOSPIROSIS IN RUSSIA: EPIDEMIOLOGICAL TRENDS ACROSS TWO DECADES** Ananyina Y. V.	Epidemiology

10:00 – 10:15	**OP 13**	**SEROPREVALENCE AND CORRELATES OF LEPTOSPIROSIS IN METRO MANILA, PHILIPPINES** Borja M. P., Napulan R. M., Gloriani N. G., Yoshida S.	Epidemiology
10:15 – 10:30	**OP 14**	**THE HANTA-HUNTING STUDY \"HANTAVIRUS INFECTION IN LEPTOSPIROSIS SUSPECTED PATIENTS IN THE NETHERLANDS.\"** Goeijenbier M., Reusken C. R., Goris M. G. A., Hartskeerl R. A., Martina B. E., Osterhaus A. D. M. E., Wagenaar J. F. P., Van Gorp E. C. M.	Epidemiology
10:30 – 10:45	**OP 15**	**AN OVERVIEW OF HAEMOSTASIS IN SEVERE LEPTOSPIROSIS: FROM PLATELET TO CLOT** Wagenaar J. F. P., Hartskeerl R. A., Van Gorp E. C. M.	Clinical leptospirosis -human
10:45 – 11:00	**OP 16**	**LEPTOSPIROSIS AND FASCIOLIASIS: IMMUNOLOGICAL INTERACTIONS IN THE CLINICAL POLYMORPHISM OF PATIENTS FROM SAO MIGUEL (AZORES, PORTUGAL)** Azevedo V., Clemente I., Carreira T., Nunes M., Belo S., Afonso A., Vieira M. L.	Diagnostics
11:00 – 11:15	**Coffee break**		
11:15 – 11:30	**OP 17**	**ESTABLISHMENT OF VALID LABORATORY CASE DEFINITION FOR HUMAN LEPTOSPIROSIS** Goris M. G. A., Leeflang M. M. G., Boer K. R., Goeijenbier M., Van Gorp E. C. M., Wagenaar J. F. P., Hartskeerl R. A.	Diagnostics
11:30 – 11:45	**OP 18**	**LEPTORAPIDE: A ONE-STEP ASSAY FOR RAPID DIAGNOSIS OF HUMAN LEPTOSPIROSIS** Savage R. (presenter - Karla Hedley)	Diagnostics
11:45 – 12:00	**OP 19**	**SIGNIFICANCE OF PCR IN MONITORING OF LEPTOSPIROSIS** Breneva N. V., Kiseleva E. Y., Balakhonov S. V.	Diagnostics
12:00 – 12:15	**OP 20**	**EVALUATION OF REAL-TIME PCR AND CULTURING FOR THE DETECTION OF LEPTOSPIRES** Ahmed A., Klaasen H. L. B. M. , Van der Veen M., Van der Linden H., Goris M. G. A., Hartskeerl R. A.	Diagnostics
12:15 – 12:30	**OP 21**	**DESIGN OF TAQMAN-BASED PCR ASSAYS TO DISTINGUISH PATHOGENIC *Leptospira* GENOMOSPECIES** Ferreira A. S., Costa P., Amaro A., Nunes M., Thompson G., Vieira M. L., Rocha T., Inácio J.	Diagnostics
13:00	**Lunch**		
15:00 – 15:30	**KL 3**	**GENETICS AND GENOMICS IN PATHOGENIC *Leptospira* spp.** Picardeau M.	Genetics / Genomics/ Proteomics

15:30 – 16:00	**KL 4**	**IDENTIFICATION OF INFECTION-REGULATED TRANSCRIPTS OF *Leptospira interrogans* SEROVAR Copenhageni BY RNA-SEQ** Sivasankaran S. K., Caimano M. J., Santiago R. B., Hinton J. C. D., Nally J. E.	Pathogenesis
16:00 – 16:15	**OP 22**	**STUDY REGARDING THE ANALYTICAL SPECIFICITY OF THE MICROSCOPIC AGGLUTINATION TEST USED IN THE LABORATORY DIAGNOSIS OF LEPTOSPIROSIS** Guţu E., Rădulescu R. A., Nicolae Ş., Ţogoe I., Răgălie A., Decei L., Negoiţă A.	Immunology
16:15 – 16:30	**OP 23**	**A NEW CANINE LEPTOSPIROSIS VACCINE PREVENTS BOTH DISEASE AND INFECTION FOLLOWING CHALLENGE WITH STRAINS FROM FOUR SEROGROUPS** Klaasen H. L. B. M., Van der Veen M., Molkenboer M. J. C. H., Sutton D. J.	Immunology
16:30 – 18:00		**Coffee break and Poster presentation (categories; Epidemiology – human, Diagnostics, Immunology, Pathogenesis)**	
20:00		**Gala dinner**	

Saturday, 2 June 2012

10:00 – 10:15	**OP 24**	**MULTIPLE BACTERIAL INFECTIONS IN CROATIAN RODENTS** Tadin A., Markotić A., Habuš J., Tokarz R., Vucelja M., Turk N., Margaletić J., Lipkin W. I.	Epizootiology
10:15 – 10:30	**OP 25**	**STUDY ON THE *Leptospira* PREVALENCE IN RATS IN MAJOR GERMAN CITIES** Mayer-Scholl A., Luge E., Hammerl J. A., Dremsek P., Plenge-Bönig A., Rietschel W., Guenther S., Ulrich R. G., Nöckler K.	Epizootiology
10:30 – 10:45	**OP 26**	**FIRST RECORD OF *Leptospira* ISOLATION FROM WILD BOARS OF SARDINIA** Pintore A., Palmas B., Noworol M., Canu M., Fiori E., Picardeau M., Tola A., Piredda I., Ponti M. N.	Epizootiology
10:45 – 11:00	**OP 27**	**PREVALENCE OF LEPTOSPIRAL ANTIBODIES IN WILD UNGULATES FROM CONTINENTAL PART OF CROATIA** Slavica A., Cvetnić Ž., Konjević D., Deždek D., Milas Z., Štritof Z., Habuš J., Turk N.	Epizootiology
11:00 – 11:15	**OP 28**	**SURVEY ON LEPTOSPIROSIS IN WILD BOARS (*Sus scrofa*) IN LOMBARDY, NORTHERN ITALY** Figarolli B. M., Gaffuri A., Alborali G. L., D'Incau M., Tagliabue S.	Epizootiology

11:15 – 11:30 **OP 29** **IMMUNE REACTIONS TO LEPTOSPIRES AND HANTAVIRUSES: DIFFERENCES AND SIMILARITIES** Immunology

Markotić A., Kurolt I. C., Cvetko Krajinović L., Žmak Lj., Janković M., Škerk V., Milas Z., Turk N.

11:30 – 11:45 **OP 30** **CLIMATE CHANGE, FLOODING AND INCREASE OF LEPTOSPIROSIS IN ALBANIA** Epidemiology

Bino S., Alla L., Rogoz E., Kaduku E., Abazaj E., Mesi A., Shestani I., Puca E., Kraja D.

11:45 – 12:30 **Closing ceremony**

13:00 **Lunch**

15:00 – 21:15 **Excursion "Taste of Konavle"**



Keynote lectures



KL 1

CHALLENGES IN ANIMAL LEPTOSPIROSIS

Ellis W. A.

c/o OIE Leptospirosis Reference Laboratory, AFBI, Vetereinary Sciences Division, Stoney Road, Stormont, Belfast BT4 3SD, Northern Ireland, UK

This paper will discuss the challenges and opportunities presented by the decline and stagnation which has occurred in animal leptospirosis research. Much of this stagnation has arisen from governments; attitudes to endemic disease, exacerbated by the economic downturn and the loss of veterinary microbiology skill base, in many countries, through changes in university curricula. The difficulties inherent in working with such a fastidious group of organisms militate against fast turn-around science. Changes in weather patterns, animal distribution patterns, animal management systems and the lack of up-to-date surveillance data plus the technical developments - or in some cases the lack of them - all indicate that it is a good time to increase animal leptospirosis research.



KL 2 GLOBAL LEPTOSPIROSIS AND TOOLS FOR THE DETECTION AND CHARACTERIZATION OF LEPTOSPIRES

Hartskeerl R.

Royal Tropical Institute, KIT Biomedical Research, Amsterdam, The Netherlands

Leptospirosis is probably one of the most neglected infectious diseases in the world. The disease is difficult to diagnose. It has a large variety of signs and symptoms and ranges from mild to fatal and, therefore, is easily confused with several other, often locally endemic and epidemic, diseases such as dengue, Hanta virus infection and other viral hemorrhagic fevers, salmonellosis, malaria, yellow fever, hepatitis and brucellosis.

Incidences established by the WHO-Leptospirosis Burden Epidemiology Reference Group (LERG) amount 5 per 100,000 population for endemic leptospirosis and 14/100,000 for epidemic leptospirosis (WHO-LERG report 2011). This implies a total of 1.7 million actual severe cases per year, which still underestimates the burden because of notorious underreporting..

Due to its protean character, leptospirosis is difficult to diagnose on clinical grounds. Clinicians might recognize Weil's syndrome with hepato-renal failures and hemorrhages, which accounts only for 5-10% of the severe cases, but other cases are likely missed.

Support of the laboratory is indispensable. The Microscopic Agglutination test (MAT) is the gold standard. However, this technique is tedious and laborious as it requires panels of viable *Leptospira* serovars, preferably local isolates. Thus the method is only performed at a few laboratories. Besides, MAT may not be as golden as can be expected from a reference test. Rapid tests such as the IgM-detecting Lateral Flow Assay (LFA) are very helpful tools for first line diagnosis. However, rapid serological tests share the disadvantage with MAT in that they only can confirm the disease at a later stage – usually one week after onset of illness - when the host has produced sufficient amounts of antibodies. Isolation of leptospires by culture provides evidence for an infection but leptospires are fastidious bacteria that grow too slowly in vitro to be beneficial for the treatment of the individual patient. PCR on early acute blood samples forms a suitable alternative. Currently, a few real-time PCRs have been well validated and indeed combine a high sensitivity with a high specificity notably at the early acute stage of the disease which is most relevant for clinical decision making.

Serological classification of leptospires identifies the serovar as the basic taxon, while molecular classification determines the *Leptospira* species. These two are distinct classification methods that hardly correspond. Serological classification is tedious and requires viable leptospires. The standard test, the CAAT, is currently confined to a few reference centers. Molecular techniques that produce digital, electronically portable data such as MLST are most promising tools for the near future.



KL 3 GENETICS AND GENOMICS IN PATHOGENIC *Leptospira* spp.

Picardeau M.

Institut Pasteur, Unité de Biologie des Spirochètes, Paris, France

As a spirochete, *Leptospira* spp. differs considerably from well-studied gram-negative and gram-positive bacteria. One centenary after of the discovery of the causative agent of leptospirosis, this zoonosis remains a major public health problem in developing countries. A significant barrier to control and prevent leptospirosis has been the limited understanding of *Leptospira* pathogenesis, due in part to the lack of genome sequences and tools to genetically manipulate this pathogen. These barriers have now been overcome. We determined the genome sequence of the saprophyte *L. biflexa*. Comparison of the genome sequence of pathogenic and saprophytic *Leptospira* strains provides an opportunity to identify features that are unique to pathogenic species. The pathogen *L. interrogans* genome contain approximately 3400 predicted coding regions, of which 656 are pathogen-specific genes, i.e. genes not present in the saprophyte *L. biflexa*. The majority of these genes has no known function, suggesting the presence of pathogenic mechanisms unique to *Leptospira*. However, any function of these putative virulence factors remains speculative until the generation of specific mutants. Our laboratory has pioneered the field of *Leptospira* genetics. The genetic tools developed at the Institut Pasteur allow the generation of mutants in *L. interrogans*. We thus obtained random transposon mutants in genes encoding putative virulence factors. Some of these mutants showed decrease virulence compared to the wild-type strain in the animal model of leptospirosis. We also generated mutants by allelic exchange in *L. interrogans*, wherein a spectinomycin resistance gene replaced a portion of the coding sequence. This analysis of *L. interrogans* mutants provides an opportunity to investigate genes that contribute to pathogenesis and will provide a better understanding of the biology of *L. interrogans*. More recently, we also used of the saprophyte *L. biflexa* as a surrogate host to characterize the role of key virulence factors contributing to leptospirosis. Further progress in our understanding of the virulence of *Leptospira* will contribute to the development of novel strategies for the diagnostic and prevention of this neglected emerging disease.



OP 1

ANIMAL LEPTOSPIROSIS IN THE RUSSIAN FEDERATION

Panin A. N., Viktorova E. V.

All-Russian State Center for Quality and Standards Animal Drugs and Feeds (VGNKI), Moscow, Russia

Leptospirosis is an important zoonotic disease caused by spirochetes of the genus *Leptospira*. Sick animals of any age, but the most severe disease occurs in young animals. In cattle the disease manifests itself in typical cases of short-term fever, icterohaemoglobinuria, necrosis of the mucous membranes and skin, abortion, and in some cases - atypical mastitis, a syndrome of loss of milk production. In pigs leptospirosis is characterized by a predominantly asymptomatic, and in pregnant sows - abortion or the birth of non-viable piglets. The sources of leptospires are essentially wild and domesticated mammals harbouring the spirochetes in the proximal convolute tubules of the kidneys and chronically excreting the leptospires with urine into the environment. The diagnosis of leptospirosis in the set of the following methods: 1) bacteriology (includes microscopy, culture isolation, bioassay), 2) serological (MAT, ELISA, PRD), 3) histological, 4), immunofluorescence, 5) molecular genetic (PCR). In general, the Russian Federation over the past 10 years was recorded from 20 to 59 of leptospirosis disadvantaged areas, where attack rates from 2500 to 3300 animals and the case was from 17 to 112 animals. Currently, up to 70% dysfunctional items accounted for the Central, Volga, North Caucasus and the West-Siberian regions. It should be noted that, as in previous years leptospirosis in animals occurs mainly asymptomatic - only 7% positive animal clinic note of leptospirosis. Clinical signs of leptospirosis recorded in the Altai region (pigs), the Astrakhan region (RS), the Krasnodar region (cattle, pigs), the Kurgan region (cattle, pigs), Lipetsk region (RS), the Volgograd region (horse), Udmurtia (pigs), Chuvashia (cattle), Omsk region (RS), Belgorod, Pskov and Tula regions (the dog).

In the Russian Federation each year to investigate the leptospirosis in the RMA 900 thousand samples of blood serum of animals. Over the past five years the number of serological tests decreased by 0.9%. To date, the number of positively reacting to leptospirosis of animals has decreased slightly - from 20% to 15%. On the territory of the Russian Federation agents of leptospirosis of farm animals and dogs are *Leptospira* serogroup Pomona, Tarassovi, Grippotyphosa, Sejroe, Hebdomadis, Icterohaemorrhagiae, Canicola; in natural foci of *Leptospira* serogroups established circulation Grippotyphosa, Pomona, Sejroe, Javanica, Icterohaemorrhagiae, Bataviae, Australis, Autumnalis. In connection with

the foregoing, the vaccination of agricultural and domestic animals against leptospirosis will significantly reduce the incidence of this disease in humans and animals. Vaccination prophylacted disease, abortion, preclude the formation of leptospira carrier state. Mass vaccination is the most economical and effective measure to combat leptospirosis. Vaccination of livestock is not interrupted by epizootic process in the economy.



OP 2

INFECTION RATES AND ETIOLOGICAL STRUCTURE OF LEPTOSPIROSIS IN RUSSIAN FEDERATION

Soboleva G. L., Malakhov Y. A., Aliper T. I.

Vetbiochem LLC, Moscow, Russia

Leptospirosis is a naturofocal zoonosis occurring all over the globe. In Russian Federation, leptospirosis is considerably widespread among both domestic and wild animals of various species. The epizootic situation in Russia remains threatening to a great extent.



OP 3 CURRENT SITUATION OF LEPTOSPIROSIS IN SARDINIA

Ponti M. N.[1], Palmas B.[1], Noworol M.[1], Canu M.[1], Fiori E.[1], Picardeau M.[2], Carboni G. A.[1], Pintore A.[1], Ruiu A.[1], Piredda I.[1]

[1] *Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

[2] *Institut Pasteur, Unité de Biologie des Spirochètes, Paris, France*

Leptospirosis is a zoonotic infection caused by pathogenic *Leptospira* species. Domestic and wild animals would be affected, but clinical signs are not always apparent. Previous studies carried out in Sardinia showed that leptospirosis affects both humans and animals and that *Leptospira interrogans* serovar Pomona was responsible for the icterus-haemorrhagic syndrome in calves or reproductive failure in swine. With regard to leptospirosis our Institution leds investigation on the basis of clinical suspicion on request from veterinarians and hospitals (medical doctors). Regarding wild species a large number of animals were tested during regional epidemiological surveillance plans. The pourpose of this paper is to report results of the investigation carried out during the period 2006-2010.

Laboratory diagnosis is based on detection of specific antibodies by MAT, by detection of bacteria in urine and organs and by molecular assay. In our study, 13085 serum samples were submitted for a leptospirosis MAT; 740 fresh organs, blood or urine samples were used for the isolation in EMJH medium; PCR protocols were applied on 444 biological tissue or fluid. *Leptospira* isolates were identified using molecular techniques as VNTR and PFGE.

Serological positivity was found on 1273 (about 10%) sera from different animal species and human. The seroprevalence was 19% in cattle, 14% in swine, 23% in horses, 4% in dogs, 2.5% in small ruminants, 1.5% in human patient and 7% in wild animals. The predominant serogroups identified in all species were Bratislava and Pomona. In cattle the most common serovar are Pomona (58%) and Hardjo (40%), in swine Bratislava (62%), Tarassovi (33%) and Pomona, in horses Bratislava (97%) and Pomona, in dogs Bratislava (71%) and Icterohaemorrhagiae, in human Icterohaemorrhagiae (70%) and Bratislava (30%), in wild animals Pomona and Bratislava. However, small ruminants showed very low serological prevalence. Isolates was obtained from 7.5% of organs and fluids cultured from domestic and wild animals. Leptospires were identified by VNTR and PFGE as Pomona (in cattle, swine and wild boars) and Bratislava (in a dog). 35% samples were PCR positive.

Our data suggest that leptospirosis in Sardinia is present in all animal species investigated and represents a risk of human infection. Continuing surveillance and case reporting are essential in order to improve preventive measures.



OP 4

PASSIVE SURVEILLANCE OF SWINE IN GERMANY FOR INFECTION WITH LEPTOSPIRES

Strutzberg-Minder K.[1], Tschentscher A.[1], Hartmann M.[2], Beyerbach M.[2], Kreienbrock L.[2]

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Leptospirosis in swine is a notifiable disease in Germany. Nevertheless no current data are available on the prevalence of this infection in swine, which are assumed to be the reservoir for the serovars Pomona, Bratislava and Tarassovi, and severe economic losses in swine reproduction are caused by leptospirosis worldwide; moreover, the Bratislava serovar is endemic in some regions. Diagnostic examinations were carried out on samples from all over Germany and laboratory data from 2003 to 2010 have now been analysed. In all, 66852 serum samples from swine in Germany were examined by MAT according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2003 to current editions 2008, available online). Samples were tested for antibodies against subcultures of reference strains from the Leptospirosis Reference Laboratory (at KIT Biomedical Research, The Netherlands) of the serovars Australis, Bratislava, Canicola, Grippotyphosa, Copenhageni, Icterohaemorrhagiae, Pomona, Hardjo, Saxkoebing and Tarassovi. All available information about the samples was taken into consideration. Overall, 40.6% (n = 27149) of all 66852 swine serum samples tested positive by MAT. Seropositivity ranged between 23.1% (n = 2217) in 2009 (total = 9583) and 56.4% (n = 5949) in 2005 (total = 10548). While 80.1% of the positives reacted with one serovar only, less than 19.9% reacted with two or more, possibly indicating an early stage of infection. The most frequently detected serovar was Bratislava, which was found in 37.5% (n = 25094) of all samples, followed by Copenhageni, 5.7% (n = 3813), Australis, 4.2% (n = 2817), Icterohaemorrhagiae, 1.2% (n = 831), and Grippotyphosa, 1.1 (n = 702). All other serovars were detected less often (< 1.0% of all samples). Analysis was begun in 2003, when 3252 samples were tested. The number of samples increased to 5893 in 2004, peaking in 2005 (10548) and 2006 (10954). From 2006 to 2009 the number of samples tested and the

percentage of positive test results ran in parallel, with both generally decreasing over time. However, the changes in the amount of positives over time were substantially affected by the number of samples testing positive for Bratislava, suggesting that this serovar is endemic in swine in Germany, as has long been indicated by serological testing, and that swine are indeed a reservoir for Bratislava, while this is apparently not the case for Pomona and Tarassovi. In general, data show that leptospires are found in German pig herds with substantial frequency. However, as data from passive surveillance is prone to selection bias, analysis of subpopulations and further information should help improve understanding of the epidemiology of leptospires in swine in Germany.



OP 5 DETERMINATION OF SPECIFIC ANTIBODIES TO *Leptospira* spp. IN POPULATION OF STRAY DOGS IN THE REPUBLIC OF SERBIA

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Leptospirosis is a worldwide spread infectious disease of mammals caused by pathogenic bacteria of genus *Leptospira*. Natural reservoir species are mainly rodents, but in cities stray dogs have important role in maintenance of infection. Because of specific ecological niche that stray dogs occupy, they present constant source of infection for human population as well as for domestic animals. In this paper we present the results of serological examination of 521 blood sera originated from stray dogs of different cities in the Republic of Serbia. Microscopic agglutination test (MAT) was used as a gold standard method for determination of specific antibodies to 7 different *Leptospira* serovars (Icterohaemorrhagiae, Pomona, Grippotyphosa, Canicola, Bataviae, Sejroe, Australis). The results showed that 36 dog sera (6.9%) were tested positive for the presence of specific antibodies to *Leptospira* spp., and 485 dog sera (93.1%) were tested negative. Among the positive sera following serovars were registered: Icterohaemorrhagiae (6.91%), Pomona (2.68%), Canicola (0.76%), Grippotyphosa (0.19%), Bataviae (0.19%), Sejroe (0.19%). Specific antibodies for Australis serovar were not detected. Titers of antibodies were in range from 1:100 to 1:30000. In conclusion, stray dogs are important reservoir species of *Leptospira* spp. They present constant ecological problem in big cities that should be resolved according to current strategies for stray dog population control and principals of animal welfare.



OP 6 SEROLOGIC EVIDENCE OF *Leptospira* spp. SEROVARS IN CATTLE IN CROATIA

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Bovine leptospirosis occurs throughout the world, but it is most commonly in warm climates. It is economically significant disease that results in abortions, stillbirths and loss of milk production. Therefore, the implementation of surveillance and control measures is of particular importance for the suppression of this important zoonosis. According to annual orders issued by Ministry of Agriculture for years 2008, 2009 and 2010, all bulls used for natural breeding and production of semen for artificial insemination must be serologically tested for leptospirosis twice each year. In addition, all cases of abortion in cattle must be reported and blood of each animal serologically tested for leptospirosis. Also according croatian legislation, after treatment we carry out serological blood tests twice at intervals of 21 days after completion of treatment. During the year 2008, 3024 (485 positive) cattle blood samples were tested for leptospirosis, in 2009, 3150 (538 positive) blood samples and 5373 (475 positive) in 2010. All sera were tested by microscopic agglutination test (MAT) for the presence of 12 leptospiral antigens. An initial sera dilution of 1:100 was considered as a cut-off point for a positive reaction. In 2008. the highest prevalence had Osijek-Baranja 126 (26%), Sisak-Moslavina 108 (22.3%), Vukovar-Srijem 71 (14.6%) and Varaždin 48 (9.9%) counties. In 2009. the highest prevalence had Sisak-Moslavina 236 (43.9%), Zagreb 92 (17.1%), Bjelovar-Bilogora 46 (8.6%) and Pozega-Slavonia 38 (7.1%) counties. In 2010. counties with the the highest prevalence were Pozega-Slavonia 154 (32.4%), Osijek-Baranja 80 (16.8%), Bjelovar-Bilogora 75 (15.8%) and Sisak-Moslavina 72 (15.2%). Positive reactions for leptospirosis during the study period were not found in the Dubrovačko-neretvanska, Lika-Senj, Primorje-Gorski Kotar and Zadar County. The most frequent serovars in cattle during the three years were Hardjo (22.32%), Sejroe (22.32%), Saxkoebing (21.93%) and Hardjobovis (21.38%). Results indicate that the cattle in the continental part of the Croatia due to leptospirosis natural foci (Lonjsko Polje i Kopački rit) and the joint holding cattle, pigs and horses in the same area are exposed to leptospira infection more than animals from the croatian coast. In order to control the spread of disease within the same breeding and on the other breedings it is necessary to continue to control the

disease, treat breedings with the aim of reducing the carrier state, as well as to the availability, change management to reduce the possibility of re-infection.



OP 7

CATTLE INCIDENTAL INFECTIONS DUE TO *Leptospira* STRAINS CARRIED BY SMALL MAMMALS IN TRÁS-OS-MONTES E ALTO DOURO REGION, NORTHERN PORTUGAL

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Cattle are considered the main maintenance host for Hardjobovis strains (serovar Hardjo). In contrast, cattle infections caused by *Leptospira* strains not adapted to bovine are considered incidental infections. Due to the traditional management system of indigenous cattle in Trás-os-Montes e Alto Douro (TMAD) region, Northern Portugal, bovine has a close contact with small mammals species. The main objective of the present study was to know the importance of *Leptospira* cattle infections due to strains maintained by all mammals of this region.

752 sera samples from cattle of TMAD region were study by the microscopic agglutination test using has live antigens the Hardjobovis strain and local strains isolated from small mammals trapped in indigenous cattle small holdings. The seropositivity rate was 61% (45+/752) and the main reactions occurred with strains from the following serogroups: Sejroe, 26.7%; Ballum, 12.1% and Pomona, 11.8%. The anti-Sejroe reactivities were the most common, nevertheless, we should assume that this finding is not only result of endemic infection of cattle by Hardjobovis strains but also consequence of the contact with Saxkoebing strains whose presence was confirmed in this ecosystem by their isolation from *Mus musculus* in this study. The seroreactivities to Ballum were equally important indicating that cattle exposure to strains maintained by rodents is common in this region, corroborated by the fact that the majority of strains isolated from small mammals in TMAD region were Ballum strains. The preva-

lence of Pomona group seroreactions showed the significance of the occurrence of incidental infections in cattle by a new strain of this serogroup isolated for the first time. This new finding demonstrates the importance of an active surveillance of leptospirosis in Northern Portugal, especially in TMAD region, in order to identify a possible change in the pattern of this zoonotic disease.



OP 8 OBJECTIVISATION OF ANTIBIOTIC THERAPY PRACTICE FOR LEPTOSPIROSIS SEROPOSITIVE DAIRY COWS

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Diagnosis of leptospirosis in dairy cows by testing blood serum is today the most frequent test method in use, weather is it about the asymptomatic or animals with the clinical manifestation of the disease, and the therapy results are subsequently based on the results of the serotesting. Antibiotic therapy spontaneously infected cows is justified in both cases, the animals with the clinical manifestation of the disease and the asymptomatic cows, but after testing the paired sera, after which they can compare the obtained values with the MAT test. Problems due to the presence of low level persisting antibodies, the absence of high antibody titer or a slower disappearance of specific antibodies in asymptomatic seropositive cows after treatment is more difficult to interpret and to plan the repeated treatment(s). Our target population are asymptomatic dairy cows among the 14 030 we have found 145 seroreactors on one of three serovars of *L. interrogans* we have used. Of the 117 cows included in the first therapy for 36 there was no need for therapy repetition, which means that the efficiency was 30.76%. The dominance of serovar Hardjo, with relatively modest antibody titre (not over 1:400), we could not define precisely, except the adaptability of the strain to other or even changed locations in the host organism and even mild immunostimulation.



OP 9 LEPTOSPIROSIS IN SHEEP: ISOLATION OF LEPTOSPIRES FROM THE GENITAL TRACT

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Serological evidence of widespread infection by leptospires belonging to the Hardjo serovar has been reported in sheep in different part of the world and field evidence has indicated that this serovar may be involved in reproductive disease. Diagnostic investigations of ovine abortions and stillbirths in Northern Ireland have resulted in the isolation of leptospires from ovine foetal tissues (Ellis at al, 1983). The kidney has been the recognised site in infected carrier animals, however, and by analogy with bovine leptospirosis many authors expect that the genital tract could also be important as a site of leptospiral localisation. In contrast to this hypothesis, many studies have failed to demonstrate genital tract infection in experimentally and naturally infected sheep. Recent developments in isolation media have made the investigation of the prevalence of leptospiral infection in genital tract possible. It is the purpose of this report to record such a study. Eighteen clinically healthy ewes, seropositive to Hardjo infection by the microscopic agglutination test (MAT) were used in the study. No titres were recorded against antigens from other serogroups. Kidney, liver, brain, oviduct and uterine scraping samples were macerated with BSA diluent and inoculated into four variant forms of semi-solid medium enriched with rabbit serum and two forms of biphasic medium with addition of charcoal. The four formulations differ in type and antimicrobial agent concentrations. Cultures were incubated at 28-30° C, and examined weekly by dark-field microscopy to detect leptospiral growth. Leptospires were isolated from kidney of four and from uterus of two ewes. All the isolates were typed as belonging to Sejroe serogroup and have been specifically identified as serovar Hardjo type Bovis by Restriction Endonuclease Analysis of genomic DNA. This is the first isolation of leptospiral strains belonging to serovar Hardjo from female reproductive tract of adult sheep. Many authors have postulated that sheep act as maintenance hosts for serovar Hardjo: the persistent of this serovar in the genital tracts would support this view. The persistence of leptospires in the genital tract suggests that veneral transmission could play an important role in the spread of infection.

OP 10

DIAGNOSIS LEPTOSPIROSIS OF ANIMAL BY POLYMERASE CHAIN REACTION

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Timely (early) diagnosis is the basis for dealing with any infectious disease. The aim was to study the use of PCR method for the in vivo diagnosis of leptospirosis. There was a comparative evaluation of the effectiveness of the method used PCR with bacteriological and serological methods in the diagnosis of leptospirosis in the early stages of infection, as well as to identify *Leptospira* carrier. According to studies by the PMA, the antibodies in the serum of experimentally infected calves and pigs appeared on the 7-30 days after infection. Cultures from the blood of calves infected with leptospirosis were isolated on a 3-14 day, from the blood of pigs - by 4-14 per day. *Leptospira* by PCR in the blood of animals found already on day 3 after infection, and within 1 month after infection. In the study of urine from infected calves in a dark field microscope *Leptospira* have been found in three samples of 372 (0.8%) for 12 and 14 days. In the study of the same samples by PCR *Leptospira* detected in 283 samples (76.07%), ranging from 5-10 days and 6 months after infection in all animals.

In the study of the urine of pigs infected with *Leptospira* detected by microscopy in 94 samples out of 553 (17%) for 10-20 days and were detected up to 3 months after infection, PCR - in 388 samples (70.16%), starting from 4-7 days and up to 6 months after infection. *Leptospira* culture is not isolated from the urine from any animal. Also, studies were carried out on samples of vaccinated and infected animals. Antibody titers in sera were almost identical. In the study of blood and urine, *Leptospira* DNA was detected only in infected animals. Results of the study samples from vaccinated animals were negative. Our studies of samples from vaccinated and infected of animals have shown the possibility of using PCR to differentiate infected animals from the induced animals with serum post-vaccination of the body. The results presented here suggest that PCR can be used along with the classical methods of research for early diagnosis of leptospirosis of animals, identifying *Leptospira* carrier, as well as to differentiate between vaccinated and infected animals.

OP 11

HUMAN LEPTOSPIROSIS IN CROATIA

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Due to observed changes in epidemiology and clinical presentation, human leptospirosis has been recently worldwide recognized as a „reemerging" infectious disease. Since the first description of the patient with Weil's disease in Croatia in 1935, till nowdays leptospirosis has been in our country the constant object of research among infectious disease specialists, epidemiologists, immunologists, veterinarians and foresters. The aim of our retrospective cohort study was to asses the epidemiological and clinical characteristics of human leptospirosis in Croatia.

According to official data, the mean yearly incidence of human leptospirosis from 1990 to 2011 was 1.63/100 000 inhabitants (range per years: 0.27-3.42/100 000 inhabitans). Among reported cases in the observed period, 22/1614 (1.36%) patients died. In addition to the majority of sporadic cases, two minor outbreaks were recorded. The clinical burden and more detailed epidemiology of 152 patients with serologically proven leptospirosis hospitalized at the University Hospital for Infectious Diseases „Dr Fran Mihaljević" (UHID) in Zagreb in the period 1997-2011 were also studied. Clinical presentations were as expected, with an overall case fatality rate (CFR) of 0.66%. The most commonly established infective serovars were Australis followed by Saxkoebing and Grippotyphosa.

In conclusion, we consider that Croatia is one of the countries with the highest incidence of human leptospirosis in Europe. In comparison with previous periods, the mean yearly number of patients with leptospirosis hospitalized at UHID decreased, but among them a rather higher rate of patients with Weil's disease and a higher CFR was observed. According to the profile of causative serovars, inland Croatia presents a specific "archaic" natural leptospirosis focus.



OP 12

HUMAN LEPTOSPIROSIS IN RUSSIA: EPIDEMIOLOGICAL TRENDS ACROSS TWO DECADES

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Natural and man-made leptospirosis foci are known to be widely spread in Russia with human cases being recognized for decades. This paper describes current trends in the epidemiology of leptospirosis and presents the existing prevention system in this country. Retrospective epidemiological analysis of data on leptospirosis incidence within the last two decades (1991-2011). Results of field investigations in the foci of infection. Sero- and genotyping of pathogenic leptospires circulating in different geographical regions of Russia. Within the two last decades the number of reported cases of human leptospirosis has dramatically declined partly due to negative trends in volume of laboratory investigations. Implementation of interventions in endemic areas of South Russia using the newly developed polyvalent vaccine resulted in a sharp decline of morbidity. Epidemiological data obtained so far testify to ubiquitous distribution and apparent epidemic and/or epizootic manifestation of infections caused by three *Leptospira* genospecies: *L. interrogans*, *L. kirshneri* and *L. borgpeterseni*. Beginning from the early 90-ies pet dogs has become one of the most important infection sources for human leptospirosis. This resulted in a shift of etiological structure with a marked increase of serovar Canicola leptospires as agents of human leptospirosis with high CFRs in urban areas. Not infrequently serovar Copenhageni and serovar Canicola leptospires manage to breach the species barrier affecting not only the principal reservoir host (dog) but also cattle, swine and horses. The latter agent was the cause of water-borne outbreaks among humans in rural areas. Pilot studies have also documented an emerging human leptospirosis agent namely; serovar Muenchen, in the European Russia, but its epidemiological and epizootological significance needs further investigation. Notwithstanding the low reported incidence rates, leptospirosis is still an important though underestimated public health problem in many regions of Russia.

OP 13 SEROPREVALENCE AND CORRELATES OF LEPTOSPIROSIS IN METRO MANILA, PHILIPPINES

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Clinically, leptospirosis can manifest as a mild acute febrile illness or a relatively severe and life-threatening icteric disease which could involve the liver, lungs and kidneys. It is endemic in the Philippines and has been one of the notifiable diseases since 1999. However, it is grossly underreported and is not considered a priority health problem. This cross-sectional study was conducted to estimate the seroprevalence of leptospirosis in Metro Manila. The survey included 413 households randomly selected through a four-stage random sampling design from 30 villages in Metro Manila. Antibody testing was done among 1,136 household members aged 10 - 65 years using the Leptospira IgG ELISA System (Immunospec Corporation, USA). The test uses microwells coated with purified *Leptospira biflexa* (serovar Patoc 1) antigen. *L. biflexa* serovar Patoc 1 is known to cross-react with most pathogenic serovars but not with animal strains. A seropositive result was defined by an optical density reading (OD) ≥ 0.50. Data on socio-demographic and environmental factors, respectively, were obtained through a face-to-face interviewing of the mothers or through an observation check list. Multiple Logistic Regression analysis was used to identify the correlates ($p \leq 0.05$) of leptospirosis. The overall seroprevalence of the disease was 26% (95% CI: 24-29%). Seropositivity was not found to be associated with sex, education, occupation, fever episodes within the past year, and proximity of residence to a market, a garbage disposal site or an open sewer. Meanwhile, the disease odds of those aged 30 years or older (OR = 1.5; 95% CI: 1.14-1.94), those living within 10 meters from a filth-filled creek (OR=1.4; 95% CI: 1.04-1.78) and those whose trash bins inside the house are uncovered (OR=1.4; 95% CI 1.06-1.91) were significantly higher compared to the younger individuals, to those who lived farther from the creek and to those with covered trash bins, respectively. The sources of leptospirosis infection are apparently the poor environmental conditions that favor the breeding and thus, proliferation of rodents within and outside the respondent's homes. Hence, public health workers should undertake advocacy campaigns to convince the relevant government units to do regular clean-up activities particularly of

the multitude of stagnant creeks that traverse through the National Capital Region. They should also inform the public about the importance of maintaining good sanitary conditions within and outside their homes as a protective measure against leptospirosis.



OP 14 THE HANTA-HUNTING STUDY \"HANTAVIRUS INFECTION IN LEPTOSPIROSIS SUSPECTED PATIENTS IN THE NETHERLANDS.\"

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Leptospirosis and hanta virus infections cause diseases with highly similar clinical manifestations. While hanta is a viral rodent-borne zoonotic disease, leptospirosis is a zoonotic disease, often rodent borne, caused by spirochetes of the genus *Leptospira*. An increase in incidence of human Puumala virus (PUUV) is recognized in countries neighboring the Netherlands. PUUV is vectored by bank voles. Therefore, sighting/contact with non-identified rodents or rodent excreta in an anamnesis of patients with symptoms of a leptospirosis infection could be indicative for an infection with PUUV as well. Furthermore, increasing evidence shows circulation of Seoul hantavirus SEOV in rats in France and Belgium and circulation of SEOV is suspected for the Southern parts of the Netherlands (serological evidence, currently being confirmed in NT, Reusken pers. comm.). It is hypothesized that because of the overlapping clinical signs and symptoms, SEOV infections are confused for leptospirosis with a certain unknown incidence. To gain insight in the potentially under diagnosis of hantavirus infections in leptospirosis negative patients, we proposed to screen leptospirosis negative sera from dutch non-travelers for the presence of hantavirus antibodies. The Royal Tropical Institute (KIT) harbors the National and International Leptospirosis Reference Centre (NRL) which performs the laboratory diagnosis of leptospirosis. The KIT has a big collection of leptospirosis negative sera (defined by negative MAT) from their NRL activities. Sera will be screened using IgG and IgM ELISA (DxSelect Focus diagnostics). This is an ELISA test for a broad range of hantaviruses. Selection criteria sera: 1) patients coming back ill from travel are excluded 2) seasonality of hantavirus infections: include period april-november 3) Sera will be selected from the following time periods: 2005 (hantavirus outbreak in Germany) 2007, 2008 (increase hantavirus cases in Europe) 2010 (hantavirus increase Germany in neighboring regions with the Netherlands). The results obtained with the generic hantavirus ELISA tests need to be validated/confirmed. Therefore sera tested positive in the hantavirus ELISA need to be further analyzed.

The reactive sera and a selection of negative sera will be further analyzed using the specific SEOV IFA (optional also specific SEOV ELISA) techniques available at the RIVM to study the circulation of Seoul virus in the Netherlands. The same will hold through for the screened samples for PUUV. Samples confirmed at the RIVM will be confirmed by virus neutralization at a third party lab. This is a collaborative project between KIT BR, RIVM and Erasmus University Virology department which will start in January 2012 with an estimated end point around april 2012 just in time to be presented at the Eurolepto meeting in Dubrovnik, Croatia.



OP 15 AN OVERVIEW OF HAEMOSTASIS IN SEVERE LEPTOSPIROSIS: FROM PLATELET TO CLOT

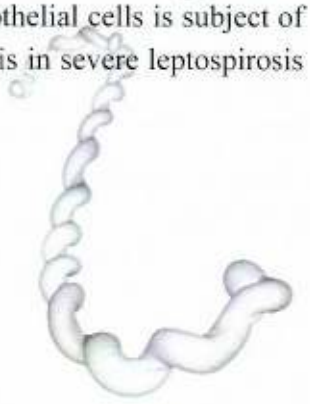
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Sepsis with multi-organ failure and haemorrhaging are the biggest threats to patients suffering from severe leptospirosis. Theoretically, bleeding can be the result of an impaired primary haemostasis, secondary haemostasis, endothelial damage or a combination. Indeed, several studies show a significant association between thrombocytopenia and bleeding. During sepsis the blood coagulation system is triggered due to the pro-inflammatory environment and endothelial cell damage. When insufficiently controlled, this can lead to the syndrome of disseminated intravascular coagulation (DIC), with bleeding and microvascular thrombosis as the clinical hallmarks. Recent work showed that overt DIC as defined by the International Society on Thrombosis and Haemostasis is an important feature in leptospirosis patients. On protein level this consumptive coagulopathy is illustrated by elevated thrombin-antithrombin complexes (TAT), prothrombin fragments 1+2 and d-dimer levels. Mechanisms that counteract coagulation activation are diminished with low antithrombin and protein C levels. Markers of endothelial cell activation such as von Willebrand factor and soluble E-selectin are strongly elevated in leptospirosis patients. If and how pathogenic *Leptospira* damage endothelial cells is subject of debate. This presentation will give a comprehensive overview of haemostasis in severe leptospirosis from platelet to clot.



OP 16

LEPTOSPIROSIS AND FASCIOLIASIS: IMMUNOLOGICAL INTERACTIONS IN THE CLINICAL POLYMORPHISM OF PATIENTS FROM SÃO MIGUEL (AZORES, PORTUGAL)

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The Archipelago of the Azores presents nine volcanic islands, and marks the western end of Europe, being São Miguel the largest and the most populated island. In this Portuguese insular region, leptospirosis is an endemic zoonotic disease, which affects humans accidentally due to a direct or indirect contact with pathogenic leptospires. Clinically, this pathology ranges from influenza-like to fulminant disease depending on different factors, although laboratory confirmation is important for accurate diagnosis. Fascioliasis is another important zoonotic disease in Azores that has been described as endemic. This food-borne disease, in Portugal including the Azores Archipelago, is caused by the liver flukes *Fasciola hepatica* and has been recognized globally as an emerging/re-emerging disease. Human infection can occur due to consumption of undercooked vegetables and/or contaminated water. The diagnosis is not easy since *F. hepatica* infestation can present a wide variety of clinical syndromes. The aim for this study is to understand a possible interaction in the humoral immune response between *Leptospira interrogans* sensu lato (s.l.) and *Fasciola hepatica* infection, in patients from São Miguel Island, presenting symptoms and epidemiological data common to both pathologies. The samples used in this study were 280 sera from São Miguel patients which were previously analysed by MAT (Microscopic Agglutination Technique) for detection for *Leptospira interrogans* s.l. agglutinins. ELISA technique was applied in the same samples for the study of fascioliasis infection, as a screening appro-

ach. The results from MAT for the 280 serum samples were the following: 124 (44%) patients were negative, 73 (26%) inconclusive and 83 (30%) positive. These sera have been submitted to ELISA and its analysis through comparison with three standard deviations, regarding the endemism of fascioliasis in Azores, showed 88 (31.4%) positive samples, 88 (31.4%) doubtful and 104 (37.2%) negative. Concerning the relationship between fascioliasis and leptospirosis, the results revealed 38 co-infected sera, 21 positive sera for fascioliasis and not conclusive for leptospirosis, 27 positive sera for leptospirosis and doubtful for fascioliasis and in 18 serum samples were observed antibodies against *L. interrogans* s.l. and *F. hepatica*. These results are promising since it appears there is a possible relationship between leptospirosis and fascioliasis infection; however, it's necessary to confirm fascioliasis results by the reference technique. Further, clinical symptoms common to both diseases will be analysed, in order to obtain a more consistent analysis for this possible association.



OP 17 ESTABLISHMENT OF VALID LABORATORY CASE DEFINITION FOR HUMAN LEPTOSPIROSIS

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Laboratory case definition of leptospirosis is scarcely defined by a solid evaluation that determines cut-off values in the tests that are applied. This study describes the process of determining optimal cut-off titers of laboratory tests for leptospirosis for a valid case definition of leptospirosis.

In this case the tests are the microscopic agglutination test (MAT) and an in-house IgM enzyme-linked immunosorbent assay (ELISA) both on single serum and paired samples using a positive culture as the reference test in the Dutch population. The specificity was assessed using panels of sera from healthy donors, cases with known other diseases and non-leptospirosis cases with symptoms compatible with leptospirosis. Cases were divided into three periods corroborating the acute phase (1-10 days post onset of illness (DPO)), the early convalescent (11-20 DPO) and the late convalescent phase (>20 DPO).

Cut-off titers for MAT and IgM ELISA were determined as 1:160 and 1:80 respectively for all three periods. These cut-off titers combined 100% specificity with a sensitivity that changed according to the stage of disease for both tests. The low sensitivities in the early acute phase are consistent with the dynamics of the humoral immune response. IgM ELISA yielded higher sensitivities compared to MAT in the acute and early convalescent stages. Moreover, the optimal sensitivity of MAT, the gold standard was < 82%, implying that a significant part of global cases is missed by this recommended test. MAT and IgM ELISA manifested partly complementary, resulting in a higher sensitivity when combining the results of these two tests. The availability of paired samples and of adequate clinical and epidemiological data are other parameters that will significantly increase the sensitivity of laboratory confir-

mation. This study enables fine-tuning of the current laboratory definition towards an improved case finding and implies that solid validation of laboratory parameters for case definition will improve both the diagnosis for individual patient care and for estimating the disease burden at a worldwide scale.



OP 18 LEPTORAPIDE: A ONE-STEP ASSAY FOR RAPID DIAGNOSIS OF HUMAN LEPTOSPIROSIS

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Leptospirosis outbreaks due to an increase in travel and recreational activities have been reported in developed and more industrial areas of the world. A lack of awareness and knowledge of the disease also delays diagnosis, increasing the severity of symptoms for the patients. The current gold-standard for diagnosis is the Microscopic Agglutination Test (MAT). However, MAT is only performed in Reference Laboratories, is time-consuming, costly and only considers certain specified serovars. Serological tests have been described but lack sensitivity and/or specificity, are time-consuming, expensive and require sophisticated equipment and skilled staff. Linnodee Ltd has developed a simple one-step antibody test for specific and accurate diagnosis of human leptospirosis. Leptorapide is a latex agglutination assay performed using 5µl of serum sample and 5µl of antigen-bound latex beads. Evidence of spirochete infection can be assayed accurately within minutes; by agglutination scored on a scale of 1 - 5 and results interpreted using a score card provided in the kit. The assay is cost-effective, requires minimal laboratory equipment and unskilled personnel, offering a practical alternative to MAT and ELISA in regions where medical support is limited. This is particularly important when considering a possible epidemic or throughput of large numbers of samples. Sera - 55 human sera samples were collected from the Leptospira Reference Unit, Hereford, UK. 52 human sera samples were collected from K.I.T Biomedical Research Institute, Netherlands. Sera (n=168) from patients with clinical presentation suggesting leptospirosis in Southern Brazil, was used to test Leptorapide in a population where the disease is endemic. IgM ELISA - The Pan Bio IgM ELISA was used as per kit instructions. A titre of 1:40 or greater was considered positive for the presence of leptospiral antibody. The MAT was performed using a series of 19 (Hereford) or 53 (Brazil) antigens. Linnodee Leptorapide - 5ml of test sera / positive control sera was mixed with 5ml of the antigen-bound latex beads. The mix was left to dry and agglutination scored on a scale of 1-5; 1 = negative, 5 = strong positive. The test has shown sensitivity and specificity values of 90.91% and 95.24% respectively, as compared to MAT and IgM ELISA. Leptorapide has been validated with a large sample size obtained from individuals originating from various

parts of the world including Brazil and India. It is currently exported to several distributors worldwide and used routinely in endemic countries such as India, Malaysia, Taiwan and Philippines and offers a cost-effective, quick and accurate alternative to the more historical methods of detection.



OP 19

SIGNIFICANCE OF PCR IN MONITORING OF LEPTOSPIROSIS

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Gold standard of leptospirosis laboratory investigation is MAT. Its main drawback is the retrospectivity. Development of molecular genetic techniques, the most accessible of which is PCR, has opened up new possibilities for monitoring of leptospirosis. Due to certain difficulties in *Leptospira* isolation, specific DNA detection is also considered as a direct evidence of infection (Leptospirosis: Laboratory Manual. WHO, 2007).

Four hundred fifty one sera from humans and samples from 368 small mammals obtained in six administrative areas of Siberia and the Far East were investigated. Laboratory studies were carried out by MAT and PCR. PCR was performed in two ways. Pathogenic *Leptospira* 16S-r-RNA and its encoding gene locus were detected simultaneously by real time PCR with „AmpliSens Leptospira-FL" (Public Entity Central Research Institute of Epidemiology, Moscow) on cycler Rotor-Gene 6000 (Corbet Research, Australia). Region of lipL32 gene was amplified by nested PCR (S.D.D. Jouglard et al., 2006). Total preparation of DNA/RNA was obtained by „RIBO-sorb" set (Moscow).

Diagnosis in humans with suspected leptospirosis was confirmed to 1.77 times higher by MAT than PCR. This fact can be explained by late medical care when the bacteremia stage was over and antibody production was started. On the contrary, the efficiency of PCR was greatly exceeded MAT in human risk groups: in workers at stock farms in 2.18 times, in contact with sick dogs in 6.67 times. The main difference between these two groups includes the fact that farm worker contacts with the pathogen have hidden and permanent nature, but dog owners are examined during the initial contact at the acute phase of animal disease. The role of PCR and MAT was similar in active identification of patients at the leptospirosis effective disease area. No positive results were observed in the control population group. Specific DNA/RNA was detected in 13.2 % blood swabs from the thoracic cavity of small mammals, whereas antibodies only in 0.47 % of the samples. Both PCR variants were demonstrated the same results during studying human and animal samples.

The efficiency of two PCR variants (real time and nested) for leptospirosis monitoring was demon-

strated. PCR is a beneficial supplemental instrument for obtaining of epidemiological data to confirm human infection with *Leptospira* and the pathogen circulation among small mammal populations in natural foci.



OP 20 EVALUATION OF REAL-TIME PCR AND CULTURING FOR THE DETECTION OF LEPTOSPIRES IN CANINE SAMPLES

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Molecular detection of leptospires in clinical samples requires development of a highly sensitive tool to confirm clinically suspected cases at an early stage of the disease. Currently, the polymerase chain reaction either conventional or real-time PCR amplifying leptospiral DNA in clinical materials is the most widely used molecular approach for the early diagnosis of leptospirosis. However, several PCR tests for the specific detection of leptospiral DNA from body fluids and tissues have been described, validated real-time PCRs detecting leptospires for veterinary purposes are not readily available. This study describes the prospective evaluation of *secY*-based SYBR Green real-time PCR on blood samples from healthy and unvaccinated experimentally infected dogs, using culture as the reference test to define infection. Furthermore both PCR and culture were applied on blood and urine samples from same dogs to investigate infection dynamics. Compared to culturing, the assay had a diagnostic sensitivity and specificity of 91.7% and 90.0%, respectively. Culturing for part is complementary to PCR and preferably both should be applied for diagnosis and vaccine challenge experiments. In a subsequent prospective study on the dynamics of experimental infections with serovar Canicola and Copenhageni and serovar complex Bananal-Liangguang in young and adult dogs, the PCR was applied on blood samples in conjunction with culturing on blood, urine and kidney samples with the following results: (i) Leptospires persisted longer in the blood of young dogs than of adult ones. (ii) Numbers of viable leptospires in the blood are rapidly reduced but DNA remains occasionally detectable up to 7 days post infection (DPI). (iii) Appearance of viable leptospires in the urine follows a biphasic dynamics. (iv) EDTA hampers effective culturing from blood samples. (v) Serovar Canicola persists longer in the blood of dogs than Copenhageni and Bananal-Liangguang. Together with a markedly higher recovery rate from kidney samples (71% compared to respectively 33% and 0% in young dogs) this

probably reflects the adaptation power of Canicola to its canine maintenance host. Appearance of leptospires in the urine samples indicates that experimental infections have been successful. PCR presents a valuable adjunct to the diagnosis of veterinary leptospirosis and the follow-up in vaccine protection experiments.



OP 21 DESIGN OF TAQMAN-BASED PCR ASSAYS TO DISTINGUISH PATHOGENIC *Leptospira* GENOMOSPECIES

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Leptospirosis is a growing public and veterinary health concern. This zoonotic disease is caused by pathogenic species of *Leptospira*. Serological approaches are commonly used for diagnosing leptospirosis and the isolation of *Leptospira* from animals showing clinical signs suggestive of acute leptospirosis constitutes a definitive diagnosis. The detection of leptospires in kidney or urine samples is also necessary to identify animal carriers. However, culture-based procedures for detecting leptospires are technically demanding and highly susceptible to failure. The aim of this study was to develop improved real-time PCR-based assays able to both detect and discriminate the most relevant pathogenic genomospecies of *Leptospira* in animal biological samples. Improved sets of species-specific TaqMan probes, and respective flanking primers, were designed 'in silico' with complementary target sequences in 16S rRNA, ompl1, *rpoB* and *secY* genes. A control PCR targeting the beta-actin gene was also developed and tested with animal tissues. An algorithm was developed allowing the detection and discrimination of pathogenic leptospires in biological samples. In a first step, a duplex including a β-actin-targeted and a pathogenic *Leptospira* catch-all probe was used for the initial screening of samples. In a second step, species were discriminated using multiplex-based reactions including the species-specific probes. Genomic DNA was extracted from *Leptospira* reference and local strains and other bacteria grown in cultures using standard methods. Multiplex real-time PCR assays were optimized for critical variables, such as template DNA and primer/probe concentrations, in order to maximize its specificity and analytical sensitivity. The developed assays have shown to be 100% specific for the most relevant pathogenic genetic species of *Leptospira*, *L. interrogans* sensu stricto, *L. borgpetersenii*,

L. kirschneri, *L. noguchii* and *L. santarosai*. The analytical sensitivity of assays ranged from one to five genomic target copies in the reaction mixture. Pathogenic leptospires were successfully detected in tissue homogenates of infected *Mus* spp., *Rattus* spp., *Dolichotis patagonum*, *Sus domesticus* and other animals, corroborating the culture-based procedures and the isolation results. However, efforts are further needed towards the optimization of methods for the extraction of leptospiral DNA from those biological matrices.



OP 22

STUDY REGARDING THE ANALYTICAL SPECIFICITY OF THE MICROSCOPIC AGGLUTINATION TEST USED IN THE LABORATORY DIAGNOSIS OF LEPTOSPIROSIS

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The aim of this study was the analytical specificity evaluation of the microscopic agglutination test (MAT) in the leptospirosis diagnosis. The standard test was performed with live antigens represented by *Leptospira* spp. (11 serovars) and the specific anti-sera other than *Leptospira*, like: *Brucella, Coxiella, Chlamydia, Francisella, Burkholderia, Toxoplasma, Trypanosoma, Mycoplasma* and *Salmonella*. Also we performed a study regarding the analytical specificity of other serological tests used in the serodiagnosis of some bacterial diseases (Rapid Agglutination Test, Slow Agglutination Test, Complement Fixation Test, cELISA), respective identification of false-positive results due to unspecific immune complexes formed between *Leptospira* antibody and commercial antigens for brucellosis, tularemia, glanders, toxoplasmosis, dourine, avian mycolpasmosis and salmonellosis. Consequently analytical specificity studies of MAT we identified false-positive reactions at the *Brucella suis* antibody that reacted with *L. interrogans* serovar Pomona at 1/1600 titre and with *L. interrogans* serovar Australis and *L. borgpetersenii* serovar Ballum at 1/100 titre, and at the *Brucella ovis* antibody that reacted with *L. interrogans* serovar Wolffi and *L. interrogans* serovar Hardjo type Prajitno at 1/400 titre and *L. borgpetersenii* serovar Sejroe at 1/100 titre. After the evaluation of the analytical specificity of CFT applied in Ovine epididymitis and Rapid agglutination test in Tularemia were observed the false positive reactions between *Brucella ovis* antigen and *L. interrogans* serovar Hardjo type Prajitno , *L. interrogans* serovar Wolffi and *L. borgpetersenii* serovar Sejroe antibody and between *Francisella tularensis* antigen and *L. interrogans* serovar Wolffi antibody. Cross-reactions were obtained for antibody from all positive sera which were tested, respective secondary reference sera and positive sera obtained after experimental and natural infections.

OP 23

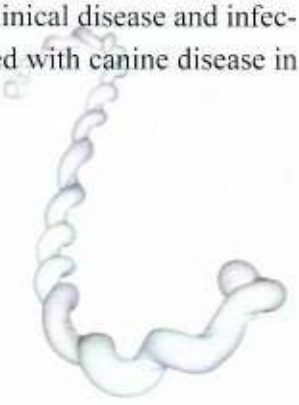
A NEW CANINE LEPTOSPIROSIS VACCINE PREVENTS BOTH DISEASE AND INFECTION FOLLOWING CHALLENGE WITH STRAINS FROM FOUR SEROGROUPS

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This report describes the results of four challenge studies which demonstrate the ability of a new, tetravalent leptospirosis vaccine to prevent both disease and infection of dogs following challenge with strains from the four main European serogroups. For each study two groups of conventional beagle dogs, free of serum antibodies against the four serogroups, were used. Two days prior to the date of first vaccination both groups received intravenous serum containing antibody against the four vaccine antigens in order to mimic the presence of maternal antibody. One group was subcutaneously vaccinated with the tetravalent Leptospirosis vaccine at the age of 6 and 10 weeks. The second group (control group) was left unvaccinated. All dogs were challenged at 13 weeks of age. For four weeks following challenge, the dogs were evaluated for signs of clinical disease, the presence of thrombocytopenia, and the presence of challenge organisms in blood or urine. At the end of the study the kidneys were examined histopathologically for any evidence of interstitial nephritis and were cultured for evidence of renal infection. The results demonstrate that vaccination of passive antibody positive pups with a new tetravalent Leptospirosis vaccine is effective at preventing the signs of clinical disease and infection following challenge with strains from the four main serogroups associated with canine disease in Europe.



OP 24 MULTIPLE BACTERIAL INFECTIONS IN CROATIAN RODENTS

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Rodent borne zoonoses represent a serious public health problem worldwide. Croatia is an important natural focus for many rodent-borne zoonoses because of diverse forest ecology and different species of small rodents. 242 rodents including bank vole (*Myodes glareolus*), yellow-necked mouse (*Apodemus flavicollis*), striped field mouse (*Apodemus agrarius*), wood mouse (*Apodemus sylvaticus*), common shrew (*Sorex araneus*), common vole (*Microtus arvalis*) and field vole (*Microtus agrestis*) were trapped in eight different parts of the continental Croatia. All animals were tested for the presence of *Leptospira* strains with renoculture method. Infections with *Borrelia, Rickettsia, Bartonella, Babesia, Ehrlichia, Borrelia RF, Anaplasma, Francisella* and *Coxiella* were determined by MassTag multiplex polymerase chain reaction assay. All positive samples were sequenced after MassTag PCR for additional confirmation. 52 rodents (21.5 %) were infected with *Leptospira*, 2 rodents (0.8 %) with *Borrelia*, 29 rodents (12 %) with *Bartonella*, 69 rodents (28.5 %) with *Babesia*, 2 rodents (0.8 %) with *Ehrlichia*, 13 rodents (5.4 %) with *Borrelia* RF, 4 rodents (1.7 %) with *Anaplasma* and 2 rodents (0.8 %) with *Francisella*. No infections with *Rickettsia* and *Coxiella* were detected and 108 rodents (44.6 %) were uninfected. Different combinations of dual infections were detected: *Leptospira* and *Borrelia* RF in 4 rodents (1.7 %), *Leptospira* and *Ehrlichia* in 1 rodent (0.4 %), *Leptospira* and *Babesia* in 8 rodents (3,3%), *Leptospira* and *Bartonella* in 2 rodents (0.8 %), *Leptospira* and *Anaplasma* in 1 rodent (0.4 %), *Babesia* and *Borrelia* RF in 2 rodents (0.8 %), *Babesia* and *Bartonella* in 9 rodents (3,7 %), *Babesia* and *Anaplasma* in 1 rodent (0.4 %), *Babesia* and *Borrelia* in 2 rodents (0.8 %), and *Bartonella* and *Anaplasma* in 1 rodent (0.4 %). Multiple coinfections with three different pathogens were detected

in 4 rodents: in 1 rodent (0.4 %) with *Leptospira*, *Borrelia* RF and *Babesia*, in 1 rodent (0.4 %) with *Leptospira*, *Borrelia* RF and *Bartonella*, in 1 rodent (0.4 %) with *Leptospira*, *Bartonella* and *Babesia* and in 1 rodent (0.4 %) with *Leptospira*, *Bartonella* and *Francisella*. This data show that infection prevalence with different bacteria that cause zoonoses in Croatian rodents is significant. We confirmed here our previous findings about the presence of multiple infections with zoonotic agents in rodents which presents an important potential for the transmission of multiple infections to humans as well.



OP 25

STUDY ON THE *Leptospira* PREVALENCE IN RATS IN MAJOR GERMAN CITIES

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Amongst wildlife species, wild and commensal rodents are considered the most important reservoirs for *Leptospira* in both rural and urban environments. The network „Rodent-borne pathogens" was established in Germany allowing synergistic investigations of the rodent population dynamics and the prevalence of rodent-associated pathogens in order to gain a better understanding of their impact on the frequency of human infections. In the current study, kidney samples of 276 Norway rats (*Rattus norvegicus*) caught in 16 different urban regions throughout Germany were examined for *Leptospira*. The majority of the samples originated from major cities such as Berlin (55 %) and Stuttgart (22 %). The samples were analysed by both a lipl32 PCR and the WHO recommended duplex PCR. In total 53 samples (19%) were positive for *Leptospira* DNA revealing the importance of *Rattus norvegicus* as a reservoir for pathogenic leptospires. Of the positive samples detected by the lipl32 PCR, 48 samples (91%) could be detected by duplex PCR confirming the lower sensitivity of the latter PCR system. Interestingly only one *Leptospira* positive sample from the commensal rats was identified as the genomospecies *L. kirschneri*. This is in contrast to previous studies in wild rodents such as *Microtus*, *Myodes* and *Apodemus* species, where the majority of samples were identified as *L. kirschneri*.



OP 26 FIRST RECORD OF *Leptospira* ISOLATION FROM WILD BOARS OF SARDINIA

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Epidemiology of leptospirosis in domestic animals in Sardinia has been investigated for a long time, but the role of wild species is not yet well known. Previous serological surveys on leptospirosis in Sardinian wild boar (*Sus scrofa meridionalis*) reported overall seroprevalences of 10%. During the last hunting seasons has been possible to perfom direct assays, collecting biological samples (kidney and urine) besides serological tests. Here we report data from a study carried out during 2009-2011 hunting seasons in North Sardinia. Total of 233 sera were screened against 8 *Leptospira* reference strains using the microscopic agglutination test (MAT), 419 renal homogenate and 73 urine samples were cultured for the isolation of *Leptospira* in EMJH medium; all kidneys and 7 urine samples were submitted by PCR protocols. Leptospires isolates were identified and submitted for typing by Variable Number Tandem Repeats (VNTR) using the loci VNTR-4, VNTR-7, VNTR-10, VNTR-Lb4 e VNTR-Lb5 and by pulsed-field gel electrophoresis (PGFE).

Serological positivity was found in 67 of the 233 serum examined (cut off value; 1:100), Pomona and Grippotyphosa, followed by Bratislava and Hardjo were the most represented sterovar. 244 samples (242 renal homogenates and 2 urine specimens) were PCR positive for species-specific genes of rRNA 16S. 33 strains from kidneys and 1 from urine submitted by culture has been isolated. Leptospires were identified and typified as *L. interrogans* serovar Pomona.

Considering the large presence of wild boards in the region and that a large number of cattle and swine herds is free ranging, data suggest that sanitary and epidemiogical health management would be improved. If also considered that during the hunting season in Sardinia usually is estimated a number of 50000 wild boars slaughtered and the meat is generally processed for human consumption not always in accordance with the rules of hygiene, we recommend adopting the required standards of hygiene and also suggest health authorities to submit official health inspection for these meats, such as required in case of domestic pigs slaughtered for human food.

OP 27

PREVALENCE OF LEPTOSPIRAL ANTIBODIES IN WILD UNGULATES FROM CONTINENTAL PART OF CROATIA

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In continental part of Croatia, over the period of seven (2002-2009) years, serum samples of 485 wild ungulates were collected and analyzed for the presence of leptospiral antibodies. Using Microscopic Agglutination Test (MAT) we searched for specific antibodies to twelve (12) different *Leptospira* spp serovars. At different sera dilution (ranging from 100 to 3200) 121 samples (24.9%) were found positive to at least one pathogenic serovar, and antibodies to nine *Leptospira* spp. serovars (Australis, Pomona, Ballum, Tarassovi, Sejroe, Grippotyphosa, Icterohaemorrhagiae, Saxkoebing, and Bataviae) were detected. In total we recorded 157 positive reactions in which the majority (N=109; 69.4%) of them were found positive at basic dilution (BD = 100), whereas 30.6% (N=48) reactions were detected positive at higher dilutions. Most frequently found serovar was Australis (33.3%), followed by serovars Pomona (21.8%) and Ballum (19.3%), while serovars Australis and Pomona recorded the highest AB titer (3200) and showed statistical significant frequency in cross-reactions (recorded in 21.8% cases). According to ungulate species, in red deer (*Cervus elaphus*) we found antibodies against six different leptospiral serovars in 24 out of 126 analyzed sera (19.04 %). The most frequently recorded antibodies were for serovars Pomona (22.3%) and Ballum (19.6%), while the highest titer was recorded for serovar Sejroe (1:800). In analyzed roe deer (*Capreolus capreolus*) 41 samples low level of leptospiral antibodies (6.07 %) was determined, with just antibodies for two serovars - Australis and Sejroe. In wild boar (*Sus scrofa*), leptospiral antibodies were detected in 101 of 286 analyzed samples (35.31 %), with antibodies for nine antigen serovars. The most frequently found serovars were Australis (48.7 %) and Pomona (22.7 %) and these serovars also recorded highest titer (1:3200). According to aerial distribution the highest percentage of positive wild ungulates samples originated from lowland habitats in regions of Posavina (Novska - 46.8%, Kutina - 45.3%) and Slavonija and Baranja (Tikveš - 43.6%).

OP 28 SURVEY ON LEPTOSPIROSIS IN WILD BOARS (*Sus scrofa*) IN LOMBARDY, NORTHERN ITALY

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Wild boar (*Sus scrofa*) is known as animal host for *Leptospira* spp.. Pomona and Bratislava are the most frequently detected serovars in Europe. The increase of population density of wild boars and the consequent migration to agricultural and urban areas have been noticed in many European countries and also in Italy, particularly in Tuscany and Sardinia regions. For this reason, wild boar is considered as a potential source of leptospirosis for humans and domestic animals. The aim of this study was to investigate the prevalence of *Leptospira* spp. in wild boar population in Lombardy, Northern Italy, and update national epidemiological data on leptospirosis in wildlife. Samples were collected from wild boar (*Sus scrofa*) during wildlife sanitary monitoring programs in Lombardy region, from 2003 to 2007. Serum samples (n=194) were tested by microscopic agglutination test (MAT) and examined for antibodies against a reference panel of 8 *L. interrogans* serovars (Bratislava, Ballum, Canicola, Grippotyphosa, Copenhageni, Pomona, Hardjo, Tarassovi); a *hap1* (hemolysis-associated protein-1) gene PCR-based commercial assay (Adiavet®Lepto, Adiagene) was applied for the detection of pathogenic leptospires in 298 tissues samples, especially kidney and urine; isolation of leptospires in EMJH semisolid selective medium was also carried out in 134 tissues samples. Sixty-four (33%) out of 194 sera examined scored positive for anti-*L. interrogans* antibodies. Antibody titres at MAT cut-off of 1:100 were detected against serovars Bratislava (24.2%), Pomona (4.6%), Grippotyphosa (2.1%), Copenhageni (0.5%). High antibody titers (≥1:400) against Bratislava were detected in 19 sera (9.8%). Leptospiral hap1 gene was detected in 15 (5%) tissues samples: 11 kidneys (3.7%), 3 urine (1%) and 1 limph-node (0.3%). Any *Leptospira* spp. strain was isolated, although glomerulonephritis, interstitial nephritis and other macroscopic lesions were observed. The results showed that most seropositivity to *L. interrogans* was due to serovar Bratislava and Pomona, whereas positivity to serovars Grippotyphosa and Copenhageni was found in low percentage. Antibody titers against Pomona reflected the circulation of this serovar in intensive swine herds, while those against Bratislava indicates that this serovar is

emerging among domestic and wild animals. The lack of *Leptospira* spp. strains isolation and the low percentage of leptospiral DNA detection could be explained by the low concentration of leptospires carried in kidney but also by the storage of samples related to the sampling conditions (hunted animals) and the long delivery times to Laboratory. Leptospirosis is a re-emerging global disease, frequently underestimate. For this reason, *Leptospira* spp. infection in wild boars should be monitored in order to confirm the role of this wild animal species in the epidemiology of leptospiral disease.



OP 29 IMMUNE REACTIONS TO LEPTOSPIRES AND HANTAVIRUSES: DIFFERENCES AND SIMILARITIES

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Leptospires and hantaviruses cause two zoonoses: leptospirosis and hemorrhagic fever with renal syndrome (HFRS) respectively, which are distributed worldwide. Both diseases may have very similar clinical picture and it is not easy always to differentiate them. Dual infections with both pathogens are recorded as well. There is significant lack of data on immune reactions to both pathogens. We will present here results of several our studies in which we analyzed and compared different immune parameters in both diseases. Sera and blood samples were collected from Croatian patients with leptospirosis and/or HFRS. The sera samples were tested on several different cytokines/chemokines, growth factors and apoptosis factors using enzyme linked immunosorbent assay (ELISA). The immunophenotypic changes in the main lymphocyte populations and activation markers were analyzed by use of whole blood two- and three-colour flow cytometry. In comparison to controls, significantly higher production of CXCL-8, CCL-2, tumour growth factor beta 2 and soluble FAS was found in patients with leptospirosis or HFRS, with no significant differences in the levels of measured parameters between the patients. Higher, but not significant levels of interleukine (IL)-10 levels were found in patients with HFRS or leptospirosis. Looking at the sera levels of several different cytokines/chemokines in two patients with dual infection (leptospirosis and HFRS), we may expect higher cytokine/chemokine response than in patients with single infection. Platelet-derived growth factor (PDGF) was decreased in the early, acute phase of HFRS or leptospirosis, while at the same time thrombospondin-2 (TSP-2) increased. The levels of Tie-2 were significantly decreased in the acute and convalescent sera of leptospirosis patients. In HFRS patients, angiopoietin-1 was elevated but not significantly. All patients showed elevated vascular endothelial growth factor levels. Interestingly, within T- lymphocytes population, we found significantly increased percentage of total activated T-lymphocytes (TCR+HLA-DR+) and helper T-lymphocytes expressing early activation marker (CD4+CD25+) in patients with HFRS in

comparison to patients with leptospirosis or healthy controls. In contrary, patients with leptospirosis had significantly increased percentage of CD4+ lymphocytes simultaneously expressing both CD45RA and CD45RO markers when compared with healthy controls. It is important to analyze and compare different immune parameters in patients with leptospirosis and/or HFRS in order to better understand their immunopathogenesis and determine parameters which may help in their differentiation.



OP 30

CLIMATE CHANGE, FLOODING AND INCREASE OF LEPTOSPIROSIS IN ALBANIA

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Leptospirosis is one of the most common zoonosis in Albania. The average incidence within 2005 – 2011 has been 2.8 cases /100.000. Climate change with heavy rainfall, flooding and extreme weather conditions have been discussed as factors to increase disease incidence and magnitude of leptospirosis outbreaks. During the last two years Albania has experienced severe flooding in different areas especially in the city of Shkodra and surrounding communes, bordering Montenegro. We have been analyzing the impact climate factors such as temperature and rainfall and flooding on leptospirosis occurrence in Albania. The temperature and rain fall data have been analyzed for the last 10 years as well as the data showing the change of habitat and reservoirs combined with leptospirosis data in humans and animals. All data were compared related to the data from Shkodra and surrounding communes. Data are showing an increase of temperatures and rainfall and especially a change in distribution of rainfall during hte last two years. Also the data are showing that leptospirosis is spread in 47% territory of the country with increase of incidence during the last two years. The most affected areas are the flooded areas of Shkoder, Fier and Lezha and also Berat and Tirana with single cases in other areas. The level of incidence has been increased from 0.1 cases/10 000 in 2005 toward 1.8 -2.5 cases/ 10 000 in 2010 and 2011. Most of the cases are concentrated during May - October always during and after the flooding and with high rainfall. There is evidence that the highest incidence is in the surorunding communes of Shkodra that were more affected by flooding. A linear increasing trend of specific burden of leptospirosis in Shkodra district compared with other districts and the total number of cases country wide seems statistically significant for years 2005-2011. ($\chi^2_{\text{linear trend}}=7.9$, $p<0.01$).The main common identified rodents are *Apodemus flavicollis, Apodemus sylvaticus* and *Rattus norvegicus*. *Leptospira* spp. have been identified through molecular techniques in both *Apodemus flavicollis* and *Rattus nor-*

Poster presentations



PP 1

MOLECULAR TYPING OF PATHOGENIC *Leptospira* STRAINS ISOLATED FROM CANINES IN BUENOS AIRES, ARGENTINA

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This worldwide zoonose is endemic in many countries, with a total of 450 cases diagnosed in humans in Argentina in the year 2011. Over the last decades many new reservoirs of pathogenic leptospires have been determined including farm animals, domestic animals and wildlife. Because of the high genetic plasticity present in this spirochete, the molecular typification of the circulating pathogenic strains in the country is relevant for the determination of surveillance, prevention and control strategies according to the actual epidemiological scenario. This study has the objective to molecularly genotype the pathogenic *Leptospira* strains obtained from clinical cases of canines in Buenos Aires by the Multiple-Locus Variable-number tandem repeats Analysis (MLVA). This technique was used with 1 set of oligonucleotides specific for the pathogenic *Leptopsira interrogans* sensu stricto. The following loci were used to discriminate the strains, VNTRS: 4, 7, 9, 10, 19, 23 and 31. Six isolated strains from canines were genotyped by MLVA obtaining the following results: strains Dogy I and Mayo II were identical to the genotype of Canicola Hond Utrecht IV. The strains Bel III and Sarmiento IV were identical to the genotype of Canicola Portlandvere. The strain Avellaneda V was identical to the genotype of Icterohaemorraghiae RGA and the strain SB VI was similar to the genotype of Pomona Pomona. These results show the wide range of genotypes present in the isolated strains of Buenos Aires. The determination of the genotypes present in other states of Argentina is important to obtain the actual panorama of pathogenic strains circulating in canines in this country. The genotypes from this study found in canines are the same isolated from cases of abortion in bovines, wildlife and humans. The vaccine used in Argentina for canines includes the serogroups Canicola, Icterohaemorraghiae and Ballum. The actualization of the vaccines for domestic animals is necessary in every country because of the constant ecological changes in the host biology and the genetic plasticity of pathogenic *Leptospira* strains. Further studies have to be carried out and more isolated strains from different states in Argentina have to be analyzed to recommend an actualization of the vaccine used these days.

PP 2

UNRAVELING *Leptospira* spp. GENOSPECIES FROM AZOREAN RODENTS THROUGH NEW MOLECULAR TOOLS

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Leptospirosis is a bacterial zoonosis that affects populations worldwide, mainly in tropical and temperate areas. The Azores Archipelago, a group of nine inhabited Portuguese islands in the North Atlantic Ocean, have been known as an endemic leptospirosis area since 1993; it was recognized as a major health problem soon thereafter. The temperate and wet climate of these islands with mild winters and the high number of infected rodents favor the survival and transmission of these spirochetes. Conventional identification and diagnostic assays for *Leptospira* are based on serological methods. Although considerable difficulties are encountered in the classification of leptospires at serovar level, this system remains as a standard to intra-species differentiation. Serovar identification has become faster with the introduction of genotyping methods that can serve as supplementary or alternative system to identify a set of serovars causing human and animal disease. These typing methods can identify differences at the serovar or serogroup level. Numerous PCR-based techniques such as random amplified polymorphic DNA (RAPD) and multiple-locus variable-number tandem repeat analysis (MLVA) have been developed and evaluated to study the population structure of bacteria and to characterize isolates even from monomorphic populations. The RAPD and MLVA typing of pathogenic leptospires could permit the quick and reliable identification of strains during outbreaks. The aim of this study was to genotype a large number of *Leptospira* isolates obtained from kidney from live-trapped rodents on Sao Miguel (SM, n=988) and Terceira (T, n=868) islands, through PCR-based assays. 1856 rodents were trapped and kidney cultured in EMJH media. The obtained isolates were genotyped by RAPD with Irep1 primer and MLVA with eight variable-number tandem repeat (VNTR) loci. Leptospires were isolated in 60% (1102/1856) of rodents. 715 isolates were genotyped by RAPD and two species were identified as *L. borgpetersenii*, Ballum serogroup (82%) and *L. interrogans* sensu stricto, Icterohaemorrhagiae

serogroup (18%), both with a similar distribution pattern regarding the reservoir species. The VNTRs analysis, specific for *L. borgpetersenii* species, was applied in 95 (9%) samples as an attempt to differentiate at serovar level. With this approach it was possible to distinguish the serovar Castellonis from Ballum and Arborea serovars. However, so far, in the "new" isolates, Castellonis serovar was not identified. The RAPD approach proved to be a simple and rapid typing tool of *Leptospira* spp., at the serogroup level. Concerning the VNTR's analysis, this tool differentiates the *L. borgpetersenii* serovars of Ballum serogroup. In conclusion these approaches demonstrated to be powerful tools in epidemiological studies and support the understanding the human leptospirosis prevalence in Azores.



PP 3

SEXUAL MATURITY STATE AS A RISK FACTOR TO *Leptospira* INFECTION OF SMALL MAMMALS INTRÁS-OS-MONTES E ALTO DOURO REGION, NORTHERN PORTUGAL

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Small mammals, particularly rodents, are significant maintenance hosts for pathogenic leptospires in the majority of ecosystems. Sexual maturity state is considered relevant in intraspecific transmission of leptospirosis due to the conviction of several researchers that sexual/venereal contact is the main transmission route. Moreover, the cumulative risk of exposure to *Leptospira* with age it is a fact that should not be negligible. Sera and kidney tissue from 282 small mammals (mainly rodents) live-trapped in Trás-os-Montes e Alto Douro (TMAD) region were analysed by microscopic agglutination test (MAT) and polymerase chain reaction (PCR), respectively. MAT was performed using as live antigens strains representative of major serogroups and additionally two representatives of strains isolated from rodents in this region. The PCR was conducted using a primer set designed from LipL21 gene sequence and Southern Blotting was carried out using a 257 bp probe. The live-trapped species were *Mus musculus* (n=231), *M. spretus* (n=23), *Rattus norvegicus* (n=18), *R. rattus* (n=3), *Apodemus sylvaticus* (n=4) and *Crocidura russula* (n=3). The specimens were included into sexual maturity classes, mature (n=133) and immature (n=149), accordingly on biometrical characteristics: colour, weight, metric measurements, and degree of development of sexual organs. The study of the variable sexual maturity state as a putative risk factor for the presence of *Leptospira* antibodies and leptospiral DNA in kidneys

were assessed by $\chi 2$ tests and nominal logistic regression analysis. Of the 282 serum samples tested by MAT, 55% (155/282) showed serological evidence of *Leptospira* infection. Additionally, 52.5% (148/282) of the individuals presented leptospiral DNA in kidneys identified by PCR LipL21 primer set. Mature animals have shown higher seropositivity rate than immature (54.2 vs. 45.8%; $P<0.01$: OR=1.88; IC=1.86-1.91), as well as greater leptospiral DNA detection (PCRLipL21) in kidneys (54.0 vs. 46.0%; $P<0.05$: OR=1.86; IC=1.83-1.89). These findings suggest sexual maturity state as a risk factor associated with leptospiral infection in small mammals of TMAD region and positively related with renal carrier state. These results strongly suggest that venereal route could be the main intraspecific way for the transmission of *Leptospira* infection between small mammals. Other facts that should be highlighted are the high prevalence of antibodies and leptospiral DNA in small mammals' population of TMAD region, indicating the potential transmission risk of *Leptospira* infection to livestock and humans due to the lack of rodents control in this region.



PP 4 SEROEPIZOOTIOLOGICAL INVESTIGATION OF LEPTOSPIROSIS IN SWINE IN CROATIA

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Leptospirosis in swine is characterised by impaired fertility, neonatal deaths, abortions and weak piglets. According to annual orders issued by Ministry of Agriculture for years 2008, 2009 and 2010, all boars used for natural breeding and production of semen for artificial insemination must be serologically tested for leptospirosis twice each year. In addition, all cases of abortion in swine must be reported and blood of each animal serologically tested for leptospirosis. During 2008, 2009 and 2010 more than 20000 swine blood samples were tested for leptospirosis. All samples were tested by microscopic agglutination test (MAT) for the presence of 11 leptospiral antigens: serovar (sv.) Icterohaemorrhagiae, sv. Ballum, sv. Australis, sv. Pomona, sv. Grippotyphosa, sv. Sejroe, sv. Saxkoebing, sv. Tarassovi, sv. Canicola, sv. Bataviae and sv. Hardjo. Initial dilution of blood samples for MAT testing was 1:100. In 2008, 10501 blood samples were tested for leptospirosis and 990 (9.43%) were positive with at least one serovar. In 2009, out of 6387 tested blood samples, 468 (7.33%) were positive, while in 2010, 3966 blood samples were tested and 236 (5.95%) were positive for leptospirosis. In 2008 Medimurje county had the highest prevalence of positive blood samples (25%). In Sisak-Moslavina there were 16.47% positive samples, in Krapina-Zagorje 13.51% and in Varaždin county 12.5% positive samples. In 2009 the higest prevalence of positive blood samples was in Osijek-Baranja county (12.27%), followed by Zagreb county (9.33%), Brod-Posavina county (8.91%) and Međimurje county (6.73%). Like in 2008, in 2010 the highest percentage of positive blood samples was found in Međimurje county (10.13%). Koprivnica-Križevci county had 9.66%, Brod-Posavina county 8,48% and Zagreb county 8.39% positive blood samples. During monitored period only few blood samples from coastal counties in Croatia were tested for leptospirosis and none of them was positive. In 2008 and 2009 the most frequent serovar was sv. Australis (55.71%/2008 and 39.66%/2009) followed by serovars Pomona (17.26%/2008 and 21.55%/2009), Icterohaemorrhagiae (10.70%/2008 and 17.24%/2009), Sejroe (7.49%/2008 and 8.74%/2009) or Grippotyphosa (4.42%/2008 and 8,99%/2009) and Hardjo (2.28%/2008 and 3.08%/2009). Sv. Pomona was the most frequent serovar in 2010 (28.9%) followed by serovars Ictero-

haemorrhagiae (23.02%), Sejroe (18.16%), Australis (10.49%), Grippotyphosa (10.23%) and Hardjo (7.93%). In 2008, 2009 and 2010 serovars Canicola and Saxkoebing were sporadically found, while in 2008 there were also a few samples positive with serovars Bataviae, Tarassovi and Ballum. Regarding the seroprevalence, leptospirosis in swine is common disease in continental counties in Croatia. Although leptospirosis in swine is in decrease, the risk of spreading it to other animals and humans still exists thus causing substantial economic losses in pig farming and threat to human health.



PP 5 SEROVAR Hardjo: POSSIBLE CAUSE OF ABORTIONS IN DAIRY COWS IN CZECH REPUBLIC

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Leptospires of the serovar Hardjo are one of the major causes of bovine leptospirosis. This infection in cattle as a maintenance host is associated with infertility, abortion, stillbirth and birth of weak calves in various parts of the world. There have been no previous reports of serovar Hardjo strains, isolated in Czech Republic. The system for routine monitoring and reporting is recently considered to be inadequate and diseases burden of leptospirosis in cattle may have been underestimated and uncertain.

The aim of our present pilot study was to establish prevalence of antibodies against serovar Hardjo in 12 districts of Czech Republic. Blood samples from 27 1aborting cows were assayed for antibodies against serovar Hardjo using commercial ELISA test (Linnodee Leptospira Hardjo ELISA). Cut-off point was estimated accordingly to the manufactures instructions. All sample stesting positive to ELISA were confirmed by MAT using the panel of 4 serovars (Icterohaemorrhagiae, Grippotyphosa, Hardjo, Pomona) and starting with a 1:10 dilution. All blood samples were concurrently tested for antibodies to infectious bovine rhinotracheitis (IBR), Q-fever and brucellosis.

The overall estimate of serovar Hardjo seroprevalence assuming random sample was 13.28 %. The district total seroprevalences ranged between 4.35% and 36.84 %, whereas the seroprevalences in four regions of Klatovy, Domazlice, Ustinad Orlici and Znojmowere 27.27%, 30.77 %, 36.84% and 25% respectively, while the seroprevalences in the remaining 6 districts were in therange 4.35%to 12.5 %.Cattle from two regions of Pisekand Jihlava, tested for serovar Hardjo antibodies found to be negative. Out of 36 samples positive by ELISA, 23 (63.89%) were confirmed by MAT. The titers of antibodies against serovar Hardjo varied between 1:20 and 1:640. The presence of antibodies against IBR was detected in 9 (2.79%) and antibodies against Q-fever were detected in 73 (22.6 %) cows. From 35 serovar Hardjo positive cows, only 3 were also positive for Q- fever.

Cattle aborting are a result of chronic leptospirosis and significant percentage of cattle infected with serovar Hardjo will be seronegative. In terms of legal requirement, the serum for MAT in laboratories within the Czech Republic is serially diluted from an initial dilution of 1:100. Of the 23 sera samples,

positively tested by MAT for serovar Hardjo antibodies, 12 (52.17%) reacted in titres ≤ 1:100. ELISA is more promising for detection of chronical leptospirosis in cattle and MAT tests should start at a titre of 1:10. Serological results indicate that serovar Hardjo infections might cause reproductive problems in high risk areas and furthermore aspects, such as monitoring and sampling strategies are addressed.



PP 6

INCIDENCE OF LEPTOSPIRAL ABORTION IN CATTLE IN CROATIA

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In Croatia, leptospirosis in domestic animals is controlled in accordance with the annual order issued by the Ministry of Agriculture. All cases of abortion in cattle must be reported and blood of each animal serologically tested for leptospirosis. In 2010, 1372 blood samples were tested. All sera were tested by microscopic agglutination test (MAT) for the presence of 12 leptospiral antigens. Serovars: Icterohaemorrhagiae / RGA, Ballum / Mus 127, Australis / Ballico, Pomona / Pomona, Grippotyphosa / Moskow V, Sejroe / M84, Saxkoebing / M24, Tarassovi / Perepelicin, Canicola / Hond Utrecht IV, Bataviae / Van Tienen, Hardjo / Hardjoprajitno and Hardjo / Hardjobovis, were used as antigens for testing the sera. An initial sera dilution 1:100 was considered as a cut-off point for a positive reaction. Positive reactions with at least one sv. were found in 90 (6.6%) samples. Highest seroprevalence was found in the counties with most significant dairy and beef production (Sisak-moslavina (13.4%); Virovitica-podravina (10%); Bjelovar-bilogora (8.4%); Osijek-baranja/Zagreb (8.1%, respectively) and Brod-posavina (7.1%)). Out of 212 positive reactions, most frequent serovars were Pomona (19.8%), Sejroe (16.5%), Saxkoebing (16.5%), Hardjoprajitno (16.5%) and Hardjobovis (16.5%). Serovars Icterohaemorrhagiae (6.1%), Grippotyphosa (6.1%), Tarassovi (1%) and Bataviae (1%) were found at lower frequencies. Our investigation indicates leptospirosis, particulary infections with serovar Pomona and serovars from serogroup Sejroe (Sejroe, Saxkoebing, Hardjoprajitno and Hardjobovis) as a significant problem in cattle production in Croatia.



PP 7

SEROPREVALENCE OF LEPTOSPIROSIS IN JACKALS FROM NATURE PARK LONJSKO POLJE AND LANDSCAPE SPAČVA FOREST

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In the year 2011, as a part of regular activities in 5 hunting grounds from Sisak-Moslavina and Vukovar-Srijem counties, blood samples of 26 golden jackals (*Canis aureus*) were submitted to laboratory. The above mentioned hunting grounds are mostly located in the Nature Park Lonjsko Polje and Spačva forest, the largest integrated oak forest (*Quercus robur* L.) in this part of Europe. The significance of Lonjsko Polje Nature Park is that throughout most of the year it is inhabited by various kinds of domestic (cattle, horses and pigs) and wildlife species. Spačva forest covers an area of approximately 40,000 acres and includes two special protected forest reserves and two significant wetland areas. All sera were tested by microscopic agglutination test (MAT) for the presence of 11 leptospiral antigens. An initial sera dilution 1:100 was considered as a cut-off point for a positive reaction. Positive reaction was detected in 4 (15.4%) bloods from 3 different hunting grounds. Three jackal bloods from two hunting grounds reacted positively to the serovar Sejroe (1:100-1:500), and one blood from the third hunting ground reacted positively to the serovars Tarassovi and Bataviae (1:100). This is the first monitoring of leptospirosis in golden jackals in the area of Lonjsko Polje Nature Park and Spačva forests. Regarding the nutritional characteristics of the golden jackals (small rodents and carrion), population density in these areas and relatively high capacity of large-distance migration, they should be considered as an important factor in the maintenance and expansion of leptospirosis in the ecosystem of the Nature Park Lonjsko Polje and Spačva forests.

PP 8

LEPTOSPIROSIS IN BUCKS AND RAMS IN CROATIA

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In accordance with the annual orders issued by Ministry of Agriculture for years 2008, 2009 and 2010, all bucks and rams used for natural breeding and production of semen for artificial insemination have been serologically tested for leptospirosis twice each year. During that period total of 36100 blood samples were examined in the Laboratory for bacterial zoonoses and molecular diagnosis of bacterial diseases of Croatian Veterinary Institute. 16218 (44.93%) of above mentioned samples were collected in the continental region of Croatia, and the other 19882 (55.07%) in the coastal region. All samples were examined by microscopic agglutination test (MAT) for the presence of 11 leptospiral antigens. An initial sera dilution of 1:100 was considered as a cut-off point for a positive reaction. Positive reaction was found in 220 (0.61%) blood samples, 212 (96.36%) of which reacted positively with only one serovar, 6 (2.73%) with two serovars and 2 (0.91%) with three serovars. 207 (94.09%) positive bloods samples came from the continental counties and the other 13 (5.91%) from the coastal region. The majority of positive samples from continental region came from three counties (Osijek-Baranja 40 (19.32%); Virovitica-Podravina 35 (16.91%) and Bjelovar-Bilogora 34 (16.43%)). The most frequently recorded serovars in this region were Pomona (33.18%), Grippotyphosa (27.19%), Sejroe (22.12%) and Australis (13.36%). Highest incidence of serovar Pomona was found in Virovitica-Podravina county (30.56%). Sv. Grippotyphosa was the prevalent serovar in Osijek-Baranja county (37.29%) and sv. Sejroe in Bjelovar-Bilogora county (41.67%). Sv. Australis was equally distributed throughout the region. Serovars Icterohaemorrhagiae, Hardjo and Ballum were found at much lower frequencies. 5 serovars (Australis, Grippotyphosa, Icterohaemorrhagiae, Pomona and Sejroe) appeared sporadically and equally distributed in the coastal region. 7 (53.85%) out of 13 positive blood samples from the coastal region came from Split-Dalmatia county. The endemic nature of leptospirosis, as well as prevalence of different leptospiral serovars within animal population depend primarily on combination of environmental factors such as geographical and climate conditions, exposure to different animal species, variety of present *Leptospira* reservoirs and farming methods. All this must be taken into consideration when assessing this surveys results, according to which the highest number of positive samples was recorded in central and eastern part of Croatia.

PP 9 SEROLOGICAL EVIDENCE OF LEPTOSPIRAL INFECTIONS IN PIG POPULATION IN POLAND

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Leptospirosis is a worldwide zoonosis caused by pathogenic serovars of spirochetes of the genus *Leptospira*. In swine, leptospirosis can demonstrate range of clinical signs varying with the infecting serovar, however majority of cases are subclinical and the only perceptible symptom can be abortions. Chronic infections and asymptomatic carrier states in swine can stay for long time unrecognized creating a significant possibility of *Leptospira* transmission to other animal species or to man. Serological screenings help to effectively control epidemiological situation in swine herds and to prevent transmission of *Leptospira* from animals to man. The purpose of this study was to investigate, by the use of serological methods, the prevalence of infections caused by selected *Leptospira* serogroups in swine population in Poland. A total of 11 622 swine serum samples were examined. The samples were collected in years 2010 - 2011 and came from 215 counties situated in all 16 provinces of Poland. All sera were preliminary examined by enzyme-linked immunosorbent assay (ELISA). The samples positive or doubtful in ELISA were investigated by microscopic agglutination test (MAT) with following live antigens: *Leptospira interrogans* serovars Icterohaemorrhagiae, Pomona, Canicola, *L. borgpetersenii* serovars Sejroe, Tarassovi and *L. kirschneri* serovar Grippotyphosa. Serum dilution (including added antigen) used during preliminary examination was 1:100. For antibody titres estimation in sera reacting with one or more serovars in the preliminary examination, two fold dilution series of these samples were prepared to titre the end point, 50% agglutination. The samples with titres >100 were regarded as positive. Of the collected sera examined by ELISA 128 (1.10 %) samples were positive, 249 (2.14 %) - doubtful and 11 245 - negative. Among ELISA-positive and doubtful sera 222 samples (coming from 14 provinces) were recognized in MAT as positive. Among MAT-positive samples 93 sera exhibited titres to serovar Sejroe, 87- to Pomona, 18 - to Grippotyphosa, 8 - to Icterohaemorrhagiae, 7 - to Canicola and 6 - to Tarassovi. Apart from it 3 sera exhibiting equal titres to more than one serovar were found. The first reacted with three serovars (Sejroe, Tarassovi, Pomona), the second with two (Sejroe, Pomona) and the third also with two Sejroe, Tarassovi). Share of MAT-positive samples among all

examined sera obtained 1.91 %. In comparison to results of similar screening organized in years 2008-2009 (0.91 %) mentioned value increased more than two times. Similarly like before the domination of samples demonstrating titres to serovar Sejroe is visible. There are currently no reports describing similarly high prevalence of positive serological reactions with serovar Sejroe in swine populations in other European countries. Reasons of the phenomenon will be analyzed.



PP 10 SEROPREVALENCE OF *Leptospira* spp. IN ARABIAN HORSES IN POLAND. THE PRELIMINARY SURVEY.

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Leptospirosis is zoonotic disease caused by pathogenic serovars of the genus *Leptospira*. In horses the acute infections occur quite rare. More often chronic or subclinical course of the disease is noted. Clinical signs, when observable, are usually connected with renal and hepatic dysfunctions, stillbirths, abortions and relatively often with recurrent uveitis. Results of serological surveys from many countries indicate horses are frequently infected by leptospires, however the positive results of this findings not often are associated with clinical manifestation of the disease. The prevalence of *Leptospira* infection in horses in Poland is unknown, however specific antibodies to many different serovars of *Leptospira* have been detected in a range of domestic (pigs, cattle, sheep, goats, and dogs) and wild species (wild boar and European bison). The aim of the present study was to determine the seroprevalence of selected serogroups of *Leptospira* spp. in Polish herds of Arabian horses. A total of 350 horse serum samples from Arabian horses (stallions and mares) in different age (from one to over ten years) were examined. The samples were collected from two horse-breeding farms. All sera were examined by microscopic agglutination test (MAT) with use of serovars Icterohaemorrhagiae, Pomona, Canicola, Sejroe, Tarassovi and Grippotyphosa. Serum dilution (including added antigen) used during preliminary examination was 1:100. For antibody titres estimation in sera reacting with one or more serovars in the preliminary examination, twofold dilution series of these samples were prepared to titre the end point, 50% agglutination. The samples with titres >100 were regarded as positive. Out of all collected sera 131 (37.48 %) samples were positive. Among this group 78 (59.54 %) samples exhibited titres to serovar Grippotyphosa, 15 (11.45 %) samples to serovar Canicola, 7 (5.34 %) samples to serovar Tarassovi, 5 (3.82 %) to serovar Sejroe and 1 (0.76 %) to serovar Icterohaemorrhagiae. Apart from mentioned samples 23 sera exhibited reactions with two serovars (Grippotyphosa - Canicola, Grippotyphosa - Pomona, Grippotyphosa - Sejroe, Grippotyphosa - Tarassovi, Sejroe - Tarassovi, Tarassovi - Canicola, Icterohaemorrhagiae - Grippotyphosa, Icterohaemorrhagiae - Canicola, Icterohaemorrhagiae - Sejroe).

Moreover, one sample reacting with four serovars (Icterohaemorrhagiae, Grippotyphosa, Sejroe, Canicola) and one - reacting with three serovars (Grippotyphosa, Sejroe, Tarassovi) was indicated. The highest titres noted during the study obtained 3200. The results of serological survey seems indicate that horses from examined studs are quite often exposed or infected with *Leptospira* spp. Most prevalent serovar is Grippotyphosa. The study will be continued with the bigger group of horses from different horse-breeding farms taking in to consideration occurrence of equine recurrent uveitis.



PP 11

CANINE LEPTOSPIROSIS: REPORT OF AN OUTBREAK

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Leptospirosis is a worldwide disease caused by pathogenic members of the genus *Leptospira* which affects numerous animal species, including dog. Humans become infected through direct or indirect contact with infected domestic and wild animal species. In dog the disease causes an acute and septicemic infection, with kidney and liver being the target organs. Considering that many clinical-pathological aspects of the *Leptospira* infection are poorly understood, it has been raised the possibility that the disease in dogs may remain frequently undiagnosed. Here we reported anatomical-pathological, serological and bacteriological results from a canine leptospirosis outbreak which was correlated with a human clinical case. We investigated in Sardinia the health conditions of the dogs housed in a kennel, in which a worker, who was employed for managing the animals, was hospitalized with severe symptoms indicative of leptospirosis and found seropositive against *Leptospira* serovar Icterohaemorragiae. To do this, 201 dogs were blood sampled and serological diagnosis of leptospirosis by using MAT with a panel of 8 *Leptospira* reference strains (Hardjo, Grippotyphosa, Bratislava, Pomona, Tarassovi, Copenhageni, Icterohaemorragiae, and Canicola) was performed. On the same blood samples the metabolic profile was also determined. Kidney, urine and liver from 5 symptomatic dogs were investigated by bacteriological cultures and PCR methods. In addition, we examined samples from 2 rats trapped in the kennel. Results. MAT results for each serovar were reported as the highest dilution of serum (from 1:100 to 1:12.800) at which ≥ 50% agglutination of organisms occurred. By using cut-off titers of ≥ 100, MAT showed that 30 of the 201 dogs were positive for one or more serovars. More in particular 10 of the 30 dogs were positive only for one antigen, 12 for 2 and the remaining 8 for 3. The most common serovars were Icterohaemorragiae, Copenhageni and Bratislava. PCR was positive in 4 of the 5 dogs while all the bacteriological cultures were negative, althought gross anatomopathological lesions indicative of leptospirosis were found in each of the examined animals. Metabolic profiles showed

variations of albumin, bilirubin, and transaminase values. Several strains of leptospires were isolated by bacteriological and PCR examinations from the kidney and the urine of the 2 rats. Identification of the isolates with VNTR and PFGE is still ongoing.

Our report confirms that kennels should be considered a public health risk as dog can be asymptomatic carrier of *Leptospira* and a complete prevention and control by vaccination cannot be carried out for all the serovars. In addition, the isolation of *Leptospira* spp. from rats, here reported for the first time in Sardinia, suggests the adoption of hygienic measures based on effective rodent host control in order to reduce the prevalence of this zoonosis in canine kennels.



PP 12 COMPLEX APPROACH FOR DETECTION OF LEPTOSPIROSIS IN EAST SIBERIA

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Only one case of leptospirosis was recorded in 2001-2010 in Irkutsk Region (East Siberia), but a lot of seropositive dogs and farm animals suggest the possibility of the pathogen circulation and human leptospirosis infections. Therefore, we conducted an active screening of leptospirosis in a group of professional risk.

Sixty six workers at five stock farms in Irkutsk region were examined. The epidemiological study of leptospirosis focus was conducted, 18 sera from individuals (random sampling) were studied, 20 rodents were captured, 12 of which were caught alive. Laboratory studies were carried out by bacteriological, serological (MAT) and molecular genetic (PCR) methods. Real time PCR with „AmpliSens Leptospira-FL" (Public Entity Central Research Institute of Epidemiology, Moscow) was used.

Fifteen human positive PCR results at three stock farms were obtained. The effective disease area at the cattle farm was identified with the double increase of antibody titers to *Leptospira* serogroup Icterohaemorrhagiae to 1:200 and 1:400 in paired sera from two milkmaids was established. Despite the absence of the expressed clinical manifestations a diagnosis of leptospirosis was confirmed in one of the women on the basis of laboratory findings. Also two infected women (mother and daughter) were revealed during the inspection of the local population, one woman had antibodies with an increase in titers of 1:400 to 1:800, the other the specific DNA. Total MAT and PCR hit ratio was 54.5 % in farm workers and 11.1 % at the random sampling in the local population. Specific pathogenic *Leptospira* DNA was revealed in the epizootological study of the rodents caught in areas of agricultural activity and in private houses (backyards). In several cases *Leptospira* was observed through direct microscopy but cultures failed to identify.

Agricultural and household leptospirosis epidemic points were effectively identified as a part of a mixed focus. Percentage of infection in the professional group was five times higher than at

the random sampling in the local population. *Leptospira* circulation among commensal and wild rodents was demonstrated. The absence of clinical picture in patients with positive results of the laboratory research implies that leptospirosis in Siberia is not so much the „neglected" but „invisible" infection.



PP 13 LEPTOSPIROSIS IN LUANDA (ANGOLA): CONTRIBUTION FOR A DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH FEBRILE ILLNESSES

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Leptospirosis is an important zoonosis of worldwide distribution, particularly in tropical countries. The nonspecific clinical presentation of the infection can lead to a misdiagnosis in these regions, where similar febrile diseases are common and can be confused with other disorders such as malaria. In Angola, the leptospirosis diagnosis is done only by the patient history and medical suspicion, which can lead to misdiagnosis contributing the increase in antibiotic treatment and resistance in the population. The aim of this work was to know the seroprevalence of the leptospirosis in patients that presents febrile symptoms and an initial diagnosis of malaria (after not confirmed), and to evaluate the *Leptospira* infection rate from rodents living close to human communities and their role as major spirochete reservoirs. Serum samples were obtained from 250 patients (109 children and 141 adults) attended at Pediatric and Military Hospitals, both localized in Luanda. A clinical and epidemiological questionnaire was applied to all patients, according to Ethics Committee of Angola Health authorization. A total of 40 rodents (rats and mice) were live-trapped and necropsied to extract the kidney. The serological evaluation was performed by Microscopic Agglutination Test (MAT), and the renal carrier status was studied by two PCR one specific of genus Leptospira 16S gene, the other specific of pathogenic leptospires (hap1). The last PCR was performed on patient serum samples. The laboratorial work was developed at the Leptospirosis Lab., (IHMT, UNL), Lisbon, Portugal. The analysis of the epidemiological data suggests that the population has high exposure to risk factors such as the contact with rodents and poor sanitation. Of the 250 sera analyzed, 11 (4.4%) showed agglutinins anti-*L interrogans* s.l. with titers (≥1:100) and about 20% (50/250) showed borderline reactivity. DNA of *Leptospira* was not detected in the studied sera. So far, leptospiral DNA was found 3/40 (7.5%) in rodent kidney samples. The kidney cultures are still ongoing. Our preliminary results showed that: i) about 25% of febrile studied patients had contact with the etiologic agent of leptospirosis; ii) the significant number of borderline sera may

be due to the indiscriminate use of antibiotics and other drugs by the population (self-medication), early in the febrile syndrome; iii) therefore, it is necessary to enhance the parameters of the clinical-epidemiological survey for the history of symptoms and therapeutic evolution in these patients; and iv) it is important to increase the population sampling, including patients from other provinces and in particular, those living in rural areas in order to understand the true incidence of leptospirosis and the potential co-infection with malaria or other febrile diseases.



PP 14 MANY-YEAR MONITORING OF LEPTOSPIRAL INFECTION IN ST. PETERSBURG, RUSSIA

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The objective of our study was to estimate the evolution of leptospirosis epidemiological manifestations in St. Petersburg (Leningrad), Russia, reasoning from many-year (1942-2010) monitoring data. We analysed our original data, published scientific papers, and research data archives of Zoonoses Laboratory at St. Petersburg Pasteur Institute that proceeded epidemiological surveillance, and serological studies on a total of about 2500 cases of leptospirosis in humans. We divided the entire period under consideration into four reference periods that differ much in their epidemiological profiles of leptospiral infection. The first reference period was that of the 2nd World war including the Leningrad blockade; the second period continued till the late 1980s, the third period lasted till the beginning of XXI century, and the fourth period is running currently. Those four periods differ significantly in the sources of disease, routes of leptospiral infection acquiring, leptospirosis etiological structure in humans and animals, high-risk groups of population, seasonality, clinical course, and outcome of the disease. The evolution of leptospirosis epidemic manifestations at the metropolis conditions under the influence of anthropogenic factor is traced clearly.



PP 15 HUMAN LEPTOSPIROSIS SEROPREVALENCE IN THE AZORES ARCHIPELAGO (PORTUGAL): AN UPDATE (2004 – 2011)

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Since the early 90\'s that Azores Archipelago (Portugal), namely São Miguel and Terceira islands have been considered an endemic area for leptospirosis. High precipitation and moderate temperature, together with wide densities of rodents, provides an extensive dissemination of these spirochetes allowing their survival in environment. In Portugal, the first isolation of Weil acutes agent disease was obtained in 1931. However, regular research on human leptospirosis started in the 1980s, becoming a notifiable disease in 1987. In Portugal, the number of official reported cases has been low when compared with other countries. In fact, leptospirosis is still not always notified nor even diagnosed due to its often unspecific clinical appearance and difficult laboratorial diagnosis. This study aimed to evaluate the current trend of leptospirosis in Azores, established on seroprevalence registered at the Leptospirosis Lab (IHMT, UNL) databases, during the last eight years. Between 2004 and 2011, a total of 1028 human sera (563♂ and 84♀) from 647 patients with leptospirosis clinical suspicion, were analyzed during the referred period, by the Microscopic Agglutination Test (MAT) in order to detect anti-*Leptospira interrogans* sensu lato (s.l.) antibodies. In the eight-year study period, 225 (35%) symptomatic patients were confirmed to have a current *Leptospira* infection. The annual distribution of leptospirosis cases, showed an average of 28 cases per year, with higher frequency in males (93%). Middle age adults (21-50 years) were most frequently infected (64%); and 29% corresponds to a population with more than 50 years old. Leptospirosis has been confirmed as a public health problem in Portugal, particularly in Azores islands. This retrospective study revealed a marked decrease of cases; however, the annual incidence rate increased (15/100 000 population) when compared with the incidence revealed in a previous study (11.1/100 000), nevertheless, it is noted the absence of fatalities in that period. The major infecting serogroup is Icterohaemorrhagiae (28%) followed by Ballum serogroup (15%), this distribution is coincident with the previous data known in these islands. According to the present series

study, leptospirosis continues predominantly affecting active adult men due to their professional and leisure activities. Finally, it is important to note that these results show an increasing attention given to this zoonosis for the population and Health care. These improvements results from the active surveillance began with the project „Epidemiology and Control of Leptospirosis in Azores" developed between 2004-2008, under USA Scientific-Cooperative Agreement No. 58-4001-3-F185. Nevertheless, we assume that it is necessary to improve diagnostic methods, including molecular techniques in order to obtain an early diagnosis, anticipating the MAT results and contributing to disease control.



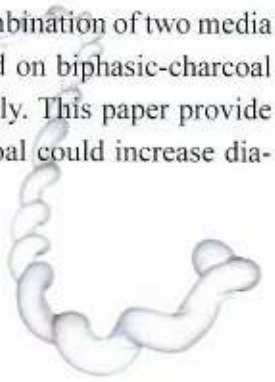
PP 16 DEVELOPMENT OF BIPHASIC CULTURE MEDIUM CONTAINING CHARCOAL FOR THE ISOLATION OF LEPTOSPIRES

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The isolation of leptospires from fresh clinical material not only implicates them, but it also allows for the identification of the infecting serovar. Leptospires being delicate and fastidious organisms may fail to grow due to there being inadequate nutrients available and or because of the presence of inhibitory substances. Each strain will have different requirements and wild organisms can be reluctant to grow in isolation media. A *Leptospira* biphasic culture medium, which is modification of Tween 80/40 medium, has been recently developed. Each tube of this medium is made up of 2 phases, a sloped solid layer overlaid with liquid layer. The solid layer contains 0.8% agar and 0.8% untreated activated charcol. It has been suggested that the charcoal facilitates the removal of the toxic products of bacterial metabolism. Therefore it may be possible to use a more concentrated inoculum, thereby increasing the number of organism present for isolation. Both the solid and liquid layers are in two variant forms: M1 and M4. M1 contain 5-fluarouracil, M4 contains 5-fluorouracil, rifampicin and amphotericin B. The aim of the study was to examine the effectiveness of incorporating this medium for the isolation of leptospires and comparison with conventional semi-solid Tween 80/40 medium used in 4 forms, varied in the content of the inhibitory substances to control contaminating microorganisms (5-fluorouracil or other antibiotics). A total of 51 urine samples were tested on two media. The combination of two media yields the highest rates 58.8%. The percentages of *Leptospira*-positive isolated on biphasic-charcoal medium and semi-solid Tween 80/40 medium were 50.9 and 41.2% respectively. This paper provide evidence that new biphasic version of Tween 80/40 medium containing charcoal could increase diagnostic sensitivity of *Leptospira* culture isolation methods.



PP 17 RAPID MALDI-TOF MASS SPECTROMETRY IDENTIFICATION OF *Leptospira* ORGANISMS

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Leptospirosis is a worldwide deadly zoonotic disease. Accurate identification of the causative *Leptospira* spp. spirochetes ascertains the pathogenic status of the isolates, identifies potential source of infection and recognises outbreaks. Species identification is currently based on technically demanding, time and resources consuming serological and molecular methods. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) recently emerged as a first-line method for the accurate identification of bacteria, yet no data issued for *Leptospira* spp. We investigated the potential of MALDI-TOF-MS for the rapid identification of *Leptospira* isolates. Starting from a 105 organisms/mL suspension, MALDI-TOF-MS yielded an unique protein profile for each one of 19 *Leptospira* species reference isolates with a 100% reproducibility over 12 repeats, allowing to create a *Leptopsira* database. MALDI-TOF-MS further accurately identified 20/21 additional reference isolates representative of various serogroups at the species level as *Leptospira interrogans* (n=12), *Leptospira kirschneri* (n=5), *Leptospira borgopetersenii* (n=3), *Leptospira noguchii* (n=1) with identification score value of 2-2.5. Furthermore, six clinical isolates previously identified by rpoB sequencing, were correctly identified by MALDI-TOF-MS as *L. interrogans* (n=5) and *L. borgpetersenii* (n=1) with identification score value of 2-2.6. Identification was achieved in 40 minutes starting from the *Leptospira* suspension. MALDI-TOF-MS could complement serological and sequencing-based methods for the first line, rapid identification of *Leptospira* isolates in the clinical microbiology laboratory.



PP 18

DEVELOPMENT AND VALIDATION OF A QUANTITATIVE PCR (TAQMAN) ASSAY FOR DETECTION OF *Leptospira interrogans* USING JOINT BIOLOGICAL AGENT IDENTIFICATION AND DIAGNOSTIC SYSTEM (JBAIDS)

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Leptospirosis is considered an underdiagnosed disease. The differential diagnosis of leptospirosis is difficult due to the varied and often "flu like" symptoms which may result in a missed or delayed diagnosis. Several PCR-based methods are currently in use but the information is still limited. In this study, we developed and evaluated a rapid and simple assay for the specific detection of pathogenic *Leptospira* spp. This diagnostic platform, referred to as the RAPID/JBAIDS, is a real-time PCR Taq-Man assay performed on a multiple use instrument that we aim to develop for the rapid detection of parasites in clinical samples from the field. The highly sensitive limits of detection (LOD); DNA detected as low as 10 fg/ml and assay can detect as few as 2 parasite in a sample make our assay ideal for providing early diagnosis of infections in infected individuals.

Freeze-Dry assay kit has been developed to use with RAPID/JBAIDS real-time PCR. Primers and probes were designed using a 132 bp target on the lipL32 gene, a sequence of the gene encoding the *L. interrogans* major outer membrane protein. Quantitation, accuracy and precision of the real-time PCR assay were determined through serial dilutions of *L. interrogans* serovar Autumnalis genomic DNA. Tenfold serial dilutions were made in duplicate, ranging from 10 ng to 1 fg, to test for sensitivity. These serial dilutions were used to assess efficiency and sensitivity of the assay and to produce a standard curve for quantification. The sensitivity of the qPCR assay was validated through 10 fold serial dilutions of genomic DNA extracted from *L. interrogans* serovar Autumnalis. The assay consistently detected as low as 10 fg of *L. interrogans* genomic DNA.

The assay showed sensitive and specific tools for diagnosis of leptospirosis. Development and validation of this highly sensitive and specific real-time PCR assay for the detection of leptospirosis will be highly useful for early diagnosis of pathogenic leptospirosis. This assay will be further evaluated for its clinical utility in detecting from clinical samples pathogenic species of *Leptospira*.

PP 19

EXPERIMENTAL INFECTION OF RABBITS WITH *L. interrogans* SEROVAR Hardjo

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By improving diagnostic methods for detecting leptospirosis, especially new laboratory diagnostic methods, a knowledge is provided on the nature and consequences of this infection and its importance both in veterinary and human medicine. New tests and procedures have been introduced in diagnostics of all the contagious diseases, including leptospirosis. The aim of this examination was to test different laboratory methods and evaluate their accuracy. In this experiment eleven rabbits were artificially infected by scarification with the live culture of L. Hardjo. The blood samples and sera were taken every second day, starting from day 21 post infection and then once a week for the following 5 weeks. The blood sera was examined for the presence of specific antibodies against L. Hardjo applying the methods of microscopic agglutination (MA) and ELISA. The blood samples were examined using the method of cultivation on agar according to Johnson supplemented with 200 µg/ml 5- fluorouracil (5-FU). L. Hardjo genome was examined by polymerase chain reaction (PCR). A pair of primers, separated from the basic structure of *Leptospira interrogans* rrs (16S) was used. The specific antibodies in rabbits against L. Hardjo were detected in 67 sera samples (36.6%). The first positive finding of specific antibodies was recorded on day 9 post infection and remained until day 17. ELISA test detected positive results in 67 samples and 18 suspected samples. The earliest positive results were recorded on day 15. The number of positive findings increased and reached its maximum on day 42. From the total of 183 blood samples, L. Hardjo was isolated in 33 (18.3%) samples, earliest on day 3, and the largest percent was recorded on day 17 post infection. Using PCR positive L. Hardjo genome was detected in 67 (56.30%) samples out 119. Positive finding was recorded already on the first day, and the highest percentage of positive findings was recorded on day 19 post infection. When the method of cultivation and PCR method were compared up to 21 days after infection for the period from infection until the application of dihydrostreptomycin a high level of linear correlation (r = 0.810) at the significance 0.01

was detected. After dihydrostreptomycin was administered 21 days upon infection, by the method of cultivation L. Hardjo was not isolated from the blood samples of rabbits. However, using PCR method L. Hardjo was detected in 23 samples.



PP 20

EVALUATION OF THE MEMBRANE IMMUNOASSAY FOR DETECTION OF SPECIFIC ANTIBODIES AGAINST LEPTOSPIRES

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Leptospirosis is a worldwide zoonosis caused by tightly coiled spirochetes with characteristic hooked ends. Clinical presentation in humans varies considerably, ranging from mild influenza-like illness to jaundice, renal failure, bleeding and sometimes also dead. Routine diagnosis of leptospiral infection is based on demonstration of specific antibodies with serological tests. Performance of the reference serological test, the microscopic agglutination test (MAT), requires significant expertise and is quite expensive. The aim of our study was to find out if leptospiral infection can be proven with simple, commercially available membrane immunoassay at the first level of serological diagnosis. In the present study 110 samples from patients with clinical manifestations suggestive of leptospirosis were tested with reference MAT and rapid membrane immunoassay test which was performed following the manufacturer`s instructions. Some patients were followed up regarding to serological diagnosis. We found 4 positive and 100 negative samples with both tests (Table 1). In 6 cases membrane immunoassay showed positive results while MAT was negative. Later we received serums of 2/6 patients which were MAT negative and membrane immunoassay positive at first presentation and found both of them to be positive with both tests. Other 4/6 patients were not observed clinicaly as leptospiral infection and no aditional serums were tested.

Results are shown below:

Microscopic agglutination test (MAT) Membrane immunoassay

	Positive	Negative	All
Positive	4 (3.6 %)	0 (0.0 %)	4 (3.6 %)
Negative	6 (5.5 %)	100 (90.9 %)	106 (96.4 %)
All	10 (9.1 %)	100 (90.9 %)	110 (100 %)

According to our findings simple membrane immunoassay can prove *Leptospira* infection without missing any patients comparing with MAT, so membrane immunoassay could be used for routine diagnosis of leptospiral infection in emergency cases, but all results should be confirmed with a reference MAT. Unfortunately, many patients were not followed up, and after first serological testing leptospirosis was excluded based on MAT results.



PP 21

EVALUATION OF TWO COMMERCIAL ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISA) FOR THE DIAGNOSIS OF *Leptospira* Hardjo IN BOVINE SERA

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Leptospirosis represents a severe problem in cattle herds. Acute as well chronic infections manifest with agalactia, insurgence of infertility, stillbirths or abortions. Therefore, besides affecting the single animal wellness leptospirosis might cause substantial losses in the overall herd production. Previous studies show that the most prevalent causative leptospirosis serovars are Sejroe (Hardjo. Hardjobovis), Pomona and Grippotyophosa (1-3). To assess for a potential "replacement" of the time consuming reference method for the detection of strain specific antibodies, we tested the performances of 2 commercial ELISA test kits and compared the results to those obtained with the microscopic agglutination test (MAT). To do so, a panel of 238 bovine sera was challenged with: 1) the Linnodee *Leptospira* Hardjo (L. Hardjo) double sandwich ELISA kit (Linnodee) targeting anti-LPS antibodies of L. Hardjo, and 2) the PrioCHECK L. Hardjo indirect ELISA (Prionics) using inactivated whole cells of L. Hardjo to target anti-Hardjo antibodies. Results were compared to the MAT ran upon serovar Sejroe only. Bovine samples included 92 (38.5%) sera derived from a national "Abortion program" and thus always associated with an abortion. The MAT detected 92 (38.7%) positive and 146 (61.3%) negative samples. Sensitivities were of 84% (77/92) and 85% (79/92) for the Linnodee and PrioCHECK respectively, while specificity reached 70.0% (102/146) for Linodee and 87.7% (128/146) for PrioCHECK. Agreement between MAT and PrioCHECK was excellent reaching 91.3% and 92.6% for positive and negative samples respectively (kappa value >0.81). Agreement between MAT and the ELISA of Linnodee was good, 80% and 77.3% for positive and negative samples respectively, but not reaching levels of the PrioCHECK (kappa values >0.54). We conclude that the PrioCHECK indirect ELISA is an acceptable performing test for the serodiagnosis of L. Hardjo in cattle at herd level, and that it can be used with a high degree of confidence in place of the laborious MAT test when a large number of samples have to be analyzed such as in large screening programs.

PP 22

SPECIFIC PROPHYLAXIS OF ABORTION IN CATTLE

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Leptospirosis, despite ample research carried out, still remains not only an economic, but also a social problem.

According to our data, the average infection rate of cattle in Russian Federation, as of 2007-2009, was 15.1% and varied between as little as 2.8% in the Southern region and as much as 29.3% in the Uralskii region.

At farms leptospirosis has been recorded not only as a monoinfection, but also as part of mixed infections, namely with infectious rhinotracheitis virus, viral diarrhoea, rota- and coronaviruses, Chlamydia, Campylobacter etc. These infectious agents, like leptospirae, provoke abortions, foetal reabsorption, formatio of a foetus papyraceus (or mummification), premature births or birth of weak offspring that was infected in utero.

The goal of the present study was to develop and assess the efficiency of a combined vaccine against infectious rhinotracheitis, viral diarrhoea, rota- and coronavirus infections and leptospirosis in cattle. This choice was determined by the fact that the above infections are widespread in nearly all of the regions of the Russian Federation.



PP 23

DETECTION OF ANTIGENS OF *Leptospira* spp. REACTIVE WITH SERA FROM MARES THAT ABORTED

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Abortion in mares may result from a variety of causes; infectious and noninfectious. One of the infectious causes is bacterial infection with pathogenic *Leptospira* spp.

According to the state annual order of the Ministry of Agriculture in Croatia, all aborted mares must be serologically tested to leptospirosis. A total of 40 aborted mare sera were chosen among those routinely examinated on leptospirosis during the year 2010 and 2011. Sera were tested by microscopic agglutination test (MAT) using 12 *Leptospira* serovars. Initial dilution was 1:50. Out of 40 sera, 21 showed a positive MAT reaction. Ten (48 %) reacted with the highest titer against serovar Bratislava, six (28.6 %) against serovar Pomona, three (14.3 %) against serovar Saxkoebing and two (9.52%) against serovar Icterohaemorrhagiae. 19 aborted mare sera were considered serologically negative with the titer < 50. Since the MAT is dependent on agglutinating antibody, predominantly IgM against LPS, this work was done to screen MAT positive and negative aborted mare sera for the detection of IgG antibodies to different *Leptospira* antigens by one and two-dimensional SDS PAGE immunoblot (1D and 2D IB). Proteins of an in vitro cultivated isolate of *L. interrogans* serovar Copenhageni, strain RJ 16441 were separated by 1D and 2D electrophoresis, visualised by SyproRuby staining (Sigma-Aldrich St. Louis,USA) and transfered to PVDF mambrane. Membranes were probed with mare sera diluted on 1:200, followed by incubation with goat Anti- Equine IgG diluted 1:800. Sera with different MAT results showed different patterns of reactivity with *Leptospira* antigens. Antigens corresponding to the molecular mass of LipL32, Lip L41 and Lip L21 were detected by 1D IB. In addition, reactive *Leptospira* proteins with molecular masses of approximately 50, 60, 80, 100 and 120 kDa also

were detected. Two-dimensional (2D) immunoblots showed better resolution of reactive *Leptospira* proteins. Negative sera also showed reactivity with *Leptospira* antigens, indicating that mares were previously exposed to *Leptospira* spp. and that 1D and 2D immunoblot can detect antibodies against *Leptospira* proteins in sera which were negative by MAT.



PP 24

USING DIFFERENT ADJUVANTS IN THE PREPARATION OF A VACCINE AGAINST LEPTOSPIROSIS IN HORSES

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Leptospirosis is a naturofocal infectious disease occurring in numerous animal species including humans, cases of which have been recorded all over the world. Infection rates in Russian Federation are on average as high as 22.3%. In some regions, however, they may reach or even exceed 40%.

Over the last several years the population of horses in Russian Federation has grown significantly as a result of increasing popularity of equestrian sport. Therefore the ultimate goal was to perfect medicines for specific prophylaxis of leptospirosis in horses and, more particularly, to test different adjuvants (aluminium hydroxide, oil and gel) used in the preparation of vaccines against leptospirosis in horses.



PP 25

DIFFERENTIAL PROTEIN EXPRESSION IN LUNG TISSUE IN A GUINEA PIG MODEL OF THE LEPTOSPIRAL PULMONARY HAEMORRHAGE SYNDROME

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Leptospirosis is a global zoonosis affecting most mammalian species. It is caused by pathogenic *Leptospira* spp. Clinical manifestations vary from chronic asymptomatic carriage to acute severe disease characterised by pyrexia, renal failure and jaundice (Bharti *et al.* 2003). More recently, a severe form of leptospirosis, the Leptospiral Pulmonary Haemorrhage Syndrome (LPHS), is being increasingly recognized in both humans and dogs (Trevejo *et al.* 1998, Schweighauser and Francey 2008). LPHS is characterized by rapidly progressive intra-alveolar haemorrhage and is associated with high mortality rates (Marotto *et al.* 1999). The pathogenic mechanisms of LPHS are poorly understood. Previous studies in a guinea pig model of LPHS demonstrated the deposition of IgM, IgG, IgA, and C3 in affected lung tissue in the absence of significant numbers of leptospiral organisms suggesting that LPHS is in part mediated by autoimmune mechanisms (Nally *et al.* 2004). Aim of the present study was to further elucidate the pathogenic mechanisms of LPHS by assessing differential protein expression in lung tissue of animals with LPHS and non-infected controls in a previously described guinea pig model of LPHS. Ethical approval was obtained for all animal work. Animals were injected intra-peritoneally with virulent *Leptospira* in EMJH medium or EMJH medium only (controls). Two-dimensional difference gel electrophoresis (2-D DIGE) was performed using lung tissue from 6 experimentally infected guinea pigs with LPHS and 6 non-infected controls. Proteins were separated on pH ranges of 3-10 and 4-7. Differentially expressed protein spots were identified (Progenesis SameSpots®) and identified via mass spectrometry. A total of 1569 (pH 3-10) and 1554 (pH 4-7) protein spots were successfully aligned between gels. Comparison of lung protein patterns revealed statistically significant ($p<0.05$; power >0.8; $q<0.01$) differential expression of 5 spots in the pH 3-10 range (4 up-regulated, 1 down-regulated in LPHS)

and of 130 spots in the pH 4-7 range (83 spots up-regulated, 47 down-regulated in LPHS). Significantly up-regulated proteins identified to date include: 60 kDa heat shock protein, laminin B1 and plastin-2. Significantly down-regulated proteins include annexin-A5-like protein, alpha-actinin-4-like isoform 1 and integrin alpha-3 isoform 2. Results of this study confirm differential protein expression in LPHS lung tissue compared to non-infected controls. Protein identification is ongoing and will further elucidate the pathogenic mechanisms of LPHS. LPHS has been described in many mammalian species and is therefore highly suited to a comparative research approach. While the guinea pig model is now well established as a model for human LPHS, lung tissue from naturally infected dogs with LPHS will also be included in future studies.



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