MEETING ABSTRACTS

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ORAL PRESENTATIONS

WG1 - THE “ONE HEALTH” CONCEPT IN THE ECOLOGY OF VECTOR-BORNE DISEASES

01 Ticks feeding on ruminants and humans in Greece

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Ticks are important vectors of disease and transmit an extensive range of viral, bacterial and protozoan pathogens to livestock in a wide variety of habitats. In recent years, diseases such as babesiosis, ehrlichiosis and anaplasmosis have all shown evidence of increased prevalence and distribution in various parts of Europe. However data concerning the prevalence of ticks and tick borne diseases present in livestock and humans in Greece are limited. In order to fill this gap we performed the current study to define the existence and prevalence of different tick species found in farm animals and humans. As regards livestock, we focused on ruminants (mainly sheep and goats and, in a lesser extent, cattle) since they are the only ones spend time on pastures. A sufficient number of farms all over the country were visited during 2 tick seasons (from March to October), taking different habitats and animal density around Greece into account. Ticks collected from ruminants originated from infected individuals who visited hospitals. In conclusion, the majority of ticks found in both animal species and humans examined belonged in the Rhipicephalus sanguineus group which is the main vector of Rickettsia conorii, while Hyalomma marginatum, the vectors of CCHF virus, were also present. The above results were more or less anticipated given the climatic conditions of the area, fact that also explains the low prevalence of Ixodes spp.

02 Risk assessment for West Nile Virus in Northern Greece (2010-2013)

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Since the large WN fever epidemic in Central Macedonia in 2010 caused by WN Lineage 2, with a total of 262 cases (197 neuroinvasive, incidence rate 1:140), WNV cases are reported every year from different areas throughout Greece (2011: 100 cases/75 neuroinvasive, 2012: 161/109, 2013: 86/51). WN fever has become one of the most important issues for the National Health Authorities in terms of vector borne diseases. From 2011 and onwards, for the surveillance of WNV in Central Macedonia, two major networks were established by Ecodevelopment in...
The role of Sergentomyia schwetzii in epidemiology of visceral leishmaniasis in Ethiopia

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Leishmaniasis is caused by a protozoan of the genus Leishmania and transmitted by the bites of phlebotomine sand flies. During the blood feeding, sand fly females inject saliva into the host thus affecting Leishmania transmission; in a naive host saliva enhances parasite virulence, in preexposed host it acts as the protective immunogenic agent by eliciting anti-saliva specific cellular and antibody immune response. Interestingly, anti-saliva antibodies in bitten hosts can be used in epidemiological studies as the marker of exposure and the risk marker of Leishmaniasis transmission. Ethiopia is endemic for visceral leishmaniasis caused by Leishmania donovani and transmitted mainly by Phlebotomus orientalis. However, the most abundant sand flies in the area belong to the genus Sergentomyia. Sergentomyia females prefer to feed on reptiles, but several studies reported mammals as the additional blood source. The main aim of this

Co-occurrence of Babesia microti, Bartonella spp., Borrelia burgdorferi s.l. and Anaplasma phagocytophilum in rodents from Lower Silesia, Poland

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This study aims to establish the relative contribution of rodent populations from diverse habitats to the occurrence of rodent-borne pathogens of public health significance. Rodents (n = 492) represented by Apodemus agrarius, A. flavicollis and Myodes glareolus, were captured in live traps in four localities of south-western Poland (2009-2012). For the analysis of co-occurrence of pathogens, Babesia microti, Borrelia burgdorferi s.l., Bartonella spp. and Anaplasma phagocytophilum, both blood and spleen samples were obtained from selected rodent specimens. The choice of genetic markers and primers was based on the literature data and our preliminary results. Conventional PCR was used for the detection of DNA of examined pathogens. Selected PCR positive products were purified and sequenced. BLAST searches were conducted in order to elucidate any homologies with previously deposited sequences in GenBank. The DNA of pathogens was detected in 66.7% of the rodents tested. We observed that among infected rodents, 40.5% were infected with at least two pathogens, while only 4.7% with all four pathogens. All three of the rodent species were infected with each of the examined pathogens. In examined rodent populations the prevalence of B. microti was 40.0%, Bartonella spp. 37.7%, B. burgdorferi s.l. 28.2% and A. phagocytophilum 17.7%.

In each of the tissue samples (blood or spleen), the prevalences of Babesia microti and Bartonella spp. were recorded as comparable, on rather high levels. Interestingly, the occurrence of these pathogens in both blood and spleen was only detected in 13% of the rodents. While examining the blood and spleen-derived DNA, it was found that as many as 92.7% of A. agrarius harbored at least one pathogen. Co-occurrence of 3-4 pathogens was most common in this rodent species (32.1% infected).

By examining the spleen and blood samples of rodents at the same time, we estimate that the prevalence of pathogens in these rodents is higher than the literature indicates. This would implicate the examined rodent species as a significant reservoir of pathogens with zoonotic potential. Additionally, the role of A. agrarius, now widespread in some regions of Europe, as a reservoir host needs to be emphasized. A. agrarius can act as a bridge between woodland habitats and periurban environments frequented by humans.

Due to the repeated outbreaks of blackflies, consequent economical losses and health problems, Serbia was considered as the most threatened European country in the past. During the last century (up to ‘60s), Simulium colombaschense caused enormous losses of livestock. Significant losses in poultry production caused by S. maculatum were also reported in 1958, while S. erythrocephalum caused severe dermatological problems in humans in 1965 and 1970.

In the last fifteen years, repeated outbreaks of blackflies and reemerging of bite related problems in humans were recorded in some parts of Serbia. The research objective was to update the knowledge of blackfly pest species distribution in Serbia, with a special attention to endangered regions in the present and the past. Samplings were conducted in the period 2003-2012. Immature stages were collected from submerged substrates: in the Danube river and its tributaries, the Nera river and the Nižava river. Adults were sampled close to the breeding sites by application of CO2 baited traps or by light traps.

In the lowlands 11 blackfly species were recorded. Two mammophilic species have been dominant: S. erythrocephalum in the Danube and S. ornatum (complex) in confluent streams. In 2010, S. erythrocephalum was recorded for the first time in the hilly area in southeastern Serbia. Periods of high adult population density of those two species coincided with the bite cases reports. In the Iron Gate region, 21 mainly mammophilic species were recorded in the Danube confluents exclusively. S. colombaschense, the main pest species in the past, was detected only in the Nera river, about 30 km upstream from the entrance of the Iron Gate.

Present state of blackfly fauna composition indicates the existing risk of outbreaks of some species in the future.

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study was to determine, whether S. schwetzi frequently bite domestic animals and thus may play some role in the pathogen transmission. Sera of domestic animals collected in three leishmaniasis foci were tested for anti-S. schwetzi IgG antibodies by EUSA using S. schwetzi salivary gland homogenate as an antigen. Altogether we tested 603 serum samples from five species: cattle, sheep, goats, donkeys and dogs. Sera of animals from nonendemic countries served as a negative control and the results were statistically evaluated. Significant seropositivity for anti-S. schwetzi IgG was found in about one third of domestic animals tested. The highest seropositivity was found in sheep (11/181), cattle (25/108) and goats (26/144), followed by donkeys (2/24) and dogs (10/37).

Our results suggest that sand flies of the genus Sergentomyia frequently bite domestic animals in Ethiopia. However, further studies are needed to investigate the role of Sergentomyia in transmission cycle of veterinary important pathogens, including Leishmania sp.

The study was supported by following grant projects: Grant Agency of Charles University 675012/2012, Czech Science Foundation 13-05292S and Bill and Melinda Gates foundation.

O6 Sustainable and multifunctional mosquito pest management: a pull opportunity and a push advice
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Since 2001, we have repeatedly detected pathogens during mosquito field studies in Austria (e.g. West-Nile-, Tahyna-, Usutu-virus and Plasmodium sp.). Recent surveillance discovered two invasive and two thermophilic mosquito species that were new for the Austrian fauna (Aedes japonicus, Ae. albopictus, Anopheles hyrcanus and Culiseta longiareolata). These species increase the possible spectrum of vector-borne communicable diseases in Austria. But until today flaviviruses and bunyavirides have only been detected in context with the endemic mosquito species. Little attention was given to control pests and to develop sustainable, useful and affordable projects for controlling the dynamic of these indigenous mosquito populations. We emphasize the need for integration of mosquito control aspects, which up to now were ignored within contemporary Austrian river construction projects and we ask for a Europe wide consideration of mosquito control within the EU-Waterframework Directive (WFD); in particular we recommend mosquito control elements within fish ladders which are prescribed for water storage structures all over Europe. We also report on a material which allows the pulling of mosquito in the dimension of landscape management. We present our experience and emphasize the great advantage of actions working in connection with constructive river engineering.

O7 Preliminary studies on dynamics of Culicoides spp. in western Romania in conjunction with some environmental factors
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Culicoides spp. play an important role as vectors for bluetongue and their dynamics is markedly influenced by the environmental changes. Based on these considerations, a study was conducted during May-June 2013 in the west part of Romania, which aimed capturing and identifying Culicoides spp. and tracking their population dynamics. We used two types of traps: (i) CDC Ondestepo mt mobile light and (ii) unconventional type (hand-made). Traps were placed in different areas and localities in the counties of Timiș, Arad and Caraș-Severin. They were followed over three consecutive nights in each location. In total 28 samples were taken.

In addition, abiotic parameters monitoring was performed (minimum temperature, maximum temperature, relative humidity and wind speed), evaluating their influence on Culicoides population dynamics. Data on atmospheric temperature (maximum and minimum temperature), wind speed and relative humidity were taken at each trap using an anemometer and an ambient thermo-hygrometer; for a more precise expression of data a conjunction with data taken from the website of the National Agency of Meteorology of the two months was made.

A total of 4354 Diptera specimens were captured during this research in Timiș, Arad and Caraș-Severin Counties, including three species having the role of potential vectors for bluetongue, namely Culicoides obsoletus 409 (9.02%), Culicoides pulicaris 239 (5.27%) and Culicoides nubeculosus 183 (4.03%). Based on the preliminary data obtained in Western Romania, it is considered that the population dynamics of Culicoides is influenced by abiotic factors such as maximum temperature, minimum temperature, average temperature, wind speed and relative humidity.

Of all the abiotic factors monitored, the average temperature, relative humidity and wind speed have a major role in the variability of the total number of Culicoides. Dynamics of Culicoides population is positively correlated with minimum temperature (11.5-16 °C).

The results obtained in previous studies conducted in this part of Romania show that the maximum temperature explained most variability in the total number of Culicoides and wind speed at least. Comparing to them, the results obtained in this study render that during May-June 2013 increased values of wind speed (1.8-2.5 m/s) and low relative humidity (60%) had a more negative influence on insect population dynamics, as compared with the maximum (30 °C) and average (15-16 ° C) temperatures.

O8 Changing epidemiology of the tick-borne bovine parasite, Babesia divergens
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Bovine babesiosis is caused by the tick-borne blood parasite, Babesia divergens. A survey of veterinary practitioners and farmers in Ireland in the 1980’s revealed an annual incidence of 1.7% associated with considerable economic losses. However, two subsequent surveys in the 1990’s indicated a decline in clinical babesiosis. Recent evidence from continental Europe suggests that, probably due to climate change, the distribution of the tick vector of B. divergens, Ixodes ricinus is extending to more northerly regions and higher altitudes. In addition, milder winters are thought to increase the window of tick activity.

In order to determine whether any such changes have affected the incidence of bovine babesiosis in Ireland, a questionnaire survey of farmers and veterinarians was carried out and compared against data from previous surveys. Our results indicate that while the incidence of clinical disease has continued to decline, cases occurred at any time of year. In contrast to previous surveys, affected farms were the same size as unaffected ones and there was no correlation between disease risk and the presence of deer on the land. Disease severity and mortality rates were increased because many infections were advanced by the time they were detected and treated. While the precise reasons for the decline in the incidence of redwater are unknown, a reversal of the trend could be devastating, as vigilance among farmers and veterinarians is flagging and the national herd is losing its protective immunity to disease.
Complement-mediated lysis of pathogens is the effective immune mechanism developed in the mammalian hosts, nevertheless, pathogens have also evolved complement evasion strategies to infect the hosts. Study of complement evasion mechanisms in various host help to understand many aspects like, pathogenesis, reservoir competence, association and host selectivity of vector-borne pathogens etc., and thus forms unique benchmarking to prevent the infection within the ‘One Health’ concept. The most effective complement evasion mechanisms employed by pathogens are: 1. binding of the host complement-regulatory/inhibitory proteins (CRPs) on their surface and 2. expression of CRP mimicking molecules. A comparative study of complement-pathogen interaction was performed, that includes three different hosts (human as susceptible, cattle as resistant and sheep as intermediate susceptible host) and a repertoire of *Borelia* (twelve species) and *Francisella* strains (three subspecies). Series of experiments like affinity ligand binding experiments, pull-down assays and mass spectrometry based protein identification revealed an array of surface proteins of *Borelia* (37 proteins) and *Francisello* (6 proteins) that bind human factor H or CRP or vitronectin to block complement activation. We also found that some stains of *Borelia* possess human CD46 mimicking molecule, while some *Borelia* and *Francisella* may express human CD59- homologous protein. Results show that these vector-borne pathogens possess multiple proteins that can bind or mimic various CRPs and block human complement. It is noteworthy that, two proteins of *Francisella* (LV5, Tu4 strains) bound vitronectin only, whereas, only two proteins of *Borelia* (of strains SKT-2 and DN127) bound ovine C4BP or factor H. It shows that both pathogens possess very few proteins that can bind ovine CRPs and insufficiently protect bacteria against ovine complement attack, which correlates with intermediate competence nature of sheep for both pathogens. None of the *Borelia* and *Francisella* strains, except *B. coriacae*, possessed factor H binding protein. This also correlates with the fact that only *B. coriacae* effectively evade bovine complement system and infect cattle. This correlates with non-competence nature of cattle for both pathogens. In summary, study of tangled association between surface proteins of pathogens, CRPs, complement evasion in various hosts and host-selectivity of vector born organisms is crucial to understand basic molecular principles of host-pathogen relationship. Moreover, identification and characterization of the molecules circumventing host complement system is also important aspects to develop therapeutic or preventive approaches.

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**WG2 - BARCODING, MOLECULAR DIAGNOSIS AND NEXT GENERATION SEQUENCING**

**O9** Vector borne pathogens and host complement system: a tangled tale

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Identification of insect vectors is primarily carried out using morphological features, which can be a time-consuming and difficult task. PCR-based approaches have been developed for the identification of a number of vector species. We have recently established matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), which has come of age for the high throughput identification of microorganisms, to identify imagos and larvae of biting midges (*Culicoides spp*.), and the technique has proven its suitability to accurately identify field-collected biting midges on a large scale. We have extended our insect vector reference database to also include culicid mosquito and phlebotome species. Currently, biomarker mass sets are determined for the imagos of 15 phlebotomines (9 *Phlebotomus* spp., 3 *Lutzomyia* spp., 3 *Sergentomyia* spp.), as well as for both imagos and larvae of 38 Central European culicid species, including all established and presumptive Aedine invasive species such as the Asian tiger mosquito *Aedes albopictus* or the Asian bush mosquito *Ae. japonicus*. In addition, biomarker mass sets are determined for indigenous and invasive container-inhabiting Aedine mosquitoes (*Ae. aegypti, Ae. albopictus, Ae. atropalpus, Ae.cretinus, Ae. geniculatus, Ae. japonicus, Ae. koreicus, Ae. philocheir, Ae. triseriatus*) allowing to identify eggs collected in oviposition traps in the framework of surveillance programs. Eggs can be identified by MALDI-TOF MS either singly or in pools (demonstrated for pools of 10 eggs, but possibly also for larger ones) for those species of which at least 3 eggs are present in the
sample. We are continuously expanding our database, e.g. to also include the remaining mosquito species whose eggs can be encountered in surveillance programs of container-inhabiting mosquitoes in Europe, i.e. species usually breeding in tree holes or rock pools in southern Europe (Ae. berlandei, Ae. echinus, Ae. gilvoculatai, Ae. mariae, Ae. pulcritarsis, Ae. zammitii, and Orthopodomyia pulcipalpis). Taken together, protein profiling by MALDI-TOF MS is a quick and inexpensive tool to accurately identify adult and immature stages of insect vectors collected in the field, and the technique has the potential to become the method of choice for a centralized, robust and high throughput screening of insect vector populations in connection with surveillance programs. The simple sample preparation can be done in peripheral laboratories and the slides be sent to the measuring laboratory. The future application of the method will include the accomplishment of the measurement with a mass spectrometry device anywhere and the identification via our online platform.

012

Multiple detection of pathogens in ticks: development of a high throughput real time PCR chip used as a new epidemiologic investigative tool
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Worldwide, ticks transmit more pathogens than other arthropods. Around 60 bacteria, 30 parasites and 100 viruses have been registered as tick-borne pathogens; a third of these pathogens are responsible for zoonoses. Usually, detection of tick-borne pathogens depends on the tick species collected: assays are performed for a restricted number of pathogens that are known to be transmitted by a particular tick species collected at a particular site. To better understand the epidemiology of tick-borne pathogens, it will be important to detect in each sample (one tick or one pool of ticks) most of the diseases they potentially transmit, regardless of the tick species. The aim is therefore to develop a new epidemiologic investigative tool which could detect high number of tick-borne pathogens by real time PCR.

We developed a chip (BioMark™ dynamic arrays, Fluidigm Corporation) targeting pathogens of worldwide distribution transmitted by ticks. The designed epidemiologic arrays may detect 48 pathogens in 48 samples corresponding to 2304 qPCR reactions on the same time. Specific primers and probe have been designed for each pathogen and their specificity has been tested in silico with Blast. To begin, we targeted: (i) 37 pathogens whose Francisella tularensis, Coxiella burnetii, Neohelichris mikurensis, 5 species of Anaplasma, 3 species of Ehrlichia, 8 species of Borrelia, 2 species of Bartonella, 4 species of Rickettsia, 10 species of Babesia and 2 species of Theileria, (ii) 5 species of ticks whose 3 species of lice and 2 species of Dermacentor. Sensitivity of primers and probe has been tested on a dilution range of reference DNAs of the targeted pathogens on a Lightcycler 480. Specificity then has been tested on a BioMark™ dynamic array. The chip was secondly evaluated on field samples corresponding to 47 pools of 25 nymphs collected in two sites in France, the Netherlands and Denmark (corresponding to 7050 nympha in total). We successfully detected and determined the prevalence of Anaplasma phagocytophilum, Neohelichris mikurensis, Rickettsia helvetica, Bartonella henseae, five different genospecies of Borrelia burgdorferi s.l., the recently identified pathogen Babrella miyamotii, and two parasite species Babesia divergens and Babesia venatorum. This fast and low-cost tool allows comprehensive testing of tick-borne pathogens and can be customized to fit regional demands or to accommodate new or emerging pathogens. The tool represents a major improvement for surveillance and future epidemiological studies.

013

Next generation sequencing as a novel tool for diagnostics of apicomplexan pathogen in ticks and mammalian hosts
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Among apicomplexan parasites, ticks are known vector of several species belongs to three protozoon genera (Babesia, Theileria and Hepatozoon). During their life cycle, tick-transmitted apicomplexan parasites alternate between asexual (in vertebrate host) and sexual (in ticks) developmental stages. The major constraint for the proper diagnostics of those pathogens is the high possibility of mix infection, both in ticks and vertebrate hosts, with several species or genotypes. The aim of this study was to apply the Next Generation Sequencing (NGS) as a method of choice for simultaneous determination of the full spectrum of apicomplexan pathogens in ticks and the mammalian hosts. Therefore, A pair of universal primers were designed to flank a 167 bp barcode region of the 18s rRNA gene of all Babesia, Theileria and Hepatozoon species. The new protocol was evaluated on DNA samples isolated from 195 dogs and 144 ticks (Rhipicephalus aruntus and R. pulchellus) collected from Northern Kenya. In total 301 sample (89%) were positive for apicomplexan infections; ranging from single to multiple infection with one species or several species and/or genotypes in a single sample. The most abundant apicomplexan pathogens were Hepatozoon followed by Babesia and Theileria, respectively. Further, the result shows that the barcode region entails enough variability that allows identifying the pathogen up to species and genotypes level. The exact methodological and results detailed will be presented later. This work was supported by the project OP VK CZ.1.07/2.3.00/30.0014.

014

Zoonotic bacterial survey assessed by next-generation sequencing
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Rodents represent one of the major sources of pathogens; most of them are vectored by ticks. Tick-borne diseases are very diverse and cause a wide range of diseases in livestock and human populations. Rodents, carrying ticks, are distributed across a vast range of natural habitats and they often live in close contact with humans and their domestic animals, exposing them to zoonoses circulating in natural ecosystems. In this study, we analyse the potential of Next-Generation Sequencing (NGS) technologies as a tool for large-scale survey of bacterial zoonotic pathogens carried by rodents. We combined two NGS approaches in order to establish a list of zoonotic bacteria and to identify their distribution in individuals of rodents in natural populations.

Briefly, RNA/DNA were extracted from the spleen of 192 rodents collected in Northeast France. RNA from all samples was pooled and submitted to high throughput RNA sequencing (RNAseq). Succeeding de novo assembly, bacterial contigs were assigned to the closest already-known taxa,
revealing a list of zoonotic bacteria for the whole sample. Parallel, DNA samples were submitted to meta-barcoding approach: each sample was amplified by PCR using universal primers tagged at the V4 region of the 16S rRNA. The amplified templates were multiplexed and submitted to 454-sequencing. The resulting dataset was demultiplexed using a home-made pipeline that assigns each read to a sample using the tagged primers, following these were processed using Mothur pipeline to construct OTUs and classify them using the RDP database. These methods allowed listing bacteria detected in each rodent and, so derive the prevalence, coinfections and bacteria interactions.

DNA/RNA of the following genera were detected by both approaches, RNAseq and DNA 16S metabarcoding: Bartonella, Leptospira, Rickettsia, Treponema, Neisseria, Spiroplasma, Klebsiella, Listeria and Shigella. Some unexpected genera were detected; such as Orientia, up to now only found in Asia or Helicobacter, generally thought to be restricted to animal guts. Several bacterial pathogens explored by RNAseq was passed undetected by 16S metabarcoding; Anaplasma, (Neo)Ehrlichia, Wolbachia, Brucella, Coxielia, Campylobacter, Mycoplasma, Salmonella, Yersinia, and Francisella. Furthermore, 16S metabarcoding allowed to specify prevalence of bacteria within our sample, and revealed high level of coinfection in wild rodents. Our data demonstrate that NGS allows having a rather complete screening of pathogenic bacteria present in animal reservoirs without any a priori on their presence, while having a price compatible with cohort studies. NGS approaches are becoming the new routine approaches in large-scale epidemiological studies.

O15 To be or not to be co-infected

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Ticks can transmit a large spectrum of pathogens including bacteria, viruses and parasites with a significant number of these pathogens being agents of emerging infectious diseases. In Europe, the most prevalent tick-borne disease is Lyme Borreliosis, caused by the bacteria Borrelia burgdorferi s.l. In most cases, Lyme Borreliosis is well diagnosed. However, beside these typical cases, patients bitten by ticks can be infected by many other pathogens (bacteria: Anaplasma spp., Bartonellosis spp., Rickettsia spp.; parasites: Babesia spp., Theileria spp.; and arboviruses: TBEV) that are more difficult to diagnose. Moreover, co-infections between several of these pathogens might also occur. Clinical surveys show that patients coinfected by several tick-borne pathogens present more severe symptoms and a longer duration of illness than those infected by a single pathogen. The overall objective of our study was to evaluate tick-borne pathogen coinfecetion in ticks and the consequence of those coinfections for human health. Using the high throughput real-time PCR chip, we detected the DNA of the 37 major tick-borne pathogens in a cohort of questing adult of Ixodes ricinus ticks collected in Ardennes (France). We identified that 60% of ticks were infected by at least one pathogen and half of the infected ticks were coinfected. We then evaluated the risk for simultaneous infection of those pathogens to humans by detecting the DNA of the most prevalent tick-borne pathogens in the blood of patients bitten by ticks. Our data illustrated the importance of coinfection, and highlighted the necessity to evaluate coinfection in the context of tick-borne diseases.

WG3 - GEOSPATIAL TOOLS IN VECTOR RESEARCH

O16 Transmission risk of Dirofilariasis in Portugal

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Dirofilariais is an important and potential fatal vector-borne canine disease, endemic in Portugal as well as in other Mediterranean countries, particularly due to its geographic and climatic conditions. Despite improved diagnostic methods and effective preventives, infections of Dirofilaria spp. continue to increase. Forty-one species of mosquitoes have been detected in mainland Portugal although only Culex theileri was found naturally infected with Dirofilaria larva. Therefore, a study was addressed to assess Dirofilaria transmission risk in Portugal based on air temperatures, estimating the potential days with temperature values compatible with the transmission of Dirofilaria larvae between mosquito and reservoirs. A degree-days model based on Dirofilaria Development Units (DDUs) was used, considering minimum and maximum daily temperatures registered in five Portuguese meteorological stations, obtained in the platform wunderground.com. Preconditions for the model were: a threshold temperature of 14°C below which Dirofilaria development will not proceed in mosquitoes; 130 cumulative DDUs for larvae to reach infectivity; and a maximum life expectancy of 30 days for mosquito vectors.

DDU was evaluated in three areas of Portugal mainland - Porto (North, 41°9’0”/8°37’0”), Lisbon (Centre, 38°43’0”/9°8’0”) and Faro (South, 37°10’0”/7°56’0”), as well as in two different Portuguese islands in the Atlantic Ocean - São Miguel, Azores (37°4’0”/25°40’0”) and Madeira (32°38’0”/16°54’0”), over the period from 2003 to 2013. The results show that the highest number of potential days with suitable conditions for Dirofilaria transmission was registered in Madeira with an average of 209.9 days/year, followed by Faro 175.2 days/year, Lisbon 163.5 days/year, Azores 140 days/year and Porto 117.2 days/year. The year 2006 was the only with a maximum number of potential transmission days (1758) across the selected stations. During the last decade, 130 DDUs were inclusively registered from June to November in Porto and Azores Island, from April to November in Lisbon and Faro, and an uncommonly extended period from April to January in Madeira Island. An average Dirofilaria seasonal risk period ranged from a minimum of 5 months/year in Porto, 5.6 months/year in Azores, 6.4 months/year in Lisbon, 6.9 months/year in Faro and 8 months/year in Madeira.

These results are in accordance with the existing prevalence data and reinforce the value of geospatial tools, mapping the risk and helping to monitor and forecast future epidemiological trends, ensuring a continued surveillance and a “One Medicine-One Health” integrated approach.

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O17 Roe deer used as indicator species for a country wide survey for the occurrence of Tick Borne Encephalitis in Austria

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Although 85% of the Austrian citizens are vaccinated against Tick Borne Encephalitis (TBE), still ~100 clinical cases are reported each year. Until now, risk maps are created by using human cases only, albeit this source is biased due to the high level of vaccinated persons, uncertainties about the location of infection and undocumented cases because of inapparent infections. To overcome this problem, several solutions are proposed: Investigations of ticks by PCR seems to be reasonable, when looking on small areas. Another possibility to gain knowledge about TBE occurrence is to investigate the sera of wildlife animals for antibodies against TBE. Due to the fact that roe deer can be found all around the whole Austrian landscape, but its habitat is restricted to a relatively small area, we chose this species as indicators for the monitoring of antibodies against TBE. Together with the hunting organisations blood samples from whole Austria was collected starting in September 2013 and screened for the
occurrence of antibodies against TBE using an indirect immuno-fluorescence assay test. A total of 928 serum samples from roe deer were collected so far. The analysis of ~100 samples showed positivity to TBE in 2 roe deer. The shooting/finding location of each roe deer was georeferenced and a geographical information system was constructed. The findings of this survey on roe deer will be used to update the TBE risk map in Austria.

O18 Methodological caveats in the environmental modelling and projections of climate niche for ticks, with examples for *Ixodes ricinus* (Ixodidae)
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There is a growing interest in inferring the associations of health-threatening arthropods to capture the climate niche to which they associate, projecting such inference on a territory. This is intended to predict the range of distribution of the tick and to understand their responses to climate scenarios, using the so-called correlative models. However, some methodological gaps might prevent to obtain an adequate background against which test hypotheses. We explore, describe and illustrate these procedural inaccuracies with examples focused on the tick *Ixodes ricinus*, and how these may affect the modelling outcomes. We aim to provide a background of rules against which develop reliable models for these parasites. The use of partial sets of occurrences of the tick might produce unreliable associations with climate because the algorithms cannot capture the complete niche to which the tick is associated. Reliability measures of the model cannot detect these inaccuracies, and undesirable estimations of the niche will prevail in the chain of further calculations. The use of inadequate environmental variables (covariates) may lead to inflation of the results of the model through two statistical processes, called autocorrelation and collinearity. The high collinearity existing in climate products derived from interpolation of climate recording stations is demonstrated, and it is explicitly advised the training of climate models with satellite-derived information of climate, of which collinearity of the time series has been removed through a harmonic regression. The high uncertainty if inference on the climate niche is applied into different time slices, like projected climate scenarios is also pointed in the results.

O19 Illegal waste sites as potential micro foci of Mediterranean Leishmaniasis
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Apart from being against the law, illegal waste dumping also poses a threat to human health and to the environment. Solid and decomposing waste is associated. Reliability measure of further calculations. The use of adequate environmental variables (covariates) may lead to inflation of the results of the model through two statistical processes, called autocorrelation and collinearity. The high collinearity existing in climate products derived from interpolation of climate recording stations is demonstrated, and it is explicitly advised the training of climate models with satellite-derived information of climate, of which collinearity of the time series has been removed through a harmonic regression. The high uncertainty if inference on the climate niche is applied into different time slices, like projected climate scenarios is also pointed in the results.

O20 Mapping and modeling *Dirofilaria* infections in Europe
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Climate change and increasing temperatures are a global phenomenon that can influence the dynamics of a number of hematophagous arthropods, vectors of pathogens with importance in human and veterinary medicine. In fact, climatic changes, together with an increase in the movement of dogs across Europe, have caused an increase in the geographical range of *Dirofilaria immitis* and *D. repens* infections. A Geographic Information System based on thermal regimen was constructed to identify areas potentially suitable for *Dirofilaria* transmission in Europe. These models are based on evidence that: i) there is a threshold of 14 °C below which *Dirofilaria* development will not proceed in mosquitoes; ii) there is a requirement of 130 growing degree-days for larvae to reach infectivity; and iii) there is a maximum life expectancy of 30 days for a mosquito vector. The output of these models predicted that the summer temperatures (with peaks in July and August) are sufficient to facilitate extrinsic incubation of *Dirofilaria* even at high latitudes. Recently, an additional model was constructed to verify the influence of temperature in the course of three decades (1980-1989, 1990-1999 and 2000-2012) on the risk of infection by *Dirofilaria* in Italy. The results showed an expected increasing trend of temperatures, an increase of the *Dirofilaria* generation numbers into the mosquitoes and a significant extension of the infection risk from 5-6 months (1980-1989) to 6.5 months (1990-1999), up to more than 7 months (2000-2001). These findings show that geospatial tools are very useful for mapping, monitoring, forecasting and surveillance of both heartworm and subcutaneous dirofilariasis.

WG4 - PHYLOGENETICS AND PHYTOGEOGRAPHY OF VECTORS AND VECTOR-BORNE PATHOGENS

O21 Phlebotomine sand flies on the crossroads of Anatolia: transmitted diseases and vectors
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The Western Palearctic (WP) is composed of Europe, Middle East and North Africa. In this territory, the Mediterranean Sea, and the land under the influence of the Mediterranean Sea is the most important geographical
character for both migration and dispersion of organisms; especially for invertebrates including sand flies. Anatolia (Asia-Minor) takes place on the crossroads of this area and these events.

The phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) are vectors of several infectious pathogens causing leishmaniasis and arbovirus infections due to phleboviruses. Several of these diseases have wide geographical distributions in the WP, and give rise to occasional epidemic outbreaks. In numerous countries, increasing risk factors are making sand fly-borne diseases a major public and veterinary health problem. Many studies on phylogenetic relationship among sand fly taxa, their distribution, population structure and diseases of phlebotomine species have been already performed, but there are still many gaps waiting to be filled up, especially, Anatolia. In this point, scientists have to discuss some deficiencies under cover of geography, history and phylogenetic studies to understand the mechanisms of distribution of both sand fly species and their pathogens in Anatolia.

In this presentation, updates in distribution of sand fly species with state of art maps of EU-VIBRONET project, possible new species, leishmaniasis and phlebovirus epidemiology will be discussed with an emphasis on several studies performed by our group between 2000 and present in Anatolia. Studies were supported by EU-FFP Edenext project, HU-Scientific Research Foundation and Turkish Scientific and Technical Research Council.

O22 A molecular survey on host specificity of feline and canine Hepatozoon in model site of northern Kenya

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Species of the genus Hepatozoon are apicomplexan parasites transmitted by variety of hematophagous arthropods to a wide range of intermediate hosts. Hepatozoon infections are broadly distributed around the world and the presence of this parasite is confirmed both in cats and dogs. Until now, two species from canines (H. canis, H. americanum) and a single species from felines (H. felis) have been reported. While the canine Hepatozoon spp. are transmitted by ticks, the vector for H. felis remains unknown. The aim of presented study was to determine the prevalence and the diversity of Hepatozoon in population of domestic cats and dogs living in close contact in rural communities of Samburu pastoralists in northern Kenya and to evaluate the existence of possible cross-transmissions between both hosts. Between years 2007-2012 in total, 135 and 258 blood samples from cats and dogs from the area of Mt. Kulal (5 localities) was collected, respectively. The DNA of blood (preserved in ethanol) was extracted using phenol-chlorophorm method and followed by conventional PCR screening. First round of PCR was done by using Piropolasmid-F and Piropolasmid-R primers, amplifying 185 RNA gene of Hepatozoon spp. (400 bp). A second PCR assay was performed in samples positive by Piropolasmid primers to amplify a larger fragment (1400 bp) of the 18S rRNA gene. All PCR amplicons coming from positive samples were sequenced and determined according to the BLAST match. In cats, this revealed 110 out of 135 (81.5%) positive samples, of which we obtained 104 sequences with an identity of 98% - 100% to an existing GenBank accession. In total, 77 sequences were confirmed as Hepatozoon felis, 26 samples was proven to be Hepatozoon sp. and a single sample was determined as Hepatozoon canis with an identity of 96%. Remaining sequences of 258 (47%) were positive and we gained 107 sequences of which 105 showed similarity to Hepatozoon canis and the remaining two proved to be Hepatozoon sp. Despite a close contact of hosts, obviously cats and dogs do not share the same parasite, which demonstrates a great majority of clearly identified Hepatozoon canis/Hepatozoon felis. Mentioned facts could be explained either by strict host specificity or by presence of different vector or by both statements.

O23 Questing for the identity of Hepatozoon in foxes

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Apicomplexan parasites of genus Hepatozoon invade the blood cells of many mammalian species, being transmitted by range of arthropods. In domestic carnivores, three species of Hepatozoon were described to date: H. canis and H. americanum in dogs and H. felis in cats. The classification of Hepatozoon in wild carnivores is still not complete due to lack of field and experimental data as well as phylogenetic studies. The aim of this study is to carry out a survey on the prevalence and diversity of Hepatozoon sp. in red foxes Vulpes vulpes. Samples of tissues were collected from dead foxes in 11 counties of Romania; 91 samples of liver tissue were examined in total. DNA extraction was performed with commercial kit according to the manufacture’s protocol. Hepatozoon sp. DNA was detected by PCR using primers amplifying 400-600 bp long part of 18S rRNA gene. These primers are commonly used for diagnostic purposes in dogs and in some studies also for detection of Hepatozoon sp. in wild carnivores. PCR products were sequenced to validate positive results of reaction. DNA of parasite was confirmed in 55% of examined samples. Recent findings classified Hepatozoon sp. in foxes and other wild canids in Europe as H. canis. However, Rhipicephalus sanguineus, the only known vector of H. canis in Europe, is absent in most of our sampling sites. Moreover, this tick is typical for the dogs but rare or even absent in foxes. In order to clarify the identity of the parasite, the next step of our study is to focus on amplification of longer or full segment of 18S rRNA gene to allow more accurate phylotypic analyses and comparison with H. canis sequences from dogs and Hepatozoon isolates from other carnivores.

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O24 Anaplasma phagocytophilum - pathogen with a zoonotic potential

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Anaplasma phagocytophilum has for decades been known to cause the disease tick-borne fever (TBF) in domestic ruminants in bovines -infested areas in northern Europe. Later studies have shown that the infection is widespread on the continent. A. phagocytophilum is able to persist between seasons of tick activity in several mammalian species and movement of hosts and infected ticks on migrating animals or birds may spread the bacterium. A. phagocytophilum encompasses multiple genetic strains, a broad host range and has pathogenic potential in several other mammalian species, including humans. Identification and stratification into phylogenetic subfamilies has been based on cell culturing, experimental infections, PCR and sequencing techniques. The clinical symptoms vary from subclinical to fatal conditions, and considerable underreporting of clinical incidents is suspected in both human and veterinary medicine. In human, the most common clinical manifestations are flu-like symptoms and are due to tick attachment. In Europe, human granulocytic anaplasmosis (HGA) was first described in 1997. Since then only few cases of HGA have been reported. The reason for the suspected underreporting may be lack of awareness among physicians, unspecific clinical signs, lack of available adequate hosts and non-virulent strains of A. phagocytophilum involved. Phylogenetic studies indicate that stains isolated from humans, dogs and horses from Europe belongs to the same clonal complex, while
similar studies on isolates from wild and domestic ruminants indicate that they are unlikely to harbour variants of *A. phagocytophilum* that are infectious to humans. In contrast, wild boar and hedgehogs are suspected to be competent reservoir hosts for human variants. However, the natural transmission cycles of various *A. phagocytophilum* strains, the involvement of their respective hosts and vectors, and in particular their zoonotic potential, have to be unravelled. Updated information concerning the zoonotic potential of *A. phagocytophilum* will be presented.

**O25**

Strain diversity of *Borrelia burgdorferi* sensu lato in Serbia

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The geographic distribution of *Borrelia burgdorferi* sensu lato species in Europe shows very dynamic spatial and temporal variations. Since different *Borrelia* species usually coexist with different clinical manifestation of Lyme borreliosis, knowledge of the geographic distribution of the pathogen is very important for understanding the ecology and epidemiology of the disease. Our previous studies, based on direct molecular methods, revealed high diversity of species of the *B. burgdorferi* s.l. complex and unexpected high prevalence of *B. lusitaniae* in bovine ruminants from Serbia. The aim of the present study was further isolation and typization of viable *B. burgdorferi* s.l. strains from vectors in Serbia as a basis for studies of biological, genetic and ecological variations.

A total of 248 adult *I. ricinus* ticks collected from 24 localities were processed for cultivation. Prior to cultivation, all ticks were disinfected, then triturated in BSK H medium. The culture tubes were incubated at 33°C for 3 months. Cultures were periodically examined by dark field microscopy. For confirmation of isolated strains, “seminedent” PCR for the flaB gene was performed. Determination of *B. burgdorferi* s.l. species was carried out by the RFLP technique, using restriction enzymes *MseI* and *DraI* on the previously amplified 5·23S rDNA intergenic spacer (Postic et al., 1994). For *Borrelia* identification by RT-PCR targeting the hbb gene protocol of Portnoi et al. (2006) was followed. Sequencing of the 5·23S rDNA intergenic spacer and flaB gene was performed for phylogenetic analysis.

Thirty-four spirochete cultures were isolated and subjected to further genotyping analyses. According to the RFLP patterns of the 5·23S rDNA intergenic spacer, specimens were determined as: *B. lusitaniae*, *B. afzelii*, *B. burgdorferi* s.s., *B. garinii* and *B. valaisiana*. For all strains identified as *B. lusitaniae*, *B. garinii*, *B. afzelii* and *B. valaisiana* according to the Msel-RFLP method, results of RT-PCR were in absolute agreement. However, Tm values for all strains identified as *B. burgdorferi* s.s. according to RFLP patterns were in the range for *B. lusitaniae* strains (64.17 to 64.58 °C). Thus, it was impossible to distinguish *B. burgdorferi* s.s. and *B. lusitaniae* strains by this method. Sequencing analysis for strains identified as *B. lusitaniae*, *B. garinii*, *B. afzelii* and *B. valaisiana* was in agreement with Msel-RFLP and RT-PCR results. In cases of uncoordinated Msel-RFLP and RT-PCR results, sequencing analysis confirmed unclear strains as *B. lusitaniae*. The results of this study showed *B. lusitaniae* to be the species with highest prevalence in *I. ricinus* ticks from Serbia.

**WG5 - RARE AND EMERGING VECTOR-BORNE PATHOGENS**

**O26**

Preliminary results on the seroprevalence of *Angiostrongylus vasorum* and co-infection with *Dirofilaria immitis* in shelter dogs from Portugal

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Angiostrongylosis and dirofilariosis have recently revived the attention of the scientific community due to their emergence in several geographical areas. *Angiostrongylus vasorum* is responsible for verminous pneumonia and also for neurological, cardiovascular and coagulation disorders while *Dirofilaria immitis* is responsible for right side heart failure. Portugal is an endemic country for *D. immitis*. However, prevalence data concerning *A. vasorum* is scarce. Considering that documented cross-reactions between these parasites may lead to incorrect or misfit diagnoses, we aimed to assess the prevalence and potential co-infections of both canine heartworms.

An epidemiological survey was conducted, involving 341 sera collected from shelter dogs housed in three coastal districts of Portugal: Coimbra, Santarem and Setubal. Sera were tested for circulating *A. vasorum* antigens by a sandwich-ELISA using mono and polyclonal antibodies (sensitivity 95.7%, specificity 94.0%, Schnyder et al., 2011) and for specific antibodies against purified *A. vasorum* adult stage antigen (sensitivity 85.7%, specificity 98.8%, Schucan et al. 2012). In order to detect the presence of *D. immitis* circulating antigens, a commercial kit WITNESS® Dirofilaria (Symbionics Corp., Europe, sensitivity 100% and specificity 98.3%) based on rapid immunomigration technology was used. Statistical analysis was performed with SPSS program and estimated prevalence was obtained using Agresti-Coulth method.

Regarding *A. vasorum*, a total of 1.17% (4/341, 95% Confidence Intervals, CI: 0.3-3.1%) of the animals were positive in both ELISAs, while 1.76% (6/341, CI: 0.7-3.9%) were only antigen-positive and 2.35% (8/341, CI: 1.1-4.6%) only antibody-positive. Positive dogs were from the three surveyed areas. Concerning *D. immitis*, a total of 7.92% of the animals were positive (27/341, CI: 5.5-11.3%), also from the three areas. Out of the 341 sera analysed, one dog was positive for *D. immitis* circulating antigen and *A. vasorum* antibody-positive only.

These results show a slightly higher seroprevalence of angiostrongylosis in Portugal in comparison with seroprevalence found in UK, Italy, Germany and Poland, probably explained by the fact that this study was performed with shelter dogs, usually not under prophylaxis and therefore at a higher risk of infection.

To the authors’ knowledge, this is the first study performed in Portugal to assess *A. vasorum* seroprevalence in dogs and *D. immitis* co-infection. We expect that the obtained data will enhance the awareness of veterinary practitioners concerning these diseases and reinforce the importance of a more targeted preventive therapy for heartworms in companion animals in Portugal.

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**O27**

*Leishmania tropica* infection in wild and domestic canines

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*Leishmania tropica* is a causative agent of cutaneous leishmaniasis in the Middle East, North Africa and some parts of southeastern Europe. It has also been described as a cause of human visceral leishmaniasis. Although cutaneous leishmaniasis caused by *L. tropica* is usually considered an anthropoontonic infection transmitted between people directly by phlebotomine sand flies without the involvement of an animal reservoir, in Israel, Jordan and the Palestinian Authority it is a zoonosis with the rock hyrax (*Procavia capensis*) as a main reservoir host. Golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) have also been found to be infected with *L. tropica* in Israel and are assumed to have a role in spreading the infection to distant locations, but clinical signs of infection in these wild canids have not been detected.
In the domestic dog, *L. tropica* infection has been reported in only a few cases from Morocco and Iran where infection was mostly described as involving the visceral organs. While some surveys describe the detection of parasitic infection from dog organs by culture or PCR without much detail on the manifestations of disease, reports from Morocco described two dogs infected with *L. tropica* with clinical manifestations similar to those found in canine *L. infantum* infection including generalized lymphadenomegalic, oncogryphosis, alopecia, keratoconjunctivitis, and also glomerulonephritis in one case. A report from northwestern Iran also described *L. tropica* in a dog with cutaneous and visceral involvement comparable to canine *L. infantum* infection. A young dog from a focus of *L. tropica* human leishmaniasis near Jerusalem, with a large proliferative red mucocutaneous lesion between the upper lip and the nose was recently found to have mucocutaneous leishmaniasis caused by *L. tropica*.

In conclusion, domestic and wild canine infection with *L. tropica* may be more prevalent in areas of endemic human *L. tropica* cutaneous leishmaniasis than currently recognized, and canines should be evaluated as possible additional reservoirs for human infection.

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**O28**

Identification of *Candidatus Neoehrlichia mikurensis* and Anaplasma species in wildlife from Croatia

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**O29**

New insights into the biology and ecology of *Acanthocheilonema reconditum* (spirurida: onchoeridieae)

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Among filarioids infesting dogs, *Acanthocheilonema reconditum* has a global distribution and epidemiological data indicate that it is the most prevalent or even the sole filarioid species-infecting dogs in some regions of the Mediterranean basin. For instance, in southern regions of Italy the prevalence is as high as 13.3%, and an annual incidence of 5.9% was estimated in naturally exposed dogs. In spite of its wide distribution and its suspected zoonotic potential, scant information is available on the biology of this filaroid. Nonetheless, recent studies have enhanced current scientific knowledge on the biology and ecology of this nematode in naturally infected dogs. Recent data indicate the absence of any defined periodicity of blood circulating microfilariae with peaks recorded either diurnal or nocturnal. With regard to the life cycle, fleas were confirmed to be vectors of *A. reconditum*, whereas the role of ixodid ticks (i.e., *Rhipicephalus sanguineus* sensu lato) as vectors of this filaroid species has been definitely rejected. The full development of microfilariae to infective forms occurs in the experimental infected cat flea *Ctenocephalides felis felis* in about 15 days. In addition, localization and size of developing larvae inside infected flea suggest that this arthropod might act as an intermediate host throughout the infection of infected fleas rather than the inoculation during the blood meal on dogs. If confirmed, this route of *A. reconditum* transmission is unique, differing from that of other filarioids affecting dogs such as *Dirofilaria immitis* and *Dirofilaria repens*, which are actively transmitted through the bites of mosquito vectors.

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**O30**

How entomological studies can help the control of mosquito-borne diseases: a five-years experience in north-eastern Italy

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North-eastern Italy is particularly suitable for mosquito survival, due to its climate, landscape and abundance of wild/domestic animals. After the emergence of West Nile Virus (WNV) in 2008, entomological studies were implemented. Here we describe how entomological data were managed to optimize surveillance and control of mosquito-borne pathogens. CDC-CO2 traps were used (May-October, 2009-2013). In one site, captures with CDC and gravid traps were organized every 2hrs for 24hrs. In other three sites pre- and post-disinfection captures were done. The mosquitoes were screened by RT-PCR for *Flaviviridae* and a sub-sample for Bunyaviridae. Host preference of *Culex pipiens* was assessed by PCR blood meal analysis. Mapping, modelling and spatial analyses were done using entomological data to identify correlations with climate, landscape, animal and human infections.

More than 700,000 mosquitoes were collected, with *Cx.pipiens* the most abundant (80%) and the only vector of WNV and USUV. Tahyna virus was isolated once in *Ochlerotatus caspius*. *Cx.pipiens* fed preferentially on birds (76%), mainly blackbird, sparrow, magpie and collared dove, but not on humans. Diel activity showed that *Cx.pipiens* changed its host searching activity according to the season and defined the favorable period for oviposition. The success of disinfection measures in reducing *Cx.pipiens* density varied according to the methods used. The contribution of density-depended control vectors population growth resulted greater than any environmental factor on its own. Overall the most significant predictors of *Cx.pipiens* dynamics included length of daylight, population...
density and temperature in the 15 days prior to sampling. Precipitation, number of rainy days and humidity had limited importance. Linear models detected significant relationships between WNV in humans and mosquitoes. Spatial analysis detected clusters of WNV occurrences for all the hosts, identifying an area where to focus surveillance and promptly detect WNV re-activation.

In long term studies the mosquito species composition, seasonality, distribution, abundance and pathogen rate of infections were assessed. The blood-meal analyses indicated possible bird targets for surveillance. The control of the efficacy of disinfestations highlighted the need for harmonic guidelines. Modelling indicated the need to incorporate density dependence in development of the key environmental factors for robust prediction of Cx. pipiens population expansion, helping to identify when and where an increase in vector population size and WNV transmission risk should be expected. The results stressed also the necessity to improve the density and the frequency of mosquito captures.

**Funding:** Veneto and Friuli Venezia Giulia regions, Italian Ministry of Health.

### 031 Increased seroprevalence of lymphocytic choriomeningitis virus infection in mice sampled in illegal waste sites

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**Abstract:** Lymphocytic choriomeningitis virus (LCMV), the prototypic member of the Arenaviridae family, was isolated from a fatal case of aseptic meningitis during the St. Louis encephalitis epidemic in 1933. Although it was among the first isolated human pathogenic viruses, LCMV remained less attractive through years due to its lack of clinical importance even though it is an important teratogen causing a severe and permanent brain injury in newborns. Recently, this old neglected virus found a new niche in immunosuppressed solid organ recipients. The natural host and reservoir of LCMV is a common house mouse, *Mus musculus*. Zoonotic transmission of LCMV to humans occurs worldwide by direct route with mice secretions.

LCMV has been isolated from several arthropods including fleas, *Culicoides* flies, *Aedes* mosquitoes and ticks, but it is believed that arthropods play a minor role in LCMV transmission. However, in habitats where mice and arthropod vectors are abundant, a vector transmission of LCMV could be important. The illegal waste sites present optimal conditions for spread of rodents, common house mice and rats in particular. Therefore, we tested 83 samples of *Mus* or *Apodemus* species sampled on illegal waste sites in Slovenia and Croatia. LCMV infection was determined with IFA and RT-PCR techniques. IFA slides and positive controls were kindly provided by Remi Charrel. The presence of IgG against LCMV was detected in 47% and 16% sera samples of *Mus musculus* and *Apodemus* species, respectively. Total RNA was extracted from spleen using RTF Pathogen Kit (Invitrek-StatReK) and tested with nested RT-PCR specific for amplification of LCMV genome. Amplicons are in process of sequencing to obtain the nucleotide sequence and confirm the presence of LCMV.

Since the seroprevalence studies in Europe revealed up to 17% prevalence of LCMV infection in mice and voles sampled in natural host habitat, we concluded that illegal waste sites accelerate the spread of rodent-borne pathogenic LCMV.

### 032 Epidemiological investigations of bovine besnoitiosis in Hungary

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**Abstract:** B. besnoiti *Nébih*, is an apicomplexan parasite of carnivores transmitted by the tick *Rhipicephalus sanguineus* trough ingestion. Infections in red foxes (*Vulpes vulpes*) appear to be mechanical by blood-sucking dipterans (tabanid and stable flies), although it is also possible iatrogenically (with hypodermic needles) and most likely with close contact between animals. Bovine besnoitiosis has been endemic to South-Western Europe for more than a century, but a significant geographical expansion was observed during the last decade to other parts of the formerly endemic countries and to countries neighboring France.

During the autumn of 2013 bovine besnoitiosis was diagnosed in a beef cattle herd in Hungary (for the first time in Central-Eastern Europe), following the import of Aubrac heifers and bulls from France in the previous two years. The preliminary serological herd screening with ELISA shows that even after the 2nd year of the presence of imported animals (with high prevalence of B. besoii infection), seropositivity among local animals, which originally belonged to the herd, is low. Based on ELISA results venereal transmission (from imported, infected bulls to local, uninfected cows) appears to be either rare or unlikely. The seroprevalence of besnoitiosis decreased significantly among calves born to the group of imported mother cows. The risk of infection seems to be high, when calves stay with their mother during suckling (for 6-7 months), and if animals are kept in the same stable (although physically separated) during the main fly season. Confirmation of the ELISA results is done with immunoblot and IFAT. All seropositive cattle are now kept at a distance of several kilometres from other groups of animals, prior to culling. Molecular and sero-epidemiological evaluation of the situation continues with the aim of preventing the spread of the disease and regaining the epidemiological status of Hungary as exempt of bovine besnoitiosis.

### 033 Occurrence of Hepatozoon spp. in red foxes (*Vulpes vulpes*) in Romania

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**Abstract:** Hepatozoon is an apicomplexan parasite of carnivores transmitted by the tick *Rhipicephalus sanguineus* trough ingestion. Infections in red foxes (*Vulpes vulpes*) have been recorded in several parts of the world, including European countries, with variable prevalence values.

The study aimed to investigate the occurrence of Hepatozoon spp. in red foxes originating from two counties (Arad and Timiș) of Western Romania. Fresh/coagulated blood samples from 28 red foxes, killed in sanitary hunting during the routine rabies monitoring, were molecularly analyzed by a conventional PCR using the primers Hep F and Hep R for the presence of Hepatozoon spp. Overall, 11/28 (39.2%) investigated samples contained the DNA of the parasite. Most of the positive samples were from Arad County (8/17), while in Timiș County only 3 out of 11 foxes tested were positive. Occurrence of *Hepatozoon canis* was reported in Romania in dogs but investigations in wildlife were not performed.

The relatively high prevalence value of the infection recorded in the current survey can be related to the common occurrence of the brown dog tick (*Rhipicephalus sanguineus*) in the area, documented by previous surveys. The high prevalence of the parasite among red foxes confirms the presence of sylvatic cycle, highlights the role of wildlife as reservoirs and potential vectors for the infection in the screened region and also the emerging of this pathogen agent in the country.
This is the first report of *Hepatozoon* spp. infection in red foxes from Romania. The findings support the global changing in vector borne diseases expansion.

**Funding:** this study was financially supported by CNCS Grant TE_277_116/2010.

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**O34**

**Laboratory evidence on vector competence of the invasive mosquito *Aedes koreicus* (Hulecoetomyia koreica) for *Dirofilaria immitis***

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**Aedes (Finlaya) koreicus** is an exotic invasive mosquito detected for the first time in Italy in 2011. Little information on its vector competence for *Dirofilaria immitis* is available. The area where this species is now established (Veneto Region, north-eastern Italy) is endemic for dirofilariosis. In this study, *Ae. koreicus* specimens were experimentally infected with *D. immitis* to evaluate the development of filarial larval stages in different body districts.

**Aedes koreicus** were reared under laboratory standard condition (temperature: 25 ± 1°C; relative humidity: 65 ± 5%; light-dark: 16:8 h). A test group (T) (n = 54 mosquitoes) and a control group (C) (n = 29 mosquitoes) were fed by an artificial feeding system (Hemotek®) using uninfected (in C) and naturally infected (in T) dog blood (3000 microfilariae/ml). Mosquitoes naturally dead and specimens killed at 1, 13, 16, 22 and 28 days post infection (dpi) were dissected; head, thorax and abdomen were examined separately. Five specimens (3 from T and 2 from C) were selected for histology. In addition, molecular confirmation by real time PCR for *Dirofilaria* were performed. Each larval stage was documented by pictures and videos.

The experiment lasted 28 days. A total of 46 mosquitoes fed in T (85%) and 24 mosquitoes in C (83%) groups. In T, 11 mosquitoes were killed and 32 were recovered dead. The mosquito mortality rate in T was 52% during the first nine days, significantly higher compared to C (8%) (p < 0.01). In total, 31 mosquitoes (67%) were infected. The average of microfilariae, L1 (sacculus stage) and L3 was 14.67, 8.56 and 3.15, respectively. Second stage larvae were observed only once (8 specimens on 13 dpi). First stage larvae were first observed on 3 dpi whereas L3 on 8 dpi. The latter were found in salivary glands and proboscis starting on 16 till 28 dpi.

**Aedes koreicus** seems to be a suitable intermediate host for *D. immitis*. Despite the low initial number of microfilariae, the infective L3 stage was observed in all body districts including the proboscis. An high mosquito mortality occurred during the first days, Nevertheless one third of them survived and became infective. This results show that *Ae. koreicus* may be involved in the natural cycle of *D. immitis*, increasing the risk of exposure for dogs and humans.

**Funding:** This work was sponsored by the Veneto Region and IZSVe.

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**O35**

**Autochthonous tick-borne encephalitis virus (TBEV) - seropositive cattle in Belgium: a risk-based targeted serological survey***

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Tick-borne encephalitis virus (TBEV) is the most important arthropod-borne virus in Europe. The Western subtype of this pathogenic neurotropic flavivirus is carried by *Ixodes ricinus*. Tick-borne encephalitis has become a considerable public health risk in several European countries, with currently 3000 hospitalized cases per year. The risk of TBEV-introduction into Belgium remains high and the presence of infected wildlife in Belgium is suspected. Domestic animals such as dogs or cattle can serve as excellent sentinels for TBEV-surveillance in order to install an early warning surveillance component for this emerging zoonotic disease of public health importance.

In a targeted, risk-based and cross-sectional sampling design, serological screening was performed on Belgian cattle (n = 650), selected from the 2010 Belgian national cattle surveillance serum bank. The three most Eastern provinces of Belgium, which are geographically situated closest to known and/or recently emerging TBEV-endemic, were targeted. These areas are also currently known as endemic for Lyme disease (*Borrelia burgdorferi*), another disease transmitted by the same tick.

Bovine sera were tested at the TBEV Belgian National Reference Centre at the WIW-IISP, by gold standard TBEV seroneutralisation test, based on the rapid fluorescent focus inhibition test (RFFIT) protocol. Using a conservative >1/15 cut-off titer for SN test, 17 bovines were seropositive and six had borderline results (1/10 < titer < 1/15). The accuracy of the RFFIT-SNT was confirmed in a mouse inoculation test and by West Nile virus and Rabies virus serology. There was a positive correlation between the neutralizing antibody titer, determined by SN, and the median survival time in mice inoculated intranasally with a mix of virus and serum. Lesions consistent of viral encephalitis were demonstrated in histopathology.

The overall bovine TBEV-seroprevalence in the targeted area was estimated between 2.6 and 4.3% and freedom could no longer be substantiated. Bovines with borderline results were often located close to confirmed seropositive animals. The geographical locations roughly coincided with the known Belgian hot spots for Lyme disease. This risk-based cross-sectional serological survey in cattle, obtained through “one health” cooperation confirms the presence of infected foci in Belgium for the first time. Given the relevance of TBEV for the food chain through consumption of unpasteurized milk and cheese and through its considerable public health burden in other European countries, further surveillance in cattle, other sentinel, ticks and humans at risk is recommended to further determine the location and size of endemic foci and the risk for public health.

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**O36**

**Use of small rodents for the surveillance of agents and vectors of tick-borne zoonoses in the northern Apennines, Italy**

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Vector borne zoonoses are emerging threats in Europe. Data collection from animals may be useful to evaluate their occurrence and intensity of transmission, and to detect their introduction into previously free geographic areas. Indeed, vertebrates serve as hosts for pathogens and for arthropod vectors, although their ecological role can vary according to the diseases.
Data collection on small rodents was used to study the eco-epidemiology of two tick-borne pathogens, *Rickettsia slovaca* (agent of tick-borne lymphadenopathy, transmitted by *Dermacentor marginatus*) and *Borrelia burgdorferi* sensu lato (agent of Lyme Borreliosis, transmitted by *Ixodes ricinus*), in the Apennine mountains, Tuscany (Italy), where human cases of tick-borne diseases were reported. Small rodents are preferential hosts for the immature stages of the two tick vectors and are involved in the transmission cycle of both diseases. In the summers from 2009 to 2012, we live trapped *Apodemus* spp. and *Myodes glareolus* from 1100 to 1650 m above the sea level (a.s.l.). Rodents were found infested by immature *I. ricinus* and *D. marginatus*. The monthly activities of these two tick species on the same hosts were different, reflecting differences in their life cycles. Although few individuals were co-infested, both tick species tended to aggregate on the same *Apodemus* spp. males. *R. slovaca* and *B. burgdorferi* s.l. were detected in rodent ear biopsies and attached ticks up to 1650 m a.s.l. In our study area, rodents might play a role as amplifiers of *R. slovaca* infection; even in the absence of the host's systemic infection, tick aggregation on the same individuals might favour the transmission of the pathogen through co-feeding. While *D. marginatus* had been found at the same location in studies carried out in 1994, *L. ricinus* was very rare or absent. Data collection on small rodents thus highlighted the recent range expansion of *I. ricinus* and *B. burgdorferi* s.l. in a previously unoccupied area. Major land use changes, the increased abundance of wildlife populations, as well as a general climate warming in the Mediterranean area, might be interacting factors affecting the altitudinal range expansion of *L. ricinus* in the Northern Apennines, and in Italy in general.

**POSTER PRESENTATIONS**

**WG1 - THE “ONE HEALTH” CONCEPT IN THE ECOLOGY OF VECTOR-BORNE DISEASES**

**P1**

Ixodid ticks in red foxes (*Vulpes vulpes*) from Romania

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Among wildlife, the red foxes (*Vulpes vulpes*) are one of the most adapted wild species to anthropic ecosystems. They are well recognized as important reservoirs for a large number of zoonotic agents in Europe, including ticks and tick-borne pathogens. Currently, there are few available data on the importance of red foxes in the eco-epidemiology of vector-borne diseases. Therefore, the aim of this study was to screen the dynamics of tick infestation in 357 red foxes from 12 Romanian counties. Tick identification was performed using the morphological keys. The overall prevalence of tick infestation was 43.7%. The 5753 collected ticks belonged to five species: *Ixodes hexagonus* (on 113 out of 156 foxes; prevalence 72.44%), *I. ricinus* (28.84%), *I. crenulatus* (7.7%), *Dermacentor marginatus* (7.05%) and *Haemaphysalis punctata* (0.64%). Co-infection occurred in 24 foxes (22 with 2 tick species; 2 with 3 tick species) with the following associations: *I. ricinus + I. hexagonus* (n = 10), *I. hexagonus + D. marginatus* (n = 5), *I. ricinus + I. crenulatus* (n = 4), *I. ricinus + D. marginatus* (n = 2), *I. hexagonus + I. crenulatus* (n = 1), *D. marginatus + I. hexagonus + I. ricinus* (n = 1), and *H. punctata + I. hexagonus + I. ricinus* (n = 1). This study indicates that foxes are hosts to a relevant number of tick species with recognized vectorial role. The provided information can facilitate the understanding of the ecology of ticks and can be the basis for studies on the epidemiology of tick-borne diseases. The high prevalence of tick infestation in red foxes, coupled with the increasing presence of this species in synanthropic environments and a more nature-oriented lifestyle of people, can pose a significant threat to human health, through the emergence of tick-borne diseases. Moreover, foxes represent a good model of sentinel species.

This research was performed as part of project IDEI PCE 236/2011.

**P2**

The role of cervids (*Cervus elaphus*) in the eco-epidemiology of some tick-borne diseases

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The maintenance of tick-borne pathogens in nature follows a variety of patterns that are always involving ticks, reservoir hosts and pathogens. The red deer (*Cervus elaphus*) is the fourth-largest deer species and inhabits most of Europe in a wide variety of habitats. In the last 50 years the red deer (*Cervus elaphus*) population remained constant in Romania, with around 30,000 individuals and no significant fluctuations registered. These animals are one of the preferred hosts for *Ixodes ricinus*, a tick with

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**O37**

Are *Babesia* a risk factor for blood products in an alpine area?


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After malaria, babesiosis is the second most common transfusion-transmitted vector-borne disease. This study investigates seroprevalence rates to Babesia divergens and *B. microti* in the Tyrol and assesses the risk of blood products being contaminated by either agent. The area of investigation comprises the Austrian part of Tyrol. A number of 988 sera were tested for IgG antibodies against *B. divergens* and *B. microti* by in-house immunofluorescence assays (IFA). IFA-slides were tested by using commercially available hyperimmune sera.

Collection of questing ticks was performed in summer 2009 by about 120 volunteers among hunters at 25 sampling sites over a period of three months by flagging. Of 988 sera, 21 (2.1%) were positive in IFA against the *B. divergens*-complex at titres of 1:128 or higher and 5 (0.5%) were positive in IFA against *B. microti*. Under the presumption of a long-lasting immune response we can expect 0.5 (±0.2, 95%) seroconversions against *B. divergens* per 10,000 persons per year. For *B. microti* the same calculation results in 0.1 (±0.08, 95%) seroconversions per 10,000 persons per year. *B. divergens* The risk of a blood donation being contaminated by *B. divergens* or *B. microti* is estimated to be 24.2 and 5.8 per 100,000 blood donations.

The presented data shows that the local population comes into seroreactive contact with at least one member of the *B. divergens*-complex and - to a lesser extent - *B. microti.* To our knowledge, it is the first demonstration of *B. venatorum* in the Tyrols. Thus, and as vector-borne diseases are subjected to dynamic changes, we recommend re-assessment of the risk of transfusion-mediated infections on a regular basis and to introduce PRT for blood components like platelets.
vectorial capacity for a wide range of pathogens; they are reservoir hosts for many tick-borne diseases; due to their movement from sylvatic to synanthropic environment and back they can influence the spreading of both ticks and pathogens. In Romania, studies about the epidemiology of tick-borne diseases in wild animals, eminently in red deer are limited. In this frame, our study aimed to describe the diversity of ticks that are parasitizing Cervus elaphus and to evaluate the molecular prevalence of Babesia spp., Theileria spp., Anaplasma spp. and Ehrlichia spp. in ticks (I. ricinus) and tissues (heart, spleen, liver) by PCR. In October and September 2012 samples from 53 animals (24 bred in captivity; 29 wild) were collected. All animals originated from Harghita County that harbours 10% of red deer population from Romania. Ixodes ricinus was the dominant tick species (99.7%). Only three specimens of Dermacentor marginatus were identified. Samples (1007 ticks, 19 heart tissues, 12 spleen tissues and 21 liver tissues) were used for molecular diagnosis. Genomic DNA extraction was performed individually on all ticks and tissue samples, followed by PCR. In ticks samples, all investigated pathogens were present: Anaplasma spp. (6.5%), Ehrlichia spp. (2%), Theileria spp. (0.8%), Babesia spp. (0.6%) and Babesia spp. (0.3%). From both heart and spleen tissue samples 3 pathogens were diagnosed: Anaplasma spp. (21%), Theileria spp. (15.8%), Ehrlichia spp. (5.3%) and Anaplasma spp. (16.7%), Theileria spp. (16.7%), Babesia spp. (8.3%), respectively. Only Theileria spp. and Ehrlichia spp. (4.8%) were present in liver samples. To our knowledge this is the first molecular study that evaluates the presence of tick-borne pathogens in ticks and tissues collected from Cervus elaphus in Romania, offering important eco-epidemiological data from public health perspective, stressing out the necessity of permanent surveillance.

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P3 Recurrent subcutaneous human Dirofilariasis due to Dirofilaria repens after surgical removal of the worm and anthelmintic treatment
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The genus Dirofilaria includes various species that are natural parasites of dogs, cats, foxes, and wild mammals, transmitted by mosquito vectors. Dirofilaria repens is commonly encountered in the subcutaneous tissue of dogs, foxes, and cats. Due to recent increase of human Dirofilaria repens infections, it is considered an emerging zoonosis. In Romania, there are only few reports regarding the infection with D. repens in dogs and in humans. Patients usually present single migratory subcutanal or subcutaneous nodule which may or may not be tender. Surgical excision of lesions and affected areas is the treatment of choice for dirofilariasis. Anthelmintics are not usually recommended.

We describe the case of a 65 years old, retired woman, without history of traveling in the past years, living in the Oradea (north-west part of Romania), in an urban area, who presented a left breast subcutaneous nodule with fever and eosinophilia in August 2012. After surgical excision, histopathology and PCR assay (Isolate Genomic DNA Kit, Bioline, UK using 125 ADN1 gene (250 bp) the diagnosis of D. repens infection was established. Patient received Albendazole 400 mg/day for 7 days after surgery. The eosinophil count became normal and no symptoms were noticed till December 2012 when another nodular lesion developed in the left breast, accompanied by eosinophilia. After the surgical removal of the second nodule, another larval worm was identified as D. repens. The patient was treated with 150 µg/kg BW of ivermectin and no other nodules appeared. Eosinophiles level remained normal during next year follow-up. This is the first report of autochthonous D. repens infection in humans in the northern part of the country and, to our knowledge, the first case of recurrent subcutaneous human dirofilariasis. The recurrence in this case is most probably the result of infection with multiple larvae, of which at least two developed into nodules at four month interval, rather than a repeated infection. We recommend the treatment with ivermectin after surgical excision to avoid recurrences in dirofilariasis.

P4 Prevalence of tick-borne pathogens in ticks from migratory birds in republic of Moldova
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Migratory birds are often implicated in transporting ticks, which can carry pathogenic agents of several human and domestic animals diseases. By themselves ticks are not highly mobile, and the only ways to expand the habitat are hosts. Birds, due to the ability to fly, have the greatest influence on the resettlement of ticks. As was documented by many authors birds can transport ticks harboring Borrelia spp., Babesia spp., Anaplasma spp. and Rickettsia spp. throughout many parts of Europe.

Migratory birds were caught in ornithological nets from March 2012 till October 2013 at natural forest reservations (Yagorilik, Prutul de Jos, Padurea Domneasca), and urbanconesthes of Chișinău and suburbs. All birds were identified to species level, and their sex. Birds were placed in bags made of thick fabric, right after birds were examined in the camp for the presence of ticks. After the examination, birds were released without being harmed. Tick species were detached from birds and stored individually in 70% alcohol. Total DNA was extracted using QIAamp DNA Mini Kit. Specific PCRs were carried using DNA to detect Borrelia, Anaplasma, Rickettsia, Neoehrlichia, Brucella species. Total of 131 ticks relating to the 3 ticks species (Ixodes ricinus n = 124, I. frontalis n = 6, Haemaphysalis punctata n = 1) were collected from 45, tick-infested birds relating to the 7 avian species. The most common host was Turdus merula. From 131 ticks in extracted DNA of 77 one or more of the following pathogens was detected: 25 ticks were positive for Anaplasm phagocytophilum, 18 were positive for Borrelia garinii, 1 was positive for B. lusitaniae 1, for B. valaisiana, 9 for Rickettsia monacensis, 2 ticks were positive for Rickettsia helvetica, 1 for Rickettsia typhi, 2 for Neoehrlichia mikurensis, 2 for Babesia microti and 2 for Borrelia miyamotoi. These included 12 cases with a mix of the two pathogens and 2 cases with three pathogens. The presence of the above pathogens in ticks collected from passerine birds corroborates the role of these vertebrates in the epidemiology and dispersion of tick-borne diseases. Some of the birds are considered as migratory or partial migratory. In addition these birds share the ecologic niche with the others migratory birds which migrate for long distances from Africa to the Europe. Our data suggest the involvement of ticks parasitic in birds in the cycle of human and cattle tick-borne diseases.

WG2 - BARCODING, MOLECULAR DIAGNOSIS AND NEXT GENERATION SEQUENCING

P5 Development and field trial of a Tams1-targeted isothermal DNA amplification (Tams1-lamp) assay for detection of Theileria annulata in cattle
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Mediterranean Theileriosis is frequently diagnosed in cattle in southern Portugal. This tick-borne hemoproteozoan disease, caused by the apicomplexan parasite Theileria annulata, poses important health problems in cattle in addition to the economic losses caused by decrease of productivity in chronic infected animals. Integrated control strategies for this disease will then benefit to the detection of cattle with chronic
infection that usually acts as parasite reservoirs constituting a risk to susceptible animals. The highly sensitive and specific molecular methods for A. phagocytophilum detection are suitable for the identification of such animals often with very low parasitaemia. The isothermal amplification methods proved particularly useful for diagnostic laboratories with fewer resources since it does not require the use of expensive and sophisticated equipment such as thermal cycler.

In the present study we develop an isothermal amplification technique targeting Tams1 gene (Tams1-LAMP) for detection of A. phagocytophilum in cattle blood samples and compared this technique with real time PCR for assessing its applicability for diagnosis. One hundred blood samples from 16 farms were collected and analyzed. Genomic DNA extracted from blood was used as template in real time PCR and LAMP reactions for Tams1 gene amplification. A DNA sample extracted from T. annulata cell culture and one from an animal with 0.03% parasitaemia were used as positive controls. DNA samples from other closely related parasites were used as negative controls. The LAMP technique detection limit has been determined to correspond to a parasitaemia of approximately 0.008% and a specificity of 100%. Real time PCR positive samples were detected in 14 farms with a total of 62% infected animals with very low parasitaemia that usually is characteristics of chronic infections. Tams1-LAMP identified 66% infected animals from the 14 farms. Cohen’s test was used to evaluate the agreement between methods and the k value found (0.98) is indicative of an excellent agreement for this group of samples. These results permitted to conclude that Tams1-LAMP is a useful molecular technique to be applicable for detecting Theileria annulata in cattle with chronic infections.

**P6**

*Borrelia turcica* in *Hyalomma aegyptium* ticks in Romania

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Testudo graeca tortoises are distributed in the south-eastern part (Dobrogea region) of Romania. *T. graeca* is a potential host for the three-host ticks, *Hyalomma aegyptium*. *H. aegyptium* ticks are important from epidemiological point of view as they constitute potential reservoirs for numerous zoonotic bacterial pathogens (*Anaplasma phagocytophilum*, *Ehrlichia canis*, *Coxiella burnetii*). However, *H. aegyptium* was reported to host less studied bacteria, non-Lyme members of genus *Borrelia*. Despite its relatively wide distribution range, the extent of co-distribution of ticks with these bacteria was not investigated in detail. The aim of the present study was to evaluate *H. aegyptium* engorged ticks collected from tortoises in south-eastern Romania for the presence of non-Lyme *Borrelia*. Between 2008 and 2013, 448 *H. aegyptium* ticks were collected from 45 *T. graeca* tortoises located in Dobrogea region in Romania. DNA extraction was performed individually from each tick using a commercial kit. For the total 78 (17.4%) *Borrelia* spp. positive ticks, PCR analysis targeting the intergenic spacer 5S-23S region, gplQ, respectively gyrB genes, and further sequencing was performed for the further identification. Sequences of gypB and gplQ genes showed 99%-100% similarities with reptile-associated *Borrelia turcica*. The most frequently infected stages were males (10.7% of the total males examined or 61.5% from the total infected ticks) followed by females (5.36% of the total females examined or 31% from the total infected ticks) and nymphs (1.34% of the total nymphs examined or 7.7% from the total infected ticks). This is the first report of *Borrelia turcica* in Romania.

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**P7**

Bead-based suspension array for detection and identification of tick-born *Borrelia* species

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In the Netherlands, screening for tick-borne pathogens is performed using real-time PCR on DNA extracted from ticks, followed by sequencing of positive samples, thereby disallowing identification in case of double-infections. Until recently, testing was performed with several reverse line blots (RLBs), each containing ~10 different probes. Advantages of RLBs are the capabilities to differentiate between bacterial species and to detect double infections, disadvantages are that RLBs are fastidious, elaborate, and time-consuming.

The aim of this work was to ‘test drive’ bead-based suspension arrays for the rapid detection and identification of tick-borne *Borrelia* species. The platform used is the flow cytometry-based xMap technology of Luminex, in which fluorescent nanoparticles (beads) are used to build multiplex assays simply by mixing different bead sets that are covered with specific probes. Each bead set contains a different ratio of red and infrared fluorophores, which enables identification of beads that pick up a third fluorophore by hybridization to a labelled target molecule.

Using standard chemistry, amminated oligonucleotides were covalently linked to carboxylated Luminex beads, resulting in bead sets with general and specific probes for the 23S-5S intergenic spacer (IGS) of *Borrelia* species. Presence of probes on the beads was verified by hybridization of complementary biotinylated oligonucleotides in presence of a mixture of 1000 beads per bead set, using a 30 minute incubation at 37°C in a NaCl/Tris buffer, followed by labelling with streptavidin-phycocerythrin. Of each mixture, a minimum of 100 beads per bead set were investigated by flow cytometry on a dedicated Luminex instrument.

To evaluate the suspension array, DNA of nearly 1000 ticks was isolated and used to generate amplicons with a universal PCR targeting the 23S-5S IGS of *Borrelia* spp. All PCR reactions were then used in a direct hybridization assay in presence of a mixture of probe-containing beads. Out of 961 ticks, 20% were found to carry one or more *Borrelia* species (resp. 15 and 5%). The most prevalent species was *B. afzelii*, followed by *B. burgdorferi sensu stricto*, *B. garinii*, *B. sensu lato* (unidentified) and *B. valaisiana*. These results show the viability of bead-based suspension arrays for (relatively) rapid detection and identification of multiple species, but at this stage need verification by a ‘gold standard’. Currently, confirmation of these results is being performed by next generation sequencing.

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**WG3 - GEOSPATIAL TOOLS IN VECTOR RESEARCH**

**P8**

*Ixodes* ticks in sheep and cattle in the Basilicata region (southern Italy)

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*Ixodes* are an important health problem for domestic and wild animals for direct damages caused by these ticks, but mostly because they are involved in the transmission of many diseases (tick-borne diseases - TBDs). Therefore, the surveys on the presence and geographical distribution of ticks in domestic ruminants need to be constantly updated.

The aim of the present study was to update the data on the presence and distribution of *Ixodes* ticks in cattle and sheep bred in the Basilicata region (southern Italy), an area with a Mediterranean climate favourable to the biology and ecology of ticks. In this region, the breeding of pasturing cattle and sheep is a widespread reality very important from an economical point of view, especially for the natural vocation of animals in using marginal hilly and mountainous pastures.
From May to September 2013, sheep and cattle farms (n = 82 and 31, respectively) were visited and ticks were collected from 20 animals per farm, preserved in ethanol 70% and then identified at species level using the morphometric keys present in the literature. A total of 2,179 ticks were collected. The following species were identified in the sheep farms (total = 1,594 adult ticks collected): *Rhipicephalus bursa* (67.1%), *R. sanguineus* (34.1%), *R. turanicus* (25.6%), *Dermacentor marginatus* (6.1%), *Hyalomma detritum* (3.7%), *Ixodes ricinus* (3.7%) and *Hyalomma marginatum* (1.2%). In the cattle farms, a total of 585 adult ticks were collected and the following species identified: *R. bursa* (61.3%), *H. marginatum* (29.0%), *R. turanicus* (25.8%), *R. sanguineus* (25.8%), *D. marginata* (9.7%), *I. ricinus* (9.7%), *Ixodes gibbosus* (6.5%) and *Hyalomma detritum* (3.2%). The results of the present cross-sectional survey confirm that the environmental and climatic conditions of southern Italy are suitable for different tick species infecting domestic ruminants that are therefore exposed to different TBDs.

**P9**

**Distribution of two invasive mosquito species in Slovenia in 2013**

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In the recent years there has been growing interest in the establishment and spread of invasive mosquito species in Europe. Apart from the most dispersed, *Aedes albopictus*, there are five other alien aedine mosquito species spreading in Europe. Their spreading represents a considerable threat for public health as they can serve as vectors of mosquito-borne diseases. A survey in whole Slovenia was performed in 2013 to determine the distribution of invasive mosquitoes. The study was based on the search for invasive mosquito larvae in artificial water containers at the cemeteries, around human dwellings and in used tires at vulcanizing companies. Additionally, a call for citizens to report any nuisance from “unusual” mosquitoes was published in local media. With the study we have revealed the presence of two invasive mosquito species in Slovenia, *Ae. albopictus* and *Ae. japonicus*. Altogether we caught 494 larvae of *Ae. albopictus* and 1318 larvae of *Ae. japonicus*. The results show that *Ae. albopictus* is present mostly in Southwestern and central part of the country, with some isolated locations in other parts of Slovenia. Nevertheless, *Ae. japonicus* was found in a large part of the investigated area. This species was previously collected from a small area near the Austrian border in 2011, and in only two years it has colonized the majority of Northeastern part of Slovenia.

In Slovenia no systematic mosquito surveillance or control is currently done. There are only some minor projects, which are carried out by local institutions at a regional level. *Ae. albopictus* and *Ae. japonicus* are spreading fast and they both present high nuisance for the humans, as well as a public health risk since transmitting vector borne diseases. Therefore an effective national monitoring program of invasive mosquito species is highly needed. With well planned, long term strategy we will be able to detect and control their occurrence, monitor their pathogens and prevent the establishment of new foci of mosquito-borne diseases.

**WG 5 - RARE AND EMERGING VECTOR-BORNE PATHOGENS**

**P10**

**Effect of PilE4 of Francisella tularensis subsp. holarctica (live vaccine strain) to brain endothelium**

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Francisella are, highly infectious vector-borne bacteria, which infect more than 250 host species, including humans. *Francisella* are able to invade multiple organs in the host body, such a skin, liver, lung and central nervous system. To invade various organs, *Francisella* have to cross the cell linings like endothelial barriers. Interaction with endothelial cells is multifaceted process, which includes adhesion and activation of signaling events. Our previous work has shown that *Francisella tularensis* subsp. *holarctica* Live Vaccine Strain (LVS) interacts and adhere to endothelial cells through PilE4 and Intercellular adhesion molecule 1 (ICAM-1) interaction. To corroborate consequences of PilE4 adhesion to endothelial cells, we incubated brain microvascular endothelial cells with recombinant PilE4 protein and assessed the regulation of several genes with qRT-PCR. Results showed that PilE4 upregulated the expression of adhesion molecules (ICAM-1 and PECAM-1), matrix metalloproteases (MMP 1, MMP 3 and MMP 9) and molecules that are involved in pathogen recognition (TLR-6, MyD88, IRAK-1 and TRAF-6) in brain endothelium. On the other hand, we found down regulation of interleukins and NF-κB. Inhibition of NF-κB indicates that Francisella might use NF-κB subversion mechanism to evade immune response. This mechanism is described in other pathogens such as *Listeria* and *Yersinia*.

Previous studies have showed that matrix-metalloproteases, mainly MMP-9, is crucial to disrupt cytoskeleton of brain endothelium to increase the permeability of BBB. In summary, work reveal hidden aspects of invasion and translocation of *Francisella* across the brain endothelium, that offers new insight into the pathobiology of neuroinvasion of *Francisella*.

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**P11**

**Rhipicephalus turanicus: from low numbers to complete establishment in Cyprus. Its possible role as a bridge-vector**

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We describe herein the abundance of *Rhipicephalus turanicus* in Cyprus, and its potential role as bridge vector carrying tick-borne pathogens among various hosts.

Following the first recording (1970-72), at low numbers, of *R. turanicus* in Cyprus, two studies took place (March 1999 - March 2001, January 2004 - December 2006), during which, ticks were collected from ruminants (goats, sheep, bovine), dogs and wild mammals (foxes, wild-rabbits and mouflons) from different sites of the island. All ticks were morphologically identified to the species level, washed in 70% alcohol, rinsed in sterile water, dried on sterile filter paper and triturated individually. Following DNA extraction ([QiAamp Tissue Kit (QIAGEN, Germany)]) all ticks were tested for *Rickettsia* species and *Coxiella burnetii* by Real-time PCR targeting the *rfa* gene and *IS1111* insertion sequence, respectively, and for *Anaplasm* species by PCR targeting the 16s rRNA gene. Samples positive for *Rickettsia* species were further amplified by PCR targeting the ompA and *ompB* genes. Of the 3950 ticks collected, 805 (20.4%) were identified as *R. turanicus*; this species was the only one, amongst the rest of the ticks (*R. bursa, R. sanguineus, Hyalomma anatolicum excavatum, H. marginatum, H. m. rupelis, Ixodes gibbosus, I. ventalli, Haemaphysalis sulcata, H. punctata*), that was collected from every host. Of the ticks tested, 125/805 (IR: 15.5%) were tested positive for *Rickettsia* species (*R. massilliae* (83/125), *Candidatus Rickettsia barbaraiae* (5/125), *Candidatus Rickettsia barbaraiae* genotype *Cretocripinsis* (26/125) and *Candidatus Rickettsia tserentii* (11/125)). The latter *Rickettsia* species showed a close relationship with *R. africae*, was described for the first time in Cyprus and was identified in *R. turanicus* only disregarding the animal host. Of the 805 ticks, 107 (IR: 13.3%) were tested positive for *C. burnetii*, and 16 (IR: 2%) were positive for *Anaplasm* species. Five ticks harboured both *Rickettsia* species and *C. burnetii*. *Rickettsia turanicus*, largely distributed in the Mediterranean sub-region, Africa and Asia, is flexible and adaptable and can be found in a rich variety of hosts (domestic and wild). It has well adapted and spread

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over a great variety of animal species in Cyprus during the last 40 years and seems to play an important role as a bridge-vector of tick-borne pathogen. The changing climate, geographical position of Cyprus (wintering area for migratory birds), make the island an important cross-over for possible dispersal of ticks and their tick-borne pathogens from East to the West.

P12
Rodents as reservoirs of human pathogens in Bulgaria

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Small mammals are reservoirs of various human pathogenic. The aim of this work was to investigate infections with human pathogens in rodents trapped in different regions of Bulgaria. A total of 284 rodents were investigated by PCR for detection of the flagellin gene of borreliae within Borrelia burgdorferi sensu lato complex, ankA gene of Anaplasma phagocytophilum and nucleoprotein gene of hantavirus Dobrava - conventional nested RT-PCR and Real Time PCR with TaqMan probe. B. burgdorferi was detected in 64/284 (22.5%) of the investigated rodents by PCR. Of them, 41 samples originated from Apodemus flavicollis, 20 from A. agrarius, and 3 from A. sylvaticus. Overall, 33 of the investigated 284 rodents were infected with Anaplasma phagocytophilum (11.6%) - 11 Apodemus flavicollis (infectivity rate 8.6% of the 128 investigated), 13 Apodemus agrarius (infectivity rate 13.5% of the 96 tested), and 1 Apodemus sylvaticus (infectivity rate 9% of the 11 tested). Hantavirus RNA was detected in 9 of the rodents. Only Dobrava-Belgrade virus but not Puomala or Saaremaa virus was detected. Almost all infected animals were A. flavicollis (8/9 PCR-positive rodents). Rodents are important reservoirs of human pathogens. In this study, active infection in rodents was confirmed by detection of microorganism’s genome. Remarkably, a high number of rodents from genus Apodemus were infected with borreliae. The high rate of detection of A. phagocytophilum in rodents from A. agrarius species suggested that this species might serve as major reservoir of human anaplasmosis in Bulgaria. Mainly infected with hantaviruses were A. flavicollis mice, known as reservoir of Dobrava hantavirus but hantavirus infections were detected also in A. agrarius mice. Medical authorities should be aware of the risk for humans.

P13
Flavivirus genome detected in Mus and Apodemus species sampled in illegal waste sites

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Tick-borne encephalitis virus (TBEV), a zoonotic flavivirus that occurs on the Eurasian continent and causes tick-borne encephalitis (TBE) in humans, is considered medically the most important arthropod vector transmitted virus in Europe. As such, TBEV is of course not a neglected pathogen. However, the knowledge about TBEV could importantly contribute to the research of other neglected vector borne pathogens. In nature, TBEV is transmitted from tick to tick via vertebrates in a process named co-feeding which is independent of a systemic viremia in vertebrates. Therefore the ticks are the reservoirs as well as the vectors for TBEV, while the vertebrate is considered the transient bridge in transmission and maintenance of TBEV. Slovenia is an endemic country for TBEV with approximately 300 cases reported annually and incidence around 14 per 100,000 inhabitants. It was shown, that TBEV in Slovenia is maintained in tick-rodent-deer cycle. TBEV viraeemic rodents of Myodes and Apodemus species were described previously in Slovenia. We were interested whether TBEV could be present in Mus species which share a living habitat with Apodemus mice in illegal waste sites. Thus we collected 83 samples of Mus and Apodemus species from different dump sites in Slovenia and Croatia. The presence of TBEV was determined using molecular techniques. Total RNA was extracted from spleen samples using RIT Pathogen Kit (Invitrek-Stratec) and tested with real time RT-PCR specific for TBEV-Eur subtype. All samples were negative, which could be explained by the fact that testing area was in the part of Slovenia with low incidence of TBE. However, distinctive samples were also tested with universal primers for amplification of flavivirus genome. Six amplicons of correct size were seen on agarose gel and are in the process of sequencing to reveal the identity of detected flavivirus.

P14
Bunyaviruses in human, animal and mosquito samples from southeast Austria

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In Austria occurrence of 40 different species of mosquitoes belonging to 6 genera has been described. AGES in 2011 initiated a nation-wide mosquito-surveillance program to identify invading species. As part of this project, pools of Culex, Aedes, Anopheles and Culiseta species collected in different parts of the country were analyzed by PCR analysis; in addition seroepidemiological testing for bunyaviruses was performed in selected regions. In order to be able to detect also new strains, a broad spectrum CODEHOP (Consensus-Degenerate Hybrid Oligonucleotide Primer) approach was used initially; presumably due to the low sensitivity of degenerated primer designs and due to dilution in insect pools, we did not pick up any new isolates so far using Orthobunyavirus, Phlebovirus or Nairovirus group-specific primers. Two bunyaviruses were found in Culex pipiens from the southern province Carinthia in 2012, detected with non-degenerate multiplex PCR, the sequences highly homologous to Italian Tahynavirus (TAVH) isolates. 245 sera collected for a study of zoonotic infections in hunters, veterinarians, farmers, and abattoir workers (conducted by the Styrian health authorities in 2003), were tested by ELISA tests, using inactivated Crimean Congo virus (CCHF) from Bulgaria as well as recombinant CCHF nucleoprotein produced in baculovirus. In addition, cell culture derived TAVH lysates as well as TAVH infected cells were used as antigens in ELISA and immunofluorescence (IF) under an experimental setup. No seropositivity for CCHF or related nairoviruses was found in these Austrian human sera originating from risk groups for zoonotic infection.

P15
Anopheles maculipennis complex-responsible for the re-emergence of malaria in Romania?

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Between 2010 and 2012, in Iași City of Romania 5 species belonging to the complex Anopheles maculipennis were identified by PCR: A. melanoon, A. latranchiae, A. atroparvus, A. meseaeand A. maculipennis sp. The last three species were involved in the transmission of malaria in Romania between 1892 and 1961. The species A. labranchiae was signalled for the first time in Romania, being considered as the main vector of malaria in Europe. The identified samples of Anopheles labranchiae were two stage IV larvae, concluding with the adaptation of the species to the climate of Iași City. Using a new mathematical model realized and implemented by ourselves, based on the construction of a function of interpolation of
Prevalence of Anaplasma phagocytophilum in ticks collected from migratory birds in Danube Delta, Romania

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Wild birds are hosts for several species of ticks, contributing to the maintenance of their local populations in delimited geographic areas. migratory birds play important roles as distributors of ticks within and between distant territories, including countries. Ticks collected from birds are responsible for hosting a significant number of human pathogens. The extensive wetland complex of the Danube Delta provides an internationally important stopover site for millions of migratory birds, belonging to 300 different species, travelling annually. The aim of this study was to detect Anaplasma phagocytophilum in feeding ticks collected from migratory birds along four migratory seasons. Ticks were collected from the birds with forceps and preserved in 96% ethanol for later examination using a separate vial for DNA extraction. Potential co-infections were also analyzed according to the history and clinical signs. Out of the 1661 suspected patients, 80 cases (study group) were confirmed by IIFT, WB, PCR, treated and monitored. Tick bite was recognized by 35 patients: 23 presented neurological symptoms, 10 cardiovascular signs, 20 rheumatologic signs, 12 dermatological signs (mostly EM) and one case presented ocular damage. The infection with a single Borrelia burgdorferi s.l. species was found in 56 cases, with two species in 22 cases and more than 2 species in 2 cases. Most common documented species (using the confirmation tests Western Blot and PCR DNA presence when it was possible): Borrelia afzelii (26) and Borrelia burgdorferi s.s. (19) and Borrelia garinii (11). Confirmed co-infections (using IIFT/ELISA/WB techniques included: Bartonella quintana/henselae (19), Coxiella virus (2), Ricettova spp. (2), Chlamydia pneumoniae (11), Mycoplasma pneumoniae (10), Ehrlichia spp. (2), Babesia spp. (1), Yersinia enterocolitica (3). Co-morbidities were also evaluated for the differentials and the presence of multiple sclerosis, SLE, rheumatoid arthritis or ankylosing spondylitis was noticed. All patients received antibiotics, supplements and sugar restricted diet. Cases with confirmed associated diseases received concomitant specific therapy. Combinations of antibiotics were in accordance with LD and documented co-infections.

According to our results existence of LD in some parts of Romania was emphasized. The most common confirmed Borrelia burgdorferi s.l. species were Borrelia afzelii and Borrelia burgdorferi s.s. Early diagnosis and appropriate treatment are associated with a good evolution and prognosis. Chronic cases with multiple co-infections required longer treatment and monitoring. Multidisciplinary team works increase the accuracy of the diagnosis. Public health information and prophylactic measures should be correctly provided for the population, in different manners.

Molecular characterization of Borrelia strains isolated from ticks in Vojvodina

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Borrelia burgdorferi sensu lato (s.l.) complex represents a group of different types of spirochetes that are present globally, which causes Lyme borreliosis. The total number of species is still not final because new genetic and antigenic isolates are still being described. The clinical picture of Lyme borreliosis in people is polymorphic and is characterized with symptoms similar to flu syndromes together with erythema migrans.
rheumatologic, cardiologic and neurological complications. Apart from people, dogs, horses, bovines and sheep can suffer from Lyme borreliosis. In Europe, the most important vector transmitting Lyme disease is the tick Ixodes ricinus. A description of different species of Borrelia in ticks has opened a completely new field of investigating the ecology of Lyme borreliosis. It is especially important to investigate a connection between different types of Borrelia, ticks as vectors and vertebrates as the reservoirs on various geographical localities.

The aim of the work is to isolate Borrelia species from the collected ticks I. ricinus from the geographical territory of Vojvodina, and to do a molecular characterization of the isolated strains of Borrelia.

A total of 12 tick pools of the type I. ricinus were cultivated in Barbour-Stoenner-Kelly-H medium with additional antibiotics and subcultivated into a modified Kelly-Pettenkofer/Preac-Mursic medium, until a clear culture is reached. For the molecular characterization of isolated strains of Borrelia, molecular methods Mlu-LRFP and real-time PCR for hbb gene were used.

In this research, out of 12 pools of ticks species I. ricinus 3 strains of Borrelia from the B. burgdorferi S. complex were isolated. All three isolates of Borrelia from the tick species I. ricinus from the territory of Vojvodina were identified as Borrelia afzelii by applying molecular methods (Mlu-LRFP and real-time PCR for hbb gene). By applying Mlu-LRFP all three isolated strains of B. afzelii were characterized as a subtype Mla. In this research, like in the previous researches, we have not proved the presence of a pathogenic species Borrelia spielmanii in ticks of the species I. ricinus.

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**P19** J ackals as carriers of Leishmania and Brucella species in Serbia

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The golden jackal Canis aureus occurs in south-eastern Europe, Asia, the Middle East, the Caucasus and Africa as well as in countries of the European continent, including Serbia. The scope of the current survey was to study the presence of two zoonotic agents, Leishmania species (vector transmitted) and Brucella species, in the jackal population in Serbia.

Animal samples were collected over a three-year period (01/2010 - 02/2013) from 48 localities corresponding to 12 regions of Serbia. Sampling took place from dead animals brought at the laboratory of Department for Animal Ecology and Geography, Faculty of Biology, University of Belgrade, by hunters. Data regarding location were recorded. At necropsy, samples from different tissues were collected (spleen, liver, hart); spleen was chosen as the tissue to work with. Samples were homogenised with micropastries (Eppendorf). DNA extraction took place from a small portion of mashed tissue using the Gene Jet Genomic DNA Purification Kit (Fermentas, Thermo Scientific). For the detection of Brucella species, a multiplex Real-time PCR protocol was used, targeting the bscp31, aikb and BMEl1162 genes of Brucella species. B. abortus and B. melitensis; B. canis was detected using a PCR protocol targeting the omp2B gene. For the detection of Leishmania species a Real-time PCR protocol targeting the SSU rRNA gene was used. A total of 216 spleen samples were tested. Most samples (196/90.7%) were collected during the hunting period (November-February). Of the samples tested, 108 animals were male and 108 were female. Fifteen (6.9%) were positive for Leishmania species, while four (1.9%) were positive for B. canis.

Jackals are known omnivores and scavengers and they usually prey on small mammals, on vegetables, fruit and garbage. Their ability to adapt to novel habitats both on rural and urban areas allows them to come into contact with animals that live in close proximity to humans, such as dogs, hence their ability to host a number of zoonotic, including vector-borne, pathogens. An increase in jackal population has lately been observed in Serbia, while complaints about damages that jackals may cause to livestock mean that their wildlife cycle may well interfere with areas of natural human activity. Although further studies are required to discern the potential epidemiologic role of the golden jackal in spreading and transmitting the above studied pathogens, local awareness of residents, veterinarians, and health professionals would prove of great importance in order to efficiently monitor these and other zoonotic diseases.

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Cite abstracts in this supplement using the relevant abstract number, e.g.: Girović et al. Jackals as carriers of Leishmania and Brucella species in Serbia. *Parasites & Vectors* 2014, 7(Suppl 1):P19