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ABSTRACT BOOK

ORAL PRESENTATIONS

OP-A01. LEISHMANIASES IN PORTUGAL IN THE XXI CENTURY

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Leishmaniasis, caused by *Leishmania infantum*, is an endemic vector-borne-zoonosis in the Mediterranean basin and Portugal. Dogs are considered the major host for these parasites, and the main reservoir host for human visceral infection. Parasites are transmitted by phlebotomine sand flies, being *Phlebotomus perniciosus* and *P. ariasi* the proven vectors in the country. In the last years the number of visceral leishmaniasis (VL) cases in children has decreased with an increase of infection in adults, commonly associated with HIV/AIDS. More than 95% of strains from autochthonous leishmaniasis cases were identified as *L. infantum* MON-1. However, *L. major/L. infantum* hybrids have already been identified in four immunocompromised patients.
The aim of this study is to update the scenario of *Leishmania* infection in humans, dogs, cats and vectors in Portugal. In the last 13 years, 199 new cases of human VL (23 in immunocompetent adults, 54 in children and 122 in immunocompromised patients) and 23 CL cases were diagnosed in the Leishmaniases Laboratory/IHMT. In 2009 a global canine survey was conducted under the scope of the National Leishmaniasis Observatory (www.onleish.org) and an overall seroprevalence of 6.31% was found in mainland. *L. infantum* infection in cats has also been evaluated and *Leishmania* DNA prevalence has ranged from 0.3% to 30.4%. Phlebotominae surveys have been carried out and vector species were found infected with *L. infantum*. In addition, *L. major* DNA was detected in one *Sergentomyia minuta*.
Data reveal that Portugal continues to be an endemic country for leishmaniases and the prevalence of canine and feline infections is a serious concern for the increase and spreading of leishmaniases. Official notification of cutaneous and visceral human clinical cases and on-going surveillance with systematic epidemiologic surveys on *Leishmania* reservoir hosts and vectors is crucial since the increased migration and travelling flow elevate the risk of introduction and spread of infections by *Leishmania* species which are only sporadically endemic or non-endemic. The development of national and international epidemiological networks would promote opportunities to advise health authorities about the most effective measures for prevention and control of this zoonosis.

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WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-A02. POSSIBLE RE-EMERGENCE OF LEISHMANIASIS IN SERBIA

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Leishmaniasis, cutaneous and visceral, was endemic in south and southeast Serbia until the middle of the 20th century, but never reported in northern parts of the country - Vojvodina region. For more than 50 years leishmaniasis is considered to be eradicated and all research on vector and pathogen was abandoned. During the past few years several cases of canine leishmaniasis were reported in Vojvodina, which suggested the possibility of disease spreading to the north and re-emergence of the risk in Serbia. Research of sand flies in Vojvodina had been conducted only between 1948 and 1951 following a massive outbreak of sand fly fever. Then it was terminated, mostly due to the low abundance and diversity of vector species. Considering the possibility of change in vector-pathogen system and a lack of knowledge on sand flies in Vojvodina, entomological surveillance was resumed in 2013. Survey was conducted from the middle of July until the end of August in 17 villages that were worst affected with sand fly fever during the 1950 epidemic. Sand flies were collected with standard CDC light traps, dry ice baited traps without light, sticky traps and mouth aspirators during 20 sampling days. Sand fly specimens were identified morphologically and molecularly by sequencing the cytochrome oxidase 1 mitochondrial gene (COI 1). DNA was extracted and tested for *Leishmania* presence with Nested PCR. Each male and female specimen was tested separately. In total, 55 specimens of genus *Phlebotomus* were sampled. Most of them (54 specimens) were *Phlebotomus papatasi,* species previously reported from the sampling area. Only one specimen was identified as *Phlebotomus tobbi,* which was northernmost record of this species in Serbia and the first record for Vojvodina region. Four specimens of *P. papatasi*, which is specific vector for *Leishmania major*, tested positive on *Leishmania infantum*. Detection of *L. infantum* in *P. papatasi* was most likely incidental and connected to recent blood feeding. Supported by nearby discovery of *P. tobbi*, proven vector of *L. infantum*, this confirms the presence of pathogen in the host and possible circulation in Serbia.

WG3: Geospatial tools in vector research

OP-A03. MODELLING THE SPATIAL DISTRIBUTION OF ASYMPTOMATIC *LEISHMANIA INFANTUM* INFECTION IN PEOPLE IN SOUTHERN SPAIN

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Leishmania infantum (*Li*) is endemic in Murcia Region (southeast Spain). A study was carried out in this region on 657 blood donors in order to: (**i**) estimate the prevalence of asymptomatic *Li* infection, (**ii**) analyse the relationship between infection and demographic, sociologic and residential environmental variables, and (**iii**) generate a regional predictive infection risk map. Donors came from 19 blood donning centers in urban and rural localities across the region; they were also interviewed by questionnaire to collect data on sex, age, place of residence, work characteristics, *Leishmania* awareness, dog ownership and, when pertinent, dog dedication, sleeping habits and canine Leishmaniasis history. Donor´s plasma was analysed for *Li* antibodies using a commercial ELISA test and whole blood was tested for *Li* kinetoplast DNA sequences using a TaqMan probe real time PCR test. The results of the study showed a *Li* prevalence of 2% (13/657) by ELISA and 8% (49/618) by PCR. PCR prevalence was 3% (9/354) in donors from urban areas and 15% (40/260) in those from rural localities. Chi-square tests indicated that PCR status among rural donors was not significantly associated to any of the variables in the questionnaire ($p>0.05$). Geographical Information Systems were used to plot the residences of rural donors on a digital map and to obtain quantitative environmental data from a 1km radius area around the residences. Compared to areas with low PCR prevalence, those with high PCR prevalence were significantly cooler, drier, had lower solar radiation and stronger winds between March and July (when sand flies are most abundant), more rain during the winter months and the ground contained a high percentage of xerosols and a low percentage of fluvisols ($p<0.05$). There was strong correlation between environmental variables and the most parsimonious multivariable logistic regression model included only the mean temperature between March and July and altitude as significant predictors of the donor´s PCR status. Odds ratios increased with decreasing temperature and were lower for the highest altitude range (>300m) compared to the lowest (0-100m). The logit function was then used to elaborate a map of Murcia Region depicting the probability of being PCR positive based on these two variables. This map will be an important reference for future studies investigating the distribution of canine Leishmaniasis, sandfly abundance, and *Li* infection rates in the population.

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WG3: Geospatial tools in vector research

OP-A04. MAPPING CANINE LEISHMANIASIS AND PHLEBOTOMINE VECTORS IN THE MT. VESUVIUS AREA, ITALY

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The Mt. Vesuvius area of the Campania region (southern Italy) is an endemic zone of canine Leishmaniasis (CanL). *Phlebotomus perniciosus* is the main vector involved in the transmission of *Leishmania infantum* to susceptible hosts. Noteworthy, the Mt. Vesuvius area has been considered a stable focus of intense *L. infantum* zoonotic transmission.
Geographical information systems (GIS) are very useful tools for mapping and monitoring the distribution of CanL and its vectors in the area in relation to environmental features. A previous entomological study conducted in the area showed a different density of *P. perniciosus* between the two sides of the Mt. Vesuvius. Indeed, higher densities of *P. perniciosus* were found on the coastal side (density = 5.8) than on the Apennine side (density = 1.4). The predominance of green vegetated environments (forest, semi-natural and agricultural areas) in the coastal side, in contrast with the predominance of artificial surfaces (namely urban environment) in the Apennine side, was considered responsible for the different *P. perniciosus* densities between the two surveyed areas.
Therefore, a serological survey was conducted using GIS in order to map the distribution of CanL along the coastal and the Apennine sides of the Mt. Vesuvius area. Sera from 505 autochthonous owned dogs were examined by IFAT to detect antibodies to *L. infantum.* A titre of at least 1:80 was considered a positive result.
Out of the 505 dogs examined, 213 (42.2%; 95% Confidence Interval = 37.9 - 46.6%) were found to have antibodies to *L. infantum*. The prevalence was not different between the two sides (42.6% in the Apennine side and 41.6% in the coastal side). The findings of the present study showed that even low densities of *P. perniciosus*, especially when associated with an urban environment, seem sufficient to ensure *Leishmania* transmission among susceptible hosts. In fact, no significant differences were found in CanL seroprevalence of the two areas. Comprehensive control programs, including the use of vaccine, are discussed to prevent CanL in such endemic areas as the Mt. Vesuvius.

WG5: Rare and emerging vector-borne pathogens.

OP-A05. CANINE LEISHMANIASIS IN ROMANIA: A REVIEW OF THE LITERATURE AND A CASE REPORT

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Leishmania infantum, a protozoan parasite, is the causative agent of canine leishmaniasis, an endemic vector-borne zoonotic disease in many European countries. Dogs are having an important role in the ecoepidemiology of this disease, as they are the most commonly described reservoirs of this parasite. In Romania, the first autochthonous case of leishmaniasis in humans was reported in 1912. Between 1944 and 1955, there were 26 autochthonous human case reports, two isolated cases and one outbreak. All reports were from counties in southern Romania (Prahova, Giurgiu and Dolj Counties). No other cases of human autochthonous visceral leishmaniasis were reported in Romania, between 1955 and 2013. Limited information is available regarding canine leishmaniasis. The first report of clinical autochthonous canine leishmaniasis was in 1935. Since then, only few sporadic reports of positive asymptomatic dogs were registered. Two studies were conducted during the human leishmaniasis outbrake and another one was done more recently, in 2012. Data regarding available vector species and their geographical distribution is similarly scarce in Romania.

In 2014, the authors reported the first clinical case of autochthonous canine leishmaniasis in the last 80 years in Romania. A 6 year old mixed-breed bitch from Vâlcea County (southern Romania), with no history of travelling abroad was registered as a patient of the Dermatology Clinic, Faculty of Veterinary Medicine Cluj-Napoca. The diagnostic was indicated by the clinical findings, the results of the complete blood count and serum biochemistry and was confirmed by skin biopsies from the ear pinnae, footpads and periorbital areas and by a rapid diagnostic test (FASTest®LEISH). This clinical report, despite of not being the first, has a great importance that resides in the autochthonous character, stressing out the importance of a targeted surveillance of *Leishmania* infection in dogs and the need of raising both medical and veterinary doctors awareness in Romania.

WG5: Rare and emerging vector-borne pathogens

OP-A06. A STANDARDIZED DECALOGUE FOR FIELD EVALUATION OF VACCINES AGAINST CANINE LEISHMANIASIS IN EUROPE

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Dogs are the main reservoirs for zoonotic visceral Leishmaniasis (also known as canine leishmaniasis – CanL), a sand fly-borne infection caused by *Leishmania infantum*. In recent years, there have been advances in diagnosis, infection/disease staging, treatment, and prevention of CanL. A major advance in prevention includes evidence that the incidence of CanL, both in humans and in dogs, can be significantly reduced by the topical treatment of dogs with synthetic pyrethroids that have a potent anti-feeding (individual protection) and lethal-by-contact activity (mass protection) against sand flies. However, available pyrethroid formulations cannot prevent all potentially infectious sand fly bites. In addition, unlike what it was observed in controlled studies pyrethroid formulations have been found not sufficiently effective in the hands of owners. Hence, the development of effective canine *Leishmania* vaccine is highly desirable in both veterinary medicine and public health as an additional control measure against the disease. An increasing number of efficacy studies have privileged the use of natural challenge consisting in the long-term exposure of vaccinated dogs in endemic settings (Phase III). The following matters should be considered key points to limit the possibility of field studies failure and to facilitate the study authorization by Authorithies: **1**) a minimum of 2-years follow up including two consecutive sand flies transmission seasons; **2**) the use of naïve puppies of the same breed; **3**) the choose of a stable endemic site to maximise the probability of achieving the elevated *L.infantum* transmission levels required for a strong natural challenge; **4**) a number of enrolled dogs proportionate to the expected incidence of infection to ensure statistical differences between the groups; **5**) the avoiding of chemical products to control flea and tick infestations that could interfere in the results evaluation; **6**) the performing of regular assessments using a standard set of diagnostic markers to detect infection; **7**) the use of parasite culture to discriminate infected dogs progressing to disease; **8**) the clear definition of clinical end points to define a dog as symptomatic; **9**) the use of xenodiagnosis to assess the infectiousness of vaccinated dogs; **10**) the avoiding of euthanasia for dogs considered negative at the end of study and their proposal for adoption.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-B01. EVALUATION OF AUTOCHTHONOUS CUTANEOUS LEISHMANIASIS CASES IN TURKEY: GENOTYPE ASSESSMENTS INDICATE THE PRESENCE OF FOUR *LEISHMANIA* SPECIES AS WELL AS HYBRIDS

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Located between Europe, Asia and Africa, Turkey represents a transition zone, inhabiting vast variety of organisms including clinically important parasitic species. Cutaneous leishmaniasis (CL) has been present in Turkey, where more than 15.000 cases have been reported between 2005 and 2012. The causative agents of autochthonous cases were initially *Leishmania tropica* and *L. infantum;* however, in recent years *L. donovani* and *L. major* have also been reported in local studies using molecular methods, but without any indication whether the cases were autochthonous or imported. This is significant when planning the treatment of patients and public health measures to prevent the transmission of infection from the vectors and reservoirs. Indeed, our preliminary studies indicated the presence of hybrid species as well, which were not cultivated with conventional formulas of cultures. Here, we present our list of 231 autochthonous CL patients diagnosed in Turkey in recent years, together with basic information about the cases and their genotypic assessments.

All patients have initially asked for medical care for their skin lesions in Turkey. Initial clinical evaluation followed by microscopic examination of Giemsa stained lesion samples and cultures in NNN medium indicated CL, which was confirmed by Real Time PCR that targeted ITS-1 region of *Leishmania* spp. Species identification was done using "mini-exon repeat (MER) PCR and sequencing" method and typing after amplification of *hsp70 gene* and *cpb* genes of *L. infantum* and *L. donovani,* if necessary.

Among 231 CL patients identified in different provinces in Turkey (Figure 1), males were more than females [127 (55.0%) vs. 104 (45.0%)], and the leading lesion site was the face

(n=168; 72.7%). Real Time PCR indicated *L. tropica* (n=168; 72.7%), *L. major* (n=29; 12.6%), *L. infantum* (n=22; 9.5%) and *L. donovani* (n=11; 4.8%). The remaining patient was found to harbour *L.donovani/infantum* hybrids, confirmed by the amplification of *cpb* genes of *L. infantum* and *L. donovani*.

Our results indicate the presence of a vast variety of *Leishmania* species causing autochthonous CL in Turkey. Indeed, the presence of *L. donovani/infantum* hybrids demonstrates the genetic exchange between *Leishmania* species in Turkey. Regarding the active roles of *L. donovani* and *L. major* also in visceral leishmaniasis (VL), an elevation of local VL cases in coming years may be expected. Large-scale field studies are urgently needed to unveil the parasitic cycle between the vectors, reservoirs and individuals in Anatolia, together with public health measures to prevent human infections.

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WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-B02. AN INSIGHT INTO THE TRANSCRIPTOME OF THE *RHIPICEPHALUS BURSA* TICKS INFECTED WITH *BABESIA OVIS* TOWARDS DISEASE CONTROL

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Ticks are vectors of a heterogeneous array of organisms, including viruses, bacteria and protozoa with considerable medical and veterinary importance. An example is *Babesia*, an intraerythrocytic protozoan parasite causing babesiosis one of the most important diseases transmitted by ticks with a worldwide distribution. *Rhipicephalus bursa* is widely distributed in the Mediterranean Area being found in a variety of hosts including lambs and is known to transmit *Babesia ovis* among other species. Tick's salivary glands are the vehicle for pathogen transmission during feeding and a barrier that pathogens need to surpass making search of these tissues an important tool for the understanding of infection mechanism. Transcriptome analysis allows finding new key molecules directly related with disease being a promising approach towards tick and tick-borne pathogens control. In this work our main objective was to obtain a *R. Bursa* differentially expressed gene profile in response to *B. ovis.*
To analyze tick gene expression in response to infection it was firstly established an infection model by inoculating cultivated *B. ovis* parasite into the trochanter region of each female tick. Ticks were allowed to feed in lambs, free of parasites at the beginning of the assay, and animal parasitaemia was followed by observation of blood smears stained with Giemsa and by PCR. Data showed that lambs were infected with *B. ovis*, validating our model of infection.
RNA was extracted from the two SGs populations, *Babesia*-infected and non-infected; RNA quality and integrity were checked on an Agilent 2100 BioanalyzerNano Chip (Agilent Technologies, Santa Clara, CA, USA) and the identification of salivary glands genes related to infection and transmission achieved by RNA-sequencing (RNA-Seq) method. The two RNA populations were sequenced using Illumina platform. A new catalogue of differentially expressed genes in SGs of *R. bursa*infected with *B. ovis* was produced. To analyze the statistically represented gene classes, the g: Profiler web server (http://biit.cs.ut.ee/gprofiler/welcome.cgi) was used and obtained genes were classified according to Gene Ontology (GO) terms description (Functional Class, Biological Process and Molecular Function) and further selected based on expression value for future functional genomic studies. This work offers new inclusive knowledge regarding molecular interactions at the transcriptomic level in vector-pathogen interface towards the development of new tick and tick borne diseases control strategies.
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WG4: Phylogenetics and phylogeography of vectors and vector-borne pathogens

OP-B03. POPULATION DIVERSITY OF *THEILERIA ANNULATA* IN PORTUGAL AND CONTROL OPTIONS OF MEDITERRANEAN THEILERIOSIS

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The tick-borne apicomplexan parasite *Theileria annulata* is responsible for tropical or Mediterranean theileriosis, an economically important disease of cattle, which is endemic in parts of southern Europe including Portugal. In other parts of the world, the parasite population is known to be genetically diverse and this is one of the reasons hampering development of a sub-unit vaccine. Studies to date have focused on *T. annulata* populations in North Africa and Asia and little is known about parasite genetic diversity in southern Europe. For this reason, a study was performed on Portuguese cattle to evaluate the genetic structure of the local *T. annulata* population and the incidence of mixed genotype infections. Blood samples were collected from infected cattle (n = 90) in four regions of southern Portugal. DNA was isolated and parasites were genotyped using a panel of microsatellite and minisatellite markers that had previously been applied to Tunisian and Turkish parasite populations.
A high multiplicity of infection was identified across the sampled cattle, each animal was found to be infected with a number of different parasite genotypes. An average of 3.06 and a maximum of 4.50 alleles per locus and per isolate were detected, indicating a large number of genetically distinct genotypes present in each animal. Genetic differentiation between parasite populations isolated from different regions was found to be limited. Nevertheless, analysis of the standard index of association (I^S_A = 0.0272) indicated that mild linkage disequilibrium existed when all samples are considered together. When analysed separately, the population in two regions was found to be in linkage equilibrium, suggesting that the parasite population may be geographically sub-structured. To explore this concept, Principal Co-ordinate Analysis was undertaken, however, no clear pattern of geographical sub-structuring could be identified within the Portuguese population. The universally high

multiplicity of infection in each animal implies that the disease is sporadic and caused by the expansion of single parasite genotypes within a locality. Our results suggest instead that the Portuguese *T. annulata* population is well established and that an appreciable level of transmission is taking place. Unfortunately, this is not well recognised in Portugal and, consequently, attenuated vaccines and anti-*Theileria* chemoprophylactic drugs, used elsewhere in the world are not presently available in this country. Unless such measures become available, disease control must instead focus on alternative strategies such as tick control and the implementation of breeding strategies to promote the development of resistant autochthonous cattle breeds.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-B04. MULTILOCUS SEQUENCE TYPING REVEALS OVERREPRESENTED GENOTYPES OF *BORRELIA BURGDORFERI* S.L. IN CLINICAL MANIFESTATIONS OF LYME DISEASE

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Lyme disease is currently the most prevalent vector-borne disease in Europe. Its clinical manifestations have the most diverse forms, affecting different organs, from skin to joints, and from heart to brain.
There are two underlying mechanisms for the variability of clinical symptoms associated to *Borrelia burgdorferi* s.l. infections: (**1**) the intrinsic genetic properties of the bacteria, and (**2**) the variation in the immune response of the human host. In this study we tried to address the first aspect, by investigating what genotypes are the bacterial isolates from the various clinical manifestations in European human subjects. To this end, we tested for associations between the genotypes of *Borrelia burgdorferi* s.l. and the major clinical manifestations of Lyme borreliosis: erythema migrans (EM), acrodermatitis chronica atroficans (ACA), arthritis (LA) and neuroborreliosis (NB). Additionally, by means of comparison with the genotypes from questing ticks, we wanted to see if these genotypes are more frequently associated to clinical manifestations simply due to their high prevalence in ticks, or due to other causes, such as increased pathogenicity.
Isolates of *B. burgdorferi* s.l. from 184 Lyme disease cases, with distinct clinical manifestations, were sequenced and typed using the established multilocus sequence typing procedure (MLST). Additionally, 201 *Ixodes ricinus* lysates of Dutch provenience were typed, using the same scheme, and an extra 140 sequence types of *B. burgdorferi* s.l. from ticks were retrieved from the scientific literature and GenBank.
Sequence analysis was performed with MEGA6.06. Within each gene 50 to 63 haplotypes could be identified. Multilocus linkage disequilibrium was assessed using the index of association implemented in START2 software. Statistical analysis was performed with RStudio0.98.501. Fisher's exact test revealed that some *Borrelia afzelii* genotypes are positively associated with ACA, while some *Borrelia garinii* genotypes are positively associated with NB. Furthermore, although hardly present in questing ticks (1.4%), *Borrelia bavariensis* accounted for more than half (57%) of the NB cases. This is evidence that different genotypes of *B. burgdorferi* s.l. have different pathogenic properties.
The phylogenetic relations between these genotypes, the mechanisms underlying their evolution and maintenance, and the possible implications for public health will be discussed.

WG5: Rare and emerging vector-borne pathogens

OP-B05. ECO-EPIDEMIOLOGY OF *BORRELIA MIYAMOTOI* AND LYME BORRELIOSIS SPIROCHETES IN A POPULAR HUNTING AREA IN HUNGARY

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Borrelia miyamotoi, the newly discovered human pathogenic relapsing fever spirochete, and *Borrelia burgdorferi* sensu lato are known to be maintained in natural rodent populations. The aim of this study was to investigate the natural cycle of *B. miyamotoi* and *B. burgdorferi* s.l. in a forest habitat with intensive forestry work and hunting in Southern Hungary.
We collected rodents with modified Sherman-traps during 2010-2013 and questing ticks with flagging in 2012. Small mammals were euthanized, tissue samples were collected and all ectoparasites were removed and stored. Samples were screened for pathogens with multiplex quantitative real-time polymerase chain reaction (qPCR) targeting a part of flagellin (flaB) gene, then analysed with conventional PCR and sequencing.
177 spleen and 348 skin samples of six rodent species were individually analysed. Prevalence in rodent tissue samples was 0.2% (skin) and 0.5% (spleen) for *B. miyamotoi* and 6.6% (skin) and 2.2% (spleen) for *B. burgdorferi* s.l. Relapsing fever spirochetes were detected in *Apodemus flavicollis* males, *B. burgdorferi* s.l. in *Apodemus* spp. and *Myodes glareolus* samples. In questing *Ixodes ricinus B. burgdorferi* s.l prevalence (23.5%) was also significantly higher compared to *B. miyamotoi* (2.9%) (p=0.03). In the ticks removed from rodents we detected 6.6% *B. burgdorferi* s.l. and 1.1% *B. miyamotoi* infection. DNA amplification of both pathogens was successful from *I. ricinus* (*B. miyamotoi* 4.8%, *B.burgdorferi* s.l. 9.7%,), while from *Ixodes acuminatus* only *B. burgdorferi* s.l. DNA was amplified (8.9%). Two *Dermacentor marginatus* engorged larva pools were also infected with *B. afzelii*.
This study is the first report of *B. miyamotoi* occurrence in a natural population of *A. flavicollis* as well as in Hungary. We also provide new data about circulation of *B. burgdorferi* s.l. in rodent and tick communities including the role of *I. acuminatus* ticks in the endophilic pathogen cycle. Our results highlight the possible risk of infection with relapsing fever and Lyme borreliosis spirochetes in forest habitats especially in the high-risk groups of hunters, forestry workers and hikers.

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WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-B06. COMPARISON OF AN IMMUNOHISTOCHEMICAL AND TWO REAL TIME PCR ASSAYS FOR THE DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO IN TICKS COLLECTED FROM HUMANS

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The objective of this study was to compare different methods used for detection of *Borrelia (B.) burgdorferi* in ticks: immunohistochemistry followed by focus floating microscopy (FFM) and two RT-PCR assays targeting the *ospA* as well as the *hbb* gene.
Hard ticks collected from humans between 01.04.2010 and 07.09.2010 in the Clinic of Infectious Diseases Cluj-Napoca, Romania, were investigated regarding genus, stage of development and sex, and then tested by all three assays. *hbb* RT-PCR allows to distinguish *B. burgdorferi* at species level, except in case of infection with *B.spielmanii*or *B. valaisiana*and *B. garinii* or *B. bavariensis*. Working with paraffin embedded tissue, an optimized *ospA* RT-PCR assay was developed with an integrated internal amplification control for detection of inhibition in the PCR assay. The degree of agreement among the three diagnostic assays was assessed byCohen's kappa, using DAG-Stat spreadsheet.
Sensitivity of the optimized duplex *ospA*RT-PCR ranged between 1-10 copies/ PCR and efficiency was found to be 99.85%. For inclusive specifity22 *B. burgdorferi* reference strains were tested; 19 *B. burgdorferi* strains were detected, but detection failed for both *B. lusitaniae* strains testedand for *B. japonica***.** For exclusivity 20 strains were tested (18 *Leptospira* strains, *Borrelia miyamotoi* and *Treponema phagedenis)* and all found negative.
136 ticks collected from humans were analyzed for *B. burgdorferi* identification by all three assays. FFM was positive in 16 of the tested ticks (11.8%). Twelve of the tested ticks (8.8%) were found positive by*hbb* RT-PCR, while 26 of the tested ticks (19.1%) were positive by *ospA* RT-PCR. Two samples positive by the *hbb* RT-PCR assay were not detected by the *ospA*duplex RT-PCR; species identification revealed that one was *B. lusitaniae* and one *B. afzelii*. The rest of

10 samples positive in *hbb* RT-PCR were identified as 8 *B. afzelii*, one *B. burgdorferi* s.s.and one *B. spielmanii/B. valaisiana*. A poor quality of agreement was found between FFM and each of the two RT-PCR assays, as assessed by Cohen's kappa, while the agreement between the two RT-PCR assays was moderate.

The present study argues for a low sensitivity of FFM and underlines that discordant results of different assays used for detection of *B. burgdorferi* in ticks are frequent, therefore, testing for *Borrelia* in ticks detached from humans to predict infection cannot be recommended.

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WG3: Geospatial tools in vector research

OP-C01. VECTORNET: A EUROPEAN NETWORK FOR SHARING DATA ON THE GEOGRAPHIC DISTRIBUTION OF ARTHROPOD VECTORS OF HUMAN AND ANIMAL DISEASE AGENTS

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Recently the VectorNet network (http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/emerging_and_vector_borne_diseases/pages/vbornet.aspx), jointly funded by the European Center for Disease prevention and Control (ECDC, Stockholm, Sweden) and the European Food Safety Authorisation (EFSA, Parma, Italy), was launched. It strengthens the existing collaboration between both institutions in the context of vector-borne diseases (VBDs) and uniquely bridges their respective areas of interest.

The main objective is to expand the network of medical entomologists and public health professionals already established during VBORNET and to include veterinary specialists working on vectors and VBDs in Europe and the Mediterranean Basin. The network will collect and make available harmonised datasets on mosquitoes, ticks, sandflies, biting midges and to a certain extent VBDs of public and veterinary importance both through active and passive data collection. A large part of the budget will be dedicated to plan and conduct complementary entomological fieldwork to fill gaps in maps.

The consortium includes a pan-European expert matrix of 20 specialists with access to key data records on vector distribution. It will act as a driver of the wider VectorNet network, which now already includes 350+ experts, listed under VBORNET, and will actively encourage additional members to register and provide data using the consolidated web-based vector-tool application.
This structure allows efficient information exchange between the VectorNet consortium and network, and the timely achievement of the specific objectives: harmonised data collection and dissemination, data validation and quality assessment, strengthened collaboration on vector surveillance, ad-hoc expert advice to ECDC and EFSA, and standardised field data collection.
The outcomes will result in better preparedness for risk assessments ensuring an improved response to VBDs. The network and its tasks will be presented.

WG4: Phylogenetics and phylogeography of vectors and vector-borne pathogens

OP-C02. ABUNDANCE AND VECTORIAL CAPACITY OF THE PHLEBOTOMINE SAND FLIES IN THE AREA OF THESSALONIKI, CENTRAL MACEDONIA, GREECE

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The purpose of the study was to assess the risk of phlebotomine sand fly vectors to transmit Leishmaniasis in Thessaloniki area, Central Macedonia Greece. For this reason, during July 2014 and for 6 successive nights, phlebotomine sand flies where collected at the most suitable sites in the designated research area, both outside and inside of human settlements, by 10 standard white-light CDC and 6 BG-Sentinel traps with CO_2. At each location both traps were mounted next to or inside of chicken pens, dog kennels, goats and cows shelters. The traps were checked each morning and the trapped sand flies were sorted out and kept either dry or in 70% ethanol. Inside of houses and other plausible sand fly shelters, collection was carried out by mouth and electric-powered aspirators. The trapped sandflies were fixed and stored in 70% ethanol. In total more than 1500 specimens were collected. The majority of insect specimens were collected around dog kennels using BG-Sentinel traps with CO_2. Sand flies were also abundant near chicken and rabbit pens, but very few specimens were collected around and inside cow barns. The head and the terminal part of the abdomen were separated, mounted on microscopic slides and used for species identification based on the morphological and anatomical characteristic features. The rest of the body was kept frozen at -20°C for molecular analysis. The species that were identified were: *Phlebotomus tobbi, P. perfiliewi, P. simici, P. papatasi, Sergentomyia minuta* and *S. dentata* with *P. tobbi* being the most prevalent (70%). As for the molecular analysis, different approaches were followed for the DNA extraction of the sandflies. The DNA samples were tested with a previously described real-time PCR assay for the detection of *Leishmania infantum*. The DNA of *Leishmania* was detected in the sand flies of the species *P. tobbi,* especially in the sand flies collected around dog kennels. The results of the study indicate that the study area, with its high sand fly abundance and occurrence of *Leishmania infection in vectors* is a potential endemic focus for Leishmaniasis.

WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-C03. HUMAN AND VETERINARY IMPORTANCE OF KNOWN AND NOVEL SAND FLY-BORNE PHLEBOVIRUSES BELONGING TO THE *SALEHABAD* SPECIES USING VIRUS DISCOVERY AND NEUTRALISATION-BASED SEROPREVALENCE STUDIES

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Phlebotomine sandflies are vectors of a large variety of arboviruses, mostly in the genus *Phlebovirus*. In the Old World, 3 evolutionary lineages are recognized as transmitted by sand flies; two of them (Sandfly fever [SF]-Naples and SF-Sicilian) are known to include viruses that are pathogenic for humans; the third one (*Salehabad* group) was long considered as non pathogenic neither for humans nor for other vertebrates, and included only two viruses, namely Salehabad virus (isolated in 1959 from sandflies in Iran) and Arbia virus isolated in 1988 from sand flies in Italy. Recently one human case of meningitis caused by Adria virus in Greece was the first evidence that a Salehabad virus is pathogenic.

The objectives were (**i**) to search for new sand fly-borne phleboviruses belonging to the Salehabad species, and (**ii**) to assess the capacity of Salehabad phleboviruses to infect humans and vertebrates by neutralisation-based seroprevalence studies in countries surrounding the Mediterranean.

Sandflies were collected in France, Tunisia, Algeria, Turkey and Iran and processed for virus discovery. Human and vertebrates sera were collected and tested for the presence of neutralising antibodies specific of recognised and newly discovered viruses belonging to the Salehabad species.

Of total of 29,664 sand flies collected (France [n = 3,087], Tunisia [n = 8,206], Algeria [n = 1,283], Turkey [n = 12,000], Iran [n = 5,089], we isolated 4 strains of viruses that were clearly distinct from but most closely related to Salehabad viruses: 3 strains of Medjerda Valley virus (MJVV) in Tunisia, and 1 strain of Adana virus (ADAV) in Turkey. Complete genome sequences were determined and used for genetic and phylogenetic studies. Adana virus and Medjerda Valley virus are new members of the Salehabad species.

Seroprevalence studies were performed onto sera from different origins.

- from Turkey we tested 1,000 human and 289 animal sera (dogs=190; goats=51, sheep=48)
- from Greece we tested 1,184 dog sera
- from Cyprus we tested 369 dog sera
- from Tunisia we tested 1,272 human and 312 dog sera

Human seroprevalence in Turkey and Tunisia were 0.7% and 1.33% against ADAV and MJVV, respectively. High rates were observed in dogs: 17.6% MJVV Ab in Tunisia, 11% ADAV Ab in Turkey, 16.3% ADAV Ab in Cyprus, and 2.6% ADAV Ab in Greece. Even higher prevalence of ADAV Ab (35%) was found in goat and sheep in Turkey.

Evidence that human and vertebrates possess specific neutralising antibodies against Salehabad phleboviruses must lead to consider them as potential emerging pathogens for which clinical studies should be initiated.

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WG5: Rare and emerging vector-borne pathogens

OP-C04. TOSCANA VIRUS: A NEGLECTED SANDFLY-BORNE PATHOGEN IN PORTUGAL

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Toscana virus (genus *Phlebovirus*, family *Bunyaviridae*) is present in several countries around the Mediterranean Basin and is recognized as an important pathogen due to its ability to originate central nervous system disease, namely meningitis and meningoencephalitis. This neurotropic virus was first isolated in 1871, in Tuscany region, Italy, in *Phlebotomus perniciosus* sand fly species (*Diptera*, *Psychodidae*) and twelve years later, in 1983, it was isolated in a patient presenting with meningitis and admitted to a hospital in the same region. Also in 1983, the virus was isolated in Sweden but from a sample of a tourist who acquired the infection in Portugal which was considered, since then, as an endemic country for Toscana virus.
In Portugal the laboratory diagnostics of phleboviruses is provided by the Centre for Vectors and Infectious Diseases Research from the National Institute of Health since 2007. A retrospective serological study showed that Toscana virus is circulating in the Portuguese population and is causing disease from north to south. The results of that study lead us also to believe that this is a neglected pathogen, based in the fact that all the recent or ongoing infections were confirmed in samples whose laboratorial diagnostic requests were specifically directed to West Nile virus (family *Flaviridae*) and Lymphocytic choriomeningitis virus (family *Arenaviridae*) and not for Toscana virus.
The number of requests for diagnostics of Toscana virus is very low in Portugal and it is indicative that we are dealing with a pathogen not yet sufficiently recognized as an etiologic agent by clinicians.

WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-C05. FILARIOID NEMATODES, EMERGING PARASITES IN THE ARCTIC

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There is recent data documenting the geographical expansion of vector-borne Filarioid nematodes of domestic and free-ranging ungulates to subarctic areas including Finland, and an array of diseases associated with them.
Infections attributable to a species of the genus *Setaria* appear to have emerged in Scandinavian reindeer in 1973 associated with an outbreak of peritonitis and the death of thousands of reindeer in Finland. Severe peritonitis and large numbers of *Setaria* sp. worms were common findings. However, the prevalence of *Setaria* sp. in Scandinavian reindeer subsequently diminished. The following outbreak of peritonitis in reindeer started in 2003 in the southern and middle parts of the Finnish reindeer herding area leading to economic losses. The focus of the outbreak moved northward approximately 100 km/year. The causative was identified as *Setaria tundra*.
In 2004 also a new/unidentified abundant microfilaria in reindeer blood was found. Adult parasites were found inhabiting the lymphatic vessels of reindeer and were identified, for the first time in Europe, as *Rumenfilaria andersoni* Lankester and Snider, 1982 (Splendidofilariinae). There were no earlier reports of lymphatic-dwelling Filarioid nematodes in ruminants. In reindeer, *R. andersoni* prevalence in the southern part of Finnish reindeer herding area was up to 95% (of 249) in 2004. The focus of the outbreak moved simultaneously with *S. tundra* to north. In moose, the observed prevalence was 10 % (of 847 animals), and in new hosts; in wild forest reindeer 69 % (of 159), in white tailed deer 15% (of 105) and in roe deer 3% (of 59). The impact of *R. andersoni* to cervid health is unknown but visible chronic changes were seenaround ruminal lymphatic vessels during reindeer slaughter. Our hypothesis is that *R. andersoni*was introduced to Finland with white tailed deer in 1935 from North America.
Mosquitoes play an important role in the transmission of *S. tundra,* but the vector of *R. andersoni* is unknown. The development of *S. tundra* to the infective stage in mosquito is temperature dependent. We demonstrated that mean summer temperatures exceeding 14°C drive the emergence of disease outbreaks due to *S. tundra*, but the morbidity manifests in the following summer if it is still warm. Our theory was realized in autumn 2014; summers 2013 and 2014 were exceptionally warm in the Finnish reindeer herding area that led to the emergence of new *S. tundra* outbreak.
We predict that global climate change will promote the further emergence of Filarioid nematodes and diseases caused by them in the subarctic ecosystem. This may have effects on public health, sustainability of free-ranging and domestic ungulates, and ultimately food security for subsistence cultures at high latitudes.

WG5: Rare and emerging vector-borne pathogens

OP-C06. CIRCADIAN RHYTHM OF *DIROFILARIA IMMITIS* AND *DIROFILARIA REPENS* MICROFILARIAE IN NATURALLY COINFECTED DOGS

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Dirofilaria immitis and *D. repens* are mosquito-borne filarioids infecting carnivores, causing a severe cardio-pulmonary affection and a dermatological condition, respectively. Both species are zoonotic and, in Europe, most human infections are caused by *D. repens*. The female nematodes are ovoviviparous and produce blood-circulating microfilariae, which are assumed by the mosquito vectors. However, microfilaremia fluctuates during the day (periodicity) under the influence of several factors; including geographic location (i.e. maximum counts differ between countries from various regions of the world). As sampling hour may be essential for a correct diagnosis and therapy follow-up, the time when the maximum number of microfilariae occurs needs to be established. The aim of the present study was to assess the periodicity of microfilariae in dogs coinfected with *D. immitis* and *D. repens* in Romania. In July 2014, two positive dogs from Danube Delta were selected and sampled every 2 hours for two consecutive days. At every sampling time 0.7 ml of blood were taken from the cephalic vein: 0.5 ml were examined using the modified Knott's test and the remaining 0.2 ml were used for subsequent molecular analyses. The total number of microfilariae / ml of blood was calculated for each of the two species (identified by morphology) and variation charts were generated. *D. immitis* microfilariae were present at every sampling time, with higher counts at night and the highest peak at 1 AM. *D. repens* showed a similar variation pattern, but was absent from all samples taken at 9 AM. The mechanism of periodicity has not yet been fully understood. Some hypothesis imply that it is determined by the feeding behaviour of vector species, while others suggest it is host-related and dependent on physiological parameters. Indeed, in our study, maximum counts correlate with the mosquitoes' activity, which was more intense at night. On the other hand, experimental data from has shown that a low oxygen pressure determines a rise in microfilaremia, so the animals' behaviour (i.e. sleeping at night) may also account for the same results. Duplex PCR reactions produced only *D. immitis* specific bands in most samples. As the number of *D. immitis* microfilariae always exceeded that of *D. repens*, the disproportion between the DNA concentrations of each species can probably cause false PCR results. The present study is the first focused on coinfected dogs, indicating that the microfilariae of both *Dirofilaria* species react to the same stimuli, probably without influencing each other.

WG5: Rare and emerging vector-borne pathogens

OP-C07. ELUCIDATING THE ROLE OF LICE AS VECTORS OF HUMAN PATHOGENS

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Human head and clothing lice (*Pediculus humanus*) are comprised of two highly related ecotypes. Clothing lice are established notorious vectors of human pathogens, whilst it is held that head lice are not disease vectors. The current upsurge of head louse infestations within Europe underscores the importance of understanding the capacity of these lice to serve as disease vectors. We undertook the study to evaluate a real-time PCR assay that could distinguish between head and clothing lice. To determine the phylogenetic grouping of lice tested and to screen lice for the three known louse-borne bacterial pathogens of clinical significance, *Bartonella quintana*; *Borrelia recurrentis*; and *Rickettsia prowazekii*.

Genomic sequencing of the human clothing louse coupled with transcriptional analysis of head louse sequences has revealed that these ecotypes differed within their Phum_PHUM540560, a gene coding for a protein of unknown function. A real-time PCR assay targeting Phum_PHUM540560 could differentiate between the head and clothing ecotypes of *Pediculus humanus*. Cytochrome b gene sequencing has revealed that *Pediculus humanus* can be divided into three genetic clades A-C, and that clade A was comprised of both head and clothing lice. Clade A has a global distribution, whereas Clade B (head lice only) can be found in America, Australia and some areas of Europe. Clade C lice are only found only in Ethiopia, Nepal and Senegal, and include only head lice. Until now, only clade A lice have been evaluated using this ecotype specific PCR. We compared clades A and C. Finally, as clothing lice are notorious vectors of disease, we screened all lice tested for presence of these organisms using pathogen-specific PCRs. A sub-set of lice was additionally tested using universal microbial primers.

A total of 112 louse pools were evaluated. Of these, 34 were clothing lice. The majority of samples were correctly identified as either head or clothing lice. A small subset (both head and clothing) failed to react with the primers/probes of this assay. These yielded products using the cytochrome b PCR, thus poor quality DNA was unlikely to account for this anomaly. Sequencing of Phum_PHUM540560 gene failed to produce quality reads, preventing deduction of the reason for their failure in the real-time assay.

Cytochrome b sequencing confirmed that all clothing lice belonged to clade A whilst all head lice were in clade C fitted with the Ethiopian origin of these lice. Pathogen screening failed to detect any *Rickettisa prowazekii* or *Borrelia recurrentis*, however *Bartonella quintana* was

detected among 7% of lice tested. Universal primers detected *Enterobacter*, *Citrobacter*, *Gordonia* and uncultivated gamma Proteobacteria.

These findings confirmed that head and clothing lice could be differentiated, however on a cautionary note, some lice failed to amplify. This assay worked for both clade A and C lice. We were unable to detect either *Rickettsia* or *Borrelia* among the lice tested, however *Bartonella quintana* was detected not only among both clothing lice and head lice, confirming recent findings by others.

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WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-D01. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR RAPID IDENTIFICATION OF INVASIVE *AEDES* MOSQUITO SPECIES

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Invasive aedinemosquito species are of increasing concern in Europe as most of them are recognized or putative vectors of pathogens. Surveillance of these mosquitoes is mainly done by collecting eggs with ovitraps. A rapid molecular identification method of eggs, preferably applicable in the field, would greatly facilitate surveillance and control. Loop-mediated isothermal amplification (LAMP) is a rapid method to amplify DNA with high specificity and efficiency under isothermal conditions. Over the last 10 years, LAMP has been used in clinical diagnosis of infectious diseases as a valuable tool to detect a variety of pathogenic microorganism and viruses. The aim of this work was to develop LAMP assays for the rapid identification of eggs of container-breeding aedine species, including the invasive *Ae. japonicus, Ae. albopictus, Ae. aegypti, Ae. koreicus*as well as the indigenous *Ae. geniculatus*. At least 6 rDNA internal transcribed spacer 1 and 2 sequences for each species from at least 3 geographic regions/populations were sequenced. SixLAMP primers targeting the conserved regions of the consensus sequences were designed for all these species, and their specificity confirmed with DNA from all other species and additionally *Ae. triseriatus* and *Ae. atropalpus*. Exceptions were the *Ae. japonicus* and *Ae. Geniculatus* LAMP assays which finally contained only 5 primersas the Loop B or the Loop F primer, respectively, contributed to nonspecific amplifications. The assays were optimized with regard to betaine and $MgSO_4$ concentrations. LAMP reactions were detected by a color change of hydroxynaphthol blue from violet to sky blue visible by the naked eye. However, egg but not adult homogenates caused the colour change already before adding the enzyme. With a preceding incubation at 92°C for 5 min, which likely denatured egg proteins that interfere with the reaction, this challenge was overcome.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-D02. IDENTIFICATION OF MOSQUITO VECTORS THROUGH DNA METABARCODING

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Mosquitoes and biting midges are important disease vectors but the vectorcompetence variesbetween species. This makes it of outmost importance to monitor the distribution of different species in order to be prepared for future outbreaks. We have used a crowd sourcing method where the public could submit collected mosquitoes to establish further knowledge of the geographical distribution of mosquito species throughout Sweden. This has resulted in data on presence of 35 species of mosquitoes from 161 locations and discovered two species new to Sweden. By using presence data combined with geographical and climate data, distribution for present species can be modeled. The morphological identification of mosquitoes is time consuming and fails to classify worn or damaged specimens. For these reasons we have developed molecular techniques to identify mosquito vectors in field samples using DNA sequencing.

Many animal species can be identified by sequencing the mitochondrial gene COI which is established as a biological barcode. In order to identify specimens collected throughout Sweden we sequenced COI and produced a database of mosquito species present in Sweden.

By Next Generation sequencing we sequenced batches of mosquitoes together. From these sequences we count sequences corresponding to each individual mosquito species in proportion to the frequency of the species in the collection. We have used our morphologically determined mosquitoes to assemble batches of known mosquitoes in order to test the validity of this method. Amplicon based sequencing using three primer pairs covering COI from batches resulted in detection of all species in the batch but the proportion of detection of each species does not fully reflect their proportion in the batch. Combining data from three primer pairs gives a resulting proportion that has a correlation of 0.82 to the input proportions. We are currently optimizing the method to even closer reflect the sampled mosquito population.

We have developed bioinformatics pipelines to handle the massive data output from next generation sequencing. These pipelines will be developed and optimized for mosquitoes but will be suitable also for surveillance of biting midges or biodiversity studies of other insects.

WG5: Rare and emerging vector-borne pathogens

OP-D03. SEROLOGICAL INVESTIGATION OF USUTU AND WEST NILE VIRUSES IN WILD AND DOMESTIC BIRDS IN NORTHWESTERN ITALY, 2012-2014

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Usutu virus (USUV) and West Nile virus (WNV) are emerging pathogens that belong to the Japanese encephalitis virus antigenic complex of the family *Flaviviridae*, genus *Flavivirus*. Both viruses are maintained in the environment through a bird-mosquito life cycle, and mammals including humans are so far regarded as incidental or dead-end hosts. In the recent years, human neurological disease cases, due to USUV and WNV, have been reported in many parts of Italy and Europe confirming the zoonotic potential of these viruses. Migratory birds are considered to be the main source for introduction of USUV and WNV from Africa to the European countries.
A sero-survey on wild and domestic birds was carried out between March 2012 and October 2014 to investigate the circulation of both viruses in Piedmont region (Northwest Italy). Samples belonging to 87 different bird species and 14 orders have been collected covering a vast part of the study area. Birds were sampled in three wildlife rehabilitation centers, three farms and in the frame of ringing campaigns conducted by volunteer-based networks in locations with a high concentration of migratory birds. In particular, 871 and 790 serum samples were tested for the presence of anti-USUV and anti-WNV specific antibodies, respectively, by serum-neutralization (SN) assay. Nine of 871 serum samples had neutralizing antibodies against USUV (P= 1.03%, IC 95% 0.47-1.95), while 15 of 790 samples tested positive for WNV (P= 1.89%, IC 95% 1.06-3.1). Neutralizing antibodies for WNV were significantly more prevalent ($p<0.001$) in trans-Saharan migrants (P=9%, IC 95% 4.1-16.3) than in resident and short-distance birds, but no migratory habit-related differences were found for USUV. Neutralizing antibodies for WNV were also significantly more prevalent ($p<0.001$) in raptors (Strigiformes and Falconiformes orders) (P=10.1%, IC 95% 4.7-18.3) than in the other orders. Antibodies in resident bird species suggest that both viruses are circulating in NW Italy. Results show that voluntary networks can be an effective complement of official surveillance protocols for the USUV and WNV early detection infections in wild birds, providing a large number of samples and reducing the costs and the labor-intensive actions specifically targeted to human health protection.

WG4: Phylogenetics and phylogeography of vectors and vector-borne pathogens

OP-D04. DETECTION OF MARISMA VIRUS AND CHARACTERIZATION OF OTHER MOSQUITO FLAVIVIRUSES IN NORTH-EASTERN ITALY

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Flaviviruses are a large group of positive strand RNA viruses transmitted by arthropods including important human pathogens such as West Nile (WNV), Japanese encephalitis, Yellow fever, Dengue, Tick-borne encephalitis and other Flaviviruses specific to mosquitoes (MFV), which have no recognized vertebrate host. Here we report the detection of a recently discovered MFV, Marisma virus (MMV) and the genetic characterization of other MFV detected during the WNV entomological monitoring in North-Eastern Italy. From 2010 to 2014, 14,825 mosquito pools were screened using a One-Step SYBR Green rRT-PCR, targeting 260bp of the NS5 gene, able to detect both WNV and generic *Flavivirus* infections. Marisma specific primers (Mar1F-Mar2R) were designed in order to amplify 822bp of the NS5 gene and to perform the phylogenetic analysis.
Overall, 105 pools showed the presence of insect-specific Flaviviruses. These were detected in mosquitoes belonging to the species *Aedes vexans*, *Aedes albopictus* and *Ochlerotatus caspius*.
Phylogenetic analysis demonstrated that the sequences detected were aggregated in five significantly separate clades. The Aedes flavivirus clade from *Ae. albopictus* showed a 92-98% similarity to a Northwest Italy flavivirus sequence (KF801612). Two different clades were found from *Ae.vexans* pools: the first showed a 85-98% similarity to Nothwest Italy flavivirus sequence (KF801607) of the same mosquito species; the second one was 100% identical to a Central Italy flavivirus sequence (GQ477000). The last two clades were found from *Och.caspius* pools: the first showed a 88-92% similarity to a Central Italy flavivirus sequence (GQ476991) and the second one was identified as MMV. Sequences analysis grouped our MMV sequences in two separate groups: the first with 97% similarity to a Northwest Italy sequence (KF882512) and 92% similarity to a Spanish sequence (JN603190), and the second one with 90% similarity to an Italian sequence (KF882512) and 91% similarity to the Spanish sequence (JN603190). Marisma virus has been discovered and described only in 2012 in Spain and it was reported only once in Italy in 2013. The increasing discovery of novel MFV highlights the paucity of our knowledge of Flaviviruses and will help to unravel their evolutionary history. Also, further characterization is required before the diverse groups of MFV can be absolutely determined to be mosquito-specific and non pathogenic for humans and other vertebrates. Our results testify the usefulness to maintain a screening targeting the Flavivirus group rather than focusing on a specific virus only.
Funding: Veneto and Friuli Venezia Giulia Regions.

WG4: Phylogenetics and phylogeography of vectors and vector-borne pathogens

OP-D05. CO-CIRCULATION OF LINEAGES AND STRAINS OF WEST NILE VIRUS IN THE MOSQUITO VECTOR *CULEX PIPIENS* OF NORTH-EASTERN ITALY

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West Nile virus (WNV) represents a serious public health concern for Europe. In Italy from 2008 to date human and veterinary cases caused by Lineage1 (WNV-1) and Lineage2 (WNV-2) were reported. Our aim is to describe the temporal/spatial patterns of lineages/strains in the vector *Culex pipiens*. From 2010 to 2014, 14,825 mosquito pools, collected during the entomological surveillance in Veneto and Friuli Venezia Giulia regions, were screened for Flaviviruses by biomolecular tools. Overall, 27 pools of *Cx.pipiens* were positive for WNV-1 and 68 for WNV-2.
In 2010, two different strains of WNV-1 were present: Italy08 strain in the Rovigo and Treviso areas and Livenza strain in the Venice area. In 2011, WNV-1 Livenza strain expanded to Treviso and Pordenone areas, while Italy08 apparently disappeared. Furthermore, a WNV-2 emerged in the Udine area, sharing high similarity with a Hungarian isolate. In 2012, WNV-1 Livenza strain continued to circulate in Venice, Treviso and Udine and another WNV-2 was found in Rovigo, with high similarity with the 2010 Greek isolate. The protein NS3 analysis of this strain showed the $H_{249}P$ replacement suggested as a possible cause of higher pathogenicity in humans. In 2013 the WNV-1 Italy08 strain re-emerged in the Rovigo area, slightly different but closely related to the original one; in addition, Livenza strain apparently disappeared and WNV-2 bursted (55 samples) with six different variants belonging to a new cluster (99% identity with the Greek and Hungarian isolates). Only one of these variants showed the $H_{249}P$ replacement. In 2014 WNV-2 continued to circulate in the Rovigo and Verona areas and expanded to Vicenza. At the same time a different strain of WNV-2 emerged in the Udine and Pordenone areas sharing high similarity with the Russia 2007 and Romania 2013 isolates. The protein NS3 analysis showed the $T_{334}S$ replacement.
The phylogenetic analyses demonstrated the high complexity of WNV. The findings highlight the fact that new introductions, likely through migratory birds, have occurred in few years (WNV-1 and WNV-2) and indicate the capability of these viruses to become endemic and to rapidly evolve and emerge in different sites. North-Eastern Italy is confirmed as a high-risk area for arbovirus introduction, emergence and endemisation.

Funding: Veneto and Friuli Venezia Giulia Regions.

WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-D06. MOLECULAR DETECTION OF *BABESIA CANIS*, *BABESIA* CF. *MICROTI* AND *HEPATOZOON CANIS* IN RED FOXES (*VULPES VULPES*) FROM BOSNIA AND HERZEGOVINA

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Red foxes (*Vulpes vulpes*) are the most widely distributed wild canid species and reservoirs of different vector-borne pathogens of medical and veterinary importance. The present study investigated the occurrence and geographical distribution of *Babesia* spp., *Hepatozoon canis*, *Anaplasma* spp., *Bartonella* spp., '*Candidatus* Neoehrlichia mikurensis', *Ehrlichia canis*, *Rickettsia* spp. and blood filaroid nematodes in free-ranging red foxes from Bosnia and Herzegovina.

For this purpose spleen samples from a total of 119 red foxes, shot during the hunting season between October 2013 and April 2014 in six different regions in Bosnia and Herzegovina, were examined for the presence of several vector-borne pathogens by conventional PCRs and sequencing.

Three species of apicomplexan parasites were identified by molecular methods in 73 (60.8%) red foxes from all surveyed areas. DNA of *B. canis*, *B.* cf. *microti* and *H. canis* was found in 1 (0.8%), 38 (31.9%) and 46 (38.6%) spleen samples, respectively. The geographical distribution of these pathogens overlapped in many sampled areas and co-infections with *B.* cf. *microti* and *H. canis* were confirmed in 11 animals (9.2%), while a single fox harboured all three pathogens (0.8%). There were no statistically significant differences between geographical region, sex or age of the host in the infection prevalence of *B.* cf. *microti*, whereas females (52.9%; 18/34) were significantly more infected with *H. canis* than males (32.9%; 28/85). The presence of DNA of vector-borne bacteria and blood filaroid nematodes could not be confirmed in our study.

This study reports, for the first time, the occurrence of *B. canis*, *B.* cf. *microti* and *H. canis* parasites in foxes from Bosnia and Herzegovina. Moreover, the relatively high prevalence of *B.* cf. *microti* and *H. canis* support the existence of a sylvatic cycle and reinforces the assumption that this wild canid species might be a possible reservoir and source of infection for domestic dogs.

WG5: Rare and emerging vector-borne pathogens

OP-E01. TICKS INFESTING HUMANS IN ITALY AND ASSOCIATED PATHOGENS

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Ticks are amongst the most important arthropod parasites of animals and display a worldwide distribution, being adapted to different environments and host species. In particular, hard ticks (order Ixodida, family Ixodidae) represent the most diverse group, in terms of biology and geographical distribution, and they may transmit a huge number of pathogens, which cause ailments in animals and humans, commonly referred as to tick-borne diseases (TBDs). The incidence of human TBDs in Italy is underestimated because of poor surveillance and the scant studies available. Accordingly, the limited data on ticks, and their associated pathogens infecting humans in Italy, may impair the understanding of the risk for TBDs in this country. To bridge this gap on knowledge, ticks (n = 561) were collected from humans in four main geographical areas of Italy (i.e., northwestern, northeastern, southern Italy, and Sicily), which represent a variety of environments. After being morphologically identified, ticks were molecularly tested with selected protocols for the presence of pathogens from the genera *Rickettsia*, *Babesia*, *Theileria*, *Candidatus* Neoehrlichia mikurensis, *Borrelia* and *Anaplasma*. Ticks belonged to 16 species of the genera *Argas*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*, with *I. ricinus* (59.5%) being the species most frequently retrieved, followed by *R. sanguineus* sensu lato (21.4%). The life stages most frequently collected were nymphs (41%) and adult females (34.6%), with an overall positivity to any pathogen of 18.1%. Detected pathogens were *Rickettsia* spp. (17%), namely *Rickettsia monacensis* (10.1%), *R. massiliae* (2.1%), *R. slovaca* (1.8%), *R. Helvetica* (1.4%), and *Rickettsia* spp. (0.5%); *Anaplasma phagocytophilum* (0.8%); *Borrelia afzelii* (0.5%) and *Borrelia valaisiana* (0.3%); *Candidatus* N.mikurensis (0.5%) and *Babesia venatorum* (0.6%). Results indicate that people on the Italian peninsula are at risk of being bitten by different tick species, which may transmit a plethora of TBD causing pathogens and that co-infections may also occur. This aspect is particularly important considering that the Italian peninsula has a great vocation for tourism, mostly during the spring and summer months, which constitutes the high-risk period for tick infestation in some areas.

WG5: Rare and emerging vector-borne pathogens

OP-E02. CHANGES IN TICK FAUNA DISTRIBUTION AND PATHOGENS PREVALENCE IN THE NORTHERN APENNINES, ITALY

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In Italy the risk posed by tick borne zoonoses is generally neglected, although an increase in human cases and tick abundance, together with their geographical expansion, are reported, as it is happening in other parts of Europe. Several factors are considered as responsible for this new condition, such as climate change, changes in land use, the increase and/or territorial recovery of wild ungulates populations.
A study was conducted from 2006 to 2012 in a mountain area in the northern Apennines, Italy. Questing ticks were collected by dragging in 35 sites close to tourist trails, between 600-1650 m above sea level (a.s.l.). With a total dragging effort of 644 × 100m transects, *Ixodes ricinus* (5477 larvae, 850 nymphs and 15 adults) and *Haemaphysalis punctata* (16697, 482, 37) were the most abundant species collected, followed by *Haemaphysalis sulcata* (107, 12, 19), *Dermacentor marginatus* (122 larvae and 3 adults) and one *Ixodes hexagonus* nymph. *I. ricinus,* whose larvae and nymphs were found up to 1650 m a.s.l., showed a predilection for beech woods and oak woods. *Haemaphysalis* spp. was particularly abundant in the southern area, characterized by mixed (oak, chestnut, hornbeam) and beech woods with scarce vegetation and exposed rocks. *Borrelia burgdorferi* s.l. was detected by PCR in 8.6% (95%CI: 5.6-12.4) *I. ricinus* nymphs (n tested=291) and different genospecies were identified: *B. garinii*, *B. valasiana*, *B. burgdorferi* s.s., *B. afzelii* and *B. lusitaniae*. *Rickettsia slovaca* showed a high prevalence (42.1%, 95%CI: 26.3-59.2) in 38 *D. marginatus* larvae tested.
Our results indicate a sharp increase in tick abundance compared to 1994-95, when in the same study area a lower density of *H. punctata* was registered and only three *I. ricinus* specimens were overall collected in 52 Km of dragging. Moreover, *B. burgdorferi* had not been detected at that time, by testing sylvatic mice ear punches.
In conclusion, we report an increasing risk of human-tick encounter and tick-borne pathogens transmission in the study area, even at high altitudes. In particular, we observed an invasion and spread of *I. ricinus*, a species traditionally infesting deciduous forests under 1300 m a.s.l., to beech woods that dominate the Appenines between 1100 -1700 m a.s.l. The recorded *I. ricinus* range expansion could have been driven by the increase population densities of wild ungulates in the study area, particularly roe deer, during the last decades.

WG5: Rare and emerging vector-borne pathogens

OP-E03. NEW INSIGHTS ON THE BIOLOGY OF THE TICK PARASITOID *IXODIPHAGUS HOOKERI* (HYMENOPTERA, ENCYRTIDAE)

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Natural enemies of ticks include the parasitoid wasp *Ixodiphagus hookeri* (Hymenoptera, Encyrtidae). This wasp was originally described parasitizing *Rhipicephalus sanguineus* in Texas (USA), as well as other ixodid species belonging to the genera *Dermacentor*, *Amblyomma*, *Hyalomma*, *Haemaphysalis* and *Ixodes*. Although the use of this wasp for the biological control of ticks has been suggested, the only successful attempt to use it to reduce a tick population of *Amblyomma variegatum* was reported in a field study from Africa. Considering that the impact of *I. hookeri* on populations of ixodids is little studied, the aim of this research was to investigate the occurrence of *I. hookeri* in a community of ticks from southern Italy. From May 2010 to March 2012, ticks (n = 6943) were collected monthly by dragging (n = 3694) and flagging (n = 3249). Specimens were identified at species level and about 10% of adults (n = 481) and nymphs (n = 305) were molecularly screened. Of the samples tested (n = 786), 3.1% (n = 25) were positive for *I. hookeri* DNA, 7.2% (n = 22) in nymphs and 0.6% (n = 3) in adults. *Ixodiphagus hookeri* DNA was only detected in *I. ricinus*. Almost all positive ticks (23/25) were collected between October and March of 2012, except one in October 2011. This study indicates that *I. hookeri* infests *I. ricinus* in southern Italy and that the parasitism is likely to be related to the tick life-cycle. Further studies focusing on the activity period of the adult wasp and its potential use in the control of ticks are needed.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-E04. METAGENOMICS REVEALS SHARED BACTERIAL COMMUNITIES IN TICKS AND RODENTS WITH POTENTIAL INTEREST FOR NEW EPIDEMIOLOGICAL CYCLES AFFECTING ANIMALS AND/OR HUMANS.

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In Europe, ticks are the first arthropod vectors of disease agents to humans and domestic animals and the incidence of tick-borne diseases (TBD) is increasing worldwide. Tick common habitats are woods, pastures and gardens and the main source of blood meals for larvae and nymphs are rodents, which represent one of the major reservoirs and source of tick-borne pathogens. Ticks and rodents are highly susceptible to global environmental and socio-economical changes, which in turn may lead to increased burden of tick-borne diseases. In recent years, new zoonotic bacteria carried by ticks and rodents have been described in Europe. Our study was based on the use of Next Generation Sequencing derived from bacterial metagenomics in order generate a global picture of bacterial sequences shared by ticks and rodents. We trapped voles and ticks in the French Ardennes, a forested region on the border with Belgium, along a transect line of ≈80 km. Along this transect, we sampled 6 sites in forested areas and 4 sites in fragmented habitats (i.e., hedge networks), with about 30 rodents *Myodesglareolus* and 30 ticks *Ixodesricinus* in each site. A multiplex strategy allowed characterizing the bacterial communities within each rodent and each tick individuals. Using this strategy, we have indeed identified known but unexpected bacteria as well as new or poorly known bacteria phylogenetically close to known bacteria transmissible to humans and/or animals by arthropods (*Bartonella, Borrelia, Mycoplasma, Neoehrlichia, Rickettsia, Orientia, Midicholria, Spriroplasma, Spirosoma*). We also derived bacterial prevalence in ticks and rodents according to forest fragmentation, and explored bacterial co-occurrence and potential co-transmission. This study demonstrated that many still unknown bacteria are carried by both ticks and rodents and could participate to unknown epidemiological cycles potentially affecting animals and/or humans. The monitoring of these bacteria deserves to be undertaken in human and/or animal populations.

WG5: Rare and emerging vector-borne pathogens

OP-E05. IDENTIFICATION OF NOVEL ZOONOTIC *BARTONELLA* SPECIES RESPONSIBLE FOR PAUCISYMPTOMATIC BACTEREMIA IN PATIENTS REPORTING TICK BITES

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Certain *Bartonella* species are known to cause afebrile bacteremia in humans and other mammals (*B. quintana*, the agent of trench fever, and *B. henselae*, the agent of cat scratch disease). Reports indicate that animal-associated *Bartonella* species may cause paucisymptomaticbacteremia and endocarditis in humans. A number of patients bitten by ticks complain of polymorphic and non-specific clinical symptoms (e.g., asthenia, fever, myalgia), for which the diagnosis is not straightforward. Due to a history of tick bites, it is common to refer to Lyme disease, but in many cases, there is no confirmation of the diagnosis by serological or DNA-based tests. Several subjective and unexplained syndromes have been attributed to tick bites, and some authors have proposed *Bartonella* spp, as causal agents.In this study, we screened for the presence of *Bartonella* in the blood of patients reporting unexplained symptoms after a tick bite. Here, we report the isolation and genomic sequencing of six *Bartonella* strains obtained by blood culture from 66 patients. Three strains were identified as *B. henselae*, and another three strains were identified as different animal-associated species (*B. doshiae, B. tribocorum*, and *B. schoenbuchensis*). This is the first time that these species have been cultured from humans, and they must now be reconsidered as zoonotic species causing undifferentiated chronic illness in humans. In conclusion, we uncovered three additional *Bartonella* species with zoonotic potential and showed the *Bartonella* spp. infection may be the cause of undifferentiated chronic illness in humans reporting tick bites.

WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-E06. REVIVE, A SURVEILLANCE PROGRAM ON VECTORS AND VECTOR-BORNE PATHOGENS IN PORTUGAL – FOUR YEAR EXPERIENCE ON TICKS

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REVIVE is a national wide surveillance program on vector and vector-borne agents implement and coordinate by the National Institute of Health (CEVDI/INSA) in collaboration with other institutions of the Health Ministry. The programme started in 2008 with the surveillance of mosquitoes and later in 2011 was extended to ticks. The main goals of this project are to collect and identify vectors, updating our knowledge in the distribution, host-associations, seasonality and abundance of the Portuguese species. Additionally this project contributes for monitoring the introduction of exotic vector species. This work regards the 4-year REVIVE studies on ticks and *Borrelia/Rickettsia* surveillance, among other tick-borne agents, discussing the established circuits, obtained results and practical interventions.

Over 29.000 ticks were collected on hosts or by flagging vegetation from 168 (60.4%) municipalities of mainland Portugal. Collection in humans reached the 583 specimens. In total, 13 autochthonous tick species were identified, including *Dermacentor marginatus; D. reticulatus; Haemaphysalis punctata; Hyalomma lusitanicum; H. marginatum; Ixodes canisuga; I. hexagonus; I. ricinus; I. ventalloi; Rhipicephalus annulatus; R. bursa; R. pusillus; R. sanguineus*. Of note is the identification of an exotic species, *Amblyomma* sp., attached to a Portuguese emigrant arriving from USA. The top three species collected during this surveillance program were *R. sanguineus* (69%), followed by *R. pusillus* (16.4%) and *H. marginatum* (9.7%). However regarding antropofilic behaviour, from the 11 species found in humans the most prevalent were *I. ricinus* (35%), followed by *R. sanguineus* (34%), and *H. marginatum* (14%). The abundance, distribution, host association and other relevant patterns are compared with previous existing records. Regarding the tick-borne agents, all ticks collected from humans and about 10% of the questing/host-attached ticks were tested for *Borrelia* and *Rickettsia* spp., among other agents. Ten bacteria were identified so far in single or multiple infection, including *Borrelia afzelii, B. garinii*, *B. lusitaniae*, *Rickettsia aeschlimannii, R. conorii*, *R. helvetica*, *R. massiliae*, *R. monacensis*, *R. raoulti*, and *R. slovaca*. The importance of including other tick-borne agents in routine screening is also discussed.

The presented data reinforces the importance of the REVIVE. The program has contributed to call attention to tick-borne diseases not only among healthcare providers but also in the populations. The workflow established, has also enabled timely screeningof ticks removed from humans, animals or in a given environment, allowing the implementation of informed prevention/control strategies and directly contributing to improve Public Health in Portugal.

Additional funding: *Coxiella* and *Anaplasma* testing was performed on behalf of the FCT project PTDC/SAU-SAP/115266/2009

WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-F01. EVALUATION OF THE EFFICACY OF OLYSET® PLUS IN A VILLAGE-BASED COHORT STUDY IN THE CUKUROVA PLAIN (TURKEY), AN AREA OF HYPERENDEMIC CUTANEOUS LEISHMANIASIS

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The aim of this study was to measure the protective efficacy of Olyset® Plus, a new long-lasting factory-treated insecticidal net (LLIN) incorporated with 2% permethrin and 1% of the synergist piperonylbutoxide (PBO), against cutaneous leishmaniasis (CL) transmission under field conditions. A village-scale trial, promoting the use of LLIN by the local inhabitants of the study area was conducted as a pilot study in a new hyperendemic focus of CL caused by a *Leishmania infantum*/*L. donovan*i hybrid parasite transmitted by proven vector species *Phlebotomus tobbi* in Cukurova Plain, Adana (Turkey) from May 2013 to May 2014. The study area comprised eight villages; two of them were selected, respectively, as: (**i**) an intervention village with Olyset® Plus net (Kizillar), and; (**ii**) a control village without net application (Malihidirli). Six villages with surrounding allopatric barriers were utilized as a buffer zone cluster between intervention and control villages. Monthly entomological surveys were performed in the intervention and control villages and Damyeri, representing the other six villages, to collect adults of *P. tobbi*. Results showed a significant reduction in cutaneous leishmaniasis incidence in the intervention village from 4.78% to 0.37%. The protective efficacy rate of LLIN was 92.2%. In contrast, incidence rates increased in the control village from 3.67% to 4.69%. We also evaluated residual insecticide levels of used nets after 6 and 12 months of usage. It was determined that the nets had retained full insecticidal strength. These results highlight the value of real-world data on bed net effectiveness and longevity to guide decisions regarding sand fly control strategies. To the best of our knowledge, this is the first field study to evaluate Olyset® Plus efficacy in a hyperendemic cutaneous leishmaniasis area.
Funding: This work was partially supported by EDENext (FP7-INCO-CT-2010-261504).

WG5: Rare and emerging vector-borne pathogens

OP-F02. DISTRIBUTION OF THE PHLEBOTOMINE SANDFLIES IN BULGARIA AND THEIR POTENTIAL ROLE AS VECTORS OF HUMAN VISCERAL LEISHMANIASIS

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Phlebotomine sand flies are known to transmit parasites, bacteria and viruses that affect humans and animals in many countries worldwide. During the XXth century they were considered to transmit human visceral leishmaniasis (VL) and sandfly fever in Bulgaria. Studies on species composition in the country indicate the presence of five species of the genus. 142 cases of autochthonous human VL were registered during the last 26 years in regions where phlebotomines were collected in the past.
Fourteen different regions of Bulgaria were sampled for sand flies during the period 2011-2014 using different light traps and CO_2 traps and 991 specimens (447 males and 514 females) were collected. Part of them were determined to species level (unpublished data) using morphological keys. Published data on the distribution of the Bulgarian sandfly species of genus *Phlebotomus* collected over the period 1909-2014 were reviewed. Both historical and contemporary collection localities for each species were plotted using the Universal Transverse Mercator (UTM) grid system on a contour UTM map. Data on human cases of VL registered during the period 1988-2014 in the National Centre of Infectious and Parasitic Diseases were processed the same way. A comparison was made between the species composition of the Bulgarian sandfly fauna and the available data for all the neighbouring countries (Greece, Macedonia, Romania, Serbia and Turkey).
UTM maps presenting the distribution records of *Phlebotomus balcanicus*, *Ph. papatasi, Ph. perniciosus, Ph. similis* and *Ph. tobbi*, together with the localities of registered human cases of VL were prepared. The two map sets representing the geographical extent of human VL (63 UTM squares) and its potential vectors (52 UTM squares) were compared. The distribution of VL in the country suggests a wider distribution of the phlebotomine sandflies and their active role as vectors. Considering the sandfly fauna of the neighbouring countries, about five more phlebotomine species may be expected to be found in Bulgaria – *Ph. alexandri, Ph. neglectus, Ph. perfiliewi, Ph. mascittii* and *Ph. simici*.

WG5: Rare and emerging vector-borne pathogens

OP-F03. SCHMALLENBERG VIRUS INFECTIONS IN SLOVENIA

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In November 2011, Germany reported the first occurrence of a novel *Orthobunyavirus*, named Schmallenberg virus (SBV). In 2013, the SBV was detected in 28 herds in Slovenia, where clinical manifestations of the disease in sheep and cattle were observed. To identify the time of the introduction of SBV infection to Slovenia, we retrospectively analysed 42 cattle blood samples randomly collected in different parts of Slovenia between June and October 2012 using ELISA for SBV antibody detection.
The first SBV antibody-positive blood samples were obtained on August 29, 2012.
Between January and February 2013, 87 cattle blood samples randomly collected throughout Slovenia were tested for SBV antibodies using ELISA in a prospective study. The results showed a high prevalence (82.8%) of antibody-positive animals. The clinical manifestations of SBV infection, such as congenital malformations, were observed in both cattle and sheep. A prospective follow-up study was performed in 2014 to determine if SBV is still circulating in Slovenia. The blood samples were collected from cattle young stock between 7 and 13 months old (n=170) in different parts of Slovenia between August and November 2014 and tested for SBV antibodies using ELISA. Only 6.47% of samples were positive, confirming the circulation of SBV. However, the percentage of SBV antibodies positive animals was much lower than in 2013. Additionally, no clinical cases of SBV infection were detected in 2014. Despite this low percentage of positive animals, we can anticipate a new wave of clinical cases in the future due to low immune status of young cattle.

Funding: This research was financially supported by the Slovenian Research Agency; program P4-0092 (Animal health, environment and food safety).

WG5: Rare and emerging vector-borne pathogens

OP-F04. PREVALENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS AND SPOTTED FEVER GROUP RICKETTSIAE IN *HYALOMMA MARGINATUM* TICKS REMOVED FROM PATIENTS AND BIRDS.

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Crimean-Congo hemorrhagic fever virus (CCHFV) was detected in *Hyalomma lusitanicum* ticks collected from deer in Spain in 2010. The finding of CCHFV in *H. marginatum* from birds in Morocco by our group strengthened our hypothesis of the arrival of the virus to the Iberian Peninsula from infected ticks carried by migratory birds. Furthermore, *Hyalomma* species are vectors of other tick-borne infections such as spotted fever rickettsioses. The aim of this study was to investigate the presence of CCHFV and *Rickettsia* spp. in *Hyalomma* ticks from patients (North of Spain) and birds (North of Spain and Morocco).
A total of 178 ticks collected in different periods of time from 2009 to 2013 and classified as *H. marginatum* were studied. Among them, 126 specimens (11 from patients and 115 from birds) were collected in the North of Spain and 52, in which our group had previously reported the infection with CCHFV, in Morocco (all from birds). Samples were screened for CCHFV and *Rickettsia* spp. using molecular biology techniques (one qPCR and four conventional PCR assays targeting the S segment of CCHFVand two nested PCRs targeting *ompA* and *ompB* rickettsial genes).
None of the samples collected in Spain yielded positive PCR results for CCHFV detection. In addition, 2 ticks removed from patients (18.2%), as well as 26 (22.6%) and 4 (7.8%) specimens from birds (collected in Spain and Morocco, respectively) were infected with *Rickettsia aeschlimannii*. *Rickettsia sibirica* subsp. *mongolitimonae* was also found in 2 ticks from birds collected in Spain (1.7%).
These results suggest that the risk of CCHFV-infected ticks attached to migratory birds to reach the North of Spain is low, although the absence of this virus cannot be confirmed. Our study corroborates the presence of *R. aeschlimannii* in *H. marginatum* from Spain and Morocco and supports that *H. marginatum* can be a potential vector of *R. sibirica* subsp. *mongolitimonae* in the Iberian Peninsula. The involvement of birds in the epidemiology of diseases such as lymphangitis-associated *rickettsiosis* (LAR) -caused by *R. sibirica* subsp. *mongolitimonae*-by the spread of ticks harboring these *Rickettsia* spp. is evidenced.

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WG5: Rare and emerging vector-borne pathogens

OP-F05. ISOLATION AND CHARACTERIZATION OF '*CANDIDATUS* RICKETTSIA VINI' OBTAINED FROM THE ORNITOPHILIC TICK *IXODES ARBORICOLA*

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Ixodes arboricola is a nidiculous tick that feeds on passerine birds nesting and roosting in tree holes or nest boxes. It is widely distributed throughout the Palearctic region. Recently, DNA of a new bacteria '*Candidatus rickettsiavini'* has been detected in *I. Arboricola* ticks from Spain, Turkey, Czech Republic and Slovakia. This rickettsia belongs to spotted fever group, which suggests its potential pathogenicity. Our aim was to isolate '*C.*R.vini' in pure culture and test whether it is pathogenic for mammals.

We collected 17 males and 115 females of *I. Arboricola* in the Czech Republic in June 2014. Male ticks containing *Rickettsia*-like structures according to the haemolymph test were treated with iodine alcohol, washed in sterile water, triturated in 500 µl of brain heart infusion broth and the homogenate was inoculated into shell vials containing a monolayer of Vero cells with medium containing penicillin, streptomycin and fungizon. After 3 days, antibiotic-free medium was used. The aspirated medium was monitored for the presence of *Rickettsia*-like organisms using Giménez staining. The positive cultures were inoculated into flasks containing a monolayer of Vero cells. A course of infection was checked by Giménez staining of scrapped cells. Three isolates of '*C.*R.vini' from three ticks were successfully established in Vero cell cultures. A cytopathic effect was observed.

Molecular characterization of these isolates was performed with targeting partial sequences of 4 rickettsial genes (*gltA, ompA, ompB, htrA)*, DNA was extracted using the guanidine isothiocyanate-phenol technique. The sequences subjected to BLAST analysis were 100% identical with those obtained from*I. Arboricola* ticks from Spain.

A suspension of ≈3000 *Rickettsia*-infected Vero cells was inoculated intraperitoneally into 2 male guinea pigs. The body temperature was measured rectally each day until 21 days post-inoculation. No significant changes in temperature or any signs of a disease were observed.

The growth of '*C.*R.vini' in Vero cells and the cytopathic effect indicates that this bacterium can exploit vertebrates as hosts. However, as guinea pigs are commonly used model to study a pathogenicity of rickettsiae, we concluded that '*C.*R.vini' is probably not pathogenic for mammals.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-F06. *BORRELIA PERSICA* IN ARGASID TICKS FROM A CAVE IN ISRAEL – A LONGITUDINAL STUDY

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Tick borne relapsing fever (TBRF) in Israel is caused by *Borrelia persica* and transmitted to humans by the bite of an infected *Ornithodoros tholozani* tick. *Ornithodors tholozani* has been described as an opportunistic feeder that feeds on animals that enter its habitat and *B. persica* has been presumed to be transmitted transovarially making the tick itself a reservoir. The aims of this study were to follow the dynamics of a soft tick population in one cave longitudinally, evaluating different life stages, prevalence of infection with *Borrelia* spp. and sources of blood meal. We applied molecular approach for species-specific identification based on polymerase chain reaction and nucleotide sequence analysis. A total of 1022 ticks (113 females, 132 males, 301 nymphs and 476 larvae) were analyzed. Tick 16S rRNA sequences were similar (99-100%) with *O. tholozani* sequences in GenBank. *Borrelia persica* DNA was found in all 3 dates of sampling in 21 ticks: 3 females, 5 males and 13 nymphs, representing a prevalence of 2%. In addition 5 other ticks (2 males and 3 nymphs) harbored borrelial DNA similar to *Borrelia theileri*. There was no significant difference in the prevalence of *B. persica* in adults and in nymphs (p=0.656), however, no infection was detected in larvae. We identified blood meals in 409 ticks, from cattle (n=220); wild boar (*Sus scrofa*; n=83), human (n=63), porcupine (*Hystrix indica;* n=2), golden jackal (*Canis aureus*; n=1), dog (*Canis lupus familiaris;* n=1), and cat (*Felis catus*; n=1). Furthermore, 38 ticks contained mixed blood meals (22 cow and wild boar; 4 wild boar and human, 5 cow and human, and 7 with cow, wild boar and human). Of the 21 *B. persica* -infected ticks, 14 (67%) had a blood meal. The presence of *B. persica* was related to the presence of blood meal in the ticks (p=0.022). A low and steady prevalence of *B. persica* was found in *O. tholozani* ticks from one cave in Israel. *B. persica* was found only in tick nymph and adult stages but not in larvae. Blood meal analysis supported the opportunistic feeding behavior of *O. tholozani* and the large number of cow blood meals probably reflected the abundance of cattle in the cave area. The absence of *Borrelia* spp. in larvae, and the fact that the majority of the *B. persica* infected ticks contained a blood meal, may imply the importance of animals in acquiring infection by the ticks.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-F07. *BARTONELLA* BACTERIAL LOADS IN CAT-FLEAS AREA FFECTED BY THE HOST'S INFECTION STATUS BUT NOT THE OPPOSITE

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Bartonella henselae, *Bartonella clarridgeiae* and *Bartonella koehlerae* are frequently reported to cause persistent infections in cats. Cats are considered to be their main reservoir, and the cat-flea, *Ctenocephalides felis*, has been experimentally demonstrated as a competent vector of *B. henselae.* Previous studies have found a lack of association between the presence (infection status) and/or level of infection (bacterial loads) of *Bartonella* spp. in cats and the presence of *Bartonella* spp. in their hosted fleas. It has been observed that infected cats can host negative fleas without an apparent association with the bacteremia level in the cat, and non-infected cats can be infested with positive fleas. The present study further explored these phenomena by quantifying bartonellae in both stray cats and their hosted fleas. Ecological factors that may influence the presence and/or the level of bartonellae were evaluated. Accordingly, blood samples and fleas were collected from stray cats from Rishon-LeZion, Israel. The *Bartonella* infection level was quantified by two independent high resolution melt (HRM) real-time qPCR assays, targeting the internal transcribed spacer (ITS) and the citrate synthase gene (*gltA*) of *Bartonella* spp. Flea DNA samples were confirmed and size-normalized by a housekeeping gene of *Ct. felis* (cytochrome c oxidase subunit 2 gene), and screened for a cat gene (cytochrome *b* fragment gene) as a marker for a blood meal size and to confirm the source of blood meal. Results indicate that female fleas can harbor higher numbers of *Bartonella* organisms than male fleas ($P<0.05$). In addition, fleas collected from infected cats harbored higher quantities of bartonellae per flea than fleas from non-infected cats ($P<0.01$). Interestingly, the quantity of bartonellae in the fleas correlated with the bacteremia level of their cat host, but did not correlated with the relative blood meal size in the fleas. Our results suggest that infection with *Bartonella* species in the flea is promoted by the presence of the bacterium in its host, and accumulation and/or augmentation events may likely occur in the flea. The bacterial loads in cats did not show any significant association with the cat age, cat gender, *Bartonella* spp., or the presence of *Bartonella*-DNA in the fleas. The latter findings can be explained by the cyclic pattern of *Bartonella* bacteremia in cats, and highlight the complex symbiotic association between cats and bartonellae.

WG2: Barcoding, molecular diagnosis and next generation sequencing

OP-G01. INTEGRATION OF TRANSCRIPTOME AND PROTEOME ANNOTATION IN THE NAÏVE *IXODES RICINUS* MIDGUT WITH GENOME SEQUENCING

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In Europe, ticks are the main vectors of human diseases and effect as well livestock, wildlife and pet animals. Nevertheless, publicly available sequence and functional information about the most important European tick, *Ixodes ricinus*, is quite limited. Hereby we present the first study about whole genome shotgun sequencing of *I. ricinus*. About 1 billion paired-end sequences were *de novo* assembled into 235,953 contigs comprising more than 392 million bases. These genomic sequences provide a first (partial) *I. ricinus* reference genome; thus allowing e.g. for homology search analyses. Furthermore, by combining genome with transcriptome sequencing, we identified 6,415 putative genes in the *I. ricinus* genome.
I. ricinus ticks transmit a wide range of pathogens, mostly bacteria (e.g. *Borrelia burgdorferi* sensulato (s.l.), *Anaplasma* spp., *Rickettsia* spp.), but also viruses (e.g. tick-borne encephalitis virus) and protozoa (e.g. *Babesia* spp.). 14% of European *I. ricinus* tick sare infected with members of the *B. burgdorferi* s.l. complex and Lyme borreliosis is the most important vector-borne disease in Europewith more than 85,000 cases annually. Interactions between *I. ricinus* and *Borrelia* in the midgut are essential for successful survival of the pathogen in the tick as well as its transmission to the host. Therefore, midgut proteins are important players in vector-pathogen interactions and potential targets for blocking feeding and transmission by vaccines. By combining protein identification by mass spectrometry with RNA-sequencing, we annotated more than 10,000 transcripts and more than 1,000 proteins expressed in the midgut of unfed *I. ricinus* ticks for function, localization and biological processes. This multiple-omics study provides important annotated data of*I. ricinus*, paving the way for further investigation of tick-pathogen interactions as well as for the identification of vaccine candidates to potentially control several vector-borne diseases.

WG5: Rare and emerging vector-borne pathogens

OP-G02. *RHIPICEPHALUS ROSSICUS*, A TICK SPECIES IN EXPANSION: NEW RISKS AND NEW OPPORTUNITIES

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Ticks are the medically most important group of arthropods in Europe, with high associated health-care costs for most tick-borne disease. While their medical importance is acknowledged and most diseases have a long epidemiologic history, most tick-borne diseases show an emerging pattern all over Europe. There are two main hypotheses put forward to explain this phenomenon: (**1**) the changes of geographical distribution of tick ranges and (**2**) the increased contact of ticks with the human population. Among ticks with recent range expansion, there is an understudied species, in Eastern Europe, which confronts us with a number of challenges. With a distribution primarily east from Romania and Ukraine, this species was known as a rare tick of small mammals and ruminants in the steppe region, known to be proven vector for Crimean-Congo Hemoragic Fever, Q-fever, tularemia and West Nile virus. Recent studies however highlighted its occurrence in SE Romania, with a dominant presence on dogs. This region is particularly interesting, as dogs commonly share high number of tick species, with at least 7 different species collected regularly. Among these, another common species is *R. sanguineus*, which is very similar genetically and morphologically, while it is vector for a suite of totally different pathogens. *R. sanguineus* is a competent vector for a number of *Rickettsia* determined diseases (spotted fever group), for *Anaplasma* spp., or *Cercopithifilaria* nematodes, none yet known to be vectored by *R. rossicus*. While *R. sanguineus* is usually a common tick of dogs, it was rarely recorded to attack humans, thus limiting its vectorial competence from the medical point of view. *R. rossicus* in opposition has been proved to readily attack humans, also. The close similarity of these two tick species, their sympatric co-occurrence in Romania and same host range however may provide a unique situation for exploring of pathogen bridging. Moreover, expanding hosts (or vectors) have been shown to possess (or transfer) more virulent pathogens, thus their study may provide new insights into vector-borne disease epidemiology.

WG5: Rare and emerging vector-borne pathogens

OP-G03. DOES INSECTIVORISM OF BIRDS AFFECT THEIR TICKS? RESULTS OF A TRIANNUAL SURVEY

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Birds play an important role in short- and long-distance transportation of ticks and tick-borne pathogens. The aim of the present study was to provide new data on the tick infestation of birds and its underlying factors. Therefore, during a three year period (2012-2014) altogether 3338 ixodid ticks were collected from 1167 passerine birds (representatives of 47, mainly or partly insectivorous species) at ringing stations in Hungary.
The most abundant species were *Ixodes ricinus* and *Haemaphysalis concinna*, with 2295 (69%) and 989 (30%) specimens (larvae/nymphs), respectively. The presence of the latter tick species on avian hosts was recorded two months earlier (i.e. from March) than their known seasonal activity in the region. Forty-eight *I. frontalis* specimens (including three adults) were also collected, the majority (79%) from Robins (*Erithacus rubecula*). This tick species occurred during all seasons (August to November and January to April). Regarding exotic tick species, three *Hyalomma* nymphs were found on a Whitethroat (*Sylvia communis*), and two *I. eldaricus* females on a Greenfinch (*Carduelis chloris*) and a Dunnock (*Prunella modularis*). Previously the latter tick species (indigenous in Crimea and the Middle-East) was not reported from Hungary. The majority of *I. ricinus* and *I. frontalis* larvae/nymphs (78% and 92%, respectively) occurred on ground-feeding bird species, whereas 73% of *H. concinna* immatures were found on arboreal birds (Fisher's exact test: $P < 0.0001$). Concerning the intensity of tick infestation, there was no significant difference between bird species that have smaller (6-30 g) or larger (31-140 g) body weight, nor between long vs. short distance migrants and local birds (Mann-Whitney U-Test: $P > 0.05$).
A high proportion of *I. ricinus*, *H. concinna* and *I. frontalis* larvae/nymphs collected from birds in the present study showed apolysis, the initiative act of moulting. The percentage of apolytic ticks on birds was the highest (35.5%) in June-July, following the regional peak activity of caterpillars (May-June). The monthly proportion of apolytic larvae and nymphs was highly related to the reported regional monthly population density of Lepidoptera (Spearman's rank correlation: $r = 0.89$). As a plausible explanation for this phenomenon, caterpillars (which constitute the highest portion of food of passerine birds), as well as other insects are known to contain arthropod moulting hormones (ecdysteroids). Literature data also attest that (1) dietary ecdysteroids enter the circulation of birds, and (2) feeding of ticks on ecdysteroid-containing blood may accelerate their development. However, further studies are needed to confirm this assumption.

WG5: Rare and emerging vector-borne pathogens

OP-G04. INSIGHTS INTO THE OCCURRENCE OF EMERGING SPOTTED FEVER RICKETTSIAE GROUP AND *ANAPLASMA PHAGOCYTOPHILUM* IN TICKS INFESTING CATTLE IN ROMANIA

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Spotted fever group (SFG) rickettsiae and Anaplasmataceae are obligate intracellular Gram-negative bacteria belonging to the order Rickettsiales. They are associated with ticks which act as vectors and/or reservoirs. Currently, they are recognized as important emerging agents of human tick-borne diseases worldwide. The very diverse tick fauna and abundance of tick populations represents potential risks for animal and human public health. However, agents within the rickettsiales group have been poorly investigated in Romania. Therefore, the epidemiology of rickettsial diseases it is not well known.
Here we present molecular evidence for the presence of SFG rickettsiae and *Anaplasma phagocytophilum* in *Ixodes ricinus* and *Dermacentor marginatus* ticks from Romania. Ticks (145 *I. ricinus* and 49 *D. marginatus*) were collected from naturally infested cattle in South-Eastern Romania. The presence of *Rickettsia* in ticks was evaluated using conventional PCR amplification followed by sequencing, while *I. ricinus* ticks were screened additionally for *A. phagocytophilum* with real-time PCR. Overall, rickettsial DNA was detected in 21.2% of the examined ticks. Sequence analysis revealed *Rickettsia monacensis* (15.9%) and *R. helvetica* (4.1%) in *I. ricinus* ticks and *R. slovaca* (14.3%) and *R. raoultii* (10.2%) in *D. marginatus*, respectively. Additional, 6.2% of *I. ricinus* ticks were positive for *A. phagocytophilum*. The tick species identified to harbor SFG rickettsiae and *A. phagocytophilum* have been reported to feed on people in Romania, *I. ricinus* being the most abundant species. Therefore, the high prevalence of zoonotic tick-borne pathogens in two widespread tick species in Romania should increase the awareness, particular of clinicians, for the risks of transmission of these pathogens to the human population.

OP-G05. FIRST EVIDENCE OF *BABESIA VENATORUM* IN CZECH REPUBLIC

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Genus *Babesia* includes apicomplexan blood parasites transmitted by ticks, which are their definitive hosts also. Babesia causes haemolytic disease which can be fatal, especially in imunosuppressed patients. We can find many *Babesia* species in Europe, three of them have zoonotic potential - *B. divergens*, *B. microti* and recently described *B. venatorum* (*B.* sp. EU1). *Ixodes ricinus* is the only vector and definitive host of these parasites. *B. microti* was the only reported babesia in Czech Republic so far.
The aim of our study was to update information about occurrence of zoonotic *Babesia* in Czech Republic. We analyzed 748 *I. ricinus* ticks collected by flagging from South Moravia during 2009-2011. We used alkaline hydrolysis to extract DNA from ticks. This method consists of boiling crushed tick in ammonium hydroxide. We amplified approx. 790 bp long part of 18S rDNA gene during nested PCR for confirmation the presence of pathogen. This program works with primers BTF1, BTR1 and BTF2, BTR2 in second reaction. To reduce specifity of PCR we decreased the annealing temperature to 55 °C. All PCR amplicons were sequenced and determined by BLAST match with GenBank.
We obtain two amplicons with 99% identity to GenBank record of *B. venatorum*. Prevalence was 0.27%. Both positive samples were collected near the water-reservoirs Nové Mlýny on the river Dyje. We did not amplified DNA of *B. microti*. Although there was not any case of human babesiosis in Czech Republic yet, this study proved that there is a risk of this infection.

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WG1: The "One Health" concept in the ecology of vector-borne diseases

OP-G06. MULTIPLE USE OF ENTOMOLOGICAL SURVEILLANCE OF VECTOR-BORNE DISEASES

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Due to low level of public awareness, poor training skills of medical personnel and limited financial resources, the routine medical diagnose praxis for West Nile virus (WNV) hasn't been applied in Serbia until the outbreak in 2012. The interests of public authorities to support the large-scale vector surveillance were also limited.
Nevertheless, taking in account the existing risks of WNV circulation, the first steps towards the medical and entomological surveillance were undertaken in 2005. Despite the number of suspected human cases, virus was never detected in humans, animals or vector. Random mosquito sampling in predefined risk areas revealed no WNV presence. In 2009, in order to increase probability of WNV detection in mosquito vector (that will raise awareness of public and politician), reverse (backward) system was set based on serological testing of suspected earlier human cases (IgG positive persons), followed by mosquito samplings conducted in the affected residential areas ("hotspots") where cases were grouped. Restricting the mosquito sampling in 2010 to 10 "hotspots", 3 sampling nights (September) and putative vector (*Culex pipiens pipiens*), 3 out of 29 tested mosquito pools were found positive to WNV. From 2011 to 2014, the surveillance was widened and sampling spots defined according to following priority: **1**) old "hotspots" included first; **2**) new grouping of serological human/horse cases (new "hotspots" added); **3**) places of interest for veterinary surveillance supplemented if possible. Interestingly, some "hotspots" provided positive pools of *Culex pipiens pipiens* in several consecutive years.
Consequently, approach to entomological surveillance has been shifted from "backward" to "forward" in order to facilitate predictive assessments to: **a**) determine start/end of transmission period; **b**) impact of weather and environmental changes on transmission; **c**) support the decision making process; **d**) evaluate vector control efficacy.
A protocol to ensure standardisation of the activities, allowing comparisons between different teams involved in operational part of the surveillance is in preparation. Selection of appropriate sampling technique and microhabitat could allow simultaneous surveillance of other vectors/pathogens (i.e. Phlebotominae/*Leishmania* spp.; Phleboviruses, *Culicoides* spp. /Blue Tongue Virus, *Culex* spp. /*Dirofilaria* spp.) and evaluation of vector control efforts.

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POSTER PRESENTATIONS

WG3: Geospatial tools in vector research

P01. VECMAP: A COST-EFFICIENT TOOL AND SERVICE FOR SPECIES MAPPING AND CONTROL (VECMAP)

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Continuously increasing globalised travel, trade and the changing environment allow certain disease-spreading insects, as well as the diseases they transmit, to travel further and with greater ease than ever before. Europe is now at risk of hitherto unfamiliar illnesses transmitted by both native as exotic vector species. Identifying and predicting the distribution of existing local vectors as well as the spread of new exotic vectors are essential steps in assessing the potential for epidemics.

In an activity supported by the European Space Agency (ESA, Noordwijk, the Netherlands), anintegrated applicationnamed VECMAP was developed that uses Satellite Navigation and Earth Observation data to populate an online database, allowing researchers to map high-risk areas. This approach greatly reduces the cost and complexity of tracking vectors compared to traditional fieldwork as all necessary data and services for species mapping are provided in one place. Currently, health authorities use on-the-ground sampling and statistical analysis to predict the most likely at-risk areas, but a lack of integration between the various services results in a highly complex system. VECMAP provides a single entry point to all the information needed to predict and prevent infection, making it possible for a group of researchers to easily collaborate on risk-mapping. VECMAP consists of a desktop application that prepares the most efficient sampling plan. Findings from the *in situ* sampling activities are submitted directly to an online database using a dedicated smartphone app. The data is automatically geo-labelled and supported by satellite imagery of the area. Afterwards, lab identification information of the captured species is directly uploaded to the central database and linked with the field findings.

The possible applications of VECMAP are numerous and not only limited to vector species and disease risk mapping.

WG1: The "One Health" concept in the ecology of vector-borne diseases

P02. SEROPREVALENCE OF CANINE VECTOR-BORNE DISEASES IN MILITARY WORKING DOGS IN PORTUGAL - NATIONAL SURVEY

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Canine vector-borne diseases (CVBDs) represent a serious threat to Public and Animal Health and are increasingly reported worldwide. Military working dogs constitute a risk group for these diseases as they make periodic fieldwork in diverse areas and spend long periods outdoors exposing themselves to vectors. In order to assess the risk of this exposed group, an epidemiological survey was conducted, involving serological analyses of military dogs from the Portuguese Air Force, kept in mainland Portugal (Aveiro, Beja, Leiria, Lisboa and Setúbal districts) and Madeira and Azores islands. One hundred asymptomatic dogs (92 male and 8 female), with ages ranging from 7 months to 11-year-old (average 5.2 years) and with an average body condition of 4.9 (1-9) were surveyed. Preventive measures applied against CVBDs are ivermectin, imidacloprid and permethrin spot-on monthly, and deltamethrin impregnated collars quarterly. Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for detection of specific antibodies against *Leishmania infantum* (Mettler et al., 2005) and *Angiostrongylus vasorum* (Schucan et al., 2012) and circulating antigens of *A. vasorum* (Schnyder et al., 2011). Commercial immunofluorescent antibody tests were used to detect the IgG-antibodies against *Anaplasma phagocytophilum* (MegaScreen® FLUOANAPLASMA ph.), *Babesia canis* (MegaScreen® FLUOBABESIA canis), *Ehrlichia canis* (MegaScreen® FLUOEHRLICHIA c.) and *Rickettsia conorii* (MegaScreen® FLUORICKETTSIA con.). Circulating antigens of *Dirofilaria immitis* were assessed using WITNESS® Dirofilaria kit. Statistical analysis was performed with SPSS® and estimated prevalences were obtained using Agresti-Coull method. A total of 49% of the dogs were positive for *R. conorii,* 16% for *A. phagocytophilum,* 7% for *E. canis,* 13% for *L. infantum,* 3% for *B. Canis* and 3% for *A. vasorum*. No positive cases for *D. immitis* were recorded. Totally 64% of the dogs were positive for one or more of the seven CVBDs evaluated. Co-infection was recorded in 22% of the dogs, 18% with double infections, 3% with triple infections and 1% with quadruple infection. Furthermore, 62% were positive to at least one zoonotic agent (*A. phagocytophilum*, *E. canis*, *L. infantum* and *R. conorii*) in every area assessed. These results reveal a high occurrence of CVBDs pathogens in military dogs and highlight the need to maintain a targeted and regular prophylaxis to diminish the consequences of an inevitable contact between working dogs and these agents.

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WG1: The "One Health" concept in the ecology of vector-borne diseases

P03. FIELD SURVEY ON THE IMPORTANCE ATTRIBUTED TO ANIMAL DISEASES, INCLUDING VECTOR-BORNE, AND ON THE RISK PERCEPTION ABOUT ZOONOSES BY LIVESTOCK KEEPERS IN SUB-SAHARAN AFRICA

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Livestock represent a vital resource for the livelihood of rural people, especially in the so-called developing countries. Within development cooperation projects, aimed to the improvement of animal production/health in African countries, field research activities were carried out in different livestock production systems (i.e. peri-urban intensive, semi-intensive, nomadic).
In order to evaluate major constraints to livestock production/health, and assess the risk perception and level of knowledge about zoonoses by livestock keepers/herders, semi-structured interviews and questionnaires were administered to 30 nomadic herders in Ethiopia (Somali region), 90 semi-nomadic livestock herders in Mali (Mopti region) and 29 peri-urban livestock keepers in Burkina Faso (Bobo Dioulasso). Questionnaires were designed and interviews performed according to standard techniques of participatory epidemiology and rural appraisal, with the help of local mother tongue interpreters/translators.
Livestock herders/keepers from the 3 study areas identified common constraints to animal production/health (i.e. lack of vet drugs, inadequate veterinary services, difficult access to markets) and common diseases (i.e. tick-borne diseases and ticks, and other diseases transmitted by blood-sucking vectors). Antrax, clostridiosis, foot-and-mouth, dermatophilosis, pasteurellosis, mastitis, intestinal and lungworms parasitoses were quoted as other important diseases.
None of the livestock herders/keepers interviewed showed to know the correct definition of "zoonosis", except 5 peri-urban farmers in Burkina-Faso with specific training/education. However, 60% of livestock keepers in Mali, 80% in Ethiopia and 22% in Burkina Faso declared to know about the existence of diseases transmissible between animals and man, although in most cases they quoted -as examples- diseases with common symptoms man-

animals (e.g. coughing, diarrhea, “malaria of animals”). Most participants knew the possible transmission routes of some diseases (i.e. consumption of animals’ products, direct contact with animals), although they did not usually apply adequate prevention measures (i.e. 93% of Ethiopian nomads, 65% of seminomadic herders from Mali do not boil milk before consumption, and about 50% of them avoid milk consumption from diseased animal or under drug treatment).

In conclusion, it can be said that although livestock herders/keepers are competent to identify major animal diseases or constraints to animal health/production -including some diseases identified as zoonoses- they do not usually apply preventive measures against zoonoses. Veterinary and public health training activities addressed to livestock keepers and to the local population are of paramount importance to create awareness on public health risks. Such joint activities – based on One World - One Health approach - should become the pillars of any cooperation activities in agriculture and livestock development projects.

WG1: The "One Health" concept in the ecology of vector-borne diseases

P04. IMPORTED MALARIA 1962 – 2014 AND LIST OF *ANOPHELES* SPECIES IN ALBANIA

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Malaria is believed to have been endemic in Albania since at least the time of Hippocrates until its eradication in 1967. In 1938 was a hyper endemic disease with a spleen and parasite rate respectively 59.2% and 16.5% and about 80% of the territory presented risk of malaria infection. The last reported endemic case of *Plasmodium falciparum* was in 1958 and *P. vivax* in 1966. Forty cases of transfusion malaria (28 from *P. vivax* and 12 from *P. malariae*), were reported until 1977 and the first imported malaria case was reported in 1962. Here we present a comprehensive report of imported malaria cases from 1962-2014 and list of Anopheles species found in the country.

Thick and thin blood smears stained with Giemsa are used for the diagnosis of *Plasmodium* species. For each case of malaria completion of a questionnaire is performed. Regarding the putative vectors, CDC miniature light traps and CO2 traps are used inside animal shelters for mosquitoes collection.

Since 1962 up to 2014 a total of 78 laboratory confirmed cases are reported. 64.1% of them acquired the infection in Africa, 32% in Asia, 2.6% in Europe/Greece and 1.3% in South America. *Plasmodium falciparum* accounted for 47.4% of the cases, followed by *P. vivax* 38.4%, *P. ovale* 11.5% and 2.6% are mix infection. Most of the cases (46.1%) are encountered recently, from 2012 to 2014, due to the people movement to Equatorial Guinea. In 2010 and 2012 two Albanian citizens travelling in malaria autochthones villages in Greece has been infected with *P. vivax* featuring the presence of gametocytes. Entomological surveys have shown the presence of 13 *Anopheles* species in the country: *Anopheles algeriensis, An. claviger, An. hyrcanus, An. marteri, An. maculipennis s.s, An. melanoon, An. messeae, An. plumbeus, An. sacharovi, An. subalpinus, An. superpictus, An. cinereus, and An. multicolour*. Among them *An.sacharovi, An. maculipennis* (*typicus*) and *An. superpictus* are known for their role in malaria transmission, in the past. Recent data suggest that *An. plumbeus* could act as vector for malaria and significantly contribute to increasing the malaria transmission risk in Central-Western Europe.

For the first time, is recorded one fatal case from imported malaria (2013) and *Plasmodium ovale* (2012). Albania is malaria-free country, only imported cases are registered. Malaria is mandatory reported disease and it is part of the National Mandatory Reporting System.

WG2: Barcoding, molecular diagnosis and next generation sequencing

P05. SYMBIOTIC ASSOCIATION OF THENOVEL "*RICKETTSIA FELIS*-LIKE" BACTERIUM, *CANDIDATUS* RICKETTSIA ASEMBOENSIS, WITH THE NEGEV DESERT FLEA *XENOPSYLLA RAMESIS*

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Two flea-borne pathogenic *Rickettsia* species have been detected in Israel to date, *Rickettsia typhi* and *Rickettsia felis.* Recently, "*R. felis*-like" bacteria have been detected in several flea species in regions endemic for *R. felis*, worldwide. In the present study, we explored the occurrence of *Rickettsia* species in fleas from the Negev Desert, Israel. Fleas collected from wild rodents were screened for *Rickettsia*-DNA by targeting the 16S rRNA (*rrs*). Molecular characterization of all the *Rickettsia* positive samples was assessed by amplification and sequencing of 5 additional genetic loci (*gltA*, *ompB*, *ompA*, *htrA* and *fusA*). Thirty-eight *Xenopsylla ramesis*, 91 *Synosternus cleopatrae* and 15 *Leptopsylla algira* flea-pools (2-9 fleas per pool; a total of 662 fleas) were screened. *Rickettsia*-DNA was detected in 100% of the *X. ramesis* and in one *S. cleopatrae* flea-pools. None of *L. algira* flea pools was found positive. The molecular identification of the positive samples showed that all sequences were identical and closely related to the "*Rickettsia felis*-like", *Candidatus* Rickettsia asemboensis (99-100% similarities in the six tested loci). To further investigate the association of this *Rickettsia* sp. with *X. ramesis* fleas, ten single *X. ramesis* adult fleas collected from wild rodents and 5 single adults, 5 larvae pools and 2 egg-pools laboratory-raised *X. ramesis* were screened by a specific PCR assay targeting the *ompA* of *Candidatus* Rickettsia asemboensis. All samples (100% in all life stages) were confirmed to be positive by the latter assay. These results suggest a symbiotic association of *Candidatus* Rickettsia asemboensis with the rodent flea *X. ramesis,* and the potential vertical transmission from the mother flea to its offspring. Further investigation is required to elucidate the ecological role of this *Rickettsia* sp. as a symbiont of *X. ramesis*.

WG4: Phylogenetics and phylogeography of vectors and vector-borne pathogens

P06. *BARTONELLA* SPECIES IN FLEAS FROM PALESTINIAN TERRITORIES: PREVALENCE AND GENETIC DIVERSITY

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Bartonellosis is an infectious bacterial disease. The prevalence and genetic characteristics of *Bartonella* spp. in fleas of wild and domestic animals from Palestinian territories are described. Flea samples (n=289) were collected from 121 cats, 135 dogs, 26 hyraxes and seven rats from northern (n=165), central (n=113), and southern Palestinian territories (n=11). The prevalent flea species were: *Ctenocephalides felis* (n=119/289; 41.2%), *Ctenocephalides canis* (n=159/289; 55.0%) and *Xenopsylla* sp. (n=7/289; 2.4%). Targeting the Intergenic Transcribed Spacer (*ITS*) locus, DNA of *Bartonella* was detected in 22.1% (64/289) of all fleas. Fifty percent of the *C. felis* and 57% of the *Xenopsylla* sp. contained *Bartonella* DNA. DNA sequencing showed the presence of *Bartonella clarridgeiae* (50%), *Bartonella henselae* (27%) and *Bartonella koehlerae* (3%) in *C. felis*. *Xenopsylla* sp. collected from *Rattus rattus* rats were infected with *Bartonella tribocorum*, *Bartonella elizabethae* and *Bartonella rochalimae*. Phylogenetic sequence analysis using the 16S ribosomal RNA gene obtained four genetic clusters, *B. henselae* and *B. koehlerae* as subcluster 1, *B. clarridgeiae* as cluster 2, while the rat *Bartonella* species (*B. tribocorum* and *B. elizabethae*) were an outgroup cluster. These findings showed the important role of cat and rat fleas as vectors of zoonotic *Bartonella* species in Palestinian territories. It is therefore advised an raise of awareness among physicians, veterinarians, and other health workers of the high prevalence of *Bartonella* spp. in fleas in Palestinian territories and the potential risk of these pathogens to humans and animals.

Funding: The study was funded by The Ministry of Foreign Affairs, The Hague, The Netherlands, project M27-072NVHU 2009 02 'Vector-Borne Pathogens in Israel and the Palestinian Authority'.

WG2: Barcoding, molecular diagnosis and next generation sequencing

P07. MOLECULAR DETECTION AND IDENTIFICATION OF SPOTTED FEVER GROUP *RICKETTSIAE* IN TICKS COLLECTED FROM PALESTINE

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Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia*. We aimed to identify and genetically characterize SFG rickettsiae in ticks from domestic animals originating from different regions in Palestine. A total of 602 ixodid ticks belonging to six species (*Haemaphysalisparva, H. adleri, Rhipicephalusturanicus, Rh. sanguineus, Rh. bursa and Hyalomma spp.)* were collected during the period of January-April, 2014 from dogs, sheep and camels. A group of 394 ticks were screened for the presence of *Rickettsiae* by PCR targeting 250bp of the*ompA* gene. Rickettsial DNA was detected in 68 (17.3%) of the 394 tested ticks. The prevalence of infection in *Rh. turanicus, Rh. sanguineus, H. parva and H. adleri*ticks was 58.8, 26.5, 10.3, and 4.4%, respectively. None of the ticks belonging to the species *Rh. bursa* and *Hyalomma spp*. were infected. Sequence analysis of amplified products revealed two species of *Rickettsia: R. massiliae and Candidatus R. goldwasserii.* The results of this study extend the knowledge of the geographic distribution of SFG rickettsiae and indicate that at least two of them are present in ticks in Palestine. However, the pathogenicity of *Candidatus Rickettsia goldwasserii* in human is not yet proven. In conclusion, clinicians should be aware of emerging tick-borne diseases in Palestine, particularly infections due to *R. massiliae*.

WG5: Rare and emerging vector-borne pathogens

P08. VECTOR CAPACITY TRAITS OF SWISS MOSQUITOES FOR WEST NILE VIRUS

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West Nile virus (WNv) has spread in Southern and Eastern Europe over the last decade. Mosquitoes are the only biological vectors of WNv. Birds act as reservoirs, and the virus can cause zoonotic neuroinvasive diseases in horses and humans. To assess the risk for autochthonous WNv transmission in Switzerland, this study was initiated to determine (**1**) population dynamics, (**2**) host preferences, and (**3**) vector competence of Swiss vector mosquitoes. Mosquitoes were collected from ovitraps, breeding sites and CDC traps (baited with CO_2 and iGU lure) at natural and suburban locations on both sides of the Alpine crest over two consecutive years. Host preferences were assessed with horse- and chicken-baited traps under field conditions, as well as by bloodmeal analysis of blood-fed mosquitoes collected at the Zoo Zürich. Vector competence for WNv was assessed for field-collected Swiss mosquitoes under a realistic midsummer fluctuating temperature regime (17-31 °C). A total of 120'742 mosquitoes were collected. The most abundant species in the natural zones were *Aedes vexans* (south) or *Ae. annulipes* and *Coquillettidia richiardii* (north), in the suburban zones *Cx. pipiens* (both locations) and *Ae. vexans* (south) or *Ae. japonicus* (north). Animal-baited experiments revealed six mosquito species that were collected in both horse- and chicken-baited traps (*Cq. richiardii, Ae. cantans/annulipes, Ae. vexans, Ae. cinereus/geminus, Culiseta annulata, Cx. pipiens/torrentium)*. In bloodmeals of a total of 385 blood-fed mosquitoes from the Zoo, 35 host species were identified (birds, mainly Humboldt's penguin and indigenous birds; mammals, mainly lama/guanaco/alpaca and human; and one reptile). The 3 most abundant species (*Cx. pipiens/torrentium*, *Ae. japonicus*, *Ae. vexans*) had taken bloodmeals from both birds and mammals. In the vector competence experiments in which mosquitoes were fed with WNv-spiked blood, WNv was recovered from pools (n=5) of head/thorax and mosquito saliva by reverse transcription quantitative PCR. *Ae. japonicus*, *Cx. pipiens* and *Cx. quinquefasciatus* were competent for both WNv strains 'New York' and 'Italy' under a Swiss midsummer regime, respectively. Viral RNA of Wnv strain 'New York' was detected in a significantly larger number of head/thorax pools than strain 'Italy' ($p<0.05$).
Taken together, our analyses on spatio-temporal occurrence of mosquito species, their host preferences and vector competence for two WNv strains give a basis for assessing the risk for virus transmission under local conditions, suggesting, at this stage, *Ae. japonicus* and *Cx. pipiens* as potential candidates for WNv vectors in Swiss context.

Funding: Swiss Federal Food Safety and Veterinary Office

WG1: The "One Health" concept in the ecology of vector-borne diseases

P09. THE EFFECT OF VARIOUS FACTORS FOR THE DEVELOPMENT OF TICKS AND TICK-BORNE DISEASES IN BOSNIA AND HERZEGOVINA

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Ticks from the Ixodidae family are very important for veterinary and human medicine, especially their vector role in transmitting diseases. The paper presents previous investigations of ticks and ticks-borne diseases in Bosnia and Herzegovina (B&H), including epidemiology of ticks, effect of altitude on tick biotopes and influences of climate conditions (air temperature, precipitation and relative humidity) in tick prevalence in the last 10 years. Conditions for tick growth in B&H are very favorable, because the percentage of forests and forest land is higher than that of agricultural land (with larger uncultivated portion). A large portion of the forest land is covered with degraded and low forests and underbrush.
Ixodes ricinus is the most common species in B&H, collected and determined in all of sampled host animal species and in all of the examined localities. Followed by *Dermacentor marginatus*, *Rhipicephalus bursa*, *Hyalomma marginatum marginatum*, *Rhipicephalus sanguineus Haemaphysalis punctata*, *Ixodes canisuga*, *Dermacentor reticulatus and Ixodes hexagonus*. Determined species of ticks in B&H are in accordance with research carried out in the area of European part of the Mediterranean region. All the nine determined species were found on the altitude below 500 m, eight species (*Ix. ricinus, Ix. canisuga, De. marginatus, De. reticulatus, Rh. bursa, Rh. sanguineus, He. m. marginatum,* and *Ha. punctata*) were observed on altitude between 500-1.000 m, while *Ix. ricinus* and *Rh. bursa* were observed on altitude above 1.000 m. In the last 10 years, the highest tick occurrence was observed during May and June 2004: temperature values were between 13.2°C and 18.4°C, relative humidity was between 70.3% and 71.4% and precipitation was between 92.6 l/m2 and 97.4 l/m2. It was shown that the variability in tick prevalence is dependent on the precipitation. The western and southern parts of B&H are with the largest levels of precipitation, and the highest variability of different tick species was observed in the area with average annual precipitation ranges from 1.200 to 1.900 l/m2. Conditions for the development of ticks in B&H investigated during the last 10 years showed their high prevalence, especially of species that are vectors of several different diseases. These results imply a necessity to continue ticks research in B&H, which should include molecular identification and epidemiology of tick borne diseases.

WG1: The "One Health" concept in the ecology of vector-borne diseases

P10. A SURVEY OF ENVIRONMENTAL AND MULTI-HOST NATURAL INFESTATION OF *RHIPICEPHALUS BURSA* IN PORTUGAL: PCR-BASED INFECTION DETECTION AND CHARACTERISATION

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Ticks are obligatory blood-sucking arthropod ectoparasites (Acari: Ixodida) of domestic and wild animals and humans, that cause direct damage due to their feeding behaviour and also acting as vectors of different pathogens, such as fungi, viruses, protozoa and bacteria. The incidence of tick-borne diseases is rising worldwide challenging our knowledge regarding the diagnosis, treatment and control options.
Rhipicephalus bursa (Canestrini and Fanzazo, 1977) is a two-host tick widely distributed in the Mediterranean of the Palearctic region, that can been found in a variety ofvertebrate hosts such as goats, cattle, equines, dogs, gazelles, hares, dears and sporadically humans. This tick species is known to transmit *Babesia ovis, B. bigemina, B. caballi, B. equi, Theileria ovis, T. equi* and *T. annulata,* among others. Despite being widely distributed and its high economic importance, its biology is hardly known. Therefore, we aimed to characterise the presence of *R. bursa* in different areas of Portugal and determine the presence of tick-borne pathogens among the captured ticks.
During our study, total of 226 ticks were collected from different Portuguese geographic areas between the years of 2007 and 2014. Ticks were removed from domestic animals or collected by flagging or dragging the vegetation and separated by instars and gender, and identified by morphological keys to the species level. After dissection, DNA was extracted from the whole intern organs and PCRs were performed to detect the presence of tick-borne pathogens including *Anaplasma* spp., *Ehrlichia* spp., *Theileria* spp, *Babesia* spp and *Coxiella* spp.
All ticks were identified as *R. bursa* from which 226 were found feeding on animals (88 males, 90 females, 45 nymphs and 3 larvae) and 40 were questing ticks (19 males and 21 females). Regarding the tick-host association, ticks were removed from domestic animals such as cattle (*Bos taurus*) (70.5%), goats (*Capra hircus*) (5.5%), sheep (*Ovies aries*) (5.5%), horses (*Equus caballus*) (13.5%) and dogs (*Canis familiaris*) (5%). The remaining ticks were collected from the vegetation. Molecular results confirmed the presence of *A. marginale, T. annulata* and *T. equi* in *R. bursa* ticks. *A. marginale* was found in ticks feeding in cattle, sheep, and goats, and among questing ticks. *T. annulata* was found in a tick feeding in cattle; and T. equi in ticks feeding in horses. Finally our study contributes for determining the risk of emerging tick-borne diseases and predicting pathogen transmission in different areas of Portugal, in order to develop effective control measures.
Funding: PTDC/CVT-EPI/4339/2012

WG1: The "One Health" concept in the ecology of vector-borne diseases

P11. TICK SPECIES (ACARI: IXODIDAE) OF GOLDEN JACKAL (*CANIS AUREUS*) IN SERBIA

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Golden jackal (*Canis aureus*) is now days one of the most numerous carnivore species in Europe with high spreading potential and synanthropic preferences. Although jackals are know to carry many species of ectoparasites e.g. fleas, mites and ticks, knowledge on ectoparasitic fauna of this species in Europe is still limited. Increasing densities and widening of populations have not been accompanied by research of ectoparasites and pathogens of interest both to animals and to humans. The aim of our study was to investigate tick fauna of golden jackals in Serbia, with emphasis on medically and veterinary important species. The research was conducted during hunting seasons in the period of 2010 to 2014. Bodies of hunted and road killed animals from 12 sites (Smederevo, Surčin, Veliko Gradište, Velika Plana, Svilajnac, Zaječar, Bela Palanka, Titel, Ćovdin, Smederevska Palanka, Kraljevo, and Negotin) all over the country were collected in cooperation with local hunting organizations and inspected for the presence of ticks. In the fur of 89 jackals, a total of 506 ticks were found belonging to five ixodid species: *Ixodes ricinus, I. hexagonus, Haemaphysalis concinna, Dermacentor reticulatus and Rhipicephalus sanguineus.* The number of ticks per animal ranged from 1 to 34. The most numerous species was *D. reticulatus* (47.23%) followed by *I. ricinus* (34.18%), *H. concinna* (17.19%), *I. hexagonus* (0.79) and *Rhipicephalus sanguineus* (0.19%). The majority of recorded ticks were adults (90.50%), while only 48 (9.50%) nymphs were recorded. No ticks of larval stage were observed. The most numerous species was *D. reticulatus* (47.23%) followed by *I. ricinus* (34.18%), *H. concinna* (17.19%), *I. hexagonus* (0.79) and *Rhipicephalus sanguineus* (0.19%). Presence of more than one tick species was recorded in 21 animals (23.6%) with the domination of *I. ricinus/D. reticulatus* co-infestation. All of these species have been known to harbor different pathogenic microorganisms.

P12. THE MADAGASCAR TORTOISE TICK *AMBLYOMMA CHABAUDI* IS HIGHLY INFESTED WITH *RICKETTSIA AFRICAE*

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The dry forest of south west Madagascar is a severely threatened habitat with the two endemic tortoise species *Pyxis arachnoides* and *Astrochelys radiata* facing threats from several human activities. The ixodid tick *Amblyomma chabaudi* is supposed to be a host-specific tick of *Pyxis arachnoides*, but there is a lack of knowledge of its potential role as a vector. The aims of the study were (**1**) to examine the occurrence of ticks in relation to the status of habitat degradation, and (**2**) the investigation of tick-borne pathogens.
The sampling of tortoises and collection of ticks was carried out in two sites with different level of human disturbance in southwestern Madagascar. The Tsimanampatsotsa National Park was defined as an area with low disturbance. The adjacent regions outside the park with obvious evidence of livestock grazing and deforestation were classified as disturbed.
Under the assumption that habitat degradation has negative effects on an animal's health we hypothesized that tortoises outside the national park would harbor more ticks.
120 tortoises were found: 78 *Pyxis arachnoides* and 42 *Astrochelys radiata*. Only one tick was feeding on *A. radiata* confirming that *Amblyomma chabaudi* is, at least the adult stage, specific to *Pyxis arachnoides*. Tick infestation rates of *Pyxis arachnoides* differed significantly between the two study sites. Thirty-six *Pyxis arachnoides* were found inside the national park, 8 of which were infested by 1 to 4 ticks resulting in a prevalence of 0.22. The prevalence outside of the park was 0.76 with 32 of 42 tortoises infested by 1 to 7 ticks. The 8 infested tortoises inside the park altogether carried 13 ticks equating to a mean intensity of 1.6 whereas the 32 infected tortoises outside of the park carried 60 ticks (mean intensity: 1.9). The mean abundance of ticks per tortoise was 0.4 inside the park and 1.4 outside of the park, respectively.
Detection of pathogens was conducted via PCR on DNA isolated from ticks using genus-specific primers. The *Amblyomma chabaudi* ticks revealed an infection rate of 100% with *Rickettsia africae*. Relapsing fever *Borrelia* and *Babesia* spp. were not found.

WG5: Rare and emerging vector-borne pathogens

P13. MOLECULAR SCREENING AND FIRST REPORT OF "NOVEL" EMERGING TICK-BORNE PATHOGENS IN SERBIA

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Ticks play an important role in disease transmission globally due to their capability to serve as vectors for human and animal pathogens. The Republic of Serbia is an endemic area for a large number of tick-borne diseases. However, current knowledge on these diseases in Serbia is limited. The aim of this study was to investigate the presence of emerging tick-borne pathogens in ticks collected from dogs and vegetation from different parts of Vojvodina, Serbia.

A total of 187 ticks, including 124 *Rhipicephalus sanguineus*, 45 *Ixodes ricinus* and 18 *Dermacentor reticulatus* were collected from dogs. In addition, 26 non-engorged questing *Ixodes ricinus* ticks were collected from vegetation, using the flaging method, from 4 different geographical regions in Vojvodina, Serbia. DNA was extracted from each tick separately and samples were tested by either conventional, nested and/or real-time PCR assays for the presence of *Rickettsia* spp.-DNA (*glt*A and *omp*A genes), *Ehrlichia*/*Anaplasma* spp.-DNA (16S gene) and *Hepatozoon* spp./*Babesia* spp.-DNA (18S gene). In addition, all *I. ricinus* DNA samples were tested for *Bartonella* spp.-DNA (ITS) and *Borrelia* spp.-DNA (*flaB* and *glpQ* genes) by real-time PCR assays.

The presence of "novel" emerging human pathogens including *Rickettsia massiliae* (in 1 *R. sanguineus*), *Rickettsia raoultii* (in 1 *D. reticulatus*), *Candidatus* Neoehrlichia mikurensis (in 3 *I. ricinus*), *Babesia venatorum* (in 2 *I. ricinus*), *Babesia microti* (in 1 *I. ricinus*) and *Borrelia miyamotoi* (in 1 *I. ricinus*) are reported in this study for the first time in Serbia. Moreover, 6 *I. ricinus* ticks contained *Hepatozoon canis*-DNA, being the first indication for the presence of this canine pathogen in Serbia. Interestingly, none of the investigated ticks was positive for *Ehrlichia canis*-DNA and no *I. ricinus* was positive for *Bartonella* spp.-DNA. Additionally, we confirmed previous findings of the emerging human pathogens *Rickettsia monacensis* (in 16 *I. ricinus*), *Anaplasma phagocytophilum* (in 1 *I. ricinus*), *Borrelia lusitaniae* (in 8 *I. ricinus*), *Borrelia afzelii* (in 5 *I. ricinus*) and the presence of one reemerging dog pathogen, *Babesia canis* (in 6 *D. reticulatus*) in ticks from Serbia.

The findings of the current study highlight the great diversity of tick-borne pathogens of human and animal importance in Serbia. Further broader study is required in order to determine the "true" prevalence of these "novel" emerging tick-borne pathogens in Serbia.

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WG5: Rare and emerging vector-borne pathogens

P14. OCCURENCE AND DISTRIBUTION OF TICKS AND TICK-BORNE PATHOGENS IN SERBIA

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Serbia is an endemic area for a number of tick-borne diseases (Lyme disease, Human granulocytic anaplasmosis, Tularaemia, Q fever, rickettsioses, CCHF, TBE). Autochthonous cases of these diseases were continuously or periodically registered, with sporadic outbursts (CCHF, tularaemia, Q fever), but the real epidemiological situation is underestimated. For their specific biology and obligate hematophagy, ticks act as vectors of pathogenic microorganisms being of great significance for both animals and humans. Here we present data on occurence and distribution of ticks and tick borne pathogens based on number of previous research. The first studies on ticks in Serbia dated back to the beginning of the XX century while systematic faunistic-ecological research of ixodid ticks started in 1980's. Up to now 27 hard tick species belonging to *Ixodes*, *Dermacentor*, *Rhipicephalus*, *Haemaphysalis*, and *Hyalomma* genera and two argasid species (i.e., *Argas reflexus* and *A. persicus*) have been recorded in Serbia. Presence of several tick borne pathogens (CCHF virus, TBE virus, *Anaplasma phagocytophilum*, *A. ovis*, *Babesia cannis*, *Coxiella burnetii*, *F. tularensis*, *Rickettsia* sp., and *Borrelia burgdorferi* s. l.) has been conformed in tick samples in Serbia. CCHF and TBE viruses have been isolated from *H. marginatum marginatum*/*I. ricinus* and *I.ricinus*/*I.persulcatus* pools respectively. In addition to microscopic techniques, molecular methods have been used for detection of other pathogens. *Anaplasma phagocytophilum* has been detected in *I. ricinus* and *D. reticulatus* ticks, *A. ovis* in *H. punctata, H.concinna* and *I. ricinus*; *Babesia canis* in *D. marginatus, D. reticulatus*, *H. concinna* and *R.sanguineus; B. burgdorferi* s.l. in *I. ricinus*; *C. burnetii* in *D. reticulatus*, *H. concinna* and *I. ricinus*; *F. tularensis* in *I. ricinus*; *R. helvetica* and *R. monacensis* in *I. ricinus* ticks. Prevalence rates vary from 1.9% for *A. phagocytophilum* in *D. reticulatus* to 63% for *C. burnetii* in *I. ricinus*. Up to 28.8% of infested ticks harboured more than one pathogenic species.

WG2: Barcoding, molecular diagnosis and next generation sequencing

P15. TICK SALIVA PROTEINS: VARIABILITY OF CODING SEQUENCES IN *IXODES RICINUS* (LINNAEUS, 1758) IN SERBIA

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Ticks are obligate haematophagous ectoparasites of reptiles, birds and mammals, being globally important vectors of human and animal pathogens, including numerous viruses, bacteria and protozoa. Understanding tick biology is essential for understanding epidemiology of tick borne diseases, as well as for adequate preventive measures to be taken. Tick saliva components have a crucial role in tick feeding, enabling successful blood meal uptake at several levels, acting not only as anticoagulants, but also as inhibitors of inflammation and modulators of host immune response. Research of saliva proteins variability, and their antigenic properties, contributes to the identification of tick bite markers and development of anti-tick vaccine.

We investigated coding sequence variability of three salivary gland secretory proteins of *I. ricinus* tick, the most abundant and wide spread species, collected in Serbia. For these three proteins, of which some primarily been detected in saliva of *Amblyomma americanum* tick, injection in host during the blood meal and existence of antigenic characteristics have been proven. Coding sequences were obtained by specific amplification with designed primers, cloning and sequencing in both directions. Intra- and inter species variability analysis was conducted, using obtained and sequences already submitted in GenBank. Certain level of conservativity of sequences suspected to be tick-specific implicates possible usage of their protein products in human and veterinary health practice. Tick-nonspecific sequences, on the other hand, are more suitable for evolutionary and phylogenetic studies, as they show variability among two groups of ticks (Prostriata and Metastriata). There is also differences between obtained sequences and sequences from other genera of ticks, consisting of three nucleotides or several trinucleotides, which could be of great importance in antigenic studies and possible future application of their protein products.

WG2: Barcoding, molecular diagnosis and next generation sequencing

P16. PRESENCE OF *BORRELIA TURDI* AND *BORRELIA VALAISIANA* IN TICKS REMOVED FROM BIRDS IN THE NORTH OF SPAIN, 2009-2011

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The genus *Borrelia* includes species responsible for severe human diseases such as Lyme disease, an illness caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. Birds are involved in their epidemiology as dispersers of infected ticks (vectors of *Borrelia* spp.) and also as reservoirs or amplifiers of the bacteria. Our aim was to investigate the presence of *Borrelia burgdorferi* s.l. in ticks collected from birds in the North of Spain.
A total of 336 ticks from 121 birds captured in Santa Eulalia (La Rioja, Spain) from 2009 to 2011 were studied. Samples were classified as 174 *Ixodes frontalis*, 108 *Haemaphysalis punctata*, 34 *Hyalomma marginatum*, 17 *Ixodes ricinus* and 3 *Ixodes* spp. DNA extracts were screened for the presence of *Borrelia* spp. by PCR targeting the 41 kDaflagelin gene and the 5S-23S rRNA intergenic spacer. *Borrelia turdi* was detected in 26 out of 336 ticks, corresponding to 22 *I. frontalis*, 2 *H. punctata* and 2 *I. ricinus*. Additionally, 1 *I. frontalis* and 1 *H. punctata* were found to be infected by the human pathogen *Borrelia valaisiana*. Moreover, 3 *I. frontalis* showed co-infection with both *Borrelia* species. The infected ticks (23 larvae, 7 nymphs and 1 female adult) were collected from 4 out of 19 bird species: *Turdus merula* (n=6), *Turdus philomelos* (n=8), *Erithacus rubecula* (n=2) and *Aegithalos caudatus* (n=1).
This study corroborates the presence of *B. turdi* and *B. valaisina* in ticks from birds in the North of Spain. We report the first detection of *B. turdi* and *B. valaisina* in *H. punctata* and *I. frontalis* ticks, respectively. The amplification of *B. turdi* in *H. punctata* and *B. valaisina* in *I. frontalis* specimens does not confirm their role as vectors. The presence of *B. turdi* and *B. valaisiana* in larvae specimens suggests the role of birds as reservoirs of these bacteria species.

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WG2: Barcoding, molecular diagnosis and next generation sequencing

P17. HOST SPECIALIZATION OF *BORRELIA BURGDORFERI* S.L. TRANSCENDS THE GENOSPECIES LEVEL

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Vertebrate reservoirs and tick vectors are essential for transmission of *Borrelia burgdorferi*-etiologic agent of Lyme disease - in enzootic cycles. Previous studies have shown that there is a specialization of the genospecies belonging to *B. burgdorferi*s.l. to the corresponding groups of reservoir hosts, with *B. afzelii*, *B. burgdorferi* sensu stricto, *B. bavariensis* and *B. spielmanii* being transmitted by mammals, *B. garinii* and *B. valaisiana* by birds, and *B. lusitaniae* by lizards. However, it is possible that there is further differentiation between the genotypes of any one of these genospecies. This aspect might impact on epidemiology of *B. burgdorferi*, as the different genotypes might also cause different clinical manifestations in human subjects with Lyme disease. In our study we tested the hypothesis of association of *B. burgdorferi* s.l. genotypes to various vertebrate hosts. To that end we analysed over 1500 engorged larvae collected from two rodent species – *Apodemus sylvaticus* (wood mouse) and *Myodes glareolus* (bank vole). These were tested for presence of *B. burgdorferi* s.l. by a duplex qPCR for *osp*A and *fla*B genes. The positive samples were further submitted to conventional PCR and sequencing of the 5S-23S rDNA intergenic spacer (IGS). Existence of population structure in the sample of *B. burgdorferi* s.l. was assessed using the software Structure 2.3.4. Sequence analysis of IGS was performed using Arlequin 3.5.1.3. Our analysis revealed further genetic diversity within the *B. burgdorferi* s.l. genospecies in the sense of genetic differentiation between the bacteria transmitted by the two rodent species. This specialization of bacteria to the different host species might have further implications on public health and control strategies.

WG5: Rare and emerging vector-borne pathogens

P18. PROLIFERATION ASSAY: COMPARISON OF WHOLE BLOOD AND PBMC CULTIVATED WITH *FRANCISELLA* AND *BORRELIA*

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Tick-borne bacteria *Francisella tularensis* and *Borrelia garinii* cause serious infectious disease of various animals and human. *Francisella* is the facultative intracellular bacterium, which is able to adhere to various types of cells, including macrophages, endothelial and epithelial, while *Borrelia* is strictly extracellular bacterium. However, the cell-mediated immune response is a crucial factor in the control of both infections. To validate the cell response to the presence of *F. tularensis* (live vaccine strain, LVS) and *B. garinii* (strain G1), the comparison of whole blood assay (WB) and peripheral blood mononuclear cells assay (PBMC) was performed. The whole blood assay offers more advantages (e.g. analysis of number of antigens in one experiment and small volume of the blood), nevertheless PBMC assay is widely used. Here, we have compared 3 days cultivation of 1:3 diluted blood and isolated PBMC (both from sheep) to assess the proliferative response in the presence of paraformaldehyde inactivated *Francisella* and *Borrelia*. The XTT colorimetric assay of co-cultivated WB with *Francisella* shown the increased proliferation activity after 24, 48 and 72 hours of 120.3%, 140.6% and 202.2%, respectively, while during the co-cultivation of WB with *Borrelia* we noticed slightly reduction of the proliferation – the proliferation activity after 24, 48 and 72 hours was 104.5%, 131.8% and 84.6%, respectively. The proliferation activity of co-cultivated PBMC with *Francisella* was after 24, 48 and 72 hours 156.1%, 146.1% and 100.4% respectively, with the *Borrelia* we observed decreased proliferation of lymphocytes in 24, 48 and 72 hours, which was assess as 101.5%, 72.2% and 84.3% respectively. The experiments were performed in triplicates at the optical density at 450 nm The whole blood assay thus may be a good alternative for the study of immune response if the blood volume is reduced.

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P19. PUBLIC HEALTH RELEVANCE OF *BORRELIA MIYAMOTOI*

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Substantial exposure to Borrelia miyamotoi occurs through bites from Ixodes ricinus ticks in the Netherlands, which also transmit *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum*. Direct evidence for B. miyamotoi infection in European populations is scarce. A flu-like illness with high fever, resembling human granulocytic anaplasmosis, has been attributed to *B. miyamotoi* infections in relatively small groups. *Borrelia miyamotoi* infections associated with chronic meningoencephalitis have also been described in case reports. Assuming that an IgG antibody response against *B. miyamotoi* antigens reflects (endured) infection, the seroprevalence in different risk groups was examined. Sera from nine out of ten confirmed B. miyamotoi infections from Russia were found to be positive with the recombinant antigen used, and no significant cross-reactivity was observed in secondary syphilis patients. The seroprevalence in blood donors was set at 2.0% (95% CI 0.4-5.7%). Elevated seroprevalences in individuals with serologically confirmed, 7.4% (2.0-17.9%), or unconfirmed, 8.6% (1.8-23%), Lyme neuroborreliosis were not significantly different from those in blood donors. The prevalence of anti-*B. miyamotoi* antibodies among forestry workers was 10% (5.3-16.8%) and in patients with serologically unconfirmed but suspected human granulocytic anaplasmosis was 14.6% (9.0-21.8%); these were significantly higher compared with the seroprevalence in blood donors. Our findings indicate that infections with B. miyamotoi occur in tick-exposed individuals in the Netherlands. In addition, *B. miyamotoi* infections should be considered in patients reporting tick bites and febrile illness with unresolved aetiology in the Netherlands, and other countries where *I. ricinus* ticks are endemic.

WG5: Rare and emerging vector-borne pathogens

P20. MOLECULAR CHARACTERIZATION OF *BORRELIA* DETECTED FROM *IXODES RICINUS* FROM EASTERN SLOVAKIA

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Lyme disease is a prime vector-borne zoonotic disease in Europe. The genetic diversity within the *Borrelia* genospecies in Europe is well known. In this study attempts were made to detect and genotype the *Borrelia* from *Ixodes ricinus* ticks. Questing (n = 436) and partially fed ticks (n = 221) from the Eastern Slovakia were screened with PCR for the presence of borrelial DNA. Simultaneously triturated tick samples were subjected for *Borrelia* culture in BSK-II medium. Only 4.8% (n = 21) of the questing and 11.6% (n = 51) of the partially fed ticks were found positive for the *Borrelia* analyzed with 5-23*S* intergenic spacer based PCR. Interestingly, further characterization by reverse line blotting (RLB - with incorporated species specific probes) revealed the presence of three genospecies viz., *B. garinii*, *B. afzelii* and *B. burgdorferi* sensu stricto. No other borrelial genospecies (*B. lusitaniae* or *B. valaisiana*) was found in RLB. None of the tick was found with mixed infection. Further characterization of the *Borrelia* with 16S rRNA, OspA and OspC genes confirmed the genospecies identification by PCR-RLB technique. As OspA gene is more heterologous, the genotyping based on this gene is suppose to have more discrimination ability, particularly for *B. garinii* strains. However, all *B. garinii* found in this study were belong to OspA – serotype-3. The efficiency of culturing (in BSK-II) was significantly lower because of the contamination load from the blood from partially fed tick gut. Only 10 isolates were successfully cultured from the infected ticks (3 of *B. burgdorferi* sensu stricto, 4 of *B. garinii*- serotype 3 and 3 of *B. afzelii*). Results indicate the presence of all three genospecies in Eastern Slovakia, where as the *B. garinii* population is homologous in this region. Regular screening for the presence of *Borrelia* in tick population and their characterization is a necessary step for understanding the risk of Lyme disease for human as well as animal in this region.

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WG5: Rare and emerging vector-borne pathogens

P21. SURVEILLANCE OF AUSTRIAN ROE DEER FOR THE OCCURRENCE OF ANTIBODY AGAINST TBE: ADDITIONAL DATA FOR RISK ASSESSMENT

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TBE vaccination in Austria looks back on a long history. The virus is well known among the Austrian citizens and about 85% of them have been vaccinated at least once in their life against TBE. Nevertheless knowledge of the occurrence and distribution of natural infection foci is of great importance.
Distribution maps of TBE, based on human cases, are used and probably deliver a coarse overview of the possible risk areas. Nevertheless these maps might be biased due to traveling habits of people, different virus strains etc. Monitoring of large areas via tick sampling is cost and time consuming and probably do not deliver accurate and reliable data. Rodent sampling might be a possibility, but similarly the sampling needs a lot of effort. The easiest way to obtain wild life samples is to investigate roe deer.
Therefore, we screened roe deer sera for the occurrence of antibodies against TBE. Roe deer have small home range and are widely distributed all over the country.
All in all we obtained 945 sera, which were screened with IFAT. Twenty-two positive and 17 samples with a borderline titre could be identified. About 40% of the positive samples and 70 % of the borderline titre sera were found in areas where no TBE occurred in humans before.
The majority of positive sera were found in known TBE areas. This supports the existing data and can help to more accurately determine the infection foci. Nevertheless about ~40% of the positive sera were originating from previously assumed “free TBE areas”. This might be hints for new or overlooked transmission foci, maybe areas of lower human visits due to lower “attractiveness”. Those areas could be reservoirs and might become important in terms of the increasing outdoor activities. But caution hast to be drawn with these data, too. Although the majority of roe deer spend their entire life close to their birthplace, movement to other places cannot be excluded. Therefore a positive roe deer sera does not necessarily equals an infection focus. Hence, the high amount of questionable sera in “previously TBE free” areas are hints for a low re-infection pressure in these areas. One might hypothesise that those roe deer were infected elsewhere and moved to this area, pretending a positive foci.
However, further investigations on roe deer, rodents and ticks are needed to confirm actual infection foci in an area.

P22. INVESTIGATIONS ON CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS CIRCULATION IN BULGARIA

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infection. CCHF virus is transmitted to humans through bites of infected ticks, mainly of the genus *Hyalomma.* The virus can also be transmitted by direct contact with infected blood or tissues of CCHF patients or viremic livestock. CCHF is severe febrile infection, often accompanied by hemorrhagic manifestations with fatality rate ranging from 5% to 30%. The aim of this study was to investigate circulation of CCHF virus in ticks, livestock, and people in Bulgaria in 2013.
RT-PCR nested and real-time methods were used to detect CCHF virus in ticks. To investigate livestock, ELISA for detection of specific antibodies was applied. For investigations of patients, both methods – RT-PCR and ELISA, were used.
Eight CCHF patients were diagnosed in 2013 giving an incidence of 0,11 per 100 000 inhabitants. Of them, seven were diagnosed by ELISA and 5 by PCR detection of the CCHF virus. All but one patients originated from South and Southeast Bulgaria: districts of Haskovo (3 cases), Kardzhali (2 cases), Yambol (1 case), and Blagoevgrad (1 case). The only district in North Bulgaria with confirmed CCHF case was Shumen (1 case). Mainly affected were people of active age – 45-65 y. Two deaths of CCHF were reported in 2013 (mortality rate: 0,03 per 100 000 inhabitants; case fatality rate: 25%). Human seroprevalence study showed higher seroprevalence in Kardzhali and Haskovo and lower in Blagoevgrad, while in Yambol and Shumen antibodies against CCHF virus were not detected in healthy individuals. CCHF virus was detected in ticks, collected from livestock in all of the districts.
Active CCHF virus circulation among vectors, reservoirs and hosts was confirmed South and Southeast Bulgaria.

WG3: Geospatial tools in vector research

P23. MAPPING OF RISK AREAS FOR VISCERAL AND CUTANEOUS LEISHMANIASIS RELATED WITH DISTRIBUTION OF VECTOR SPECIES IN WESTERN PART OF TURKEY USING GEOGRAPHICAL INFORMATION SYSTEMS AND REMOTE SENSING

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Leishmaniases are present in two clinical forms, visceral and cutaneous, in Turkey. While the annual number of recorded visceral leishmaniasis (VL) cases is around 50-60 and reported from most of the regions of Turkey, cutaneous leishmaniasis (CL) cases are generally present in Southeast Turkey, with a tendency of spreading throughout the country. The aim of the present study was to carry out an entomological survey and to produce leishmaniases risk maps using data related to probable vector species and geographical parameters in a selected study site in the western part of Turkey. The entomological survey was carried out around the Kusadasi town and rural areas of Aydin province, where VL, CL and canine leishmaniasis (CanL) are endemic. The study area was 48x88 km^2 and was divided in 66 squares of 16 km^2 each. At least one location was chosen in each square during the fieldwork. The detailed ecological information was also collected for each location. The results of the entomological studies indicated that the main vector species of leishmaniases in the study area are *Phlebotomus tobbi* and *P. neglectus* for VL and CanL, and *P. similis* for CL. The maps were produced to show the distribution of vector species in the study area using geographical information system (GIS) and then the risk maps were developed based on known distribution of these three species for the western part of Turkey. Methodology of the development of the risk maps was based on univariate, multivariate and linear regression analyses. Altitude, aspect, Normalized Difference Vegetation Index (NDVI) and Land Surface Temperature (LST) values were used as parameters of the analysis. Altitude and aspect were derived from SRTM data set, NDVI and LST values were calculated from Landsat TM and MODIS data of the study area. The potential distribution areas of the sandfly vector species and the use of GIS allowed the identification of the leishmaniasis risk levels, which may provide useful information to guide the control program interventions.

WG3: Geospatial tools in vector research

P24. *DIROFILARIA* INFECTION IN DOGS FROM THE CAMPANIA REGION OF SOUTHERN ITALY

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Climate variability, global changes and the increase of the movement of pets across Europe are influencing the distribution pattern of many vector-borne infections. A straight forward example is the change of *Dirofilaria immitis* and *Dirofilaria repens* distribution in many European countries, including Italy. *D.immitis,* endemic only in northern Italy in the past few decades, has now spread all over the country and nowadays canine heart worm infection is more frequently diagnosed also in central and southern Italy. *D.repens* is more homogeneously spread across Italy, with higher prevalence values in central and southern regions.

The aim of this study was to update the data on canine dirofilariosis in the Campania region of southern Italy, where heart worm and subcutaneous dirofilarial infections were considered a minor problem by clinicians and parasitologists so far. Blood samples from asymptomatic dogs (no.=450) were randomly collected between 2008 and 2014 at various veterinary clinics from the region, and then analysed using the modified Knott technique for microfilariae detection.

Microfilariae of *D.immitis* were detected in 10 dogs (2.2%; 95% Confidence Interval= 1.1-4.2%); microfilariae of *D.repens* were detected in 10 dogs (2.2%; 95% Confidence Interval= 1.1-4.2%); 1 dog was co-infected with *D. immitis* and *D. repens* (0.2%; 95% Confidence Interval= 0.01-1.4%).

The anamnestic data of dogs revealed that only 2 of the 7 dogs (positive to *D. immitis*) were not natives of the Campania region, whereas the other positive dogs were native subjects never moved outside the region according to the knowledge of the owner. The results of this study show the existence of autochthonous foci of canine *Dirofilaria* infection in the Campania region of southern Italy.

WG5: Rare and emerging vector-borne pathogens

P25. *DIROFILARIA IMMITIS*, A SILENT AGENT OF PULMONARY EMBOLISM AND SUDDEN DEATH IN A CAT FROM PORTUGAL

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Cardiopulmonary dirofilariosis is a severe and life-threatening disease, with increasing prevalence and geographic distribution in the last years. Portugal is historically considered an endemic country for canine dirofilariosis due to its temperate climate. However, available data concerning *Dirofilaria immitis* circulating antigens in cats from Portugal is scarce and low, ranging from 1.2% - 1.4% in the Centre to 4.8% in the South of Portugal. A higher prevalence of 15% was registered in Central and Northern Portugal for *D. immitis* antibodies detection.

We report the case of a 6-year-old neutered male cat, European short hair, found dead by the owners with no apparent causes. He was a seemingly healthy catdespite being positive for feline leukaemia virus. On necropsy, performed at the Lisbon Veterinary Faculty (FMV-ULisboa), three adult dead nematodes (size 27.2cm, 21 cm and 13.4 cm) were found blocking the pulmonary artery. There were also some immature worms in the lumen of the right ventricle and in both lungs. Severe pulmonary oedema, moderate hydro pericardium, passive hepatic congestion and mild anaemia were also detected. Nematodes were later analysed under the microscope and identified as *D. immitis.* It was assumed that the cat died of circulatory collapse and respiratory failure due to an acute thromboembolism of the pulmonary artery, secondary to parasitic infection.

The few published reports of feline dirofilariosis suggest that it is a relatively rare cause of death. However, cats play a central role in the transmission cycle of this disease that seems to be a growing problem. Taking into account the frequent subclinical course of the infection, the potential for serious or even fatal consequences and the lack of approved treatment in cats, preventing Dirofilariosis is absolutely crucial. We hope this report will help to increase the awareness of general community and practitioners to the need of routine checks and targeted preventive therapy for heartworms in cats from Portugal.

WG5: Rare and emerging vector-borne pathogens

P26. THE OCCURRENCE AND GEOGRAPHICAL DISTRIBUTION OF CANINE DIROFILARIASIS IN BOSNIA AND HERZEGOVINA

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We conducted the first formal study of canine dirofilariasis in Bosnia and Herzegovina (B&H) aimed to detect the presence of canine dirofilariasis and to provide a rough estimate of its geographical distribution. Blood samples were collected from 418 dogs (242 male and 176 female), aged between 12 months and 14 years, from multiple locations around the country during the period from May 2008 to November 2009. All samples were tested by Knott's test and ELISA test (PetChek HTWM, PF, Idexx Laboratories). The proportion of dirofilariosis positive dogs was calculated and stratified according to *Dirofilaria* species found and climate of the sample origin. Differences in the proportions of positive dogs of the various subcategories (strata) were tested using chi square test at a level of statistical significance of 5% (i.e. α=0.05).
Two different filarial species were identified: *Dirofilaria immitis* and *Dirofilaria repens*. From the overall number of dogs examined, 18 (4.3%) were positive by the Knott's test. *D. immitis* was found in 10 (2.4%) dogs, and *D. repens* in 8 (1.9%). Serological testing (ELISA test) established *D. immitis* infection in 12 dogs (2.9%). One sample that was negative with ELISA test was positive for microfilaria by the Knott's test. Irrespective of *Dirofilaria* species, 5.1% (9/176) positive samples were found in female and 4.9% (12/242) in male dogs. The highest proportion of dogs with dirofilariasis was found in areas which have mesothermal climates with mild winters and dry, warm summers. In the northern parts of our country with moderate continental climates, dirofilariasis was found in a smaller proportion of samples, while in the middle of the country, with a mountain continental climate, no positive samples were found. All but one positive sample belonged to dogs that never traveled outside of B&H. Our results show that canine dirofilariasis is present in B&H as autochthonous infection. Statistically significant differences were established for all tested pairs of dirofilariasis proportions belonging to investigated areas with different climate conditions.

WG1: The "One Health" concept in the ecology of vector-borne diseases

P27. VISCERAL LEISHMANIASIS CAUSED BY *LEISHMANIA INFANTUM* MON-1 IN A CAT WITH INVASIVE NASAL SQUAMOUS-CELL CARCINOMA

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Zoonotic leishmaniasis caused by *Leishmania infantum* is a serious zoonosis in the Mediterranean Basin. This is the first case of viscerocutaneous leishmaniasis caused by *L. infantum* associated with an invasive squamous cell carcinoma in a domestic cat from Portugal. Cat initially presented a single cutaneous lesion in the right nostril. A fine needle aspirative biopsy was performed and *Leishmania* amastigotes were observed in macrophages without observation of cells compatible with neoplasia. Despite treatmentwith allopurinol, the cat worsened and one year after presented a crateriform non-encapsulated and baddly delimitated mass in the nasal planum, with naso-oral fistulation and nasal destruction. Histologically, the skin mass consisted with a nasal squamous cell carcinoma. *Leishmania* parasites were detected by histophatological examination, culture and PCR on skin mass, lymph nodes, liver and spleen and anti-*Leishmania* antibodies were detected by IFAT. Isolated parasites were identified as *Leishmania infantum* zymodeme MON-1, the most common aetiological agent of human and canine leishmaniasis in the Mediterranean Basin.
From a clinical point of view, this case reinforces the importance to systematically include leishmaniasis among the differential diagnoses of feline pathologies, namely in cats from endemic areaswith cutaneous lesions.

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WG1: The "One Health" concept in the ecology of vector-borne diseases

P28. MOLECULAR IDENTIFICATION OF ANAPLASMATACEAE PATHOGENS IN RODENT POPULATIONS FROM SOUTH-WESTERN POLAND

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This study aims to analyze the occurrence of Anaplasmataceae pathogens, *Candidatus* Neoerlichia mikurensis (CNM) and *Anaplasma phagocytophilum* among rodent populations. Rodents (n=161) represented by *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus,* were captured in live traps in four localities of south-western Poland (2013). Both blood (n=132) and spleen (n=161) samples were obtained from rodent specimens. The choice of genetic markers and primers was based on the literature data and our preliminary results (*msp*2, 16S and *gro*El genes). PCR methods were 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.
All obtained samples were simultaneously tested with the use of all genetic markers. DNA of pathogens was detected on comparable level in blood (14.3%) and spleen (14.4%) samples based on *msp*2 gene, while in 21.2% and 28% concerning 16S gene respectively. There was observed statistically significant difference between the trapping locations and the detection levels of Anaplasmataceae. With regard to the rodent species no significance was recorded.
According to recent literature data *msp*2 gene is usually used for Anaplasmataceae screening in environmental samples and then other markers (i.e. 16S and *gro*El) are used for its confirmation. However in case of this study almost half of the PCR-positive samples for *msp*2 gene were also positive based on 16S gene. Additionally several positive PCR products were obtained using 16S gene only. Therefore *gro*El gene was used to examine any positive isolates. Most of the selected positive samples were identified as Candidatus *Neoerlichia mikurensis* based on the obtained sequence analysis. Our results indicate that CNM is a common rodent-associated pathogen in southwestern Poland what is in accordance with recent European studies.

This research was partly done within the project "Academy of Development as the key to strengthen human resources of the Polish economy" co-financed by the European Union under the European Social Fund".

P29. ACUTE PHASE PROTEINS AFTER experimental INFECTION WITH *ANAPLASMA PHAGOCYTOPHILUM*

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The bacterium *Anaplasmaphagocytophilum* may cause disease in several mammalian species including humans. In ruminants, the disease is named tick-borne fever (TBF). The infection is common on *Ixodesricinus* infested pastures in Europe and represent not only a welfare challenge, but may also cause severe economic lossesespecially in the sheep industry. Several strains/variants of the bacterium have been characterized, which may cause different clinical manifestations and involve several reservoir hosts. Diagnosis of TBF is normallybased on blood smear microscopy, PCR-analyses and serology. However, acute phase proteins are getting more common as a diagnostic tool in both medical and veterinary medicine. The present study monitored the change in the acute phase proteins during an experimental *A. phagocytophilum* infection. A total of 12 lambs were utilized whereas sixwere infected with the variant M73220 (Gen Bank acc. no.) of *A. phagocytophilum*on day 0. Blood samples were collected frequently throughout the experimental period of 43 days. Clinical and hematological parameters were recorded, in addition to acute phase proteins such as ceruloplasmin (Cp), haptoglobin (Hp), serum amyloid A (SAA) and albumin. The result showed that Cp, Hp and SAA values increased, while albumin decreased in *A. phagocytophilum* infected lambs. Hp and SAA showed a rapid and pronounced increase in concentration, with maximum levels on days 7and 8, respectively, returning to normal levels on days 16/17. However, Cp values in infected lambs had a more moderate change with an increased level on day 8, which remained elevated until day 24. The present study indicates that acute phase proteins could be used to monitor an *A. phagocytophilum* infection.

P30. THE SPREAD OF *COXIELLA BURNETII* IN RUMINANT HERDS AND TICKS IN ESTONIA

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The obligate intracellular bacteria *Coxiella burnetii* is a zoonotic pathogen that causes disease called Q-fever. This disease is accompanied by reproductive disorders in domestic ruminants and atypical respiratory symptoms in humans. Ruminants are considered as the main source of *C. burnetii* infection for humans, but the transmission pathways in domestic ruminants and transmission to humans are still not fully understood. The most known infection routes are through external waste excretions from infected animals and via aerosol by inhalation of air contaminated with these bacteria. Though considered rare, one way to transmit the disease is via arthropods, particularly ticks.

In Estonia there has been no Q-fever related research going on until just recently. To date there has been reported no human case of this disease, but there exist also the incapacity to diagnose the agent by medical doctors in the country. Because of the increase in travelling of the people as well as of livestock, we expected the pathogen to exist also in Estonian ruminant herds.

In our study samples of cattle, sheep and goat herds were tested for *C. burnetii* antibodies with LSIVet TM Ruminant Q Fever - Serum/Milk ELISA. The total bacterial DNA from milk samples and the DNA samples of *Ixodes ricinus* and *Ixodes persulcatus* collected from four different counties were tested by PCR for *C. burnetii* specific multicopy insertion sequence IS*1111.*

As a result, *C. burnetii* antibodies were detected in cattle and sheep but not in goats. Tank milk samples from eight dairy cattle herds were detected PCR-positive. All the tested ticks appeared to be negative for *C.burnetii* specific genetic material.

WG2: Barcoding, molecular diagnosis and next generation sequencing

P31. MOLECULAR INVESTIGATION OF *BORRELIA BURGDORFERI* SENSU LATO IN SERUM OF SEVERAL ANIMAL SPECIES FROM MAINLAND PORTUGAL

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Spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex are causative agents of Lyme borreliosis (LB), the most common tick-borne zoonosis in the northern hemisphere. Several wild and domestic animals species are susceptible to *B. burgdorferi* s.l. infection although many affected animals remain asymptomatic or may have generic clinical signs. *B. burgdorferi* s.l. has been detected in ticks from Portugal and thus this finding presumed exposure of animals to this zoonosis. Moreover, studies have suggested a potential role of some animal species in the epidemiological cycle of LB.

In the present study, we assessed the occurrence of *B. burgdorferi* s.l. in 113 serum samples from wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and horses by using two nested-PCR assays targeting the 5S–23S intergenic spacer region and *fla* gene. DNA extraction from sera was performed using QIAamp DNA Mini Kit (Qiagen). *Borrelia* DNA was detected in two sera samples, one from a wild boar and one from a horse, by both nested-PCR techniques. Sequencing results permitted to identify *B. burgdorferi* s.l. genospecies. The results confirm the presence of *B. burgdorferi* s.l. in wild boars from Portugal. Moreover, this is the first report of molecular detection of *B. burgdorferi* s.l. on a horse from Portugal.

Efforts are required between all actors involved in animal health surveillance of both domestic animals and wildlife to better understand the epidemiological and clinical significance of LB in Portugal and, also to overcome the under diagnoses and under report of this disease.

WG5: Rare and emerging vector-borne pathogens

P32. THROMBOELASTOGRAPHIC EVALUATION OF COAGULATION PROFILE IN DOGS WITH EHRLICHIOSIS

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The aim of this study was to evaluate the coagulation profile by using thromboelastography (TEG) in dogs with naturally occurring ehrlichiosis. For this purpose, dogs with or without clinical signs were screened for rickettsial antibody of *Ehrlichia canis* (*E. canis*) using Anigen Rapid Test for *Anaplasma marginale*, *E. canis*, *Dirofilaria immitis*, and *Borrelia burgdorferi sensu lato*. Dogs were screened to exclude *Leishmania infantum* by *Leishmania* Ab Test Kit (Anigen® Rapid). Co-infections were excluded from the study. *E. canis* positive dogs (n=18) were divided into two groups; subclinical (n=9) and clinical form (n=9). Dogs were examined for coagulation status by peripheral platelet count and TEG parameters (TEG®5000-Analyzer) evaluating clot kinetic (R time-reaction time, K time-kinetic time), clot strengthening (alpha angle and G value), platelet function (maximum amplitude-MA and coagulation index-CI), and clot stability (LY30). For dogs (n=18), mean values (±StandDev) of platelet count, R and K times, alpha angle, MA, G, and CI were 127±128 $x10^3/\mu L$, 2.1±1.4 min, 1.7±1.2 min, 70±13 degree, 58±12 mm, 8.0±3.5 $Kdyn/cm^2$, and 2.4±3.3, respectively. There were not statistically differences on the mean values of parameters between groups, but individual TEG values as compared to reference range published for healthy dogs indicated abnormal findings. Thrombocytopenia was more prominent in dogs with clinical form (88±60 *vs.* 159±166 $x10^3/\mu L$). R time was lower in dogs with clinical (n=4) and subclinical form (n=4). K time was lower in dogs with clinical (n=6) and subclinical form (n=5). Alpha angle was higher in dogs with clinical (n=5) and subclinical form (n=3). MA was lower in dogs with clinical (n=2) and subclinical form (n=2). G value was higher in dogs with clinical (n=2) and subclinical form (n=4). CI was higher in dogs with clinical (n=2) and subclinical form (n=3). TEG results were compatible with hypocoagulation and platelet dysfunction in two dogs with clinical signs, and hypercoagulation in a subclinical dog. Circulating platelet count was correlated positively ($p<0.05–0.001$) with alpha angle, MA, G value and CI, and negatively ($p<0.05$) with R and K times as well as LY30. Serum BUN and Cr levels were within reference ranges, but ALT and ALP enzyme activities increased in dogs with clinical ehrlichiosis. These results showed that coagulation status might have dynamic changes in dogs with ehrlichiosis. Based on the TEG value, it might be speculated that clot stability, clot kinetic, clot strengthening, and platelet function may be changed in dogs with ehrlichiosis, regardless of clinical form.

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