Book of abstracts

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Genes, Ecosystems and Risk of Infection

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> Abstracts

Oral presentations

Session 1

Genes

Mosquito-borne viruses in south Moravia (Czechland) - summary of an EDENext study

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In the years 2011-2014, we examined 51,440 female mosquitoes (in 1,024 pools; 50,496 Culex modestus, 944 Cx. pipiens) inhabiting reed-belts of south-Moravian fishponds. While Sindbis alphavirus RNA was not detected in the mosquitoes, RNA of West Nile flavivirus lineage 2 (WNV-2) was found in 13 pools of Cx. modestus caught in 2013. From five of these positive pools, WNV-2 was additionally isolated on suckling mice (SM). One pool of Cx. modestus contained RNA of Usutu flavivirus (USUV), but the isolation attempt on SM was unsuccessful. Using SM inoculated intracerebrally, we examined 8,100 Anopheles hyrcanus (in 114 pools) collected in the same habitats, 3,132 Cx. modestus (62 pools), 550 Cx. pipiens (10 pools) and 41 Uranotaenia unguiculata (2 pools). Two strains of Tahyna orthobunyavirus (California encephalitis group) were isolated from An. hyrcanus collected in 2013. A total of 740 male Cx. pipiens (in 16 pools) were examined by RT-PCR, but no flavivirus RNA was found in them. No flavivirus RNA was detected in 14,986 female Cx. pipiens (305 pools), 282 Culiseta annulata (8 pools) and 42 Anopheles maculipennis group (4 pools), overwintering in cellars in south Moravia during four winters from 2011 to 2014. Serosurvey (plaque-reduction neutralization test) of 146 common coots (Fulica atra) for WNV and USUV in 2011 revealed 18 birds reacting against WNV. The WNV seropositive samples were then titrated in parallel for antibodies against tick-borne encephalitis virus, WNV and USUV to exclude cross-reactivity. Two birds (1.4%) had the highest titers of antibodies against WNV and nine birds (6.2%) were specifically seropositive for USUV, while in seven birds the infecting flavivirus could not be differentiated. Our results indicate that WNV and USUV infections co-occur in common coots examined in Moravia, the USUV infection being more common. We autopsied nine birds found dead in south Moravia in the years 2011 and 2012: four blackbirds (Turdus merula), two sparrowhawks (Accipiter nisus), two sakers (Falco cherrug) and one kestrel (Falco tinnunculus), and carried out virus isolation attempts from their internal organs. Usutu virus killed two blackbirds. Mosquito-borne West Nile, Usutu and Tahyna viruses occur in south Moravia recently.

May complete genome-based distances be of help for taxonomical classification of members of the genus Phlebovirus within the Bunyaviridae family ?

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The Bunyaviridae family is constituted of 5 genera. Species demarcation is difficult and has been defined by serological criteria (such as cross-neutralisation and cross-hemagglutination-inhibition tests). For 3 genera (Orthobunyavirus [OBV], Hantavirus [HV] and Tospovirus[TV]), genetic distances (AA sequences of nucleoprotein (N) are one of the criteria that is taken into account for classification into species; for HV, glycoprotein sequences are also considered). Due to the lack of sequence data until recently, there is no cut-off value defined for species demarcation among the members of the Nairovirus [NV] and Phlebovirus [PhV] genera. For the latter, species are defined by the serological relationships, and are distinguishable by four-fold differences in two-way neutralization tests

It is obvious that supplementary criteria would help species demarcation within the genus Phlebovirus , and that sequence data that have been massively produced in the last 4 years are promising candidate to achieve polythetic species definition.

In this study we compiled all complete AA sequences available that are presented as: number (nb) of genbank sequences / nb of new sequences (determined by our group) / total nb of sequences analysed / nb of pairs of distances used for calculations: For N (87/10/97/4657), Ns (80/11/91/4096), Gn (62/12/74/2702), Gc (62/12/74/2702), and L (60/11/71/2485) complete AA sequences were used for studying the distribution of amino acid evolutionary distances for each of the 5 genes, independently. Intra- and interspecies cut-off values were calculated using 3 different sets of data: (i) set#1 was species recognized by ICTV, (ii) set#2 was set#1+tentative species listed by ICTV, (iii) set#3 was set#1&2+newly discovered viruses not yet listed by ICTV. N sequences allowed to discriminate intra-species from inter-species whereas Gn, Gc and L sequences allowed to discriminate intra-species, interspecies excluding tick-borne viruses, and tick-borne viruses against mosquito-borne and sandfly-borne viruses. Ns sequences were not suitable.

Possible cut-off values were determined and accordingly we propose (i) to create a tick-borne clade within the genus Phlebovirus, (ii) to revisit the putative classification proposed by Palacios et al for newly sequenced phleboviruses, and (iii) to modify the cut-off values proposed in 2009 based on limited number of complete sequences.

Transcriptomic studies of Ixodes ricinus salivary glands lead to the identification of vaccine candidates against tick and tick-borne pathogens

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Ixodes ricinus, the most widespread and abundant tick in Europe, frequently bites humans and is the vector of several pathogens including: viruses, parasites, and bacteria. It has been reported that the transmission of tick-borne pathogens is directly linked to tick saliva which contains key components underlining this action. Ultimately, tick salivary secreted molecules facilitate pathogen transmission by modulation of inflammatory, immune, and haemostatic processes at the tick-host interface. One of the promising approaches for the prevention of tick-borne pathogen transfer includes the development of vaccine strategies targeting conserved sialome components of ticks that play key roles in vector infestation and/or vectorial capacity. To identify such components, we identify some differentially expressed transcripts in I. ricinus salivary gland (SG) based on pathogens infection. We used next generation sequencing techniques to compare gene expression profiles of Bartonella henselae-infected and non-infected I. ricinus females SG. Among 24,539 isotigs identified, 829 and 517 transcripts were either significantly up- or down regulated respectively, in response to tick bacterial infection. Currently, our attention is paid mainly to secreted proteins which have shown to be up-regulated during the infection of the tick. Among them, the most up-regulated transcript encoding BPTI/Kunitz family serine protease inhibitor (IrSPI) has been studied in depth. RNA interference of IrSPI in Ixodes females affected both feeding and bacteria transmission. Temporal and spatial expression pattern of IrSPI has been examined using qRT-PCR and tissue specific RT-PCR followed by in situ hybridisation. Immunization of mammal's models with the recombinant IrSPI is currently ongoing in our laboratory. Mining the I. ricinus salivary gland transcriptome is shedding light onto molecular interactions between ticks and tick-borne pathogens. As well as tick feeding biology, which may ultimately lead to uncovering novel vaccine candidates for tick control.

Using population genetics to assess tick dispersal, from the mainland to the landscape scale : a review of current knowledge and its utility to design tick-control methods

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Assessment of tick dispersal is a central issue for the understanding of the eco-epidemiology of tick-borne diseases and the development of effective control methods. Tick dispersal is assumed to be due to host movements. However, because (1) Ixodes ricinus -the most common European tick vector- uses a high diversity of hosts, (2) these hosts show a wide range of dispersal distances (ranging from a few hundred meters for rodents to hundreds of kilometers for birds) and (3) the relative contribution of the different host species to tick feeding is unknown, estimating tick dispersal is a challenging task. Population genetics have been used to assess gene flow and thus dispersal at different scales, from the whole distributional range of I. ricinus, to local areas, using a variety of genetic markers. The sequencing of mitochondrial and nuclear genes has revealed that the Eurasiatic populations do not group according to their geographical origin, suggesting a high level of gene flow, probably due to migrating birds or to the movement of large mammals, including domestic livestock, between countries. Only the North African populations are genetically highly divergent from the other populations, suggesting inefficient geneflow between these two groups. At a smaller scale, the analysis of microsatellite loci among populations along 60 km long-transects located on both sides of wide rivers have revealed that there are no barriers to geneflow, highlighting the role of birds or large mammals in the dispersal of ticks. Genome wide SNP (Single Nucleotide Polymorphism) identification using Restriction-site Associated DNA Sequencing (RAD-Seq) was performed on a regional scale, where mountain groups and rivers have already been shown to be barriers to movement for many species; however, even here, extensive geneflow was observed for ticks. Finally, another set of SNPs based on the sequencing of Restricted Representative Libraries (RRL) were also used to investigate geneflow at the landscape scale (from tens of meters to a few kilometers). No isolation by distance has been detected at this scale either, also suggesting high levels of geneflow between populations. The contribution of those results for the design of control methods against ticks will be discussed.

Modelling the effects of tick-host interaction on pathogen dynamics: TBE as a case study

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Tick-borne encephalitis (TBE) is an emerging vector-borne zoonotic disease reported in several European and Asiatic countries with complex transmission routes that involve a number of key vertebrate host species other than a major tick vector. Understanding and quantifying the interaction between ticks and main hosts involved in the TBE virus (TBEv) cycle is crucial in estimating the threshold conditions for TBEv emergence and spread. Some hosts, such as rodents, act both as feeding hosts for ticks and reservoirs of the infection. Other species, such as deer, provide important sources of blood for feeding ticks but they do not support TBE virus transmission, acting instead as dead-end (i.e., incompetent) hosts. We used eco-epidemiological models to explore the dynamics of tick populations and TBEv infection in relation to the density of two key hosts, deer and rodents. Both host may act as tick amplifiers, but at high densities may also dilute pathogen transmission. Model outputs were validated with empirical data regarding the effect of host densities on tick population dynamics and TBE virus infection from selected European foci in Italy and Slovakia. In addition, we investigated the effect of using various models to describe tick aggregation on TBEv dynamics in a long-term study site in Trentino (Northern Italy). Specifically, we modelled the number of ticks per rodent host by using Negative Binomial, Poisson-LogNormal and Power Law (PL) distributions. PL model seems to better describe the strong heterogeneity observed in our data. Using a stochastic model, we observed that TBEv infection is highly dependent on the capability of the implemented model to describe tick burden on rodents. Specifically, we found that the epidemic threshold and the prevalence equilibria obtained in epidemiological simulations with PL distribution are a good approximation of those observed in simulations feed with empirical distribution.

Session 2

Ecosystems: vectors

Could biting midges, Culicoides nubeculosus and Culicoides sonorensis (Diptera: Ceratopogonidae) serve as Leishmania vectors?

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Biting midges of the genus Forcipomyia (Diptera: Ceratopogonidae) have been implicated as vectors of Leishmania enriettii complex. Recently, several authors reported Culicoides PCR-positive for Leishmania infecting humans, however, we highlight that PCR alone has considerable limits for studying the role of bloodsucking arthropods in the epidemiology of leishmaniasis and light microscopy remains the gold standard method. Here, we assessed susceptibility of two colonized midges, Culicoides nubeculosus and C. sonorensis, to Leishmania infection. Midges were experimentally infected with two strains from the L. enriettii complex, L. australiensis originally from red kangaroos (LV756) and L. enriettii from guinea pigs (LV90), and two Leishmania species infecting humans, L. major (FVI) and L. infantum (CUK3). A permissive sand fly Lutzomyia longipalpis was used as a positive control. Engorged females were microscopically examined at different time points post-bloodmeal to observe the localization of Leishmania in the gut. We showed that in C. nubeculosus all four Leishmania strains tested produced only light infection in the abdominal midgut. In contrast, C. sonorensis was able to support late-stage development of both strains from the L. enriettii complex; in 20% of females parasites migrated anteriorly and colonized the stomodeal valve, which is one prerequisite for successful transmission. Additionally, C. sonorensis and L. longipalpis were used for xenodiagnosis experiments on guinea pigs and hamsters infected by South-American strain of L. enriettii (LV90). We demonstrated that infected guinea pigs represent a very good source of infection for both vectors (infection rate up to 80%) and parasites disseminated to inner organs. Contrastingly, hamsters self-healed after one month post-infection and only L. longipalpis females were able to take parasites from their skin. In summary, we showed that Palaeartic species of biting midge, C. nubeculosus is not susceptible to any Leishmania tested. Importantly, the New World species C. sonorensis is refractory to Leishmania infecting humans but susceptible to parasites of Leishmania enriettii complex. Future transmission experiments are needed to confirm the vector competence of this species.

Host preferences and circadian rhythm of Culicoides (Diptera: Ceratopogonidae), potential vectors of African horse sickness and bluetongue viruses in Senegal

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Midges in the genus Culicoides Latreille are small biting dipterans distributed worldwide. Several species are vectors of viral and parasitic pathogens. Their impact is mainly on animal health: in particular, they are responsible for the transmission of two dreaded viral epizootic diseases in horses and ruminants, respectively African horse sickness (AHS) and bluetongue (BT). In Senegal, since the last epizootic outbreak of AHS in 2007, extensive investigations have been undertaken to gain better knowledge about Culicoides species involved in the transmission of the virus. The purpose of this study is to compare the host preferences and to describe the circadian rhythm of potential vectors of AHS and BT viruses.
The study took place at the Thiès national stud farm between August 29 and September 28, 2013. Culicoides were collected using two baited traps (horse and sheep) conducted in three sessions. Each session consisted in two days of trapping (twice 24 consecutive hours) separated by a three-hour interval. The trapped insects were collected inside the netting tent every three hours for 10 to 15 minutes using an electric vacuum aspirator with a fine mesh collection bottle. Captured individuals were morphologically identified and counted by species and sex.
This study shows that C. oxystoma and species of the sub-genus Avaritia (C. imicola, C. bolitinos and C. pseudopallidipennis) have a preference for horses compared to sheep (96 to 98% of females were collected on this host), and are mostly crepuscular; C. oxystoma may be active during the day while C. imicola, C. bolitinos and C. pseudopallidipennis are mostly nocturnal.
This work allowed to gain knowledge on the host preferences of potential vectors of AHS and BT viruses in Senegal and to characterize their circadian activity. Thus, it shows also the importance to investigate vector control methods against species exhibiting a diurnal biting activity.

Sandflies in the Cevennes Region: 3-year to investigate the parameters affecting their spatiotemporal dynamics

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Phlebotomine sandflies are hematophagous insects (Diptera, Phychodidae) active during night and dusk. They are abundant in peri-urban and rural environments, often close to human and animal populations. In the Old World, the species belonging to the Phlebotomus genus are known to be vectors of pathogens, such as the Leishmania genus, the causative agent of leishmaniases, Bartonella bacilliformis (Carrion´s disease) and phlebovirus. Although the leishmaniases are extensively studied in Europe and especially in the Mediterranean basin, the biology and ecology of sandflies remain poorly known. The current extension of Leishmaniasis distribution underlines the need to increase knowledge on these insects. In France, five species are described: Phlebotomus ariasi, P. perniciosus, P. mascitii, P. papatasi and Sergentomyia minuta. In the Cevennes area, P. ariasi is the main vector of Leishmania infantum and P. perniciosus is the second one.
The objective is to determine the parameters involved in the spatiotemporal organization of sandfly populations along a 14 km transect located in the region of «Le Vigan» (Cévennes, France) where canine leishmaniasis is endemic.
The abundance and distribution of sandfly species collected from May to October between 2011 and 2013 were confronted with climatic and ecological data. 15,488 individuals (7,949 males and 7,539 females) were trapped and morphologically identified. We found P. ariasi (93.23%), S. minuta (6.18%), P. perniciosus (0.48%) and P. mascitii (0.11%).
These species have different distribution. P. ariasi and P. perniciosus are ubiquitous but more abundant in stations with livestock (rabbits, chickens, sheep...). On the contrary, S. minuta preferred «wild» area, distant from human presence. Undoubtedly, the environment is one of the key points of the species organization but what about the climatic parameters?
The presence and the abundance of sandfly populations appear to be influenced by various parameters among them three have a significant impact: temperature and altitude with a positive correlation and humidity with a negative one. Surprisingly, slopes and wall orientation do not have influences, suggesting that additional environmental factors impact P. ariasi dynamics. The identification of parameters affecting the distribution and dynamics of sandflies is one of the first steps for the understanding of Leishmania transmission.

Mechanistic modelling of midge vector population dynamics

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Vector abundance and seasonal dynamics are important determinants of variation in the risk of vector-borne infections. As well as producing seasonal and spatial variation in the ratio of vectors to hosts, vector dynamics also govern the time delay between the acquisition and transmission of a pathogen by successive generations or life-stages. Hence, predicting vector seasonal abundance is crucial for effective disease mitigation strategies.
Culicoides biting midges are important in light of their potential for vectored disease transmission, especially of Bluetongue Virus and Schmallenberg Virus in livestock. Whilst it is clear that environmental drivers such as temperature, humidity and photoperiod affect annual midge abundance, it is not generally understood how these variable drivers impact upon the midge population dynamics.
In this talk we will present a mechanistic population model for Culicoides midges that incorporates the relationships between environment and demographic rates. In this stage-structured model, we parameterise the environment-driven development rates using a mixture of lab and field data. We demonstrate that the model accurately predicts UK dynamics. In particular, the model output exhibits the characteristic annual peaks in abundance in the spring and autumn, with similar lengths of annual adult presences compared to field data.
Building upon this model framework, we investigate the efficiency of alternative timing and intensity of vector control and consequences for the establishment of transmission using R0 modelling frameworks. We demonstrate that targeted control may reduce the average vector abundance, but the timing and magnitude of the seasonal biannual midge population peaks may change and increase, depending on the control effort. We discuss these results in the context of potential disease management.

Fur or feather? Feeding preferences of species of Culicoides biting midges in Europe and its consequences for pathogen transmission

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SourceURL:file://localhost/Users/Joaquin/Desktop/Dropbox/creta%20culicoides.doc
Understanding the feeding preferences of haematophagous insects is critical to depicting amplification and transmission networks of pathogens and identifying key vector species for surveillance programs. Biting midge species from genus Culicoides are important vectors of pathogens to humans, livestock and wildlife. Here, we review the current knowledge on the molecular identification of the vertebrate hosts of species of Culicoides in Europe. The analyses of 1360 individuals belonging to 31 species identified 45 vertebrate host species, including 33 species of birds and 12 species of mammals. Although information is still limited for a significant fraction of competent vectors of Culicoides-borne pathogens, results from molecular studies on the feeding pattern of females of species of Culicoides support the fact that: (i) most of the studied biting midge species are able to feed on several vertebrate species; (ii) although some species feed primarily on either mammals or birds, this is not a strict behaviour, with at least some feeding also on blood from animals of the non-preferred vertebrate group; and (iii) taxonomically or phylogenetically related species tended to feed on the same Classes of vertebrates, which could be useful to infer the feeding pattern of Culicoides species, when no empirical information is available.

Deer and rodents as determinants of tick density and Borrelia burgdorferi s.l. prevalence in ticks in the Netherlands

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Many tick-borne diseases are dependent on wildlife hosts, which, however, differ in their importance for feeding ticks and in their ability to transmit pathogens. The dilution effect hypothesis predicts that wildlife species richness is negatively correlated with vector population densities and pathogen infection prevalence. To test this prediction, we compared Ixodes ricinus densities and B. burgdorferi s.l. prevalence between 20 forest fragments across the Netherlands, that varied in wildlife community composition. We used drag sampling of ticks, molecular screening of Borrelia within ticks, live trapping of small mammals, and camera trapping of other wildlife. We found that tick densities and nymphal infection were lowest in forest fragments with few or no deer. In forest fragments with deer, nymphal I. ricinus density increased with numbers of larvae found on rodents, due to either high rodent density or high larval burdens. In these fragments, nymphal infection with Borrelia declined with deer density. Neither nymphal density nor nymphal infection were correlated with mammal species richness. These findings indicate that the relative abundance of rodents and deer determine both tick densities and B. burgdorferi s.l. prevalence in these forests. We found no evidence for a dilution effect of mammal species richness. However, the correlation between deer density and nymphal infection prevalence suggests encounter reduction of nymphal and adult I. ricinus with rodents and birds, which is one of the proposed mechanisms behind the dilution effect. Our findings imply that regulating deer in order to lower risk of Lyme borreliosis could have the opposite effect, and that preserving mammal species richness need not reduce disease risk.

Seasonal activity of Ixodes ricinus and its dependence on weather factors in the course of the year: results of a multi-annual study under quasi-natural conditions

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Ixodes ricinus (Acari: Ixodidae) is the most important vector tick in large parts of Europe transmitting the agents of Lyme borreliosis, tick-borne encephalitis, and other pathogens of medical and veterinary relevance. Although it is a comparatively well-investigated tick species, some basic questions on its ecology are still not satisfactorily answered, for example the influence of weather on the changing level of questing activity in the course of the year. This study took a novel quasi-natural approach using ticks in field plots located in a forest in the environs of Berlin (Germany). From 2008 to 2014, observations were made usually 3 times a week during the growing season (March to October) and mostly once a week during the cold season (November to February). The results of this high-frequency monitoring give an unusually clear picture of the strongly varying activity pattern of the nymphs and adults of I. ricinus over 7 years. Although the general pattern of seasonal tick activity strongly varied from year to year even in the same location, there were also certain seasonal events that tended to recur in different years. Main emphasis of this contribution is the analysis of (i) periods with distinct changes (decrease and subsequent increase) of the level of tick activity within short periods of time in different seasons, mostly in spring and summer, (ii) single episodes of winter activity, which are quite rare in central Europe, and (iii) the beginning of tick activity in spring. It seems that (ii) and (iii) are mainly driven by ambient temperature.

Session 3

Ecosystems: transmission

Evaluation of collection methods for Phlebotomus-borne viruses detection: isolation and viral RNA integrity performance

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Phlebotomus-borne (PhB) viruses are RNA viruses involved in human disease outbreaks. Because several emerging viruses have been recently isolated from sandflies, an important task is to monitor the spreading of PhB-viruses. To investigate on collection and storage methods of phlebotomine vectors for reliable RNA detection and virus isolation, experimental viral infections of colonized sandflies were carried out and different laboratory conditions were tested. Laboratory-reared Phlebotomus perniciosus was experimentally infected with Toscana Phlebovirus (TOSV, ISS-Phl3 strain) using membrane-feeding technique. After the infected blood meal, engorged females were stored by two methods: 1) alive sandflies (AS) were immediately frozen at ?80°C, mimicking the golden standard CDC light trap collection procedure; 2) insects were put onto sticky traps left at room temperature, collected daily up 144h and stored dry at -80°C. Batches were separated into dead sandflies contaminated by alcohol necessary for removal from papers (DS-A) and those in which contamination was avoided (DS). Specimens, collected individually or in pools, were homogenized and used both for virus isolation in VERO cells and genome identification by Reverse Transcription-PCR on N gene region, and Real Time-PCR on TOSV L region. TOSV isolation and RNA detection was 100% for the AS specimens, as expected. In DS specimens the TOSV isolation on VERO cells was elevated at 24h but less satisfactory thereafter, suggesting that the virus could be fairly isolated from infected wild specimens only if they were frozen 24h from death. No TOSV isolation was obtained from DS-A specimens at any collection time. As regards RNA detection, for all conditions examined Real Time-PCR gave positive results until to 6 days after the infection. Recent investigations have indicated that virus diversity in the Mediterranean basin is higher than initially suspected, therefore a standardization of reliable sandfly collection and storage methods is important to harmonize entomological surveys. We confirmed that live specimens are the best target for both viral isolation and RNA identification. However sticky traps are suitable for partial genome viral identification but less so for viral isolation.
This study was carried out in the frame of FP7-UE EDENext collaborative project, Contract Number: 261504.

Why Hantavirus prevalence does not always increase with host density: modeling the role of host spatial behavior and maternal antibodies

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For wildlife diseases, one often relies on host density to predict host infection prevalence and the subsequent force of infection to humans in case of zoonoses. Indeed, if transmission is mainly indirect, i.e. through the environment, the force of infection is expected to increase with host density, yet the laborious field data supporting this theoretical claim are often absent. Hantaviruses are among those zoonoses that have been studied extensively over the past decades, as they pose a significant threat to humans (especially the Sin Nombre virus in North America). In Europe, the most widespread hantavirus is the Puumala virus (PUUV), which is carried by the bank vole (Myodes glareolus) and causes Nephropathia Epidemica in humans. Extensive field campaigns have been carried out in Central Finland to shed light on this supposed relationship between bank vole density and PUUV prevalence and to identify other drivers for the infection dynamics. This resulted in the (surprising) observation that the relationship between bank vole density and PUUV prevalence is not purely monotonic, contrary to what previous models predicted: a higher vole density does not necessary result in a higher infection prevalence, nor in an increased number of humans reported having NE. In this paper, we advance a novel individual-based spatially-explicit model which takes into account the immunity provided by maternal antibodies and which simulates the (flexible) spatial behavior of the bank voles, both possible causes for this discrepancy that were not taken into account in previous models. We show that the reduced prevalence in peak years can indeed be attributed to transient immunity and albeit to a lesser degree, to the density-dependent spatial vole behavior. The applicability of the model is not limited to the study and predict PUUV (and NE) occurrence in Europe, as it could be easily adapted to model the spatial dynamics of other (hanta-) viruses, either with indirect or direct transmission.

Temporal variation of Dobrava-Belgrade virus (Bunyaviridae, Hantavirus) seroprevalence in a yellow-necked mice population in northern Italy

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Dobrava-Belgrade virus (DOBV) is the most pathogenic hantavirus in Europe with a case-fatality rate of up to 12%. Here we present the prevalence of antibodies to DOBV in a population of Apodemus flavicollis in the Province of Trento (northern Italy) from 2000-2013. Over the 14-year study period, 2189 animals were live-trapped and mean hantavirus seroprevalence was 3.15% (S.E.=0.3 %), ranging from 0% (in 2000, 2002 and 2003) to 12.5% (in 2012) with an abrupt increase from 2010.
Climatic (temperature and precipitation) and host (population density; individual body mass and sex; and larval tick burden) variables were analyzed with Generalized Linear Models using multi-model inference to select the best model. Mean annual precipitation, annual maximum temperature and individual body mass were found to have a positive effect on DOBV seroprevalence. We discuss possible conditions that may explain the observed pattern. We are also exploring whether contact rates differed among individual yellow-necked mice and how host heterogeneities may influence potential DOBV transmission using network theory.

Delayed and direct impacts of rodents and climate on human tularemia in Finland

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Tularemia, caused by the bacterium Francisella tularensis, is usually divided into 4 subspecies. F. tularensis tularensis (type A) and F. tularensis holarctica (type B) are sources of human infections. The former is found in lagomorphs (hares and rabbits) in North America and is highly virulent for humans and domestic rabbits. The latter is less virulent and occurs in beavers, muskrats, and voles in North America and in hares and rodents in Eurasia.
The incidence of human tularemia in Finland is high at the European scale. In epidemic years, up to one thousand cases have been diagnosed. The distribution of human tularemia cases in Finland is spatially uneven. Also, our screening of tularemia in voles indicates a large degree of focality.
Several lines of evidence suggest that voles (and lemmings) are the most important reservoir of tularemia in Fennoscandia, including Finland. We analysed long-term regional rodent fluctuations and summer weather data in relation human tularemia in Finland. The multiannual dynamics of tularemia in humans are clearly linked to vole cycles. In humans, tularemia epidemics peak in the late summer - early autumn of vole population decline years, i.e. one year after the vole population peak. On the other hand, weather conditions during the epidemic summer determine the magnitude of the human peak. Tularemia epidemics occur only when both temperature and precipitation in summer are close to long-term average values. Both temperature and precipitation extremes reduce the number of cases. Mean weather values optimize the development conditions for mosquitoes, which are the sources of tularemia infection in humans. What, then, is the connection between voles and mosquitoes? Is the bacterium shed to the environment via vole excreta and from vole carcasses in rodent peak years, only to be assimilated by mosquito larvae from surface waters transfer it to adult mosquitoes? Our results help to predict the risk of tularemia in the focal regions of Finland.

Testing mechanisms of a dilution effect for Puumala hantavirus: results of a large field experiment

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The hypothesis that the number (i.e. richness) or diversity of species within a community can reduce the prevalence of an infection of interest has been widely promoted in recent years. Commonly referred to as a «Dilution Effect», such a mediating effect of species diversity on infection dynamics has been hailed as a means to unite the conservation and public health benefits of maintaining biodiversity. Much of the dilution effect literature has focussed on vector-borne parasites, and Borrelia burgdorgeri (the causative agent of Lyme disease) in particular, although there is passionate debate regarding the validity and interpretation of the data used to support the hypothesis and the mechanisms through which it is enacted. In contrast, while there is increasing evidence that a dilution effect may also exist for several environmentally transmitted infections, including rodent-borne hantaviruses, the potential mechanisms driving the effect have been largely unstudied. Here, we report on the results of a large field study which tested if and how the presence and abundance of non-host species altered direct and indirect interactions between bank voles (Myodes glareolus), the reservoir host for Puumala hantavirus (PUUV), a system for which there is evidence of dilution. We found evidence that indirect contact rates between bank voles are altered by the proportion of non-host species within a community, but not by the absolute number of non-host individuals present. These results suggest that community diversity can mediate a dilution effect in environmentally transmitted infections, as non-host species can alter the interactions between host individuals that result in parasite transmission. We discuss the implications of these results for PUUV dynamics, environmentally transmitted infections more broadly, and the ecological implications of heterospecific effects on social interactions within a species.

Application of a Transdisciplinary Study Design for Understanding Dengue Transmission in Dhaka, Bangladesh

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To understand dengue transmission, it has been realized that successful solution must cross disciplinary boundaries. In our research, a transdisciplinary research approach was considered where different scientific perspectives and community peoples' knowledge about dengue were elicited to explain dengue transmission in the City of Dhaka, Bangladesh.
A baseline entomological survey during monsoon 2011 was conducted in 12 wards to determine Aedes abundance and socioecological risk factors. A total of 3,651 immature mosquitoes were counted in 1,501 containers in 826 premises. There were 9 types of containers yielded 82.1% of all pupae of all containers surveyed, reflected the positive correlation between container abundance and pupal productivity. A two-step cluster analysis has revealed that two different types of ecological clusters are responsible for immature Aedes productivity.
A baseline seroprevalence survey conducted during pre monsoon 2012 indicates a seroprevalence of dengue virus specific IgG rate among 1127 samples is 80%. Neutralization tests on a randomly selected sample of size 100 indicated the absence of any other flaviviruses than dengue. Multivariate logistic regression model showed that age and indoor potted plants are the most significant risk factors for DENV seroprevalence upon adjusting other explanatory variables.
To examine the KAP of local community members regarding dengue transmission, a random sample of 300 household heads was surveyed. KAP survey results indicated that most community members heard about dengue (91.3%) and knew (93.7%) that mosquitoes act as the primary vector. Multivariate logistic regression modeling revealed that the respondents in age group 45-60 were 2.83 time more likely to have positive attitudes towards undertaking precautionary measures than the respondents in age groups less than 25 years. Our transdiscipliray study design enabled to capture multidimensionality in dengue transmission, as well as to determine the need for effective dengue transmission knowledge and awareness enhancement campaign for community specific target groups.

A Resource-based habitat approach for modelling vector-borne disease risks

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Landscapes, including land use and cover composition and structure, are important drivers for vector-borne disease risk. There is a clear need to assess landscape suitability for the emergence and spread of these diseases. Since vector-borne pathogens rely on at least one vector and one host species, disease occurrence is linked to areas where habitats of these species overlap functionally. These areas do not necessarily coincide with specific vegetation types, which hampers the correct identification of areas at risk. Current modelling approaches neglect components of the functional habitat of vectors or hosts, and hence of the pathogen. Empirical-statistical methods do not explicitly incorporate biological mechanisms, whereas current mechanistic models are rarely spatially explicit; both methods ignore the way animals use the landscape (i.e. movement ecology). Applying a functional concept for habitat, i.e. the resource-based habitat concept (RBHC), can solve these issues. RBHC offers a framework to identify systematically the ecological resources necessary for the completion of the transmission cycle and to relate these resources to (combinations of) landscape features and other environmental factors. The potential of RBHC as a framework for identifying suitable habitats for vector-borne pathogens is illustrated with the case of bluetongue virus, a midge-transmitted virus affecting ruminants. The concept facilitates the study of functional habitats of the interacting species (vectors as well as hosts) and provides new insight into spatial and temporal variation in transmission opportunities and exposure that ultimately determine disease risks. It may help identifying knowledge gaps and control options arising from changes in the spatial configuration of key resources across the landscape. The RBHC framework may act as a bridge between existing bottom-up mechanistic modelling approaches, that do not include landscape factors at all, and top-down satellite image-based approaches that are based on statistical inferences only.

Session 4

Ecosystems: change

Rift Valley fever in Senegal, 2013: a changing epidemiology?

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Rift Valley fever (RVF) is a zoonotic mosquito-borne infection caused by a Phlebovirus (Bunyaviridae). The main vertebrate hosts are ruminants which are also the source of infection for humans. These infections mostly occur at the occasion of animal cares (abortions) or when slaughtering viremic animals.
RVF is widespread in sub-Saharan Africa. Most epidemics occurred in arid or semi-arid areas, during the rainy season, often associated with heavy rainfall or increased surface water (irrigated crops, dams...). The first major epidemic for West Africa was reported in the Senegal River Valley, 1987-88. Since then, several epidemics have been reported in the Senegal River Valley and south-eastern Mauritania. However, since 2010, a series of RVF outbreaks have been occurring with a wider geographical scope: from Atar (northern Mauritania, 2010) to Dakar (Senegal, 2013), i. e., 1,000 km away.
Here, we report the main features of the 2013 epidemic in Senegal. We implemented a set of surveys in the confirmed RVF outbreaks in 49 villages from 5 regions of Senegal: village-level participatory disease searching, serological (n = 1,532 samples), and animal-productivity surveys (104 ruminant herds).
Though the first RVF foci were reported in wildlife in northern Senegal at the epicentre of the usual RVFV activity region, virus transmission remained at a low level in this region. Productivity losses were limited in extensively-managed ruminant herds. On the other hand, heavy economic losses were observed in intensive dairy farms in the Thiès and Dakar regions, with mass abortions and mortality in adult cows of exotic breeds. Also, some human cases were reported in these regions, first time ever in Senegal.
The analysis of serological prevalence rate revealed a strong shift of RVFV transmission toward Dakar and Thiès regions. In these densely-populated areas, most people were unaware of the zoonotic risk. Moreover, the Aïd el Kebir celebration occurred during the RVFV transmission peak, thus exposing many people to the virus when millions of sheep were slaughtered on the same day, a portion of them being probably viremic.
We discuss the public-health significance of this unusual epidemiological pattern and review possible RVFV control and preparedness options in this context.

Assessing the risks of Puumala virus emergence from immunogenomics of its reservoir, the bank vole Myodes glareolus, and its links with environmental variations

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Although the hantavirus Puumala (PUUV), the agent of nephropathia epidemica (NE) in humans, is prevalent throughout Europe, geographical variations in the incidence of reported human cases are observed. This heterogeneity is partly explained by the geographic distribution of the main host-reservoir of PUUV, the bank vole Myodes glareolus. However relationships between vole population characteristics (especially genetics) and NE incidence remain poorly understood.
These last years, we have investigated the interplay between immune gene polymorphism in bank vole populations and the epidemiology of PUUV. Spatial variation in M. glareolus immune responses to PUUV could affect PUUV replication and excretion in the environment, as well as its genetic diversity, what could ultimately shape NE incidence in Europe.
Using candidate loci selected from the medicine literature, we have proposed the hypothesis that the selection pressure exerted by PUUV on bank voles might have led to the selection for tolerance, ie processes that limit the damages caused by a pathogen burden, in PUUV endemic areas. This hypothesis is based on two observations: the slight impact of PUUV infection on the fitness of voles and the negative impacts of mounting immune responses (immunopathologies). More recently, we have developed a genomic approach using high throughput sequencing of RAD (Restriction-site Associated DNA) markers to deepen our investigation of this tolerance of bank voles to PUUV. The challenges were to identify a large set of genes that might be involved in these mechanisms of tolerance, and to emphasize their links with environmental components.
These works have provided a fine description of the spatial immunogenetic heterogeneity of bank voles in Europe. We have demonstrated the relative role of selective processes and environmental variables in shaping this immune diversity and its spatial distribution. We have also provided evidence that support the hypothesis of a balance of resistance/tolerance to PUUV in bank voles. It seems to be mediated by some genetic variability in inflammatory responses and defenses against helminths. Overall, our results suggest that close interactions between landscape features, co-adaptation with PUUV and coinfection with helminths influence the distribution of PUUV, and as such its risk of emergence in Europe.

Autochthonous Chikungunya transmission and extreme climate events in Southern France

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During fall 2014, Health authorities reported a cluster of 11 autochthonous cases of chikungunya in the city of Montpellier, which occurred during September and October in the vicinity of a recently imported case. We present a close monitoring of the adult population of the chikungunya vector Aedes albopictus through weekly sampling in Montpellier over the entire mosquito breeding season (i.e., May to November 2014) that showed an unexpected pattern. Although mosquito densities were steadily declining after peaking in late August, extreme rainfall events flooding the area at the end of September and beginning of October (Weeks 39 and 40), with up to 252 mm of rain falling in just 3 hours (recorded on 29th September in Montpellier), resulted in an explosive mosquito population growth, which extended into October, surpassing the abundance peak recorded earlier in August. This event may have contributed to increasing and extending the period of transmission.
This is at odds with a common belief among the scientific community and public health officers that «heavy rainfalls produce a flushing effect of immature mosquitoes (larvae and pupae) in breeding containers, diminishing the mosquito abundance», hence diminishing disease transmission. Our empirical data rather suggest that heavy rainfall events do not in fact decrease but instead might increase the risk of chikungunya transmission.
Extreme weather events such as the one described here are envisaged to become increasingly likely as a result of ongoing climate change, with consequences for the distribution and dynamics of vector-borne diseases. Our results should serve as a warning for those involved in the surveillance and control of vectors and vector-borne diseases in the context of global change.

Forest fragmentation and metapopulation dynamics of bank voles govern the persistence and spread of hantavirus Puumala in Western Europe

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Hantaviruses are one of the main zoonotic infectious agents considered as emergent and?or re-emergent worldwide. Puumala hantavirus (PUUV), which is carried by the bank vole, is responsible for nephropathia epidemica (NE) in Europe, a benign form of haemorrhagic fever with renal syndrome (HFRS). NE incidence presents a high spatial variation throughout European continent and also at small geographical scales. Understanding this spatial variation is of important concern for establishing risk maps and predicting risk change under global change. Bank vole metapopulation dynamics is thought to be critical for PUUV epidemiology, as it may affect virus persistence and transmission within and between host populations. In addition, vole metapopulation dynamics may depend on landscape characteristics, particularly on the spatial arrangement of forest habitats, which constitute the optimal habitat for bank voles. Although often mentioned, this hypothesis of strong links between landscape features, vole metapopulation dynamics and PUUV epidemiology has not yet been directly addressed. This was partly explained by the difficulty to monitor numerous vole populations in different landscape contexts using standard Capture-Mark-Recapture methods. Herein, vole metapopulation dynamics was inferred using the population genetic approach. Genetic diversity was examined as a good proxy for estimating population size, isolation and migration. We considered 36 populations sampled in areas exhibiting contrasted landscape structure and PUUV prevalence, in Finland, Belgium and France. Statistical analyses revealed that vole population genetic diversity was negatively correlated with latitude. This pattern was expected under the phylogeographical scenario of postglacial recolonisation of Europe from Southern refuge during the last cold Quaternary period. Once this geographic effect was removed, vole population genetic diversity was highly positively correlated with PUUV prevalence and with the abundance of forest habitat. This suggested that the persistence of PUUV is enhanced in lowly fragmented forests where vole metapopulations are highly connective and experience low extinction rates. Differences in the metapopulation dynamics of voles across European regions could thus partly explain why PUUV is not found in the whole distribution range of the bank vole. Consequently, habitat changes, in particular forest habitat fragmentation, could significantly impact PUUV geographical distribution and risk for NE emergence in humans.

Modelling the effect of environmental drivers on mosquito populations. How might changing temperature affect seasonal dynamics?

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It is thought that climate change may bring greater unpredictability and fluctuation in weather conditions. Whilst it is widely predicted that increased temperatures would lead to increased mosquito populations and incidence of mosquito-borne diseases, there is little concrete quantitative evidence to support this prediction. Mathematical models can be used to predict the effects of climate on seasonal vector abundance, though most models implicitly assume that all life stages are affected equally by environmental drivers. We challenge this assumption by explicitly modelling all stages of the vector population, incorporating the differential effects of climate on each stage. This will improve understanding of mechanisms underpinning climate change impacts on vector seasonality.

We develop a temperature-dependent, stage-structured delayed differential equation (DDE) model for the West Nile Virus vector, Culex pipiens. We investigate the effect of variation in seasonal temperatures on several aspects of mosquito abundance by varying the mean, amplitude, phase shift and sharpness of the temperature function. In doing this we aim to mimic both current and potential future seasonal weather patterns and understand their effects on mosquito abundance. The impact of projected climate warming on seasonal abundance patterns is investigated using the UK Climate Impact Programme temperature projections. It is observed that increases in both the mean and amplitude of seasonal temperature fluctuations lead to increases in the size of the annual peak mosquito population. The sharpness and timing of the peak in summer temperatures also play a large part in determining maximum mosquito abundance. In particular, situations arise where a late, sharp peak in temperature leads to higher peak abundance than a broader peak of the same amplitude centred at the same time.

Our results show that even relatively small changes in the temperature profile can lead to substantial changes in our predictions of vector population dynamics. The form of this change is not always straightforward to predict. This highlights the need for a flexible modelling approach, capable of incorporating multiple processes with competing effects on mosquito populations. Our DDE modelling framework affords such flexibility and can be extended to understand impacts of climate change on mosquito-borne infections.

Identifying main drivers and testing control strategies for CCHFV spread

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Crimean Congo Haemorrhagic Fever (CCHF) is an emerging zoonotic disease. The causative agent is a virus (CCHFV), mainly transmitted by ticks of the species Hyalomma marginatum in Eastern Europe and Turkey. We have developed a mechanistic dynamic model to test potential scenarios for the control of pathogen spread. Design of a model to apply to a vector-borne disease is based on coupling a population dynamics model, here applied to the tick H. marginatum, with a model of CCHFV transmission. Our model takes into account the major processes involved: (i) hatching, questing, development, feeding, egg laying and mortality for tick population dynamics, (ii) transmission, acquisition, transovarial transmission and persistence in hosts for pathogen dynamics. It also considers the influence of abiotic (temperature and vapour saturation deficit) and biotic factors (host, i.e. hare and cattle, densities) on pathogen spread. Model outputs were compared with data either collected in Central Anatolia (Turkey) or reported in the literature and showed that the model is able to reproduce realistically the observed dynamics for tick population and pathogen spread. This modelling allowed us to calculate the basic reproduction number (R0) for CCHFV infection, based on the estimation of the next-generation matrix. Using R0 as output variable, the model was thereafter used to test control strategies and especially the effect of various acaricide treatments, differing by their starting date, their duration and the number of applications. Simulation results indicate that such treatments could have valuable effects provided that the acaricide is applied early (day 100 in the year), regularly (at least 3 times a year) and over several years. Furthermore, a global sensitivity analysis was carried out to calculate Sobol sensitivity indices for abiotic and biotic factors. It showed that, even though temperature has a strong impact on model outputs, host densities also play a role. In particular, one way to decrease pathogen spread could be to reduce wildlife host densities, especially hares, which are hosts for immature stages. The kind of model we have developed provides insight into the ability of different strategies to control pathogen spread.

The impacts of climate change on the habitability of Aedes albopictus: challenges in global forecasting

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The Asian tiger mosquito, Aedes albopictus, is a highly invasive vector species. It has accumulated a series of adaptations providing it with substantial physiological and ecological plasticity. It is a proven vector in the field of dengue, chikungunya and West Nile fever and has the potential to transmit a further 24 arboviruses. It has recently expanded its geographical range threatening many countries in the Middle East, Mediterranean region, Europe and North America.
In this study, we investigate theoretical limitations of this expansion and the extent of vector adaptability to changing environmental conditions. We develop an environmentally-constrained mathematical model of the population dynamics of Ae. albopictus incorporating a range of drivers, such as temperature, precipitation, population density and photoperiod. We put together a comprehensive database of physiological parameters linking survival, development time and fecundity with environmental conditions.
Bayesian parameter inference was performed to arrive at a posterior sample of parameters accounting for both laboratory and field observations. In this current setup, the model demonstrates exceptional predictive capacity over the Emilia-Romagna region of Italy from where parameter values are inferred. Based on the model, we discuss the limitations of extrapolating meaningful predictions for outside the region of inference in such cases where evidence is bounded with carefully controlled laboratory experiments and field observations from a limited geographic range and limited environmental characteristics. In order to extend model applicability over the globe, we perform sensitivity analysis on certain environmental conditions, specifically on the diapausing behaviour of Ae. albopictus at different latitudes.
As a result, we present a guideline on performing global analyses based on local observations and limited prior knowledge, demonstrating the advantages of adopting a Bayesian framework. With this, we incorporate implicitly many unobserved variables, such as differences between laboratory and field conditions, and microclimatic conditions around breeding sites. Using the model, we chart a global habitat suitability map - a risk-map for Ae. albopictus establishment - highlighting regions most exposed to diseases transmitted by this vector. With this study, we aim to improve the understanding and management of current and future risks of Ae. albopictus-transmitted infectious diseases.

Simulation of the impact of future climate scenarios on the distribution of leishmaniasis vectors in the Mediterranean basin

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Due to climate change, the geographical distribution of sandflies during the last decades has shifted northward from latitudes below 45?N in south Europe (Martens and McMichael 2002) to latitudes just above 50?N (Naucke et al., 2008). Recent studies show that some phlebotomine sandflies were recorded in several parts of Germany and Belgium (Naucke et al., 2008, Mencke; 2011). In Central Europe, some autochthone leishmaniasis cases are being recorded in regions traditionally regarded as leishmaniasis-free countries (Gogoa?e et al., 2013, Tánczos et al., 2012). In this study we attempted to predict current distribution of six leishmaniasis vectors in the Mediterranean basin and forecast species geographical shift under future climate scenarios using ensemble ecological niche modeling approach.
Species records were obtained from scientific surveys published in the research literature between 2006 and 2012. A series of climate metrics, describing temperature and precipitation in the study area under two climatic scenarios for 2020, 2050 and 2080, were obtained from WorldClim database.
A consensus model was derived from six varieties of modeling approaches (regression, machine learning and classification techniques) in order to ensure valid prediction of distribution of vectors under different climate scenarios.
Model performance was generally high for the included species with a specificity (True Negative Rate) ranging from 81.03% to 96.52% (mean=86.94%) and a sensitivity (True Positive Rate) ranging from 87.93% to 100% (mean=96.98%). Our work evidenced the hypothesis of the wide spread of Leishamania vectors under climate change scenarios. All the studied species are prospected to gain new areas that are actually not suitable for vectors' survival.
Phlebotomine sandflies are prospected to invade extra-Mediterranean regions especially Western and Central Europe, while Leishmania vectors will lose areas for suitable conditions in North Africa and Middle East. These changes in geographical distribution are more intense under the pessimistic scenario compared to the optimistic one.

Session 5

Risk of infection

Evaluation of the efficacy of olyset® plus in a village-based cohort study in the cukurova plain, turkey, in 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 piperonyl butoxide (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. donovani hybrid parasite transmitted by proven vector species Phlebotomus tobbi in Cukurova Plain, Adana, Turkey, between May, 2013 and May, 2014. The study area comprised eight villages; two of them were selected as an intervention village with Olyset® Plus net (Kizillar) and 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 Phlebotomus 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 six 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.

The use of insecticide impregnated nets against Culicoides spp.: state of the art and progress in the project Edenext

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The family Ceratopogonidae includes species of the genus Culicodes which are important transmitters of several arboviruses of importance, such as Bluetongue, African horse sickness, Epizootic haemorrhagic disease and Schmallenberg viruses. Nowadays, the control of these vector species is practically non-existing, mostly due to the lack of specific products and control methods targeting either adults or larvae.

In the framework of the project EDENext, a series of trials for testing the efficacy in controlling Culicoides populations associated to farms were performed using commercially available and self-prepared impregnated insecticide nets. In this work, we present a summary of the main results of those trials.

In 2011 we tested Deltamethrin impregnated nets (ZeroVector- Vestergaard, 4.4g/kg ± 15%) either in laboratory and field conditions. Results from laboratory tests showed that C. imicola, C. obsoletus and C. newsteadi were highly sensitive to this insecticide. Field trials showed a lower efficacy, however, the percentage of Culicoides that survived in the trap control treatment was 22.7% higher than the Culicoides that contacted with the impregnated net. In 2012 we tested in the field self-prepared Cypermethrin impregnated nets (0.5g/L ± 1%; Arpon G®). Results obtained showed that Culicoides spp. survival was not significantly different between impregnated nets and control (non-impregnated nets). In 2014 we tested in the field commercially available Deltamethrin nets (0.4%
Deltamethrin- ZeroFly Livestock- Vestergaard). Results showed a low efficacy and no detrimental effect was observed on the Culicoides spp. that passed throught the insecticide impregnated net.

We discuss among other aspects, the difficulties for controlling Culicoides spp. by using commercially available impregnated nets due to its small size (1-3 mm) and the effect of impregnated nets on non-targeted arthropods.

Field efficacy of pour-on 1% flumethrin treatment on the tick infestations in cattle in a CCHF hyper-endemic region in Turkey

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The study was conducted between April and August 2013 in 2 villages of Yozgat province with more than 500 grazing cattle. Animals were divided in 3 groups; Gr1: Owner (long distance) grazed (n=32), Gr2: Common village herd (n=38), Gr3: Common village herd (n ? 500). The cattle in Gr3 were treated monthly with pour-on 1% flumethrin starting on 10 April and ending on 10 July 2013. All cattle in Gr. 1 and Gr. 2 and representative number of animals in Gr. 3 were sampled biweekly (8 samplings). All ticks were removed and identified at species level.
A total of 805 individual samplings were conducted during the 8 field visits. A total of 5734 ticks (98.6% were H.marginatum) were collected off which 5300 (11.8 tick/cattle) were from untreated controls and 434 (1.2 tick/cattle) from flumethrin treated animals.
It is concluded that starting from the first week of grazing, periodic applications of 1% flumethrin is very effective in controlling tick infestations on cattle. If left untreated each cattle may feed at least 36 female H. marginatum ticks, which theoretically may produce 76000000 larvae per village per season.
Although commercial flumethrin producers claim 6 week of effectiveness, it should definitively not be expected at field conditions. On day 21 after treatment individual infestations with low number of ticks started to occur in this study. It might be wise to advise to decrease application interval to 20-25 days if total tick control is aimed.

Control of the Asian Tiger mosquito, Aedes albopictus (Skuse, 1894): what's wrong?

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Insecticidal aerosols dispensed from hand-held thermal-foggers or vehicle-mounted cold-foggers (ULV) are widely used to counter populations of Aedes aegypti and Ae. albopictus, the principal urban vectors of dengue, chikungunya, Zika and yellow fever, and are frequently the method of choice during public health emergencies.
We used two techniques?ovitraps baited with hay infusion and «B-G Sentinal» adult traps?to monitor Ae. albopictus populations on a 24-hour cycle in three residential areas in Nice, France. The impact of treatments with deltamethrin was evaluated by comparing trap results in treated vs untreated areas for five days before and 5 days after each treatment. Four such experiments were conducted with vehicle-mounted ULV machines using the maximum permitted application rate (1gm ha-1) and two (on a much smaller scale) with hand-held thermal foggers.
Results with vehicle-mounted ULV were disappointing: on no occasion was there any discernable change in oviposition rate or in catch of adult females, nor was there any change in their physiological stage (parous vs nulliparous). Curiously, however, there was a marked reduction in the catch of adult males. Applications by hand-held thermal fogger, however, appeared highly effective, with more than 90% reduction of males and females.
In the laboratory, topical application by micropipette as well as by insecticide-impregnated papers (the «WHO Tube Test») demonstrated that the local Ae. albopictus population was highly susceptible to deltamethrin (compared to other susceptible strains) but that females required a significantly higher dosage than males.
Our study is the first to use direct measures (rather than caged mosquitoes) to evaluate impact on the wild Ae. albopictus population. It is possible that we will obtain a more favorable impact of ULV with higher application rates. Unfortunately, however, our disappointing results are wholly in line with epidemiological evidence: there is no place anywhere in the world where the use of ULV has had any discernable impact on the transmission of chikungunya or dengue. Given the increasing prevalence and incidence of these urban diseases it is clear that new and innovative approaches are urgently required.

Crimean-Congo haemorrhagic fever virus ? The first line of protection is understanding

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Crimean-Congo hemorrhagic fever virus (CCHFV) is considered to be one of the major emerging disease threats spreading to and within Europe. CCHFV infections in humans can cause a deadly hemorrhagic fever with high fatality rates up to 80%. Every year more than 1000 human CCHF cases are reported from countries of Southeast Europe and Turkey. Most humans are infected by tick bites or by crushing infected ticks, but infections are also possible through contact with blood of viremic animals and humans. No vaccine prophylaxis and therapeutic interventions are available.
Ticks of the genus Hyalomma function as vector as well as natural reservoir of CCHFV. Within the tick population the virus can be transmitted by horizontal and vertical routes. Domestic ruminants and wildlife animals play a crucial role in the life cycle of the ticks, and in the transmission and amplification of the virus. Since infected animals do not develop clinical signs, CCHFV infections can only be detected by diagnostic tools. However, there are only few assays available for the detection of CCHFV specific antibodies in animals and data about diagnostic sensitivity and specificity are incomplete.
In Europe, human CCHF cases occur regularly in Albania, Bulgaria and Kosovo. Apart from those countries with endemic areas, the real distribution of CCHFV, the infection rate in animals and CCHF case numbers are fairly unknown. Investigations of serum samples from animals for CCHFV specific antibodies can identify risk areas for the circulation of CCHFV what is crucial for a focused and targeted implementation of public health measures.
A highly sensitive and specific ELISA was developed and validated to detect CCHFV specific antibodies in cattle. In addition, two commercially available assays (for testing human serum) were adapted for use in animals. By using these assays, new risk areas with prevalence rates up to 80% were identified in Europe. Further, the occurrence of the vector was demonstrated in some regions for the first time. This knowledge and a risk classification will assist decision makers and public health authorities in deciding on effective countermeasures and outbreak prevention.

The joint risk score for vector-borne diseases used for early detection

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Background
Risk-based surveillance for vector-borne animal diseases (VBD) and zoonoses with the aim for early detection should benefit from continuously gathered data. Passive surveillance does not allow a quick detection of VBD and zoonoses which cause few, mild or nonspecific symptoms. E.g. detection of bluetongue virus took several weeks and Schmallenberg virus even months. In this paper we present a method to combine models for risk of release and potential spread of VBD using information from animal health data or syndromic surveillance for the early detection.
Methods
The methodology presented combines predicted prior probabilities for an ongoing outbreak with additional evidence for reporting of syndromes in a region and time frame using a Bayesian framework. The prior probabilities of an on-going outbreak in a region and time-frame are based on introduction risks and the models for establishment and spread. These prior probabilities are combined with data on animal health into a posterior probability. The resulting posterior probability of different pathogens and regions can be compared to determine which pathogen or region could be prioritized for active surveillance.
Results
We show that we could compare the risk of two viral diseases of horses (African Horse Sickness and Equine Encephalosis) in France. We could distinguish a difference in risk of an ongoing outbreak of either disease, allowing to prioritise surveillance activities. We also show under which circumstances simulated BTV outbreaks in Germany, The Netherlands and Belgium were detected earlier than with current surveillance.
Conclusions
The combination of predicted probabilities of release and spread combined with evidence from animal health data can make early detection more efficient. For zoonotic diseases this could present a method for early detection helping to mitigate the impact of public health.

Clues and tools to model spatial distribution of zoonoses based on the tip of the iceberg, the human cases

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Zoonoses are complex systems that involve the pathogen, the host, the vector and humans. Spatial distribution models are often based on human data, as they frequently are the most readily available data, including through passive surveillance, if the disease cases are correctly diagnosed and reported. Conversely modeling the distribution of the host or vector requires massive sampling while searching and modelling the pathogen itself necessitates vector, host or human blood samples.
Human disease cases may be seen as the tip of the iceberg, with the undetected zoonotic cycle hidden under the water surface. The question thus arises to know how much can be learned about the immersed part of the iceberg based on the emerging part. A set of tools and a new framework are here suggested to better address the issues raised by the use of human disease case data for modeling zoonoses. Hantavirus and tick-borne encephalitis are examined in diverse environments and at diverse scales to illustrate these concepts.
Our framework is based on the concept of risk assessment that is a combination of hazard (defined as pathogen circulation in the wild) and exposure (defined as people entering into infected landscape). Results suggest that the combination of hazard and exposure is needed to improve the predictive power of models calibrated using human disease case data. The framework is also useful to investigate how factors are involved in the various parts of the disease transmission system.
The tools presented range from linear regression to niche models and from the landscape to the European scale. A multilevel approach is also advised in many circumstances. For large scale models, in this case a European scale model of hantavirus, three scenarios of variable response are identified, which bear diverse consequences for modeling and modeling results interpretation.

Getting prepared: Climate-based forecasting of Puumala hantavirus disease risk through space and time in Western Europe

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Predictive frameworks for wild zoonotic infection occurrence are needed for proper risk assessment of environmental changes and targeted prevention strategies. Puumala virus (PUUV) is the most common hantavirus in Europe. This virus is specifically hosted by bank voles (Myodes glareolus) and causes a form of haemorrhagic fever with renal syndrome in humans, called nephropathia epidemica (NE). In the last decade, intermittent epidemic foci of NE have changed significantly in size and frequency in Western Europe. Consequently, within the EDENext project, a multidisciplinary approach was used to build and evaluate the potential of climate and land use variables in predicting occurrence of NE epidemics throughout the Western-European region in space and time. Hence, allowing the construction of risk maps and visualisation of NE risk foci for the coming year.
Geo-referenced NE case data between 2004 and 2010 linked to high-resolution spatial polygons from Belgium, France, Germany and The Netherlands were used to construct a NE model dataset. Potential explanatory variables (climate, land use and remote sensing derivatives) were selected based on a literature study and previous modelling work. The final parameter selection was done by a bootstrap approach and a mixed geo-statistical space-time Poisson model was used for prediction of the probability of NE occurrence in each of the polygons of the study region. Model evaluation was based on an out-of-sample dataset from 2011 and 2012.
A climate-based model frame was selected to predict the region wide space-time trend in NE occurrence. Beech occurrence was selected as the most dominant purely spatial determinant of NE occurrence. Our model frame enables forecasting probability of NE occurrence in the coming year throughout the Western-European region with high specificity (96%) and moderate sensitivity (60%). Predictions improve further when correcting for risk overestimation in years directly following local high NE outbreaks. This study is one of few examples of applied forecasting of zoonotic disease emergence, based only on environmental triggers.

GERI 2015

Genes, Ecosystems and Risk of Infection

> Abstracts

21-23 April 2015

Aquila Atlantis Hotel,
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Posters

Session 1

Genes

Recombinant Phlebotomus perniciosus salivary proteins as markers of host exposure to visceral leishmaniases vector

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Phlebotomus perniciosus is the main vector of Leishmania infantum, the causative agent of zoonotic visceral leishmaniasis. The main reservoir of the disease is dog and therefore measuring canine exposure to sand fly bites is important for estimating the risk of L. infantum transmission. As screening of specific anti-saliva antibodies is limited by the availability of salivary gland homogenates (SGH), utilization of recombinant salivary proteins is a promising alternative. The reactivity of six bacterially-expressed P. perniciosus salivary proteins, yellow-related protein rSP03B, apyrases rSP01B and rSP01, antigen 5-related rSP07, ParSP25-like protein rSP08 and D7-related protein rSP04, was tested with sera of mice and dogs experimentally bitten by this sand fly using immunoblots and ELISA. In immunoblots, both murine and canine sera positively reacted with yellow-related protein, both apyrases and ParSP25-like protein. The reactivity of yellow-related protein and apyrases in ELISA significantly correlated with the canine antibody response against SGH. Recombinant yellow-related protein and both apyrases were identified as the best candidates for evaluating the exposure of mice and dogs to P. perniciosus bites. These three recombinants were subsequently used to determine the exposure of different species of reservoir hosts to sand fly bites in L. infantum focus in the south-west of the Madrid region. Sera from hares, wild rabbits and dogs captured in the study area presented higher anti-saliva antibody response in comparison to negative control sera. Canine antibody response against all three recombinant salivary proteins tested positively correlated with SGH, in hares and rabbits a significant positive correlation was found between SGH and yellow-related protein rSP03B and apyrase rSP01B. Data confirmed the exposure of hares, rabbits and dogs to P. perniciosus bites in the context of an outbreak of human leishmaniasis in Spain, highlighting their involvement in Leishmania transmission by supporting their role as potential reservoirs. This novel methodology represents a promising tool for further epidemiological studies that would help to design better strategies for the control of leishmaniasis in this area and other foci.
This work was supported by EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504.

Microevolution of Puumala hantavirus during a complete population cycle of its host, the bank vole (Myodes glareolus)

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Microevolution of Puumala hantavirus (PUUV) was studied throughout a population cycle of its host, the bank vole (Myodes glareolus). We monitored PUUV variants circulating in the host population in Central Finland over a five-year period that included two peak-phases and two population declines. Of 1369 bank voles examined, 360 (26.3%) were found infected with PUUV. Partial sequences of each of the three genome segments were recovered (approx. 12% of PUUV genome) from 356 bank voles. Analyses of these sequences disclosed the following features of PUUV evolution: 1) nucleotide substitutions are mostly silent and deduced amino acid changes are mainly conservative, suggesting stabilizing selection at the protein level; 2) the three genome segments accumulate mutations at a different rate; 3) some of the circulating PUUV variants are frequently observed while others are transient; 4) frequently occurring PUUV variants are composed of the most abundant segment genotypes (copious) and new transient variants are continually generated; 5) reassortment of PUUV genome segments occurs regularly and follows a specific pattern of segments association; 6) prevalence of reassortant variants oscillates with season and is higher in the autumn than in the spring; and 7) reassortants are transient, i.e., they are not competitively superior to their parental variants. Collectively, these observations support a quasi-neutral mode of PUUV microevolution with a steady generation of transient variants, including reassortants, and preservation of a few preferred genotypes.

Bacterial zoonotic pathogens survey in wildlife: a comparison of two next-generation sequencing approaches (RNA-seq and 16S metagenomics)

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Rodents are major reservoirs of pathogens responsible for a wide range of zoonotic diseases. The circulation of such diseases might be influenced by the microbiota of each host in its population. Thus, the assessment of the microbial diversity at the host population level is crucial for monitoring endemic infections. Different NGS approaches have been employed to characterize the microbial communities; yet, their relative efficacy has not been assessed. Here, we compare two NGS approaches, the RNA-sequencing (RNA-Seq) and the 16S-metagenomics, in their performance to survey zoonotic pathogens.
With this aim the nucleic acids were extracted from the spleens of 192 rodents from France. The RNA extracts were pooled, randomly retro-transcribed and then RNA-Seq was performed using HiSeq (Illumina). The sequences obtained were de novo assembled and succeeding contigs were taxonomically assigned to the closest already-known OTU, revealing a list of pathogens for the pooled RNA sample. The DNA extracts were analysed using two different protocols of the 16S-metagenomics approach: the V4 region of the gene coding for the 16S rRNA was amplified for each sample using tagged/indexed universal primers. Both tagged-/indexed-amplicons were multiplexed and, respectively, sequenced with the 454 GS-FLX (Roche) and the MiSeq (Illumina). Using the tagged/indexed primers, the resulting datasets were demultiplexed to assign each read to a single rodent. These were further processed with a pipeline developed on Galaxy, which implemented the Mothur software to obtain OTUs and classify them using the Ribosomal Database Project. This 16S-approach enabled us to describe the bacterial content of each individual rodent.
Altogether, 45 pathogenic bacteria genera were detected in the rodent samples. The list of pathogenic bacteria disclosed by RNA-Seq was comparable to that detected by the 16S-metagenomics processed with MiSeq. Conflictingly, 21 of these pathogens went unnoticed when the 16S-approach was processed with 454-pyrosequencing. Besides, the numbers of reads obtained for each genus did not correlate across the different NGS approaches. We conclude that RNA-Seq and 16S-MiSeq are comparably sensitive in bacteria detection, although only 16S-MiSeq permits identifying bacteria in each individual host reservoir allowing deriving within-host bacterial associations and the prevalence of bacteria in a host population.

Molecular detection and characterisation of Babesia species in ticks and rodents in SW Slovakia

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Babesiosis caused by intraerythrocytic parasites of the genus Babesia is an emerging zoonotic disease with a natural enzootic cycle involving ticks and vertebrate hosts. In Europe, rodents are important reservoirs of B. microti. In this study the presence of this pathogen was monitored in rodents, rodent-attached ticks and in questing ticks in two areas of SW Slovakia. By PCR-amplification of the hypervariable 18S rRNA gene region Babesia parasites could be detected in 84 (0.6%) out of 5,149 questing ticks (Ixodes ricinus and Haemaphysalis concinna). Phylogenetic analysis showed that most of these Babesia species clustered with B. microti, B. venatorum, B. canis, and B. capreoli when amplified from I. ricinus. However, Babesia sp. 3 (isolate N149/BT3) might represent a novel species as it did not cluster with any previously defined species. Babesia parasites amplified from H. concinna seem to segregate into two clusters, Babesia sp. 1 and Babesia sp. 2, each of which might represent a novel species. Isolates of these two clusters show a 100% sequence identity with those recently identified in Russia (Babesia sp. Irk-Ip525, Babesia sp. Irk-Ip256 and Babesia sp. Kh-Hc222). A total of 416 rodents belonging to five species, of which Apodemus flavicollis and Clethrionomys glareolus prevailed, were trapped. Rodents were infested with ticks: Ixodes ricinus (94.1-99.5%) and Haemaphysalis concinna (0.5-5.9%). In total, 0.3% of rodents (A. flavicollis, M. arvalis) were infected with B. microti. Rodent-attached I. ricinus were infected with B. microti while H. concinna were infected with Babesia sp. 1. The same strain of B. microti identical with zoonotic Jena strain was identified in both I. ricinus and rodents. All B. venatorum isolates obtained from questing I. ricinus were identical to B. venatorum strain recognized as an agent of human babesiosis. The present study, may shed an additional light on the epidemiology of hemoprotozoan parasites in Slovakia. Knowledge about the prevalence of these infectious agents is an important prerequisite for risk assessment of diseases. Further screening of rodent tissues and molecular identification of piroplasms strains is in progress.

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Modelling the spatial distribution of mosquitoes at different geographical scales

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Mosquitoes are known to be vectors of a large number of pathogens around the globe and are considered as prime candidates for transmitting (re-)emerging vector-borne diseases in Europe. Risk maps are a useful tool to assess and visualize the risk of establishment and spread of vector-borne diseases. Knowledge of the spatial distribution of mosquitoes is essential to create such a map.
During the EDENext project, we applied various statistical models to identify suitable habitats for mosquitoes, at different geographical scales. A first study aimed to investigate the microhabitat preferences of Aedes albopictus in urban areas. Data on egg abundance were collected by means of ovitraps in an urbanized area, the campus of the University of Rome, La Sapienza. The small size of the campus (22 hectare) allowed us to classify the land cover in detail on a high resolution image. A spatial statistical model was applied to evaluate the relationship between egg abundances and different land covers. Another study was conducted in the Netherlands at 1 km2 resolution. This study aimed to explore the macrohabitat suitability of several species of mosquitoes using environmental satellite data. Trap data for different mosquito species came from the surveillance program carried out by the Dutch National Centre for Monitoring of Vectors. Three different statistical models suitable for occurrence data (i.e., non-linear discriminant analysis, random forest and generalised linear model) were applied to data on Culiseta annulata, Anopheles claviger and Ochlerotatus punctor. The results were compared with regard to: (i) environmental variables associated with occurrence, (ii) habitat suitability maps, (iii) model evaluation.
An application of these modelling techniques is provided also for Anopheles plumbeus, a mosquito species potential vector for malaria and known for the nuisance that it causes to humans. The resulting habitat suitability map was validated against nuisance reports and showed a good agreement with the areas where most nuisance was reported.
Our studies show how statistical models can help in investigating the spatial distribution of mosquitoes at different geographical scales. The resulting maps are useful tools in risk assessment for mosquito-borne diseases.

The complete sequence of a West Nile virus lineage 2 strain detected in a Hyalomma marginatum marginatum tick collected from a song thrush (Turdus philomelos) in Eastern Romania in 2013 revealed closest genetic relationship to strain Volgograd 2007

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Background: Romania has a long-standing history of West Nile virus (WNV) infections, including severe outbreaks of human West Nile neuroinvasive disease in 1996 and since 2010. Several partial human- and mosquito-derived WNV sequences have been established. Nevertheless, the complete sequence of the main WNV strain circulating in Romania since 2010 has not been determined as yet. Also the role of ticks as WNV vectors had been poorly investigated to date.
Objective: The objectives of this study included the determination of the complete sequence of a WNV lineage 2 strain detected in a tick, and its molecular and phylogenetic investigation. The role of ticks in WNV introduction and maintenance is discussed.
Methods: Bird samples collected in the Romanian Danube Delta were investigated for the presence of WNV nucleic acid. A total of 32 ticks which were found randomly on the birds were tested as well. One positive sample was subsequently analysed in detail by several PCRs, sequencing as well as BLAST, Pfam and Prosite, and MEGA 5 computer programmes.
Results: While all bird samples and most of the collected ticks tested negative for WNV nucleic acid, one tick sample proved positive for WNV lineage 2. This tick, identified as Hyalomma marginatun marginatum, was found on a juvenile song thrush (Turdus philomelos) which had been captured on 27.08.2013. From the infected tick a complete WNV sequence was generated, and pathogenicity and neuroinvasiveness markers were identified. Phylogenetic analysis of various complete and partial WNV lineage 2 sequences exhibited its close genetic relationship with the Russian and Romanian human- and mosquito-derived WNV sequences obtained between 2007 and 2013, all of them belonging to the Eastern European lineage 2 WNV cluster.
Conclusion: Infected ticks on migrating birds may carry (new) pathogens to other areas much more efficiently than their avian hosts. The determination of the complete sequence of the main currently in Romania circulating WNV strain revealed a close genetic relationship to the neuroinvasive Russian WNV strain Volgograd 2007. Based on these sequences, future evolution of the Eastern European lineage 2 WNV cluster can be monitored.

Distinct Anaplasma phagocytophilum genotypes and other pathogens (Candidatus Neoehrlichia mikurensis, Babesia microti) associated with Ixodes trianguliceps ticks and rodents in Slovakia (Central Europe)

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Rodents are important reservoir hosts of tick-borne pathogens. Anaplasma phagocytophilum is the causative agent of granulocytic anaplasmosis of both medical and veterinary importance. Circulation of human pathogens Candidatus Neoehrlichia mikurensis and intraerythrocytic protozoan parasite, Babesia microti have been recently proposed. The aim was to identify the presence and genetic diversity of pathogens circulating in the natural foci between the rodents, ticks and to study their ecological association. A. phagocytophilum was detected in questing I. ricinus ticks from all studied sites and in host feeding I. trianguliceps ticks, as well as in rodent biopsies, whereas A. phagocytophilum was not detected in rodents in those sites where I. trianguliceps ticks were absent. Moreover, Bayesian phylogenetic analyses have shown the presence of two distinct clades and tree topologies were concordant for all four investigated loci. Importantly, the first clade contained A. phagocytophilum genotypes from questing I. ricinus and feeding I. ricinus from a broad array of hosts. The second clade comprised solely genotypes found in rodents and feeding I. trianguliceps. N. mikurensis was detected in questing I. ricinus ticks, spleens of rodents and feeding I. ricinus and I. trianguliceps ticks from rodents. The 16S rRNA and gltA sequences of N. mikurensis obtained in this study confirmed high degree of homology. DNA of. B. microti was found in biopsies of rodents, feeding and questing I. ricinus ticks. None of the 112 I. trianguliceps ticks were infected with B. microti. BLAST analysis of B. microti nucleotide sequences confirmed the presence of two genotypes; ?Jena" and ?Munich" strains. Embryos of rodents were also positive for pathogens. In this study we have confirmed that A. phagocytophilum strains display specific host and vector associations also in Central Europe similarly to the situation in United Kingdom and that A. phagocytophilum genotypes associated with rodents are probably transmitted solely by I. trianguliceps ticks, thus implying that rodent-associated strains may be not of risk for humans. Results also confirmed the importance of rodents in the circulation of both emerging pathogens in the natural foci.
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Soricomorph-borne hantaviruses in Finland: genetic diversity and serological diagnosis

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Hantaviruses are emerging viruses carried by rodents, soricomorphs (shrews and moles) and bats. In Finland, Puumala virus (PUUV) was for years the only hantavirus detected. In 2009, however, Seewis virus (SWSV) was reported from archival common shrew (Sorex araneus) samples collected in 1982 in Finland. To elucidate the diversity of hantaviruses in soricomorphs in Finland, 180 individuals were screened, representing seven species captured from 2001 to 2012: hantavirus RNA was screened using RT-PCR, and hantaviral antigen using immunoblotting with polyclonal antibodies raised against truncated SWSV nucleocapsid (N) protein. The overall hantavirus RNA prevalence was 14% (26/180), antigen could be demonstrated in 9 of 20 SWSV RT-PCR positive common shrews. Genetic analyses revealed that four soricomorph-borne hantaviruses circulate in Finland, including Boginia virus (BOGV) in water shrew (Neomys fodiens) and Asikkala virus (ASIV) in pygmy shrew (Sorex minutus). Interestingly, on two study sites, common shrews harbored strains of two different hantaviruses: Seewis virus and a new distinct, genetically distant (identity 57% at amino acid level) virus (tentatively named Uurainen virus) which clusters together with viruses in the basal phylogroup I of hantaviruses with 62-67% identity at amino acid level. This is the first evidence of coexistence of two clearly distinct hantavirus species circulating simultaneously in one host species population. The findings suggest an ancient host-switching event from a yet unknown host to S. araneus. Deeper phylogenetic analyses of partial S and M segment sequences showed that SWSV in Finland represents a unique genotype in Europe. As dominating shrew hantavirus in Finland, serological assay of SWSV was established and performed on the patient serum with NE-like symptoms. Among a panel of 486 suspect of hantavirus infection in Finland with confirmed circulation of SWSV in the nature, no evidence of SWSV infections was found. In addition, we could demonstrate a cross-reaction of human anti-PUUV serum and N protein of a shrew-borne hantavirus.

Poster 1.9.

NOT PRESENTED

Putative New West Nile Virus Lineage in Uranotaenia unguiculata Mosquitoes, Austria, 2013

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West Nile Virus is maintained in an enzootic cycle between mosquitoes and wild birds. In Russia, Uranotaenia unguiculata mosquitoes have been described as hosting lineage 4 WNV strains. Therefore, mosquitoes collected in Austria and the Czech Republic were investigated for the presence of WNV, focusing on lineage 4 viruses. In this study a putative new WNV lineage was identified in a pool of Ur. unguiculata mosquitoes collected in Austria in 2013. The complete polyprotein coding sequence was determined and phylogenetic analyses were performed.
Mosquitoes were trapped at Lake Neusiedl-Seewinkel National Park in Burgenland State (Austria) and Sedlec (Czech Republic). Mosquito species were determined according to morphologic criteria with a total outcome of 94 female Ur. unguiculata mosquitoes. The mosquito pools were screened for the presence of flaviviral RNA, using universal flavivirus primers within the nonstructural protein 5 (NS5) encoding gene. One mosquito pool proved positive for WNV. The complete polyprotein coding sequence including partial of 5´and 3´noncoding ends of this novel strain was obtained. Phylogenetic neighbor-joining trees were generated with MEGA5 software, using ClustalW alignments, 1,000 replicates for bootstrap testing, and evolutionary distances computation with the p-distance model. Furthermore characterization of the encoding polyprotein was performed. The complete polyprotein gene sequence of the detected WNV strain from Austria (WNV-Uu-LN-AT-2013) shares a maximum identity of ?83% with lineage 4 WNV strains isolated from Ur. unguiculata mosquitoes in Russia. At the amino acid level, the entire polyproteins of WNV-Uu-LN-AT-2013 and the lineage 4 strains from Russia share ?96% identity. Compared with the Russian lineage 4 strains, a 1,813-nt fragment of the NS5-coding sequence of a putative lineage 6 WNV, isolated from Culex pipiens mosquitoes in Spain, shares slightly higher nucleotide and amino acid identities with WNV-Uu-LN-AT-2013. The envelope protein carries 1 putative N-linked glycosylation site at asparagine residue N-154, which has been associated with increased WNV pathogenicity and neuroinvasiveness.
We suggest that the new WNV-Uu-LN-AT-2013 strain from Austria either constitutes a new lineage (lineage 9) or can be grouped into lineage 4 as sublineage 4c, with the strains from Russia and Spain as sublineages 4a and 4b, respectively.

Poster 1.11

NOT PRESENTED

Poster 1.12

Molecular phylogeny of the whole Apodemus genus based on the complete mitochondrial genome and two nuclear genes

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The Apodemus genus is composed of 20 different species distributed throughout the Palearctic region. It is subdivised into two subgenera: - the Sylvaemus subgenus corresponding to the majority of the species living in the Western Part of the Palearctic region; - the Apodemus subgenus, associating most of the Asian species.

Although these rodent species are very common and are spread in many European and Asian habitats, their evolutionary history and their phylogenetic relationships are still largely unknown. Previous studies developed on a restricted number of genetic markers and a subset of the Apodemus species gave partial information concerning these aspects.

However, many gaps still remain even if such information would be particularly important not only in fundamental taxonomy but also in the field of epidemiology. Indeed many Apodemus species are reservoirs of different pathogens, like the Hantaviruses. A better knowledge of their evolutionary relationships would help to better understand virus spill-overs, which seem to exist among several Apodemus species.

The aim of our study was to analyse for the first time in a single research, the totality of the 20 Apodemus species, using as genetic markers, the whole mitochondrial genome as well as two nuclear genes. The phylogenetic analyses based on 20 000 nuclear and mitochondrial DNA base pairs, give a clear pictures of the phylogenetic relationships existing among these species. These results will be compared with the different Hantavirus strains already identified on this rodent group.

Evolutionary biology and comparative genetic structure of the yellow necked fieldmouse (Apodemus flavicollis) and the stripped field mouse (Apodemus agrarius) throughout their distribution area. The answer from the microsallite nuclear markers

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The Yellow Necked fieldmouse (Apodemus flavicollis) and the Stripped fieldmouse (Apodemus agrarius) are largely distributed in the Western Paleartic region. These rodent species are of particular interest as they are the reservoirs of different Hantavirus, respectively the Dobrava, the Kurkino and the Saarema strains. Different genetic sublineages geographically structured also appear to exist within these three hantavirus genotypes.

Previous phylogeographic studies developed on the two Apodemus species also evidenced the existence of different genetic lineages geographically structured. However, these studies were only based on a single mitochondrial marker and on a limited sampling. More precise studies developed on a larger number of genetic markers as well as a better sampling covering their whole distribution areas, are therefore essential to better understand their phylogeographic structures.

A good knowledge of the distribution of the different genetic lineages existing within the Hantavirus genotypes as well as within their hosts, can allow developing co-phylogeographic approaches. These methodologies can help to better understand co-evolution processes among the hosts and their parasites. If a congruence exist among the distribution of their genetic lineages, this can notably allow to better understand the distribution of the hantavirus lineages in Europe. This aspect is particularly important on the epidemiological point of view as some genotypes (e. g. the Dobrava one) appear much more pathogenic than others.

The aim of our study was to improve our knowledge concerning the phylogeographic structure of the two Apodemus species, using a set of 9 microsatellite markers as well as a large sampling covering their whole distribution areas. The obtained results helped to develop co-phylogeographic analyses between these hosts and their hantaviruses.

Poster 1.14

NOT PRESENTED

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 Myodes glareolus and 30 ticks Ixodes ricinus 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 pilot 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.

Molecular studies regarding the: Borrelia burgdorferi, Tick Borne Encephalitis Virus (TBEv) and Crimeean Congo Hemorrhagic Fever virus (CCHFv) in the Romanian ticks

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Our studies undertaken between 2006-2012 during the EDEN project (Emerging Vector-borne Diseases in a Changing European Environment, FP6 GOCE-CT-2003-010284) have shown that the most frequent species of ticks in Romania is Ixodes ricinus due to favorable vegetation and climate conditions for this parasite. It was found that I. ricinus is the main vector in Romania for the Borrelia burgdorferi s.l. and for the TBEv, however previous analysis didn't show the presence of the two concomitant infectious agents into I.ricinus coming from the same habitat. Three Romanian counties were selected as ticks (all species) sampling sites (3 sites in Sibiu county, 2 in Tulcea county in the Dobrogea and 1 in the Giurgiu county), with this occasion we collected ectoparasite ticks from the reptile fauna too. Among the reptiles from this region, the Testudo graeca ibera (TGI) is a well represented species. Samples of ectoparasites obtained from TGI collected in the years: 2006-2007 during the EDEN project and 2014 (April-June) showed that the majority of ticks are represented by Hyalomma. Over 300 Hyalomma ticks were collected and analyzed by Real-Time PCR (using the TicKitqPCR detection concept - MEN-UEFISCDI PN II ,,Partnerships in priority areas" program, National Research Grant No. 295/2014") that will give us results on the CCHFv presence.
The I. ricinus pools, collected from the Sibiu county (area considerated ,,endemic» to the TBE and Lyme borreliosis) and from the other 2 counties have been subject to total RNA extraction followed by Real-Time PCR analysis. This technique confirmed the TBEv and B.burgdorferi s. l. concomitant presence in a large number of ticks. Total RNA were extracted and analized by in house real-time PCR reagents (included in the TicKitqPCR detection concept) for the CCHFv presence in the Hyalomma sp.pools.
The results strengthen the concern that already exists in Romania, for the enhancement of the control measures for the tick population but also for the means of active information of the human population about the danger of the diseases transmitted by ticks.

Poster 1.17

NOT PRESENTED

Genetic mapping of pathogenic hantaviruses in Europe

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Hantaviruses are enveloped (-) RNA viruses belonging to the family Bunyaviridae, genus Hantavirus. They circulate worldwide among mammal reservoirs (Rodentia, Soricomorpha and Chiroptera). Although apparently apathogenic in their reservoir hosts, they frequently provoke diseases in humans such as Hemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus Cardio Pulmonary Syndrome (HCPS). In Europe, Puumala virus (PUUV) is widely distributed except along the Mediterranean basin and associated with a mild nephropathia epidemica (NE). The prevalence of Dobrava virus (DOBV) is increasing in Central-Eastern Europe and the associated HFRS has the highest mortality (Belgrade genotype). Tula virus (TULV), previously reported as asymptomatic, has recently shown pathogenicity in humans. Seoul virus (SEOV), traditionally considered an Asian virus, has been detected in rats as well as in severe humans cases. We have explored the genetic diversity of European hantaviruses using the resequencing microarray (RM) approach. Among the 800 sequences present in a panviral RM, 52 were strategically chosen to cover the diversity of Hantavirus genus, in particular the most frequent rodent-borne species. Rodents of Muridae and Cricetidae families were captured in endemic regions for hantaviruses: France, Germany, Scandinavian and Siberian regions, Balkans, United Kingdom. RNAs from animal tissues or from infected cell supernatant, were reverse transcribed, amplified then hybridized on the microarray. Hybridization provided sequence information with a level of efficiency proportional to the divergence between the tested virus and that tiled on the array. Despite the presence of mismatches, BLASTN analysis allowed identification as precise as geographical variants up to viruses 21% divergent from the tiled sequence. Further phylogenetic analysis described the regional genetic diversity of hantaviruses such as PUUV, TULV, DOBV-SAAV, including those absent from the microarray such as Topografov virus (TOPV) from Siberia. The microarray was also able to precisely detect and characterize

hantaviruses unknown when the chip was conceived such as the SEOV emerging in United Kingdom.
Resequencing microarray is a promising approach for surveys of hantaviruses in Europe and other endemic areas, in different reservoirs, as well as in humans.

Session 2

Ecosystems: vectors

Modelling the dynamics and spatial distribution of Culicoides (Diptera: Ceratopogonidae) biting midges, potential vectors of African horse sickness and bluetongue viruses in Senegal

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In Senegal, the latest epidemic of African horse sickness (AHS) occurred in 2007. It caused the death of 1,169 horses in several regions and considerable economic loss, estimated to 0.9 billion FCFA (1.4 million euros).The vectors responsible for biological transmission of the virus belong to the genus Culicoides (Diptera: Ceratopogonidae). In Senegal, entomological studies on Culicoides conducted four decades ago were limited in scope and they did not specifically target the species in the vicinity of the animals whose health could be impacted. Thus several studies were initiated in 2011 for a better understanding of the Culicoides of Senegal and their involvement in the transmission of AHS.

A one-year monthly trapping campaign using two light traps for three consecutive nights in five sites of the Niayes region was carried out in 2011-2012. It enabled capturing over 224,000 Culicoides belonging to at least 24 species and showed that in addition to C. imicola, considered a major vector for the AHS virus, C. oxystoma may also be involved in the transmission of this virus in Senegal. Besides updating the list of Culicoides species, this study also allowed to describe their seasonal dynamics in Niayes area.

In 2012, a nation-wide Culicoides trapping campaign was organized to better describe spatial distribution of Culicoides in Senegal. Two successive collection nights were carried out in 98 sites in 12 (out of 14) regions of Senegal at the end of the rainy season (between September and October). More than 1,367,000 Culicoides belonging to at least 35 species were collected in this spatial survey. These data were used to model the spatial distribution and abundance of the potential vectors of AHS and bluetongue viruses in Senegal according to climatic and environmental data.

Overall, this work allowed updating the list of Culicoides species of Senegal, describing their dynamics, characterising suitable habitats and mapping abundance of the potential vectors of AHS and bluetongue viruses in Senegal.

Seasonal dynamics of six phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by Leishmania infantum

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Phlebotomine sand flies (Diptera, Psychodidae) are the unique haematophagous insects proven to transmit human leishmaniases. Among the over 800 phlebotomine sand fly species estimated to exist, only a hundred species of Phlebotomus and Lutzomyia genera are proven or suspected vectors of human disease in the Old and New Worlds. Leishmania infantum is an agent of visceral and cutaneous leishmaniasis in the Mediterranean region, where a dozen of Phlebotomus species have been implicated in the parasite transmission; 8 of them have been definitely incriminated as vectors following standard criteria. The activity period of Mediterranean adult sand flies is seasonal. As insect biology is deeply influenced by climate variables and, in particular, seasonal phenomena are very sensitive to small variations in temperature, phenological observations may provide high resolution of ongoing changes. Current information on sand fly seasonal dynamics in the Mediterranean region is patchy, being the available data disperse in space and time. Therefore, an accurate baseline assessment of present biological characteristics of vectors is required for monitoring leishmaniasis transmission in a context of global climate changes. A prerequisite for such studies is that assessments are performed repeatedly during relatively short time and throughout a wide geographical area, in order to avoid general (e.g. ongoing temperature increase) or particular (e.g. local weather events) confounding parameters. In 2011-2013 EDENext partners from Mediterranean countries endemic for L. infantum have carried out investigations in representative collection sites using standard sand fly trapping (CDC light traps and sticky papers) performed during a minimum of 2 nights/month for at least 2 consecutive years. About 93,000 sand fly specimens were collected and identified, resulting in the accurate description of seasonal dynamics (6 species) and 24-hour activity (5 species) of L. infantum vectors over a wide geographical range spanning from Portugal at west to Georgia at east: P. perniciosus (Portugal, Spain and Italy); P. ariasi (France); P. neglectus (Greece); P. tobbi (Cyprus and Turkey); P. balcanicus and P. kandelakii (Georgia).
This work was supported by EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504

Natural infection and insecticide susceptibility status of wild caught sand flies in rural areas of Antalya, Mediterranean Region of Turkey

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Sand fly-borne diseases like leishmaniasis and phlebovirus infections have been seen in Turkey endemically. The application of insecticides is mainly targeting mosquitoes/flies control. However, no study was performed about the susceptibility of sand flies against pyrethroids in Turkey.

This study was carried out in Antalya province located in The Mediterranean Region of Turkey. CDC's bottle bioassay has applied for pyrethroid susceptibility by using different dosages. The tests were repeated 5 times with 100 specimens per bottle. In addition, WHO's Cone test were performed using two different bed nets (Olyset Plus® with PBO and Permanet®) to understand their efficacy. Following the standards of WHO testing procedures, all specimens were dissected, mounted and identified. After dissection, 50 pools containing sand fly bodies were generated. DNA extractions were made using commercial kit and previously described conventional PCR of ITS-1 region was performed.

P. neglectus (38.82%), P. alexandri (21.66%) and P. tobbi (20.44%) were dominant species among 10 Phlebotomus species. Totally seven different active ingredients were tested and knock down times were calculated using Probit Analysis. For the lowest dosage of insecticides, KdT100 value were noted as 36 minutes for permethrin (0.05%), 60 minutes for cypermethrin (0.05%) and 42 minutes for deltamethrin (0.05%). By the end of 24-hour period, no sand fly was alive in bottle assay while 46% and 0% of sand flies were alive in cone test-Permanet® and cone test- Olyset Plus®. Four pools containing Phlebotomus neglectus and one pool Phlebotomus tobbi were found to be positive for Leishmania spp.

In conclusion, no resistance was determined in bottle assay but upcoming resistance with prolonging death time and knock down times were noted. Cone test results have showed that Olyset Plus® has more knockdown effect than Permanet®. Even no resistance was detected in this study; related to the presence of important vector species of Leishmania infantum and detection of natural infection in the area, the status of susceptibility of sand flies needs to be monitor regularly in the region.

This study is supported by The Scientific and Technological Research Council of Turkey (Project No: 112T270).

A study on nocturnal activity of sand flies in a leishmaniasis endemic village located in Aydin province of Turkey

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The nocturnal activity of sand flies in an endemic focus for canine and cutaneous leishmaniasis in western part of Turkey was studied using CDC light traps. The traps were set up in different micro-localities, such as human house, cattle shed, chicken coop and sheep area within 60-70 meters in diameter. In the study night, a total of 263 sand flies, six species belonging to Phlebotomus and two species belonging to Sergentomyia genera, were caught. P. tobbi was found to be abundant species (60.46%) while P. neglectus/syriacus was second one (21.29%). The activity of sand flies started at 21:00-22:00h, reached maximum between 01:00 and 03:00h and steadily decreased thereafter and finished at 05:00h in all micro-localities. Female sand flies was always 2-3 times in high number than males. The study was showed the highest activity of probable vector species, P. tobbi, in the area and proved the importance of the studies on seasonal and daily activity of sand flies in endemic areas.
This work was partially supported by EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504

Feeding patterns of the invasive Asian tiger mosquito Aedes albopictus and native mosquito in Southern Europe: implications for the transmission of human and avian pathogens

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The invasive Asian tiger mosquito Aedes albopictus is dramatically expanding its distribution range, being catalogued as one of the 100 world's worst invasive alien species. In Europe, this species has been incriminated in the transmission of both endemic and exotic pathogens, potentially creating novel epidemiological scenarios in the invaded areas. Here, we tested this possibility by using two molecular approaches to identify: i) the blood meal source of Ae. albopictus and the native Culex pipiens from rural and urban habitats from Italy and Spain and ii) the avian malaria parasites Plasmodium and Haemoproteus, harboured by Ae. albopictus and different native mosquitoes species from Italy. We found that contrary to Cx pipiens that fed largely on birds, Ae. albopictus showed a clear antropophilic behaviour in urban habitats where 92% of the blood meals had a human origin. In rural habitats, rats were the most common hosts of Ae. albopictus. In addition, avian malaria parasites were isolated from three mosquito species, with Cx. pipiens (30%) showing the highest parasite prevalence followed by Culex hortensis (9%) and Ae. albopictus (5%), while non parasites were found in Anopheles maculipennis nor Ochlerotatus caspius. Overall, our results support that the risk of spread of avian parasites by Ae. albopictus in Europe would be minimal, although it may play an essential role in the transmission of exotic pathogens potentially introduced in Europe and transmitted between humans, such as Dengue virus and Chikunguya virus. These results also highlight the bird species that can be involved in the transmission cycle of arboviruses transmitted by Cx. pipiens.

Role of the introduced Siberian chipmunk (Tamias sibiricus) on the risk of exposure to Lyme borreliosis in a periurban forest (Sénart, Ile-de-France)

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The introduction of a new host species can increase health threats by adding a new reservoir or by amplifying the circulation of native pathogens. We studied the role of the Siberian chipmunk (Tamias sibiricus barberi), a small ground squirrel introduced from Korea to France in the late 1960's, on the risk of Lyme borreliosis in a periurban forest near Paris (Sénart, Ile-de-France) from 2005 to 20011. This disease is a zoonosis caused by bacteria that belong to the Borrelia burgdorferi sensu lato complex and are transmitted by bites of hard ticks, especially by Ixodes ricinus ticks in Europe. In Sénart, we found that chipmunks were highly infested by I. ricinus and infected by B. burgdorferi sl (over 50%). We calculated the contribution of chipmunks to the risk of exposure to Lyme borreliosis. First, we estimated the number of questing infected nymphs produced by Siberian chipmunks based on the density and infection prevalence of hosts. Second, we built a model of contribution based on high-throughput sequence diversity of two Borrelia genes: the housekeeping gene rplB, and the infection-related gene ospC. With both approaches we found that chipmunks contributed over 8 times more to the risk than sympatric bank voles (Myodes glareolus) and wood mice (Apodemus sylvaticus), two native competent reservoir hosts. The genetic data further underlined the existence of different cycle of transmission among rodents. Finally, we mapped the risk of exposure to Lyme borreliosis across the entire area of the Sénart forest based on an intensive questing nymph sampling (ca 19,000 nymphs). The presence of chipmunks was not directly related to increase spatial risk, problably due to other confounding factors. Nevertheless, the map is an important tool for stakeholders in identifying hotspot areas of transmission. In conclusion, thanks to intensive fieldwork, high-throughput sequencing and modeling, we demonstrated that introduced chipmunks contributed to the risk of exposure to Lyme borreliosis, probably by increasing nymph infection prevalence rather than density. They are infected by genetically differentiate Borrelia compared to other rodents. The consequences of such differentiation on the pathogenicity for human remain to be evaluated.

Apparent absence of a barrier to nuclear gene flow in Central Finland between two mitochondrial DNA clades of the bank vole (Myodes glareolus, the reservoir host of Puumala hantavirus)

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Combining nuclear and mitochondrial DNA (mtDNA) markers has highlighted the limitations of studies using only mtDNA markers to depict phylogeographical histories. These last ten years, many conflicting geographic patterns between mitochondrial and nuclear genetic markers have been identified, hereafter named cytonuclear discordance. The most common form of such discordance is ?asymmetric movement of mtDNA' that can go up to a complete replacement of the mtDNA of the native taxon with selective advantages of the introgressed mitochondrial type. Previous studies showed that Finnish bank voles exhibit two mtDNA clades: the ?GLA' (mtDNA of Myodes glareolus, specific to Southern Finnish bank voles) and the ?RUT' mitotype (mtDNA of M. rutilus, common in Northern Finnish bank voles and M. rutilus). The replacement of the northern Finnish bank vole (M. glareolus)'s mtDNA with that of the red vole (M. rutilus) could be explained by a selective process favouring the mtDNA of M. rutilus, a species more adapted to cold temperatures.
In this study, we examined whether some cytonuclear discordance existed in the Finnish bank voles. We used extensive sampling (442 specimens of M. glareolus and 39 of M. rutilus) and diverse genetic markers differing in rate of evolution and parental inheritance (the cytochrome b mt gene, and 17 unlinked microsatellite loci) to assess the evolutionary processes shaping the distribution of these two mitochondrial lineages. Genetic analyses revealed discordance between the mitochondrial and nuclear data. Mitochondrial analyses confirmed the occurrence of two major haplotypes in Finnish bank voles that correspond to two distinct mitotypes (?GLA' & ? RUT'). In contrast, clustering analyses based on the 17-microsatellite loci failed to separate specimens from both mitotypes and to detect any spatial genetic structure in nuclear markers among vole populations, except isolation by distance. This particular pattern hides any signal of barrier to gene flow at the level of the mitochondrial contact zone in Central Finland. The cytonuclear discordance observed therefore raised the question of an alternative hypothesis to the ?contact zone' due to post-glacial recolonisation history. Our results allowed to revise the scenari of mitochondrial introgression and postglacial recolonisation of Finland that were previously proposed in the literature.

Mosquito Surveillance in Finland

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Data is currently being gathered on the mosquito species found in Finland and their distributions around the country. This work is ongoing, but preliminary results will be presented. Around 40 species of mosquito are present in, or have been reported from, Finland, but few recent studies have been made in this region (the last, more comprehensive, study was made in the 1970's and the data is out of date). During the summer months, mosquito numbers can be very high due to the warm temperatures and the abundance of breeding habitats, and they are considered to be pests of humans and livestock due to their persistence in biting. All species in this region are known to be either zoophilic or anthropophilic in feeding preferences, with large potential for transmission of a number of viruses, both described and as yet undescribed. The second phase of the study is to look at the mosquitoes role as vectors of mosquito borne viruses, though this data may not be presentable in April.
To date, several novel mosquito borne viruses from Finland have already been identified and isolated by Prof. Olli Vapalahti's research group at Helsinki University, including Orthobunyaviruses (Möhkö virus and Inkoo virus), an Alphavirus (Sindbis virus), and Flaviviruses (Lammi virus, Hanko virus & Ilomantsi virus). Further collections will be made in 2015 to screen mosquitoes for these known, and novel mosquito borne viruses.

Free-ranging ungulates as hosts of ixodid ticks and tick-borne pathogens in the Malé Karpaty Mts (South-Western Slovakia)

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Free-ranging ungulates are obligate hosts of Ixodes ricinus. In collaboration with local hunters, spleen samples and feeding ticks were collected from game animals shot in deciduous forests in the Malé Karpaty Mts during 2011-2014. Tick-borne microorganisms were detected in biological samples by PCR: rickettsiae (PCR, targeting the gltA gene followed by sequencing), borreliae (PCR, 5S-23S rRNA, followed by RFLP), Coxiella burnetii (PCR, com1 gene), Anaplasma phagocytophilum (real-time PCR, msp2 gene), Cand. Neoehrlichia mikurensis (CNM) (real-time PCR, groEL gene) and babesiae (PCR, 18S rRNA gene, followed by sequencing). Ninety-three ungulates of five species - roe deer (Capreolus capreolus), red deer (Cervus elaphus), mouflon (Ovis musimon), fallow deer (Dama dama) and wild boar (Sus scrofa) were screened for tick-borne microorganisms. Ticks were obtained from all examined species. Ixodes ricinus dominated (1524 ind.: 90.5% larvae, 8.2% nymphs, 1% females, 0.3% males) but Hemaphysalis concinna were also present (206 ind.: 82% larvae, 17.9% nymphs). In feeding ticks, overall prevalence of rickettsiae (R. helvetica, R. monacensis, Rickettsia sp.) was 13.2%. R. helvetica was detected in all developmental stages of I. ricinus, R. monacensis only in larvae. Prevalence of A. phagocytophilum in adult and subadult I. ricinus feeding on deer species was 78.0%, of piroplasms (Theileria sp. and B. venatorum) 36.4%. C. burnetii was present in 2.6% of I. ricinus removed from mouflons, fallow deer and roe deer. Presence of CNM, Borrelia garinii, B. afzelii and B. valaisiana was sporadically detected in subadult and adult ticks. Screening of ungulate spleens revealed the presence of A. phagocytophilum (66.7%) and Theileria sp. (31.8%). Phylogenetic analysis using partial sequences of the 18S rRNA gene showed that the isolates from feeding ticks and deer spleen clustered with Theileria sp. Spleens and blood of game were negative for rickettsiae, C. burnetii, CNM and borreliae. The results show that free-ranging ungulates are good sentinels for transport of ticks infected with the screened microorganisms, but are probably reservoirs only for A. phagocytophilum and Theileria sp. in the study area.
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Rodents as reservoirs of Bartonella species in Eastern Slovakia

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Bartonella are gram-negative pathogenic bacteria, referred to as emerging vector-borne pathogens. They are transmitted by various blood-sucking arthropods, and infect a wide range of mammalian hosts, including humans. As rodent-borne bartonellosis has been reported several times in humans, the appraisal of molecular diversity and epidemiology of Bartonella in this group of hosts is necessary. The aim of this study was to identify the most common strains of Bartonella infecting different species of rodents in Slovakia, to estimate their molecular diversity and, by comparison with published literature, their potential to threaten humans. Altogether 432 rodents were live-trapped at Rozhanovce, Eastern Slovakia, between the years 2011-2012, and they were examined for presence of Bartonella using a previously published PCR protocol targeting the 16S-23S intergenic spacer region. Among the three commonest rodent species, the highest prevalence of Bartonella was recorded in Apodemus flavicollis (32,97 %), followed by Clethrionomys glareolus (14,77 %), and Apodemus agrarius (8,9 %). We have observed great genomic divergence amongst Bartonella strains, sometimes with multiple infections present in one host. Analysis of 16S-23S ITS sequences indicated a high prevalence of Bartonella taylorii, with great diversity within this species. Single clades of B. grahamii and B. elizabethae were also identified, and several sequences formed a separate clade which could not be assigned to any known species of Bartonella. To confirm the molecular diversity of obtained strains, we have additionally analysed three housekeeping genes (gltA, rpoB, groEl) and related the variation observed in them to the results obtained in the analysis of 16S-23S ITS gene. This is the first molecular study of Bartonella sp. in Slovakia; due to high prevalence and diversity of bacteria observed in rodents, it confirms the role of this group of mammals in epidemiology of bartonellosis in this region.
The research was supported by EU project FP7-261504 EDENext, VEGA 0/0060/14, VEGA 2/0059/15 and TD1303 COST Action.

Is boar population influencing on tick-borne diseases spread?

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Objectives: Tick-borne encephalitis (TBE), caused by tick-borne encephalitis virus, which is transmitted by hard tick Ixodes ricinus is still a problem in Europe. In 1990?2007 in Europe, 157 584 cases were reported. In 1990?2007, there was an almost 320% increase in the incidence compared to the years 1976?1989. In Poland, the number of cases of tick-borne encephalitis registered by the National Institute of Hygiene in 2012 was 189 cases, and in 2013 225 cases.
A significant increase in the incidence of TBE in the north-eastern Poland since 1993 has been observed. Podlasie region has had the highest incidence of TBE ? in 2012, 101 cases were reported, which represents 53.4% of all reported cases in Poland, and in 2013 111 cases, which represents 49.3% of all cases reported in Poland.
Reasons of these phenomena may be various. One of them could be changes in wild animals population. Therefore we aimed to search for the relationship between wild animals population changes and increase in TBE incidence, based on boar population.
Methods: To statistical analysis were subjected the numbers of boar population in 5 counties of Bia?ystok, Siemiatycze, Hajnówka Grajewo and Kolno in 1995-2006.
Results: In counties with the highest incidence of TBE: Bialystok and Hajnówka two completely different trends in the number of boars were observed. Increasing trend in the number of wild boars was observed in Bialystok area, in contrast to the county Hajnówka where downward trend was observed. No significant differences were observed in the other counties.
Conclusions: Based on the analysis of trends, it can be concluded that the imposition of the boar population factor in Bialystok county could cause larger areas of wildlife habitat, and thus contribute to an increase in host ticks and increase in the number of ticks, what influence on number of TBE cases. Increase in boar population in Bialystok may be caused by growth of corn and potatoes fields, lack of regulation of the population of wild animals. This is not observed in Hajnowka region, where National Bialowieza Park exists with no access to human interference.

Investigations on sand fly bionomics and Leishmania natural infections in Eastern Sicily, Italy, with particular reference to Phlebotomus sergenti

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Visceral and cutaneous leishmaniasis (CL) by Leishmania infantum have long been known to be endemic in Sicily. Catania province, sited on the eastern coast of the island, represents also an old endemic territory for Phlebotomus sergenti, the sandfly vector of CL by L.tropica. Because of the increasing human migration to Sicily and the related risk for the introduction of exotic Leishmania, an investigation on sandfly fauna composition, seasonal dynamics and Leishmania infections was addressed in CL endemic territories. Four urban (Catania city) and 11 periurban/ rural sites, located in a previously recorded P.sergenti area, were investigated. Sandflies were collected by sticky traps from April through December and by CDC light traps from June through October 2013. Live females were dissected and examined for Leishmania promastigotes identified by ITS1 PCR- RFLP, whereas dead females were analyzed by Leishmania nested(n)-PCR.
Out of 4105 specimens collected, 2545 (62.0%) were Sergentomyia minuta. Among 1560 Phlebotomus specimens, P.perniciosus (65.4%) and P.sergenti (20.8%) were the most prevalent followed by P.papatasi, P.neglectus and P.mascittii. P.sergenti was confirmed to be endemic, being recorded in 3 urban and 5 periurban/rural sites of which those located at the slopes of mount Etna were the most productive. The comparative dynamics of P.sergenti and P.perniciosus showed that they peaked in different months, July and August respectively. Females captured alive from P.sergenti-positive sites (143 P.sergenti and 34 P.perniciosus) were found negative at dissection. In a P.sergenti-negative site dominated by P.perniciosus 3/118 (2.5%) specimens of this species were detected with promastigotes identified as L.infantum. Out of 175 dead females from all sites, 17 (9.7%) were found positive by Leishmania n-PCR, of which 13 were P. perniciosus and 4 P.sergenti. While the vector role of P.perniciosus in the L.infantum transmission was confirmed, that of P.sergenti remains unclear. The detection of Leishmania DNA in this species suggests an implication in parasite circulation, however P.sergenti does not seem to play a role as an active vector of CL, which has been recently confirmed to be caused by L.infantum in the Catania area.
This study was carried out in frame of FP7-UE EDENext collaborative project, Contract Number: 261504.

Microevolution of bank voles (Myodes glareolus) at neutral and immune-related genes during a multi-annual complete dynamic cycle : consequences for Puumala hantavirus epidemiology

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Because of the (re)-emergence of many vector-borne infectious diseases, understanding the ecological and genetic factors influencing the geographic distribution of zoonotic agents and their reservoirs has become an active research area. The Puumala hantavirus (PUUV), carried by the bank vole Myodes glareolus, is responsible for nephropathia epidemica (NE) in Europe. Temporal variation of NE occurrence is related to fluctuations of rodent population densities. We therefore tested the hypothesis that density fluctuations could affect the allele frequencies of immune genes involved in the outcomes of PUUV / M. glareolus interactions, i.e the probability of becoming infected. Immunogenetic polymorphism could be involved in changing the epidemiology of PUUV throughout vole cycles.
We studied a bank vole metapopulation in a study area of 25 km2 in Central Finland (Konnevesi) between 2005 and 2009. We genotyped bank voles at microsatellite markers and candidate genes (Tnf, Tlr-4, Tlr-7, Mx2 and Drb). PUUV serological and genetic results had previously been published.
Our results showed that in rather homogenous boreal forests, there are no spatial barriers having influence on gene flow at this geographic scale. Some spatial structure could only temporarily be observed, probably as a result of clustering of family groups. Despite a strong cyclic population crash in 2006, the metapopulation genetic diversity remained similar through time, showing that bank voles have high reproduction rate and efficient dispersal. On the whole, candidate gene polymorphisms did not significantly discriminate PUUV seropositive and seronegative bank voles. However, two Mhc-Drb alleles showed significant associations with PUUV infection. Finally, we found no signature of selection, either in space or time.
A larger and more contrasted sampling zone, with endemic and non-endemic regions, or new high-throughput sequencing techniques applied to this dataset, could allow for detecting genes evolving under selection during vole density cycles.

Field voles Microtus agrestis as reservoirs of Bartonella spp.

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Bartonella spp. are intracellular bacteria that cause chronic infection of the erythrocytes of their mammalian hosts. Typical reservoir hosts for Bartonella spp. are cats, dogs, ungulates and small rodents, between which infections are transmitted via fleas, ticks and other blood-sucking arthropod vectors. Several Bartonella species are known to infect humans, causing e.g. cat scratch disease, with symptoms including lymphadenopathy and endocarditis. In recent years, diagnostic advances have led to the identification of isolated incidents of human Bartonella spp. infection in Finland. However, as Bartonella spp. are known to colonize a number of wild rodent species, and they have been detected in questing ticks, it is plausible that these diagnosed cases are only a realised fraction of human infections and thus an underrepresentation of the true zoonotic potential of Bartonella species. In this study, we aim to determine the role of field voles Microtus agrestis, periodically the most numerous small rodent in Northern Europe, as reservoir for Bartonella spp., and how infection prevalence varies both spatially and temporally with the population fluctuations of field voles. To do so, we carried out molecular screening of 679 field voles, trapped from 14 sites across the southern half of Finland over 3 years. We identified four species: Bartonella taylorii, B. doshiae, B. grahamii and a novel species of the same genus. Bartonella spp. were found in field voles throughout the entire study area, excluding the two northeasternmost locations. The prevalence of Bartonella spp. ranged between 14-42 %, and was positively associated with vole densities six months prior to infection. Our findings indicate that Bartonella spp., also the human pathogen B. grahamii, are highly prevalent in natural populations of rodents which frequently come into contact with humans especially during times of high population densities. The fact that human cases are only rarely reported suggests that Bartonella spp. infections in humans are either underdiagnosed, and/or that Bartonella spp. are emerging zoonotic pathogens in Northern Europe.

Survey of Wild Birds for West Nile Virus in The Danube Delta Biosphere Reserve - Romania, 2011-2014

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Between 2011-2014 3,394 samples were tested for West Nile virus (WNV): bird sera - 1,564 samples, all were screened by ELISA, and 1,203 were subjeced to plaque reduction neutralization test (PRNT90); swab and organ samples - 1,791 samples consisting of cloacal swabs, pharyngeal swabs, brain tissue samples, and samples of internal organs; ectoparasites - 39 samples. Swabs and organ samples as well as ectoparasites were tested by various WNV-specific qRT-PCRs and RT-PCRs; positive and questionable results were verified by sequencing.

The total seroprevalence rate by ELISA was 11% (173 positive sera / 1,564 sera tested) and 7.15% by PRNT90 (86 positive sera / 1,203 sera tested).
The seropositive resident birds present clear evidence of local circulation of WNV. Whether the seropositive migratory birds were infected outside the study area and their chicks were subsequently infected by maternal transmission through eggs, could not be determined unambiguously. The WNV antibody prevalence differed to that in a former study (2005-2008, Danube Delta: 15.8% positive by ELISA and 1.8% by PRNT), yet, the WNV prevalence was higher than in other European countries.
In the current study (2011-2014) 20 different species of birds were found seropositive for WNV, and 8 of them for the first time in Romania: Golden Oriole, Marsh Warbler, Common Whitethroat, Reed Bunting, Sparrowhawk, Song Thrush, Great Spotted Woodpecker and Savi's Warbler.
No pharyngeal swabs, cloacal swabs, internal organs or brain tissue samples (1,791 samples collected in 2012 and 2013) were found positive by PCR, however, one ectoparasite (tick) proved WNV-positive.
One of the hypotheses was the possibility of viremia in birds after prolonged efforts such as migration, but also nesting, severe winter weather, etc. The fact that none of the 1,791 samples tested positive by PCR lead us to the conclusion that the investigated birds have not been chronically infected with WNV; however, our finding does not exclude persistent infection of certain other species of birds with WNV.
In our study we investigated the transmission of WNV in birds during winter. The negative PCR results indicate that the virus is predominantly not overwintering in birds but in mosquitoe.

Bionomics of Obsoleus complex species and other livestock associated Culicoides in laboratory conditions

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Biting midges (Diptera; Ceratopogonidae) are related to the transmission of several important diseases, such as Bluetongue, African Horse Sickness, Epizootic Haemorrhagic Disease and Schmallenberg. The basic bionomics of the major vector and non-vector Culicoides species associated with farms is still understudied. In this study we contribute to the knowledge of the sub-adult development in laboratory conditions of the Obsoletus complex species and other Culicoides species farm-associated, such as Culicoides circumscriptus, C.newsteadi, C.paolae and C. cataneii (or C.gejgelensis variation). Insects were collected from field between spring and autumn 2014 in a cattle farm (Son Valls) located in Mallorca (Balearic Islands, Spain). Gravid females were kept individually in cardboard boxes and moistened cotton wool, while filter paper provided a surface where eggs were laid. Eggs were transferred to Petri dishes with 2% Agar gel medium. Obtained larvae were reared in agar medium containing the nematode Panagrellus redivivus. Pupae were transferred again to cardboard boxes till adults emerge. We present results of bionomic variations between the different Culicoides species such as number of eggs laid per females, percentage of hatch, time development from larva to pupa, percentage of pupation and adult survival.

Where are all the boars. An attempt to gain a continental perspective.

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The presence of the species Sus scrofa (wild boar) can be an important component of the ecological and epidemiological systems within which vector-borne diseases persist. Wild boar are hosts to a number of vector species, and they can impact on disease cycles as attractants of key vectors or as reservoirs of pathogens. Information on wild boar distribution and abundance could therefore make an important contribution to models of vector-borne disease risk.
The many studies that have focussed on the distribution, abundance and habitat-use of wild boar were generally carried out in relatively small areas such as national parks or at country level. Given the broader, continental scale focus of EDENext studies, a number of project partners requested that the project Data Management Team attempt to produce a continental scale distribution and abundance map for this species.
This study combines a review of the existing literature along with abundance related data from a range of sources, including national hunting organisations, international and national distribution databases, to attempt to gain a continental perspective of boar distribution and abundance.
To create the final European 1km boar map the combined quantitative data described above were constrained using a habitat suitability mask derived from the GlobCover land cover database. A number of spatial distribution modelling tools available from the VECMAP Modelling suite were used to produce three final modelled distribution outputs for Europe. These comprise a 1km probability of presence/absence layer; a 1km abundance index based on presence and habitat availability; and a 1km ranked abundance map based on regional abundance studies and national hunting figures.
The mapped outputs will all be made available to registered users of the EDENext data website (www.edenextdata.com)

Identifying the last bloodmeal of questing wood tick nymphs (Ixodes ricinus L.) by DNA amplification: three approaches tested

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Tick-borne disease risk can be modelled more accurately when the main hosts of the most common European tick vector, I. ricinus, are known for a given area. However, I. ricinus is known to feed on more than 300 species in Europe, and estimating their relative importance from field observations (which includes live-trapping of hosts to count ticks) is time-consuming and likely to be highly biased, since individual ticks spend only a few days a year feeding. On the other hand, I. ricinus ticks are easily collected from vegetation while questing; therefore, more recent studies have focussed on using molecular markers in these individuals to identify their last bloodmeal. Here we present three different protocols that we optimized to detect bloodmeal sources and discuss the quality of the results: Reverse Line Blot Hybridization (RLBH), Next Generation Sequencing (NGS) and, for the first time, High Resolution Melting Analysis (HRMA). Regarding RLBH and NGS, we managed to limit contamination and increase the quality of results, but some uncertainty remained. Instead, using HRMA, we showed that with six newly designed host-group primers (Muroidea, Soricidae, Passeriformes, Canidae, Caprinae, Artiodactyls), we could successfully amplify 20 target host species and genera. When first tested on a limited number of questing nymphs, the new protocol showed high sensitivity (bloodmeal sources were identified in 65.4 % of nymphs), reliable mixed bloodmeal identification and high identification success (35 out of 42 amplified bloodmeals were identified to genus or species), and low contamination levels. In order to improve the cost-effectiveness and productivity of the HRMA protocol, we then automated both the extraction method and PCR reaction setup for an additional 741 nymphs. Although mean sensitivity decreased to 21.5 % (159/741 nymphs), identification success and contamination control were maintained. HRMA results confirm the importance of Apodemus spp. as larval hosts (58/173 bloodmeals), but also indicate that the domestic dog, C. l. familiaris (37/173) may support larval populations. In conclusion, we present the pros and cons of these and other published techniques and the prospects for improvements. We also discuss the epidemiological implications of the results.

Altitudinal ecological gradient study of Aedes albopictus in Albania

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Introduction

The first record of Aedes albopictus in Europe was in Albania in 1979 (Adhami & Reiter, 1998), although it is quite possible that the species was present at least a decade earlier, some 20 years before the species was first detected in Italy in 1991. Nowadays it is widespread and common throughout the country, including at high altitude. Here it is presented a three-year study of the abundance and diapause of Ae. albopictus eggs at different altitudes on Dajti mountain in central Albania.

Material and Methods

Fourteen stations were established to monitor oviposition activity, ten along a vertical gradient from 253 to 1200 meters, and four stationary at 158m to compare different habitat types. Five ovitraps, lined with heavy-weight seed germination paper were operated at each site. Monitoring started in April 2011 and continued at weekly intervals until December 2013. Temperature was recorded for each station by data-loggers (LogTag® model TRIX-8, www.logrecorders.com) at a frequency of 20 minutes interval. Eggs were counted and identified to species level based on their color, size, shape and surface sculpting. About 1500 eggs have been collected weekly in each of the three altitudes 158m, 333m and 541m, from week 26 to week 44. Subsequently the eggs were hatched in lab condition and the un-hatched ones were bleached to determine the onset of the diapause. Data analyses have been carried out by using R statistical software and a random effects regression model was devised to predict mean number of eggs by mean temperature.

Results & Conclusions

Aedes albopictus was present all through the studied altitudes up to 1200 meters. In terms of altitude the species seems well established with an abundant population up to 550 meters and particularly pervasive in gardens, in urban and suburban areas. At the lowest altitudes 158m, oviposition continued from May to early December but the period of activity declined with altitude, so that at the altitude of 762 meters the species appeared to be univoltine. Diapause began in week 31 at all the three altitudes, and more than 50% of the eggs were dormant by week 38.

Determination of the species of the sand flies-the vector of cutaneous leishmaniasis that is endemic in Mersin, Turkey

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The aim of this study was to determine the sand fly fauna in various areas of the province of Mersin where cutaneous leishmaniasis caused by Leishmania tropica are seen and to determine the potential of Phlebotomus and other species for vectoring besides showing the presence of this parasite species directly and with molecular biological methods.
The number of sand flies caught in the field studies performed in Anamur, Silifke and Mut are 197, 573 and 160, respectively. In Mersin totally nine Phlebotomus- P. sergenti (%30), P. alexandri (%18), P. neglectus/syriacus (%12), sergentomyia minuta (%12), P. tobbi(%11), P. papatasi (% 10), P. simici (%3), P. halepensis (%1), P. mascitti (%1), P. brevis (%1)- and one Sergentomyia species were detected. Of 428 female sand flies, 326 were identified and were grouped according to their blood-sucking / egg-presence properties and 29 pools were prepared by putting 3 to 19 members in each of them. Leishmania DNA was investigated in the DNA samples found in these pools by real time PCR method using ITS1 gene region. DNA isolation was performed by using ZR Insect/Tissue DNA Kit-5TM (Cat. No. D6015).
In this study, the sand fly fauna in Mersin where CL is an endemic disease was determined. P. sergenti was identified as the dominant species and it was concluded that P. sergenti might be the probable vector of L. Tropica.

Seasonal tick infestations of grazing cattle in two provinces with low and high CCHF incidence in Turkey

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Periodic sampling with 15-20 days intervals of grazing cattle was conducted in Kirklareli (European part, Trace) and Yozgat (Central Anatolia) provinces.
A total of 595 cattle were sampled and 10327 ticks (17.36 tick/cattle, 87.4% infestation) were collected in Kirklareli between April and December 2013. Nine species of ticks were recorded. Tick composition was as 65.45% H. marginatum, 18.37% Rh. turanicus, 6.65% H. scupense (adults and immatures), 5.04% Rh. bursa, 2.65% Haemaphysalis spp., 1.34% Rhipicephalus immatures, and few I. ricinus and D. marginatus.
A total of 459 cattle were sampled and 4652 ticks (10.14 tick/cattle, 91.29% infestation) were collected in Yozgat between April and August 2013. Almost all ticks were H. marginatum (98.45%), followed by small number of Rh. turanicus (1.38%), and H. excavatum (0.13%). Only single examples of I. ricinus and D. marginatus were collected.
Although Kirklareli is a place with sporadic cases of CCHF, the number of H. marginatum per animal was much higher than Yozgat where CCHF incidence is very high. It can be explained with the low rate of human-tick contact or low viral prevalance in the ticks in Kirklareli. On the other hand, although H. marginatum population is higher than other ticks in both sites, in places with high CCHF incidence (Yozgat) it is dominating overwhelmingly and the ratio of H. marginatum to the other ticks is 38.49, while in places with very low CCHF incidence (Kirklareli) there are considerable numbers of Rhipicephalus spp. and other tick species and the ratio is only 1.90. There may be a need to further discuss the possible influence of that difference on CCHF epidemiology.

Session 3

Ecosystems: transmission

Poster 3.1.

NOT PRESENTED

Changes in the distribution of canine babesiosis and their vector D. reticulatus tick in Baltic countries

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Canine babesiosis is one of the most important a worldwide spread tick-borne infection of dogs caused by hematozoan parasites of the genus Babesia. During the last decades spreading of canine babesiosis caused by B.canis to former non-endemic areas has been reported in Europe. In Lithuania the first cases of canine babesiosis caused by B. canis were registered in last decade. Recently, cases of autochthonous babesiosis caused by B.canis in a dog were reported from Latvia. To assess the risk of B.canis infection for dogs in Baltic countries we examined current distribution and habitat preference of Dermacentor reticulatus ticks in Baltic States and investigated the presence and prevalence of B.canis in D.reticulatus in different areas of Lithuania and Latvia. Questing ticks were collected from 79 locations in Lithuania and 27 locations in Latvia during 2013?2014 years. Tick sampling was conducted in different habitats: on meadows, in river valleys, in mixed deciduous and coniferous forest, ecotones between forested and grassy areas, city parks and near agricultural field. Relative tick abundance at locality was estimated. A total of 2606 D.reticulatus have been found in 58 sites in Lithuania, and 183 in 12 locations in Latvia where before haven't been registered. New areas with D. reticulatus occurrence in Latvia were detected by at list 50 km further north from northern Lithuanian border. All sites with high abundance of D. reticulatus ticks were localized in open areas close to water basin and mixed forest. The highest relative mean abundance was detected in ecotones. A total of 1389 D. reticulatus ticks from 25 locations of Lithuania were screening for Babesia parasites. The overall infection rate was 1.15% (16/1389) and ranged in different locations from 0 to 6.7%. Babesia positive ticks were found in western, north-western, central, and north-eastern parts of Lithuania. Our study providing evidence that D.reticulatus has extended its range in the surroundings of its former habitats in Baltic countries and to confirm geographical expansion of B. canis in Europe and risk of canine babesiosis for dogs in Baltic countries.
The study was partially supported by the Research Council of Lithuania (grant MIP-053/2013).

Epidemiological investigation on the spread of Phebotomus-Borne viruses in Europe

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To monitor the spread of Phebotomus-Borne (PhB)?viruses in Europe, a field sandflies collection was carried out by PhBD-EDENext partners; specimens were sent to ISS-Italy for PhB-viruses detection. During 2011-2013, 7646 sandflies were collected from 21 sites by 7 partners (from west to east: IHMT-Portugal, ISCIII-Spain, IRD-France, ISS-Italy, EUMS-Turkey, HUESRL-Turkey and NCDC-Georgia). Collections included 4 monospecific sites for P.perniciosus, P. perfiliewi, P.ariasi and P.papatasi, and 3 multispecific-sites for P.kandelakii, P.sergenti, P.tobbi and P.(Larroussius) spp. A total of 15 PhB?viruses were isolated from 3913 sandflies. In 2011, no isolates from Georgia (P.kandelakii 65%), Portugal and Italy (P.perniciosus and P.perfiliewi) were obtained. In 2012, from 900 P.perfiliewi collected in Fermo, Marche region, Italy, 7 PhB-viruses isolates were obtained. By phylogenetic and serologic analysis, 6 of them appeared to be a novel Phlebovirus, named Fermo virus, (Naples serocomplex), and one was identified as Toscana virus (TOSV)-lineage A. Minimum Field Infection Rates (MFIR) for the novel and TOSV were 0.67 and 0.10, respectively. In 2012, 4 PhB-viruses were obtained from 366 P. perniciosus collected in Fuenlabrada, Madrid, Spain and 2 PhB-viruses from 244 phlebotomine specimens [P.tobbi 49% and P.(Larroussius) spp. 26%)] collected in Cukurova plain, Turkey. The phylogenetic analysis of the Spanish isolates showed the presence of 3 Arbia virus strains, Salehabad group, and one TOSV-lineage B circulating in Spain. MFIR for the Arbia and TOSV were 0.82 and 0.28, respectively. The molecular and serological analysis of the 2 Turkish isolates identified the presence of Arbia virus in Cukurova plain, MFIR being 0.82. No isolation was obtained from P.ariasi, P.sergenti and Phlebotomus spp collected in France, Georgia and Portugal, respectively. In 2013, ISS received specimens by Georgia (P.kandelakii), Portugal (Phlebotomus sp.) and Spain (P.perniciosus). Two Ph-B viruses isolates were obtained from 597 Spanish specimens. Molecular and serological identification confirmed the presence of TOSV-lineage B in Fuenlabrada focus and showed the detection of a Phlebovirus belonging to the Naples serocomplex, clustering with Granada, Massilia and Arrabida viruses. MFIR for both isolates was 0.16.

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Poster 3.4.

NOT PRESENTED

The importance of conjunctival swab sampling in early diagnosis of canine Leishmaniasis: a two year follow-up study in the Çukurova Plain, Turkey

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The diagnosis of canine leishmaniasis (CanL) in symptomatic and asymptomatic dogs is very important and problematic public health aspect in Turkey. A longitudinal study was carried out on community dogs in the selected villages where cutaneous (CL) and visceral (VL) leishmaniasis are endemic to determine the prevalence of CanL in the area and to evaluate the diagnostic performance of non-invasive conjunctival swab nested PCR (CS n-PCR) by four consecutive samplings between July 2011 and June 2013. Indirect Fluorescent Antibody Test (IFAT) was performed for comparison. The consecutive blood and CS samples from representative number of dogs (80-100 dogs/each sampling) were collected in the 6 villages located in Cukurova Plain of Turkey. Lymph node aspiration samples were also taken from the dogs having lymphadenopathy.

In four samplings, 338 (132 of them were sampled two/four times) blood and 676 conjunctival samples were collected but only 14 dogs could be sampled four times. A total of 10 (2.95%) seronegative dogs in IFAT but positive in CS n-PCR turned to seropositive in subsequent samplings. A total of 11 Leishmania strains among 33 lymph aspiration samples were isolated, and 9 and 2 of them were identified as Leishmania infantum MON1 and MON98 by MLEE analysis, respectively. The average CanL ratio was found to be 27.18% (between 7.14% and 39.13%) by IFAT and 41.74% (between 29.03% and 46.66%) by CS n-PCR in the whole study area. A group of seropositive dogs with negative CS n-PCR results was also detected in the study. The findings showed that (i) although circulation of the dogs is very high in the villages in Cukurova Plain, the disease prevalence is high and stable, (ii) CanL can be diagnosed in early stage by CS n-PCR, (iii) the natural infection rate is at least around 3% within two years, (iv) and the parasite strains isolated from dogs, human and sand flies needs to be compared.

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Prevalence of Anaplasma phagocytophilum in ticks and rodents along an urban - natural gradient in SW Slovakia

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Granulocytic anaplasmosis caused by the obligate intracellular bacterium Anaplasma phagocytophilum is an emerging zoonotic disease with a natural enzootic cycle involving ixodid ticks and vertebrate hosts. Prevalence of A. phagocytophilum was monitored in questing Ixodes ricinus and in rodents and rodent-attached ticks in an urban-suburban habitat (Bratislava forest park) and natural habitat (non-fragmented forest at Fugelka) in SW Slovakia during 2011-2013. Estimates of wildlife densities in the sites were taken from hunter`s yearly reports. A. phagocytophilum was detected by RealTime-PCR targetting the msp2 gene. Overall prevalence of the bacterium in questing ticks significantly differed between the two habitats ? it was higher in the urban-suburban site (7.2%; 153/2117 ticks) compared to the natural site (3.1%; 71/2257 ticks). Significant differences in prevalence of the bacterium in questing ticks were also found between years at both sites. In Bratislava, the lowest prevalence was observed in 2013 (4.3%; 95%CI: 2.9-5.7) and the highest in 2012 (9.3%; 95%CI: 6.2-12.7), whereas at Fugelka the lowest prevalence was in 2011 (2.4%; 95%CI: 1.7-3.2) and the highest in 2013 (4.8%; 95%CI: 2.7-6.8). A total of 416 rodents belonging to five species were trapped in the study sites. Apodemus flavicollis and Clethrionomys glareolus prevailed. Rodents were infested with I. ricinus (94.1-99.5%) and Haemaphysalis concinna (0.5-5.9%). Only 0.2% of rodent spleen samples and 1.1% of host-attached ticks were infected with A. phagocytophilum. Prevalence of A. phagocytophilum in questing I. ricinus correlated neither with relative abundance of ticks nor with abundance of potential reservoir hosts (roe deer, red deer, fallow deer) in the area. The infection rate in questing ticks in our study is consistent with previous studies on A. phagocytophilum prevalence in Slovakia (1.1%-8%). Considering studies on circulation of A. phagocytophilum between Ixodes trianguliceps and rodents in Europe, the low infection rate in rodents and the fact that no I. trianguliceps has been found in the study sites we can summarize that rodents are not reservoirs of A. phagocytophilum in these study sites. Molecular identification of A. phagocytophilum strains is in progress.

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Several Anaplasma phagocytophilum epidemiological cycles in France revealed by sequencing

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Molecular epidemiology represents a powerful approach to elucidate the complex epidemiological cycles of multi-host pathogens, such as Anaplasma phagocytophilum. A. phagocytophilum is a tick-borne bacterium that affects a wide range of wild and domesticated animals. Our objective was to investigate the existence of different transmission cycles among both wild and domestic hosts. First, we characterized genetic diversity of A. phagocytophilum in populations of French cattle and compared the observed genotypes with those found in horses, dogs, and roe deer to determine whether genotypes of A. phagocytophilum are shared among different hosts. We sampled 104 cattle, 13 horses, 3 dogs, and 40 roe deer. We used multilocus sequence analysis on nine loci (ankA, msp4, groESL, typA, pled, gyrA, recG, polA, and an intergenic region) to characterize the genotypes of A. phagocytophilum that were present. Phylogenic analysis revealed three genetic clusters of bacterial variants in domesticated animals. The two main clusters included 98% of the bacterial genotypes found in cattle, which were only distantly related to those in roe deer. One cluster comprised genotypes only found in cattle, while

the second contained genotypes shared by cattle, horses, and dogs. The third contained all roe deer genotypes and three cattle genotypes. These results suggest that roe deer do not contribute to the spread of A. phagocytophilum in cattle in France. Then, we investigated A. phagocytophilum genetic diversity associated with ticks (Ixodes ricinus), roe deer or rodents that were sampled in the same locality to determine if roe deer were the main reservoir host. We detected 56/75 positive roe deer, 35/1837 positive nymphs and no positive rodent (0/218 Apodemus sp., 0/44 Myodes glareolus). We sequenced three loci (ankA, msp4 and groEL) in all infected deer and in 22 infected ticks by 454 sequencing to take into account co-infections. We observed many co-infected samples and very few genotypes are found in both ticks and roe deer. These results suggest that at least one other reservoir host is present in the site. Our study highlights the presence of several clusters of A. phagocytophilum genotypes that could correspond to distinct epidemiological cycles, potentially involving different reservoir hosts.

Rickettsia spp. and Coxiella burnetii in ticks and rodents in urban/suburban and natural habitats of Southwestern Slovakia

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Rickettsia spp. of the spotted fever group are obligate intracellular bacteria transmitted by ixodid ticks. Currently 26 Rickettsia species with validated and published names have been reported, some of them are pathogens of humans. Coxiella burnetii is the causative agent of Q fever, a worldwide zoonotic disease. Domestic ruminants are the most important recognised reservoirs of C. burnetii; they are often asymptomatic carriers, but the agent may also cause abortion in these animals. Ticks may also act as reservoirs of C. burnetii in nature. This study investigated infections of questing ticks, feeding ticks removed from rodents, and infections of rodents with Rickettsia spp. and C. burnetii in urban/suburban, and forest habitats in Southwestern Slovakia. Totally, 3289 Ixodes ricinus (2412 nymphs, 877 adults) and 67 Haemaphysalis concinna (46 nymphs, 21 adults) ticks were collected by blanket-dragging the vegetation in Bratislava and Fúge?ka, respectively from early May 2011 to October 2013. A total of 416 rodents (Apodemus flavicollis, A. sylvaticus, Microtus arvalis, M. subterraneus, Clethrionomys glareolus) were live-trapped. Four hundred and fourty-one I. ricinus and 37 H. concinna ticks were removed from rodents.
Rickettsia spp. was detected in extracted DNA using PCR-based methods amplifying partial regions of the gltA and 23S rRNA genes. C. burnetii was detected by PCR-amplifying a partial region of the com1 gene. Rickettsia spp. was identified in questing (7.3% in Bratislava, 7.1% in Fúge?ka) and feeding (8.3% in Bratislava, 7.3% in Fúge?ka) I. ricinus ticks and in one spleen of A. flavicollis. Sequencing showed infections with R. helvetica, R. monacensis and unidentified Rickettsia spp. C. burnetii was found only in two spleen samples of C. glareolus. The results showed circulation of pathogenic rickettsial species among I. ricinus as a vector in urban/suburban and natural habitats in Slovakia. Rodents seem to act as carriers of Rickettsia-positive ticks, but not as reservoirs of SFG Rickettsia.
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High virulence of Leishmania infantum isolates from the human visceral leishmaniasis outbreak in Madrid, Spain, assessed by a natural transmission model

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A leishmaniasis outbreak is occurring in south western Madrid region, Spain, being the parasite and vector involved Leishmania infantum and Phlebotomus perniciosus respectively. The main goal was to perform natural transmissions in the hamster model using either a L. infantum isolate from P. perniciosus collected during the entomological survey conducted in 2012 close the outbreak area (POL2FL6: IPER/ES/2012/POL2FL6) and a well characterized L. infantum strain (JPCM5: MCAN/ES/98/LLM-877). Colonized P. perniciosus were fed on a mixture of defibrinated rabbit blood and cultured L. infantum promastigotes (2.5x107) through a 3-day chicken skin membrane. Infected sand flies were subsequently fed on hamsters to initiate parasite transmission. In addition, other hamsters were intraperitoneally (IP) inoculated with 1x107 promastigotes. Hamster infections were monitored by clinical examination, serology, culture and parasite burden by limit dilution assay, Giemsa-stained imprints, and PCR for parasite DNA detection in spleen and liver tissues. Additionally, histopathology of several organs and xenodiagnoses were performed. Transmission of L. infantum was achieved with JPCM5 strain and POL2FL6 isolate both by P. perniciosus infective bites or IP route. However, high virulence of POL2FL6 strain was highlighted by the worse clinical outcome of disease, higher parasite detection in spleen and liver, higher parasitic loads and positivity of Leishmania serology when compared with hamsters infected with JPCM5 strain. Histopathology studies confirmed the wide spread of POL2FL6 parasites to salivary glands, stomach, intestine, mesenteric glands, lung, kidney, adrenal glands, liver, spleen, brain, bone marrow, skin and reproductive organs of hamster. Transmission by bite of POL2FL6 isolate generated a slower progression of clinical disease than IP infection, but both groups were infective to P. perniciosus by xenodiagnosis as soon as two months post-infection. Conversely, hamsters inoculated with JPCM5 were not infective to sand flies. A visceral leishmaniasis model that mimic the natural conditions of transmission in nature was set and allowed us to compare Leishmania strain virulence and infectivity to sand flies. The virulence of isolates that are circulating in the focus has been demonstrated. These findings would contribute to a better understanding of the outbreak epidemiology.
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Validation of the diagnostic performance of conjunctival swab Leishmania nested-PCR in dogs from different settings of Mediterranean canine leishmaniasis

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Recent studies have established that conjunctival swab (CS) molecular analysis can be useful for Canine Leishmaniasis (CanL) diagnosis in naturally Leishmania-exposed dogs because both sensitive and noninvasive. These findings suggested a careful evaluation of the diagnostic performance of CS nested(n)-PCR analysis during EDENext project. A comparative performance was evaluated by performing a larger validation study in dog groups from Mediterranean endemic settings of Italy, Portugal, Turkey, and (analyses still ongoing) Georgia. In Italy, CS n-PCR was evaluated as an early diagnostic marker at different stages of infection in a heterogeneous canine population (273 dogs) living in 4 CanL endemic areas of central Italy. CS n-PCR showed the best relative performance (76.4%), with a high concordance to standard IFAT serology (k 0.75). The highest positivity rates were found in asymptomatic infected dogs (84.2%) however, the sensitivity was not associated with the presence of clinical signs. In Portugal, 320 randomly selected dogs from 4 kennels of the Metropolitan Lisbon Region were enrolled during October 2011. ELISA prevalence was 13.4%, whereas CS n-PCR prevalence was 35.6%. CS n-PCR scored the most sensitive assay in asymptomatic animals (36.6%). In Turkey, 100 dogs were randomly chosen in 6 villages of Cukurova Plain; 4 samplings were done before and after transmission seasons 2011 and 2013. The overall concordance between IFAT and CS n-PCR tests was 65%. Moreover, a group of seronegative asymptomatic dogs showed CS n-PCR positivity and they were found seropositives in subsequent samplings. Taken together, our findings can be summarized as follows: i) CS n-PCR positivity appears to occur at high rates in dogs living in settings with elevated CanL prevalence; ii) CS n-PCR seems to be effective in early detecting Leishmania contacts in dogs exposed to parasite transmission before seroconversion or specific clinical signs; iii) CS n-PCR showed the best performance in comparison with other noninvasive tests; iv) CS n-PCR performance applied on random canine populations did not differ significantly from standard serological evaluation, but in asymptomatic dogs CS n-PCR resulted the most sensitive assay.
This study was carried out in the frame of FP7-UE EDENext collaborative project, Contract Number: 261504.

The prevalence of Ljungan virus and human parechovirus specific antibodies in early childhood

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Ljungan viruses (LV) are picornaviruses belonging to the Parechovirus genus. The virus was first isolated from bank voles in 1990's in Sweden. LV infects primarily rodents and its capacity to infect humans and cause disease remains to be determined. Human parechoviruses (HPeV), other members of the Parechovirus genus, are well-known human pathogens. To study seroprevalence during childhood, a panel of plasma and fecal samples were serially collected from 29 children (age range 0 ? 9.1) participating in the Finnish DIPP study was screened and titrated for IgG antibodies against LV-1, LV-2 and HPeV using immunofluorescence assay (IFA). LV-RT-PCR was carried out from fecal samples collected prior and after LV seroconversion.
Out of the 29 children 17 (59%) experienced both LV-1 and LV-2 seroconversions at the mean age of 3.0 (3.04; range 0.6-9.1 years) and 3.0 (2.97; range 0.3-9.1 years), respectively. In four of the cases (24%) LV-1 and LV-2 seroconversions appeared at different time intervals. HPeV seroconversions occurred at the mean of 2.0 (2.07; range 0.3-4.2 years) and LV and HpeV seroconversions occurred at the same time point in seven (41%) cases out of 17 LV seroconverted children. Over 70% of the LV-positive children had seroconverted before the age of 3-4 years (N=12; 71 %). No LV RNA was detected in the fecal samples most likely due to sparse sampling interval.
The different ages of LV and HPeV seroconversions suggests that Finnish children were exposed to distinct LV or antigenically related LV-like viruses causing LV seroconversions. The study showed seroconversions of LV-1 and LV-2 in early childhood distinct from HPeV seroconversion. The data suggests that exposure to LV or an LV-like parechovirus occurs in early childhood, and the epidemiology is likely to involve human-to human transmission chains resembling those of human parechoviruses rather than zoonotic rodent-borne transmissions events.

Diversity and dynamics of Puumala viruses in France

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Puumala hantavirus (PUUV) is the agent of nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS) in Europe. NE incidence presents a high spatial variation throughout France and also at small geographical scales. Understanding this spatial variation is of important concern for establishing risk maps. By contrast with NE, the geographical distribution of the wild reservoir of PUUV, the bank vole, is rather continuous. A missing piece of the puzzle is the current distribution of PUUV in France which has been overlooked until now and remains poorly understood. Recent investigations reported a positive correlation between PUUV positive bank voles and NE incidence in NE endemic areas. High PUUV seroprevalence has yet been observed in areas with no reported human cases (peri-endemic and non-endemic areas). In this work, we investigated the genetic diversity and the genetic structure within and between NE endemic, peri-endemic and non-endemic areas, by focusing on three regions: French Ardennes (endemic and periendemic areas), Franche-Comté (endemic area) and Orléans (non endemic area). Our analyses revealed different sub-lineages of PUUV in France, with significant differences at the genetic level. Phylogenetic analyses showed a strong spatial structure between the three regions, but also at the smaller scale of the French Ardennes. Different sub-lineages of PUUV were circulating very locally within the Northern forest, which is a highly NE endemic area.
Investigations are underway to determine the virological and / or environmental and sociological factors involved in the NE incidence between endemic and non-endemic regions in France in order to reduce the risk of HFRS outbreaks.

Poster 3.13.

NOT PRESENTED

Natural Leishmania infection of Phlebotomus sergenti (Diptera: Phlebotominae) in a highly endemic focus of cutaneous leishmaniasis in Sanliurfa, Turkey

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Sandflies (Diptera: Phlebotominae) were surveyed for Leishmania in various villages of Sanli urfa in Southeast Turkey. A total of 424 sandflies were collected by CDC light traps. Phlebotomus sergenti Parrot (49,6 %) and P. papatasi (Scopoli) (48,1%) were the most abundant species, followed by P. alexandri Sinton (1,05 %), P. perfiliewi Parrot (0,4 %), Adlerius sp. (0.2 %) and Sergentomyia theodori Parrot (0,4 %). A total of 196 female sand flies were grouped in pools of 10 specimens each and 4 pools of P. sergenti were found positive for Leishmania DNA, detected by using ITS-1 primer set. This is the first molecular detection and identification of Leishmania tropica within naturally infected Phlebotomus sergenti from the most important focus of anthroponotic cutaneous leishmaniasis in Turkey. The high frequency of P. sergenti together with natural infection by the parasite make this species the probable vector of L. tropica in Sanliurfa.

Seroprevalence of four distinct sandfly-borne phleboviruses (Toscana, Sicilian, Arbia and Adana) in dogs from Greece and Cyprus using neutralisation assay reveals massive circulation of Sicilian virus

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OBJECTIVES: The aim of this study was to use sera, collected in dogs from Greece and Cyprus, as sentinel for the circulation of sandfly-borne phleboviruses belonging to the 3 recognized antigenic complexes, of which 2 are known to infect humans and cause disease.

INTRODUCTION: Sandflies (Phlebotomus spp, Sergentomyia spp) are present in Greece and Cyprus, where sandfly fever outbreaks have been described since 1937 (Tesh and Papaevangelou, 1977). Before 1990, the presence of sandfly fever viruses was established by plaque reduction neutralisation test with rates ranging from 8.5% to 24.7% depending on the virus and the geographic area (Tesh et al 1976, Antoniadis et al., 1990). The presence of Toscana virus was suggested in serological studies since 1990, but not formally established by virus isolation or sequence-based evidence until very recently (Anagnostou et al 2014). Other phleboviruses (Corfu and Adria) were identified in Greece.

METHODS: A total of 1,692 sera were collected from dogs living in Greece (n=1,250) and Cyprus (n=442), which were geolocalized for mapping. Sera were tested for neutralising antibodies (NT Ab) against 4 phleboviruses (Toscana, Sicilian, Arbia and Adana); the Greek sera were not tested for Adana because of limited quantity. Neutralisation (NT) assay was done using two-fold serial serum dilutions (1:20 to 1:160), and 1000 TCID50 of each virus strain.

RESULTS and DISCUSSION:
In Cyprus, NT Abs were found in 8.4% for Toscana, 5.4% for Arbia, 16.3% for Adana, and 60.2% for Sicilian. In Greece, NT Abs were found in 4.4% for Toscana, 2.6% for Arbia, and 72% for Sicilian Maps reflecting the geographic distribution of NT Abs are presented. Dogs can be infected with sandfly-borne phleboviruses and used as sentinels for epidemiological studies (Sakhria et al, 2014). There is no cross-reactivity between the 4 tested viruses Phleboviruses belonging to the 3 antigenic complexes are circulating in Greece and Cyprus Toscana virus is present in Greece and Cyprus; human patients with fever and neuroinvasive diseases should be tested for them Sicilian virus is massively circulating in both countries and merits to be investigated in febrile human patients.

Life-long shedding of Puumala hantavirus in wild bank voles

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The knowledge of viral shedding patterns and viremia in the reservoir host species is a key factor in assessing the human risk of zoonotic viruses. The shedding of hantaviruses (family Bunyaviridae) by their host rodents has widely been studied experimentally, but rarely in natural settings. Here we present the dynamics of Puumala (PUUV) hantavirus shedding and viremia in naturally infected, wild bank voles (Myodes glareolus). In a monthly capture-mark-recapture study, we analyzed 18 bank voles for the presence and relative quantity of PUUV RNA in the excreta and blood from 2 months before up to 8 months after seroconversion.
The proportion of animals shedding PUUV RNA in saliva, urine, and feces peaked during the first month after seroconversion, but continued throughout the study period with only a slight decline. The quantity of shed PUUV in RT-PCR positive excreta was constant over time. In blood, PUUV RNA was present for up to 7 months but both the probability of viremia and the virus load declined by time.
Our findings contradict the current view of a decline in virus shedding after the acute phase and a short viremic period in hantavirus infection ? an assumption widely adopted into current epidemiological models. We suggest the life-long shedding as a means of hantaviruses to survive over host population bottlenecks, and to disperse in fragmented habitats where local host and/or virus populations face temporary extinctions. Our results indicate that the kinetics of pathogens in wild hosts may considerably differ from those observed in laboratory settings.

Poster 3.17.

NOT PRESENTED

Newly emerging tick-borne infections, their prevalence and genetic variability in northern Italy

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A number of recently identified Ixodes ricinus transmitted pathogens have been studied and their potential for future further emergence evaluated in northern Italy. Our study was carried out in the Valle dei Laghi (northeastern Italian Alps) where a total of 2033 questing I. ricinus ticks (1706 nymphs and 327 adults) were collected by dragging from 2011 to 2013. In addition, feeding ticks were collected from humans and from hunted or live-trapped wildlife animals. Of the ticks collected from hosts, 617 were larvae collected from wild ungulates, birds and rodents. Ticks were than screened for the detection of the following pathogens: Anaplasma phagocytophilum, Babesia spp., Borrelia spp., Rickettsia spp and Neoehrlichia mikurensis. Moreover, we studied genetic variability of A. phagocytophilum, B. burgdorferi s.l. and its ecological associations with hosts and vectors in the area. In ticks collected by dragging we found an high prevalence of Borrelia sp (22,18%) while Rickettsia sp and Anaplasma phagocytophilum were detected with a prevalence of 9,19 % and 1,86 % respectively. A high degree of variability was observed among the pathogens detected in the larval ticks. Larvae from wild ungulates were infected with A. phagocytophilum (5.3%), B. venatorum (0.5%), R. helvetica (7.9%) and R. monacesis (2.6%). Larvae from rodents were infected with B. venatorum (2.3%), B. afzelii (7.9%), R. helvetica (2.6%), R. slovacca (0.3%), R. monacensis (2.3%), N. mikurensis (5.8%) and larvae from birds were infected with B. capreoli (1%), B. garinii (18.7%), B. afzelii (1.1%), B. valaisiana (14.3%), B. turdi (6.6%) and R. helvetica (5.5%). Additionally, we compared genetic variability of different A. phagocytophilum strains identified in feeding ticks collected from roe deer, rodents, birds, sheep's, dogs and humans. Our results showed two distinguished enzootic cycles and provide new insights into the ecology of this pathogen in Europe compared to what observed in USA.

PCR prevalence of rodent-borne Ljungan virus across Europe

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Since its discovery in Swedish bank voles (Myodes glareolus) in the 1990s (Niklasson et al., 1999), interest in the Ljungan (picorna-)virus (LV) has grown as a result of a suggested association of the virus with some human pathologies, and because serological and neutralization tests have confirmed that humans are exposed to LV (38% in Jääskeläinen et al., 2013). LV infection induces fetal malformations and diabetes-like symptoms in laboratory mice, and since LV also causes disease in some wild rodents, it may have a role in small mammal cycles. Hence, LV epidemiology is potentially of global interest. Although LV has been noted in single populations of several rodent species in Denmark, the USA, Germany, Italy, Finland and the UK (Johansson et al., 2003; Hauffe et al., 2010; Kallies, 2010 and references therein; Jääskeläinen et al., 2013; Salisbury et al., 2014), this is the first systematic screening of LV across the EU, especially in the bank vole, but also in other small mammals, including shrews and commensal species. In all, 15 different species from nine European countries were sampled as part of the EU FP7 project EDENext. Using an LV-specific RT-PCR method (Donoso-Mantke et al., 2007), 1509 liver samples stored at -80°C were screened for LV, including 831 bank voles. All amplified fragments were sequenced for confirmation. LV-positive samples were found in all countries with significant sample sizes, and in most species, including house mice, but not black rats. Overall PCR prevalence in bank voles was about 16% (range 0-50% per population). We added eight new species to the list of LV hosts, including the red squirrel (Sciurus vulgaris), and a number of voles and shrews. Our study suggests that LV has a wide geographical and host distribution.

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Longevity of Puumala hantavirus in winter conditions in Finland

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In Europe, the most common hantavirus is Puumala virus (PUUV), which causes a mild form of hemorrhagic fever with renal syndrome (HFRS). Hantaviruses are transmitted to humans via inhalation of aerosolized excreta from their rodent reservoir hosts. Intraspecific hantavirus transmission occurs through either direct contact or indirectly via inhalation of virus-contaminated excreta, and infectiousness of PUUV can be retained outside the host for at least two weeks in room temperature. Knowledge of viral transmission patterns and survival of the virions outside the host is critical for assessing the human infection risk of PUUV. Here, we tested the effect of cold temperatures on the longevity of PUUV infectiousness outside the host. The study was performed in outdoor enclosures over approximately four winter months in boreal Europe. The source of the virus (i.e. donor voles) was naturally infected voles originating from two different sites in Central Finland. The infected donor voles were kept in the cages in the enclosures for 12 days, and virus shedding was confirmed by detecting the presence of PUUV RNA in urine samples collected from these voles. Consequently, PUUV-negative recipient voles were placed into the cages and exposed to the virus-contaminated beddings for three days immediately, at four and seven weeks after the removal of the donors. After the exposure, the recipient voles were placed in individual isolation cages, in which the infection was left to develop for two weeks. PUUV-infection status was studied in the recipient voles before and after exposure by detection of antibodies and RNA in blood samples. PUUV variants in each infected recipient were identified by sequencing. PUUV was shown to remain infectious throughout the experimental period of seven weeks, and thus to persist extended periods of time outside the host in northern cold conditions. Intriguingly, the study also suggested that PUUV variants may differ in their virulence, as only few of the variants detected in the donor voles were actually transmitted to recipient voles. Overall, our results provide data to improve epidemiological models for hantaviruses, and emphasize the influence of climatic factors in predicting the risk of zoonotic infections.

Neglected tick-borne pathogens in the Czech Republic - a summary of the EDENext prevalence study

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Ixodid ticks (in Central Europe mainly Ixodes ricinus) present a significant health risk for humans as vectors of pathogens of which the most important are tick-borne encephalitis virus and Borrelia burgdorferi. Nevertheless, this vector species harbours also neglected or emerging pathogens - Anaplasma phagocytophilum, Rickettsia monacensis, R. helvetica, 'Candidatus Neoehrlichia mikurenis' (CNM), Babesia microti, B. divergens and B. venatorum (EU1). Within the scope of EDENext project we aimed at determination of the minimum prevalence rate of A. phagocytophilum, Rickettsia spp., CNM and Babesia spp. in host-seeking Ixodes ricinus ticks in three different South-Moravian ecosystems: natural (game preserve in Pohansko), urban (castle park in Valtice) and agricultural (pastureland in Suchovské mlýny) by using molecular techniques. A total of 2473 host-seeking I. ricinus ticks (1817 nymphs, 310 females and 346 males) were collected by flagging low vegetation from 2011 through 2014. All ticks were analyzed individually for the presence of selected pathogens using TaqMan Real-time PCR and conventional PCR according to approved EDENext protocol. Here we summarize overall prevalence data in three ecosystems: NATURAL (2011 - A. phagocytophilum 2.4%, Rickettsia spp. 4.0%, CNM 2.4%, Babesia spp. 1.6%; 2012 - A. phagocytophilum 0.0 %, Rickettsia spp. 3.8%, CNM 2.5%, Babesia spp. 0.0%; 2013 - A. phagocytophilum 2.2%, Rickettsia spp. 5.6%, CNM 11.6%, Babesia spp. 0.9%), URBAN (2011 - A. phagocytophilum 3.0 %, Rickettsia spp. 3.8%, CNM 0.8%, Babesia spp. 1.9%; 2012 - A. phagocytophilum 6.0%, Rickettsia spp. 9.3%, CNM 2.7%, Babesia spp. 6.6%; 2013 - A. phagocytophilum 3.3%, Rickettsia spp. 4.9%, CNM 2.6%, Babesia spp. 0.7%) and AGRICULTURAL (2011 - A. phagocytophilum 2.1 %, Rickettsia spp. 7.3%, CNM 6.9%, Babesia spp. 2.6%; 2012 - A. phagocytophilum 2.5%, Rickettsia spp. 8.4%, CNM 1.7%, Babesia spp. 4.2%; 2013 - A. phagocytophilum 0.5%, Rickettsia spp. 4.7%, CNM 4.2%, Babesia spp. 4.2%). Results for 2014 are being finalized. Sequencing of positive PCR products confirmed B. microti, B. venatorum and B. capreoli in unfed ticks. This work has contributed to the knowledge of ecology and diversity of neglected tick-borne pathogens in various ecosystems. Results might help to complete the map of endemic sites which pose public health risk.

Poster 3.22.

NOT PRESENTED

Susceptibility of domestic pigeon (Columba livia L.) to West Nile virus lineage 2 infection

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A lineage 2 strain of West Nile virus (WNV) emerged in Hungary in 2004. It became resident in central Europe and in the east-Mediterranean region, and caused outbreaks of West Nile encephalitis in humans and in animals. WNV is usually transmitted between natural avian hosts by mosquito vectors. However, fatal WNV infections were frequently observed in birds of prey, therefore an alternative route of infection via consumption of infected prey carcasses were hypothesized. Because WNV-affected captive raptors were frequently fed with pigeons, this species was suspected as a potential source of infection.
Commercially reared homing pigeons (rock dove, Columba livia L.) were quarantined in mosquito-free facilities for two weeks. Seronegative birds were placed in BSL-3 animal house facilities and were experimentally infected with a field isolate of WNV (strain 578/10, horse, Hungary, 2010). In a first experiment pigeons were infected with 1 to 10.000 TCID50 of WNV and were observed for 21 days. Oral and faecal samples were collected individually every day, blood samples were collected weekly. Animals were put down on day 21, carcasses were necropsied and organ samples were collected. In a second experiment pigeons were infected with 1.000 TCID50 of WNV. On every second day oral and faecal swabs were collected, two birds were sacrificed, necropsied and organ samples were collected.
Within both experiments all pigeons remained clinically healthy. Birds infected with 1.000? 10.000 TCID50 of WNV became seropositive on day 7; those infected with 100 TCID50 became seropositive on day 14; while the ones infected with 1?10 TCID50 remained seronegative. Birds infected with higher doses of WNV have been shedding the virus by oral discharges and / or faeces between days four and twelve after infection. Virus RNA was detected in the visceral organs of infected birds even 21 days after infection.
The results of the study indicate that pigeons are susceptible hosts of the lineage 2 WNV strain which is circulating in central Europe. The birds carried the virus for at least three weeks. Therefore they might be sources of non-vectorial transmission to predatory birds.
This study was supported by the EU grant FP7-261504 EDENext.

Puumala hantavirus infection alters the odour attractiveness of its reservoir host

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The random-mixing assumptions of many parasite-transmission models are challenged if healthy individuals can alter their behaviour to reduce their risk of infection. Some pathogens reduce the attractiveness of their hosts' excretions, for example, potentially altering contact rates and thus the predicted force of infection for pathogens transmissible by contact with excretions. For bank voles (Myodes glareolus), contact with contaminated urine is an important route of transmission for Puumala hantavirus (PUUV), however, it is not known whether PUUV infection changes the voles' urinary odours or their attractiveness. We used a Y-maze to test whether PUUV infection alters the attractiveness of male bank voles' urine. We presented wild-caught PUUV-free male and female bank voles with PUUV-infected conspecific urine, uninfected urine and a water control, and measured the relative and absolute latency to first visit, number of visits, and total time bank voles spent investigating each treatment over 30 min. PUUV infection significantly altered the bank voles' initial response to conspecific urine, with fewer visits and less time spent close to infected urine relative to uninfected urine, and less total time spent near the infected urine than the uninfected urine or control. These strong preferences weakened over the 30-min trial, however, partly due to a general decline in male activity, and there were no absolute differences between the treatments overall. This suggests that PUUV infection does change the attractiveness of bank vole urine to conspecifics, and we discuss the implications of these results for random-mixing assumptions.

Poster 3.25.

NOT PRESENTED

Selective predation on hantavirus-infected voles by owls and confounding effects from landscape properties?

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Ostfeld and Holt (2004) suggested that through reducing disease host densities or selectively taking infected individuals from the population, predators may protect human health. We explicitly tested whether Tengmalm's owl (Aegolius funereus) selectively preys on hantavirus-infected individuals of its staple prey: the bank vole (Myodes glareolus), the host of Puumala hantavirus which causes a form of hemorrhagic fever in humans. We compared sero-prevalence in bank voles cached by owls in nest boxes to sero-prevalence in voles we trapped around each nest box and refuted the hypothesis. For further interpretation and an extended analysis of the data, we investigated whether forest landscape structure could account for the observed patterns in sero-prevalence. Our results suggest a complex relationship between zoonotic disease prevalence in hosts, predators, and landscape structure that offers great future research potential to shed further light on the role of predators for human health.

The role of birds in the natural cycle of Rickettsia spp. and Coxiella burnetii in Slovakia

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Ixodid ticks (Acarina: Ixodidae) are known as primary vectors of many pathogens causing diseases of humans and domestic animals. Ixodes ricinus tick is a common ectoparasite in Europe and birds are often hosts of subadult stages. From 2012 to 2013, 225 birds belonging to 36 species were caught and examined for ticks in two different study areas of Slovakia, in Bratislava (forest suburban habitat) and Prievidza (forest-steppe natural habitat). A total of 509 specimens of I. ricinus were collected (429 larvae, 79 nymphs and one female). Altogether 30% of bird species were infested by ticks, some birds carried more than one tick. The most infested bird species was the Song Trush Turdus philomelos (3 infested/3 examined) and Great Tit Parus major (30 infested/37 examined). Each tick was analyzed individually for the presence of Rickettsia spp. and Coxiella burnetii by PCR-based method. Totally, 6.6% and 2.8% of ticks were infected with Rickettsia spp. and C. burnetii, respectively. Bird species Parus major was carrying the most frequently Rickettsia - and C. burnetii - infected ticks. Rickettsia helvetica was predominantly detected in ticks (5.9%) whereas R. monacensis (0.5%) was only sporadically detected. Rickettsia spp. was detected in 8.9% and R. helvetica in 4.2% of bird blood samples. C. burnetii wasn't detected in any of examined blood samples. Our study highlights the role of birds in the natural cycle of Rickettsia spp. that are of medical and veterinary relevance. According to infection detected in ticks feeding on birds, infection detected in blood samples and our previous results we consider birds as carriers of infected ticks with a role in the geographical distribution and maintenance of Rickettsia spp. in nature.
Acknowledgements: The study was supported by VEGA 2/0061/13, FP7 project EDENext (No. 261504), and SRDA-0280-12.

Circulation of Rickettsia spp. and Coxiella burnetii in central part of Slovakia

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Geographic distribution and activity of infected ticks are important for maintaining in the epidemiology of tick-borne rickettsioses. Humans are accidental hosts that become infected when ticks containing virulent rickettsiae in their salivary glands take blood meal and inject the rickettsiae into the feeding site. The aim of this study was to investigate presence of rickettsiae and Coxiella burnetii in ticks, rodents and birds in natural forest habitat in the middle part of Slovakia. Rodent trapping during yielded 57 rodents (45 Apodemus flavicollis and 12 Myodes glareolus), which were infested with total of 92 ticks. From 167 birds of 35 species caught were collected 95 ticks. Only two species of ticks were found on hosts - Ixodes ricinus (all birds and 56 rodents) and Haemaphysalis concinna (1 larva on one A. flavicollis). By dragging of white blanket over vegetation were 605 ticks of five species (323 I. ricinus, 181 Dermacentor marginatus, 95 H. concinna, 5 H. inermis and 1 nymph Rhipicephalus truncatus) collected. Rickettsial infection was determined in 7.9% (48/605) in questing ticks (39 D. marginatus, 9 I. ricinus ticks). The presence of Rickettsia spp. was confirmed also in 6.5% (6/92) ticks from rodents, 8.4% (8/95) ticks from birds, 9.6% (16/167) bird blood samples and none rodents tissue samples. DNA of 4 different pathogenic tick-borne species, R. helvetica, R. monacensis, R. slovaca, R. raoultii, and to this time unidentified species of Rickettsia were identified. C. burnetii was not detected in tested samples. Our results confirmed circulation of rickettsiae in model study area.
Acknowledgements: The study was supported by VEGA 2/0061/13, FP7 project EDENext (No. 261504), and SRDA-0280-12.

Transmission dynamics of Borrelia bacteria in a bird tick community

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We examined the Borrelia burgdorferi s.l. circulation in a tick community consisting of three species (Ixodes ricinus, I. frontalis, I. arboricola) with contrasting ecologies, but sharing a common host: the great tit (Parus major), one of the most common birds of European gardens and woodlands.
Field data show that the birds hosted Borrelia-infected larvae of both I. frontalis and I. ricinus, indicating the facilitation of Borrelia transmission. The low, but significant numbers of Borrelia in unfed I. arboricola ticks collected from bird nest boxes, provide the first evidence that it is competent in maintaining Borrelia over long periods of time. Aside from the known avian genospecies (B. garinii and B. valaisiana), several less dominant genospecies were observed in the three ticks, including B. turdi and mammalian genospecies.
In laboratory experiments, we imitated the natural situation during the bird's post-fledging period, in which Borrelia-naïve juvenile birds are repeatedly exposed to infected I. ricinus nymphs. Birds developed systemic infections of the avian genospecies. Although birds showed a very low competence to facilitate the transmission of mammalian genospecies, a low number of birds remained permissive for B. afzelii. Infected birds were able to transmit Borrelia to naïve I. frontalis and I. arboricola individuals, but latter tick species were not able to transmit the bacteria to a new host.
When using the great tit as a host, transmission cycles are driven by I. ricinus, and are not maintained by the ornithophilic ticks (I. frontalis and I. arboricola). However, spill-over of the bacteria from I. ricinus to the ornithophilic ticks seems to be common in the wild. Vector-competence experiments with ornithophilic ticks and other avian hosts (e.g. thrushes and finches) may result in different transmission outcomes.

Session 4

Ecosystems: change

Changing farming, bush encroachment, and itck-borne disease risk in Southern Norway

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Tick-borne diseases are an emerging problem in Norway, and Scandinavia in general, which lies at the margin of the distribution of Ixodes ricinus, the main tick-borne disease vector for humans in Europe. Over the recent decades, the distribution of I. ricinus has been expanding northward and upward in the region, with a corresponding effect on tick-borne disease incidence. This represents a serious public health concern. Previous studies in Europe have indicated that land cover and land use impact tick distribution and abundance, and/or the incidence of tick-borne diseases. Several environmental factors may have contributed to modifications in tick habitat suitability in Southern Norway, including changes in climate and land cover. This study focuses on the encroachment of areas previously sparsely vegetated by bushy vegetation, as one specific factor which may create more favourable conditions for ticks or their hosts. Landscape changes are documented using a long time series of high resolution remotely sensed data, processed to characterize both continuous and categorical land cover features (land cover classes as well as forest fraction). Vegetation changes are compared to three different datasets: human cases of Lyme borreliosis, Anaplasma serologies in sheep, and field-sampled tick data, including serologies.The first two datasets have a temporal depth corresponding to the remotely sensed data. Results indicate that bush encroachment and agricultural land abandonment have favoured tick abundance and disease transmission.

Dynamics of West Nile Virus transmission in wild birds: effects of landscape and mosquito community

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The distribution and incidence of many zoonotic vector-borne diseases has increased dramatically in the last 30 years, mainly as a result of social, demographic and environmental transformation. Although biodiversity has been suggested may affect transmission and incidence of diseases, little is known on how anthropogenic landscape transformation may affect pathogen transmission. Moreover, it is known that virus amplification is affected by the characteristics of the vector species (e.g. feeding preferences), vertebrate host traits (e.g. age and sex) and abiotic factors (e. g. landscape). In this study, we evaluated how anthropogenic modification of landscape may affect biodiversity patterns, including vector community composition and abundance, and how these factors could affect the transmission of the flavivirus West Nile Virus (WNV). WNV is a mosquito-borne pathogen whose enzootic cycle is maintained in birds and mosquitoes, but in certain circumstances spillover events lead to outbreaks affecting humans and horses. We sampled mosquitoes and house sparrows (Passer domesticus) in 45 localities grouped in trio (15 urban, 15 rural and 15 natural) from Southern Spain. We analysed, first by ELISA and then confirmed by virus-neutralization test (VNT), the presence of WNV antibodies in 2609 house sparrows. On average 2% of the house sparrows were positive by ELISA and 0.73% by VNT to WNV. Prevalences were higher in adult birds and larger in natural and rural areas than in urban ones. The results for flavivirus antibodies (ELISA positives) or WNV neutralizing antibodies (VTN positives) were qualitatively similar. Mosquito community composition and the abundance of potential WNV vectors differed among urban, rural and natural areas. Moreover, the prevalence of WNV in sparrows was higher in those localities where the abundance of competent vectors of WNV was higher, especially in the areas characterized by the mosquito species Culex perexiguus, an important enzootic vector. These results support that anthropogenic changes in landscape use, probably through their subsequent effects on biological diversity and/or vector population, may strongly influence the transmission dynamic of WNV and other related flaviviruses.

Impact of landscape connectivity and fragmentation on Puumula virus genetic diversity and structure

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Microevolutionary processes (drift, migration and selection) shaping Puumala hantavirus (PUUV) and its primary host, the bank vole, were studied in a fragmented landscape of Ardennes, France. We monitored the genetic diversity and the spatial distribution of PUUV lineages, together with the spatial genetic structure of neutral and immune genes of its primary host, the bank vole, in relation with forest fragmentation. The genetic diversity of the bank vole was weakly structured in space for both neutral and immune genes, revealing high gene flow and large population sizes of bank voles over the studied area. Vole populations in tiny fragments however evidenced signature of genetic drift and genetic isolation. On the other hand, the genetic diversity of PUUV lineages was highly structured in space and according to the fragmentation of the forest; each forest fragments harbouring one specific lineage. This result suggests high genetic drift and low gene flow between PUUV lineages occurring in different forest fragments. Thus, over the same area, the genetic diversity of the virus is mainly shaped by genetic drift and isolation, while the genetic diversity of its host is mainly shaped by gene flow and population exchanges. These contrasted patterns of microevolution have important consequences for the opportunity of coevolution between PUUV and the bank vole in fragmented landscapes.

Canine antibodies against salivary recombinant proteins of Phlebotomus perniciosus: a longitudinal study in an endemic focus of canine leishmaniasis in south Italy

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Canine leishmaniasis (CanL) is a widespread zoonosis caused by the protozoan parasite Leishmania infantum. CanL is endemic in more than 70 countries including Italy, where the main vector is Phlebotomus perniciosus. During blood feeding sand flies deposit into the host skin immunogenic salivary proteins which elicit a specific antibody response. These anti-saliva antibodies enable to estimate the host exposure to sand flies and also the risk for Leishmania infections. However, the use of whole salivary antigens has several limitations and therefore recombinant salivary proteins have been produced and tested as a tool to replace whole salivary gland lysates. In this work, we selected the two most antigenic P. perniciosus salivary proteins, 43 kDa yellow protein (rSP03B) and 35.5 kDa apyrase (rSP01) and used them in a longitudinal field study. Sera from dogs naturally exposed to P. perniciosus over two years in a site endemic for CanL were tested by ELISA for the IgG antibodies against whole saliva, single salivary recombinant protein (rSP03B) and a combination of two salivary recombinant proteins (rSP03B+ rSP01). Dogs were also tested for L. infantum positivity by serology, culture and PCR and classified as Leishmania exposed, sub-patently or actively infected. We found a significant association between active CanL infection and the anti-P. perniciosus IgG amount, which suggests that ELISA test based on antibodies against sand fly salivary proteins could be a useful indication of the risk of Leishmania infection. Importantly, canine antibody response against recombinant salivary proteins was highly correlated with antibody response against whole saliva. The kinetics of antibody response showed for both, whole saliva and rSP03B, a similar pattern and was clearly related to the seasonal abundance of P. perniciosus. In conclusion, these results suggest that P. perniciosus rSP03B protein is a valid alternative to whole saliva and could be used in large-scale serological studies. This novel method could be a practical and economically-sound tool to detect host exposure to sand fly and the Leishmania infection risk. This work was supported by EDENext, a collaborative project of 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504

Investigation of spatial and temporal distribution in abundance of Ixodes ricinus and prevalence of tick-borne pathogens in different habitat types of Slovakia in frame of the EDENext project

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Ixodes ricinus is a vector of multiple microbial pathogens of medical and veterinary importance. Due to global climatic and socio-economic changes ticks have spread to new areas and became more abundant in urban and peri-urban sites, leading to increased risk of exposure of humans and domestic animals to infected ticks. Spatial and temporal changes in abundance of I. ricinus and their infection with Anaplasma phagocytophilum, Candidatus Neoehrlichia mikurensis, Rickettsia spp., Borrelia burgdorferi s.l. and piroplasms were studied in three sites of Slovakia with different levels of anthropogenic impact. Across each site, questing ticks were dragged with a 1 m2 blanket (3 × 100 m transects plus random samplings) in April-June and September-October 2011-2013. Genomic DNA was extracted from individual ticks by commercial kits and screened by PCR for A. phagocytophilum (real-time PCR amplifying a fragment of msp2 gene), Cand. N. mikurensis (real-time PCR, groEL gene), Rickettsia spp. (PCR, gltA gene, sequencing), B. burgdorferi s.l. (PCR, 5S-23S rRNA gene, RFLP analysis) and Babesia spp. (PCR, 18S rRNA gene, sequencing). A total of 10,181 questing ticks with dominance of I. ricinus (96.64-99.24%) were collected - 4,265, 3,719 and 2,197 in the urban/peri-urban, natural and agricultural site, respectively. Ixodes ricinus relative abundance, subadult/adult ratio as well as representation of individual pathogens and prevalence of infected ticks varied between sites and years. Overall prevalence of A. phagocytophilum-infected ticks ranged from 0.65% (agricultural site) to 10.60% (urban/peri-urban habitat). Prevalence of Rickettsia spp.-infected ticks was highest in the agricultural site (9.10%) and lowest in the urban/peri-urban site (6.36%). Prevalence of Cand. N. mikurensis-infected ticks ranged from 1.09% (urban/peri-urban site) to 2.12% (natural habitat) and prevalence of B. burgdorferi s.l.-infected ticks ranged from 11.61% (urban/peri-urban site) to 18.03% (agricultural site). Overall prevalence of ticks infected with piroplasms was similar in the urban/peri-urban and natural site (1.73% and 1.74%, respectively) and lower in the agricultural site (0.09%). The results indicate that habitat type, host spectrum and local microclimatic conditions affect the abundance of I. ricinus populations and the diversity of tick-borne pathogenic microorganisms.

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Evolution of Leishmania infantum infection rates and host-feeding preferences in Phlebotomus perniciosus of the focus of human leishmaniasis in Madrid region, Spain: 2012 to 2014

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Since 2010 it has increased the number of cases of human leishmaniasis in south western Madrid, Spain where the prevalence of canine leishmaniasis is even lower than the detected in nearby areas. Phlebotomus perniciosus is the vector identified, with densities of around 150 flies/m2 using sticky traps.
The evolution of Leishmania infantum infection rates and blood meal preferences in P. perniciosus along the transmission seasons of 2012, 2013 and 2014 were studied. The entomological survey was performed from May to October in 4 stations located neighbouring the urban area of the focus. Twenty sticky traps (20 x 20 cm) and two CDC light traps placed in every station were monthly used during two consecutive nights (CDC traps replaced every day).
In the seasonal study of 2012 were dissected 735 P. perniciosus females and 18 of them were found infected with L. infantum (2.4%). In 2013 a number of 863 P. perniciosus females were dissected with 57 of them infected, giving a rate of infection of 6.6%. During 2014 were dissected 1604 P. perniciosus females with 41 of them found infected (2.6%). All these collections conducted during the three years mentioned above were only done with CDC traps. All the isolates were characterized by PCR of ITS regions as L. infantum.
Blood meal identifications were conducted in P. perniciosus females collected with both sticky and CDC traps. Amplification of a fragment of 359 bp of vertebrate cytochrome b gene, sequencing and comparison with sequences deposited in the GenBank was performed. The analysis of blood preferences revealed that sand flies feed mainly on rabbits (Oryctolagus cuniculus) follow by hares (Lepus granatensis). Characterization of promastigotes isolated from sand flies fed on both lagomorphs demonstrated that were infected by L. infantum. These data are in concordance with the information obtained from direct xenodiagnosis in hares and wild rabbits from the focus that proved that they are infective to colonized P. perniciosus.
In conclusion, these findings provide significant epidemiological information which is closely linked to the spread of human leishmaniasis in the focus.
This study was in the frame of FP7-UE EDENext, grant 261504

Influence of climatic factors on TBE incidence in the north-eastern Poland

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Objectives
In Poland, the Podlaskie region is an endemic region for tick-borne diseases. A significant increase in the incidence of tick-borne encephalitis (TBE) during last 20 years is observed. Similar changes in TBE epidemiology were observed in other European countries therefore they can not be explained simply by improvement of diagnostic methods. The aim of the study was the analysis of possible influence of meteorological factors changes on TBE incidence.
Methods
We analyzed data from 6 counties in Podlaskie region (bia?ostocki, suwalski, hajnowski, grajewski, kolne?ski, siemiatycki) in 1970-2008. The analyzed data included: mean, minimal, maximal air temperatures (measured at 2 m above ground level), temperature amplitudes, rainfall, number of days with snowfall and duration of snow cover presence.
Results
The most significant changes were observed in mean, minimal and maximal temperatures in second and third dekad of April as well as in third dekads of October, second and third dekad of November. The statistical analysis showed negative correlations between TBE incidence and the number of days with snow cover presence and with snowfall in April.
Conclusions
Increase in the TBE incidence in Poland in last twenty years can't be explained by only improving the diagnosis and detection of the disease. The climate in Podlasie voivodeship in 1970-2008 was warming. The highest impact on the increasing TBE incidence seems to have the air temperature in the spring, because it can synchronize the activity of larvae and nymphs. Stabile temperature conditions (low temperature variation, that is, the difference between the maximum and minimum temperatures during the day) foster tick activity. In 1970-1989, 1990-1999 and 2000-2008 decrease of the average annual rainfall and in 2000-2008 increase in rainfall in the summer months was observed. Influence of other climatic factors (relative humidity, snow cover time, the number of days with snowfall, snow depth) on the TBE incidence is small. Climate change is one of the factors contributing to the increased TBE cases.

Poster 4.8.

NOT PRESENTED

Modelling the roles of climate, landscape and host factors in the distribution and seasonal abundance of Culicoides vectors across Europe

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Arboviruses transmitted by Culicoides biting midges (Diptera: Ceratopogonidae) have caused significant disruption to ruminant production in northern Europe over the past decade. Incursions of several strains of bluetongue virus (BTV), which cause bluetongue (BT) disease, have been particularly serious, and have followed a northward progression. The timing of BTV transmission in temperate regions is partly determined by the presence of adult Culicoides females; therefore the abundance and seasonal dynamics of Culicoides vectors are important determinants of the risk of BTV infection within a geographical region. Understanding the drivers of Culicoides distribution, as well as the timing of seasonal peaks of abundance, is therefore important for predicting, and mitigating, future incursions, and these drivers are likely to involve a combination of abiotic (climate, landscape) and biotic (host) factors.

Obtaining such an understanding for BTV is particularly challenging, however, because population sizes of their Culicoides biting midge vectors can vary by several orders of magnitude within a short space of time (weekly) at the same location. Accurately predicting the presence of a vector and the timing of seasonal peaks therefore presents a statistical challenge due to zero-inflated and overdispersed data. Here we present a novel Bayesian framework, which addresses these problems. Our modelling approach uses a statistical Poisson-Gaussian mixture model with flexible components for estimating the timing, magnitude and length of seasonal peaks in abundance. These components can be linked with environmental variables to provide inference on the drivers of seasonal variation in Culicoides abundance, and to predict seasonal patterns at unobserved sites.

Using this model, we analysed time-series National surveillance datasets from light-suction trapping regimes collected across the UK and Spain over a period of 5 years, to investigate the climate, landscape and host factors driving the distribution and seasonal dynamics of several Culicoides species. We identified significant relationships between environmental variables and the maximal abundance and seasonal patterns in abundance for the C. obsoletus complex, C. imicola, C. pulicaris, C. newsteadi and C. impunctatus. We also explore the potential for prediction of Culicoides seasonal abundance across Europe to apply within models of Culicoides-borne disease risk and spread.

Temporal dynamics of Puumala hantavirus infection in cyclic bank vole populations

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In the boreal zone of Northern Europe, bank vole (Myodes glareolus) populations undergo cyclic fluctuations that are reflected to the incidence of Puumala hantavirus (PUUV) infections, i.e. nephropathia epidemica (NE), in humans. In this study, we sought for seasonal and multiannual patterns in PUUV infection dynamics in bank voles.
We monitored PUUV infections in a monthly capture-mark-recapture study of bank voles for seven years, and for the first time, patterns of PUUV infection dynamics were also studied in detail during winter, when the human NE incidence peaks in the boreal zone. We demonstrated that the numbers of infected bank voles were the highest during winter, although the total host density peaked already in fall.
The prevalence of infection followed a regular seasonal pattern irrespective of density cycle phase. We found no apparent connection between host density and PUUV transmission; in comparison to years of increase in host abundance, the infection prevalence did not respond to the higher host abundance in peak years. On the other hand, during peak years, young animals were more often protected by maternal antibodies, and therefore further analyses are required to disentangle the possible delayed effects of host density and the effect of maternal antibodies on PUUV infection dynamics. Our data provide a solid basis for developing models to predict periods of high human NE risk in the boreal zone of Europe, where NE poses a significant public health threat.

The impact of ecological factors on the prevalence of tick-borne pathogens in endemic and non-endemic regions in Estonia

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Tick-borne diseases have become a great health concern in the Northern Hemisphere. Estonia, located in the northeastern part of Europe, is currently a high risk area for tick-associated illnesses. Ecological changes are considered to be the main driver in the emergence of these infectious diseases, as tick-borne pathogens (TBP) are greatly dependent on the environmental conditions. There are several ecological factors that may influence the prevalence of TBP in their natural foci, for example, climate, vegetation, vector host community composition and density.
The purpose of the study was to estimate the impact of ecological factors on the prevalence of tick-borne pathogens. Three endemic and two non-endemic regions were chosen based on the national TBP infection statistics. The sites were used to capture rodents and collect questing ticks from the vegetation from 2012 to 2014. In addition, the composition of local flora and fauna was described, and weather conditions were recorded during the trapping of small mammals.
A total of 4643 ticks were collected during the study, either from vegetation or removed from rodents. Our preliminary results showed that the average tick abundance in questing and on-host subsets was considerably higher in endemic regions (pIt was hypothesized that endemic and non-endemic regions may differ in the possible co-feeding incidences. This dissimilarity occurred mainly in spring, when rodent abundance was low in all locations, but tick abundance varied between sites.

Spatiotemporal prevalence of cowpox virus in Finnish field voles

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In northern Europe, rodent populations display cyclic density fluctuations, which are often associated with the human incidence of zoonotic diseases they spread. Field voles (Microtus agrestis) are periodically one of the most abundant rodent species in northern Europe. However, little is known of the viruses they carry, let alone of how they are maintained in nature or of the dynamics of their transmission to humans. The orthopoxvirus group includes several important pathogens that affect livestock animals and humans. Of these, infections by cowpox virus have suggested to be increasing globally following the cessation of cross-reactive smallpox vaccinations. Cowpox has been identified as having high seroprevalence in small rodents, which implies that they have a major role as reservoir host. To evaluate the role of field voles as a reservoir for cowpox virus in Finland, and how the risk of its zoonotic transmission to humans varies over space and time, we screened 709 field voles, trapped from 14 sites over 3 years, for antibodies against cowpox. The prevalence of cowpox antibodies was relatively high (range 0-47 %) but variable among sites and phases of the vole cycle. Seropositive field voles were encountered only in southeastern Finland. Within these sites, antibody prevalence showed delayed density dependence in spring and direct density dependence in fall. Seroprevalence was overall higher in spring than in fall. The pattern of density dependence, along with earlier findings demonstrating that cowpox reduces field vole survival, indicates that cowpox may contribute to the regulation of cyclic vole populations. Our findings highlight the significance of regular rodent monitoring as a tool for assessing spatiotemporal variation in the risk of not only cowpox infections in humans, but also that of other zoonotic pathogens.

Poster 4.13.

NOT PRESENTED

Ixodes ricinus and transmitted pathogens: a changing hazard for the European citizens

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Ixodes ricinus is the principal vector of a number of viral, bacterial, and protozoan zoonotic diseases agents in Europe. Increase in abundance, variation in spatial distribution and prolongation of the questing activity of this tick have been reported in recent years due to changes in climate, land use, wildlife management and socioeconomic factors. As a consequence of such changes, increasing abundance of tick populations have been observed in urban and peri-urban areas in many European countries. Public parks, gardens, peri-urban leisure-time areas and cemeteries are becoming hot spots for tick borne diseases as humans and domesticated animals can be frequently exposed to the bites of infected ticks. In urban habitats, small mammals, hedgehogs, squirrels, birds, companion animals (dogs, cats), but also larger mammals as roe deer and wild boars, play the major role as tick hosts and reservoirs of tick-borne pathogens. Presence of ticks infected with tick-borne encephalitis virus and high prevalence of ticks infected with Borrelia burgdorferi s.l., causing Lyme borreliosis, have been reported from urbanized areas in Europe. Emerging pathogens, including bacteria of the order Rickettsiales (Anaplasma phagocytophilum, «Candidatus Neoehrlichia mikurensis,» Rickettsia helvetica, and R. monacensis), Borrelia miyamotoi, and protozoans (Babesia divergens, B. venatorum, and B. microti) have also been detected in urban tick populations. Understanding the ecology of ticks and their associations with hosts in a European urbanized environment is crucial to quantify parameters necessary for risk pre-assessment and for the identification of the most appropriate public health strategies for control and prevention of tick-borne diseases.

Long-term trends of Nephropathia epimidemica and geography of rodents cycles in Finland

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We report on long-term patterns of nephropathia epidemica (NE) in Finland in relation to the regional population cycles of the bank vole (Myodes glareolus), the host of Puumala hantavirus (PUUV). The Finnish Forest Research Institute has run a nationwide rodent monitoring program for decades. We know the geographic patterns of vole cycles in Finland.
There are clear trends in the Finnish NE data. Until recently, the long-term increasing trend was apparently partly based on better awareness and diagnostics of the disease. The increasing trend was clear also in local datasets and in low vole years. However, a very interesting feature took place in the late 1990's when the epidemiology of NE on a national level changed from relative temporal stability to strong, multiannual cyclicity.
The change in NE dynamics is obviously due to the change in the geographic synchrony of vole population fluctuations in Finland. In the southern half of Finland, where most human NE data come from, a vole cycle usually lasts for 3 years. From the 1970s to the late 1990s the geographic synchrony of a vole peak was not as extensive as in the 2000s. Earlier, the vole peak occurred simultaneously in western and southcentral Finland, a year later in southeastern Finland, and one year even later in northcentral Finland. In other words, there was a vole peak somewhere in the country every year, and therefore a steady accumulation of NE cases. In spite of the stable national pattern, local NE dynamics showed strong fluctuations due to the local vole cycles. In late 1990s a change in geographic synchrony took place, and through the 2000s the vole peak has occurred simultaneously through most of the southern half of Finland. As a result, the number of human NE cases that earlier was divided over 3 years, now occurred in the same year. The latest change began in the 2010s when the magnitude of vole peaks declined. Concurrently, also the number of NE cases declined.
The Finnish rodent monitoring serves as an excellent early warning system in the country with the highest incidence of hantaviral disease in Europe.

Temporal changes in rodent- and tick-borne diseases in Europe: how are they linked?

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Generalist rodent species, such as Apodemus flavicollis and Myodes glareolus, play an important role as reservoirs of a number of directly transmitted zoonotic pathogens, such as hantaviruses and other rodent-borne pathogens. At the same time, these species are also reservoirs of emerging tick borne diseases, such as Tick borne encephalitis and Lyme borreliosis. In general, rodent-borne and tick -borne diseases cannot be easily controlled with the massive use of rodenticide, acaricide or large-scale animal culling. Instead, preventing and reducing the exposure of humans to such pathogens and their vectors in hot spots of infection during periods when these hazards are higher, is a more realistic and sustainable option. However, these recommendations are only possible if such hazards are predictable.
The incidence of rodent and tick borne diseases varies spatially and temporally in European citizens, and therefore understanding the drivers of such variations is relevant for identifying early warning predictors of the changing risk. In particular, understanding the consequences of rodent fluctuations not only on directly transmitted pathogens but also on tick -borne infection risk can provide a framework for prevention of multiple pathogens. Therefore, we established a joint multiannual monitoring survey in three European countries (Italy, Slovakia and Finland). Collection of questing ticks and rodents were carried out at our study sites for four years. Serological and molecular investigations were carried out both on rodents and ticks collected either from vegetation and directly from the hosts to identify their infectious status and prevalence of infection for several pathogens. Findings and criteria for selection of early warning predictors will be presented and discussed.

West Nile Virus Transmission in Mosquitoes in Danube Delta in the Context of Weather Factors

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Mosquito collections were performed, in a Danube Delta site, from May till October, during 2011-2013 years. Mosquitoes were identified, grouped into pools, and processed for molecular detection of West Nile virus (WNV) genome by RT-PCR and sequencing. WNV infection rate in Culex pipiens and Cx. modestus, main WNV vectors in Danube Delta, was expressed as its maximum likelihood estimate (MLE) for each collecting interval. Mosquito abundance index was calculated as the number of mosquitoes captured per bird-baited trap per night. Average air temperature and precipitation amounts per each 10 days periods of the months March-September were used in the analysis. Negative Binominal Model that appropriates for a counting mode was used to evaluate possible linkages between the temperature / precipitation and the mosquito population size / infection rate. Since the number of infected mosquitoes contains excess zero-count data, Zero-Inflated Negative Binominal (ZINB) Model was applied as well. The linkages were calculated for the real time (lag 0) and for three lag times of the weather parameters: 10 days earlier (lag 1), 20 days earlier (lag 2) and 30 days earlier (lag 3). Significant positive linkages Significant positive linkages ($p< 0.001$) were detected between the temperature and mosquito population size for lag 1, lag 2 and lag 3. The linkages between temperature and infection rates were positive and significant for lag 2 and lag 3. Monthly average temperatures in the area during the hot seasons of 2011-2013 years were high and above the monthly perennial averages. The highest temperatures were measured in 2012, with anomalies of 2.3-3.1°C from April to July. These results strengthen previous knowledge which showed that increased temperatures may cause an upsurge in WNV transmission. Negative significant ($p< 0.001$) results were detected between precipitation and infection rates for lag 1, lag 2 and lag 3. In the study period, the monthly rainfall amounts were below the monthly perennial averages. Drought in the area was associated with dominance of Cx. pipiens versus Cx. modestus population. Based on these results we assume that the early rise of temperature and the decrease in rainfall contributed to increased vector population and WNV amplification.

Malaria in Romania ? The past and the present

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Romania had very large malaria endemic areas where as many as 300,000 new cases appeared yearly. The old investigations on the abundance and distribution of malaria vector species in different environmental conditions and the frequency and gravity of malaria cases in different areas led to the «malaria stratification» in Romania (Zotta 1938) on ecological criteria (the concept later launched by WHO in the ?70s). The historical malaria vectors distributed as mixed populations in Romanian endemic areas were Anopheles maculipennis s.s., A. messeae and A. atroparvus. Every main malaria endemic areas (flood areas, inner plains, hills and tablelands) was dominated by one of these species. A. sacharovi was the main vector in the hyper-endemic Black Sea coast and lagoon area and has not been recorded after malaria eradication in 1962. The Danube Delta remained all the time an area with abundant ?anophelism without malaria » because of the short lifetime of anopheline females in that area not reaching the development of sporozoites. The former endemic territories remained receptive for malaria introduction after the eradication mainly because of the characteristics of anopheline populations (presence and abundance of vectors as before malaria eradication, their dynamics and vectorial capacity, infectivity with imported Plasmodium strains, resistance to insecticides). The permanent surveillance of these characteristics in correlation with the evolution of the environmental, social and economical conditions led to the evaluation of the increasing risk of malaria re-introduction in Romania and the main factors involved. The stratification of the present risk areas of malaria re-introduction has been elaborated and mapped. They generally overlap the former malaria areas. The new aspects are linked to the present environmental changes. A. daciae has an extended distribution and higher densities than A. messeae everywhere. A. atroparvus, the main vector in Romania, has higher abundant populations and its distribution extended over all the risk areas. The permanent surveillance of the factors influencing malaria re-emergence risk is needed to prevent and control its re-appearance, and all the data, included by us in a pre-operational service for access, will improve the public health policies for the protection of the human population.

First evidence of Seoul hantavirus in the wild rat population in the Netherlands

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Background:
Hantaviruses are a globally distributed group of rodent- and insectivore-borne RNA viruses. Seoul hantavirus (SEOV) is the only known hantavirus with a potentially world-wide distribution due to the ubiquitous occurrence of its reservoir hosts, the brown and the black rats. Nevertheless, prior to the last decade, human and wild rat infections of SEOV were for the most part detected only in Asia with human infections in Europe limited to laboratory rat handling. During the recent years, however, SEOV has been detected in wild rats in France, Belgium, and the UK, and in pet rats in Sweden, England and Wales. Human cases of severe SEOV-caused HFRS have been reported from France and from United Kingdom. This study proves the occurrence of SEOV in the wild rat population in the Netherlands.

Methods:
Rat hearts were vortexed together with 1 ml of PBS and centrifuged. These samples were assumed to have a dilution equivalent to serum dilution of 1:25. The samples were initially screened by a) enzyme-linked immunosorbent assay (ELISA), b) indirect immunofluorescence assay (IFA), and c) immunoblotting (IB), and confirmed by focus-reduction neutralization test (FRNT) and by RT-PCR.

Results:
Two out of 16 samples, #22 and #33, were found clearly positive by all 3 screening methods; rat #84 was positive by ELISA and IFA, but negative in IB. To finally confirm the hantavirus-reactivity and a SEOV-specificity, we tested the three positive samples #22, #33 and #84 by FRNT. The samples #22, #33 and #84 all showed high titers of SEOV-specific neutralizing antibodies. A hantavirus genus-specific nested RT-PCR was applied as described earlier. Samples #22, #33 and #84 all showed positive results, while the serologically negative sample #31 was found negative.

Conclusions:
This is the first discovery of SEOV-specific antibodies in wild rats in the Netherlands. The recent years discoveries of SEOV circulating in wild rat populations in France, Belgium, the UK, and now also in the Netherlands, together with the cases of severe human SEOV-infections so far confirmed in UK and France, strongly emphasize the importance of further studies all over Europe.

Session 5

Risk of infection

EDENext models for public health: two cultures, one goal. Case study of hantavirus disease in Germany

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A great diversity of disease risk models have been produced over the course of the FP7 projects EDEN/EDENext, improving the general understanding of the influence of environment on disease. However, their use by public health practitioners has been limited. To promote more used and useful models, we used a social science approach to investigate how public health practitioners perceived modeling outputs, as well as how models could (or not) provide answers to questions posed by both practitioners and the general public. Our basic questions therefore relate to how qualitative social scientific knowledge can be integrated into quantitative risk model approaches ? and vice versa. C.P. Snow's concept of Two Cultures shows that two different disciplines are rarely able to talk to each other, though doing so would clearly generate new unexpected knowledge. We took up the challenge ? and were surprised to find out how many different ways we identified to create win-win situations for modelers, social scientists and public health practitioners. We assessed what was needed to improve communication between modelers and practitioners to enable a more efficient use of modeling results. Typical modeling outputs from diverse EDEN/EDENext case studies were also used to assess various aspects of their comprehensibility and usefulness for public health practitioners. This allowed us to highlight issues with current modeling outputs and to outline ways in which they may be made more useful. In a second phase, risk perception surveys were used to identify specific knowledge gaps and questions that can be answered by mapping and modeling, and answers are offered based on two EDENext models of hantavirus in Germany. Results offer promising perspectives for more efficient implementation and use of modeling results for public health risk assessment and communication.

Risk of infection in different genotypes of Ixodes ricinus ticks

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Ixodes ricinus is the most widely spread tick species in Europe and is involved in the transmission of a number of diseases to animals and humans. Aim of our study was to evaluate correlation between different tick genotypes and infection rates in both endemic and non endemic locations in sympatric zone with closely related tick species I. persulcatus in Baltic countries. In total 718 I. ricinus ticks were collected from 17 different locations in three Baltic countries: Lithuania, Latvia and Estonia. We have investigated genetic diversity among collected ticks by using DNA sequencing of the mitochondrial control region, 16S rRNA gene and cytb gene fragments and evaluated prevalence of tick-borne pathogens in these populations. Borrelia burgdorferi s.l., Babesia spp., and Analpasma spp. was screened, infection rates calculated and correlation with different I. ricinus genotypes evaluated.

Poster 5.3.

NOT PRESENTED

Poster 5.4.

NOT PRESENTED

Epidemiology of imported cutaneous and visceral Leishmaniasis in Italy: implications for an endemic country

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An increase of imported leishmaniasis cases in western European countries was reported in the last decades. The trend was associated to increasing travel and ecotourism in endemic destinations, military operations and immigration. In endemic countries leishmaniasis is usually well diagnosed, however parasite identification is necessary to distinguish between autochthonous and imported cases. Indeed, without appropriate surveillance, new Leishmania species/genotypes may be introduced and transmitted locally by phlebotomine vectors with eco-epidemiological implications. Our aim is to report on surveillance of imported cases referred to the Leishmania Identification Reference Centre of Rome over the past 3 decades. Samples received from suspected imported cases from 1986 to 2012 were analyzed and conclusions were based on clinical, epidemiological and diagnostic findings. Fifteen Italian regions with 34 regional diagnostic centers were involved in the study; different identification methodologies were routinely employed: a) two molecular techniques, for both Leishmania diagnosis and typing (SSU rDNA n-PCR and ITS-1 n-PCR-RFLP) applied on samples; b) MLEE analysis on Leishmania isolates. Altogether 105 imported cases were diagnosed, of which 35.2% from Lombardy and 27.6% from Latium; 36 were visceral (VL) (16 HIV+) and 69 cutaneous (CL) cases; 85 (52 CL) were from the Old and 20 (17 CL) from the New World; there was a range of 1-20 cases/year. Nine Leishmania species were identified. High importation rate until 1995 was associated to the increase in Mediterranean Leishmania-HIV co-infections in that period. Following HAART treatment, VL imported cases became occasional in Italians. On the other hand, a steady increase of imported CL was observed from areas of Old and New World. Characteristics of the patients are changing: there are more immigrants classified as VFR and Italian tourists. This report evaluated the incidence of imported leishmaniasis, which appears a tropical disease steadily imported into Italy. The positive trend probably depends on better diagnosis, but we suspect that many CL cases remained unrecognized. Given the low incidence of imported cases, the risk of emergence of exotic species/genotypes appears to be limited.
This study was partially supported by FP7 UE EDENext project, Contract No. 261504.

The potential use of Wolbachia as a mosquito biocontrol strategy for Japanese encephalitis. First steps; colonisation, characterisation and Wolbachia transinfection experiments with the major Japanese encephalitis virus vector, Culex tritaeniorhynchus

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Japanese encephalitis is endemic across large areas of Asia and the Pacific, with an estimated 3 billion people living in at-risk regions and current incidence estimates ranging from 50,000-175,000 human cases per year. The disease is caused by Japanese encephalitis virus (JEV), a zoonotic flavivirus transmitted by mosquitoes, primarily of the species Culex tritaeniorhynchus, and maintained in an enzootic transmission cycle involving birds and pigs.
JEV transmission risk is currently limited to countries in Asia and the Pacific where the virus is endemic, however, established populations of Culex tritaeniorhynchus have also been reported in many other regions worldwide, including parts of the Middle East, Africa and Eastern Europe. Although JEV vaccines have been developed, humans are dead-end hosts, with onward transmission occurring enzootically, and there are problems with the cost, doses required and lack of cross-protection for all genotypes of JEV. Therefore, human vaccination is not a viable option for eradication. The mosquito's vital role in the transmission cycle means control of the vector species represents a more promising disease control strategy. However, the current methods, including intermittent irrigation of rice fields and space spraying with insecticides, have their own inherent problems, including logistical difficulties and development of insecticide resistance.
Recently, significant progress has been made in the potential use of the bacterial symbiont Wolbachia for mosquito biocontrol, particularly in the production of dengue virus-refractory mosquito populations. This has been applied in wild populations through open releases of Wolbachia-infected Aedes aegypti in several dengue-endemic countries, including Brazil, Indonesia and Vietnam. JEV and dengue are closely related viruses, and the successful establishment of a suitable Wolbachia strain in Culex tritaeniorhynchus populations, as the major JEV vector species, could lead to a significant reduction in transmission.
In order to prepare for Wolbachia transinfection experiments and to investigate whether there are potential differences between populations of Culex tritaeniorhynchus from JEV endemic countries and those where JEV is absent, wild-caught mosquitoes from India (JEV endemic) and Greece (JEV absent) were colonised and characterised. The progress of Culex tritaeniorhynchus embryo microinjection experiments, attempting to generate lines stably infected with Wolbachia, will also be reported.

MediLabSecure : Implementing a network of virology and entomology laboratories for a one health approach of vector-borne viruses in the Mediterranean and Black Sea regions

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Background As (re-)emerging viruses are threatening global health, the EU-funded MediLabSecure project (2014-2017) aims at enhancing the preparedness and response to viral threats by establishing an integrated network of virology and entomology laboratories in 19 non-EU countries of the Mediterranean and Black Sea areas in partnership with Institutes in 3 south European countries. The MediLabSecure project is reinforcing the laboratory and epidemiology networks established by the EpiSouth Plus project (2010-2013) by involving partners from animal virology and medical entomology laboratories additionally to previous partners in human virology and public health. The project also aims to enhance the integration of surveillance between laboratories and central national surveillance systems.
Methods Participating laboratories were selected based on the responses of potential participants from each beneficiary country to a questionnaire assessing their activities and capacities. One laboratory per field of study (human virology, animal virology, medical entomology) and per country should be identified. The first meeting involving all the Heads of selected laboratories will be held in January 2015 in Paris. Additionally, each laboratory will respond to a complementary questionnaire assessing their capacities and needs regarding biosafety and diagnostic methods for emerging vector-borne and respiratory viruses in order to adapt accordingly the upcoming training sessions.
Results A total of 47 laboratories from the 19 beneficiary countries were selected to actively take part in the Medilabsecure network. The first «Heads of labs» meeting is intended for project partners and head of laboratories to meet and exchange on the objectives and future steps of the project as well as on theirs needs and experiences. Considering these interchanges and the responses of the «Needs assessment» questionnaire, tailored trainings sessions will be organized, enabling laboratories to implement harmonized and up-to-date techniques to perform (1) laboratory diagnosis of vector-borne and other relevant emerging viral diseases such as West Nile, Dengue, Rift Valley Fever, Chikungunya, Ebola virus disease and (2) mosquito species determination.
Conclusion By enhancing diagnostic capacities and regional multidisciplinary cooperation, the Medilabsecure network could represent the cornerstone of a corporate preparedness and response to vector-borne and respiratory viral threats in the Mediterranean and Black Sea regions.

Systematic risk assessment comparing seven emerging vector-borne animal diseases

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Background
Incursions of vector-borne animal diseases animals in recent years in North-Western Europe, such as bluetongue serotype-8, have shown the need for preparedness for these diseases. This requires insight in the probabilities of entry, establishment and spread as well as the impact of an outbreak. Therefore, the risk of seven vector-borne animal diseases was assessed for the Netherlands with the aim to prioritize diseases for preparedness and to identify common parameters that contribute most to the introduction risk of vector-borne diseases for the Netherlands.
Methods
The vector-borne animal diseases selected for the risk assessment were tularaemia, bovine babesiosis, epizootic haemorrhagic disease serotype 6 (EHD), Crimean-Congo haemorrhagic fever (CCHF), Rift Valley fever (RVF), West Nile (WN) and African Horse sickness (AHS). These include protozoan, bacterial and viral diseases that are either transmitted by ticks, biting midges or mosquitoes, five of which are zoonotic.
The MINTRISK calculation methodology1 was used for the risk assessment. MINTRISK provides a semi-quantitative estimate of the risk of vector-borne animal diseases allowing for comparison. The method provides a questionnaire in which semi-quantitative answers need to be given and the uncertainty in the answers is to be made explicit. Using Monte Carlo simulation the overall risk estimate is determined, along with the risk contribution of the different answers and uncertainty intervals.
The initial risk assessment was based on literature study, which was discussed with experts from outside the project group. After these discussions the assessment was finalized.
Results
The diseases differ widely in the probability of introduction and the impact of a potential outbreak. Tularaemia and bovine babesiosis are occasionally observed in the Netherlands, hence the probability of introduction is high (more than once per ten years). The other diseases are less likely (1De Koeijer, A., et al., 8th Annual Meeting EPIZONE, September 2014, Copenhagen, Denmark.

Entomological surveillance and Public Health: first detection of West Nile Virus in Piemonte and Liguria and Aedes albopictus in Valle d'Aosta (North western Italy)

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Since 2011 IZSPLVA, through research projects funded by the Health Ministry and European Regional Development Fund, has been implementing an entomological surveillance plan for West Nile Virus (WNV) in its territories (North western Italy), considered WNV free, in addition to the activities supported by the Health Ministry at national level and mainly focused in endemic areas. This work reports results of entomological surveillance carried out in 2014. According to risk factors, 46 collection sites (34 in Piemonte and 6 in both Liguria and Valle d'Aosta regions) were selected. From July to November, mosquitoes were fortnightly trapped using CO2 CDC dry ice-baited traps, BG-Sentinel traps and Gravid traps, identified to species level by morphological standard classification keys, pooled and analyzed by Real Time RT-PCR distinctive for WNV Lineage 1 and 2 and Usutu virus (USUV). To detect the introduction of exotic Aedes spp. mosquitoes, ovitraps were also placed. A total of 20036 mosquitoes were collected and 916 pools tested. WNV Lineage 2 was detected in 3 pools of Culex pipiens: 2 in Piemonte (Alessandria province) and 1 in Liguria (Genoa). This is the first report of WNV circulation in both regions. Moreover USUV was detected in two pools of Culex pipiens, collected in Piemonte (Alessandria) and Liguria (La Spezia). Control measures against WNV were quickly implemented, coordinated by IZSPLVA together with veterinary and human local health units, as provided by national legislation (screening of equine blood samples and blood transfusion). Local disinfestation protocols were applied by IPLA too. From ovitraps, 677 mosquitoes emerged and were identified as Aedes albopictus, including the first finding of this species in Valle d'Aosta. The plan shows high sensitivity to detect the presence of WNV before the appearance of clinical cases in humans and animals and highlights the importance of entomological surveillance to detect the introduction of exotic vectors and virus introduction. This approach allows to promptly implement control measures aiming to protect human and animal health through veterinary and human Authorities. USUV circulation, reported in our territories since 2011, should receive more consideration, given the recent report of neuroinvasive human cases in Croatia.

Acrodermatitis Chronica Atrophicans - various faces of late form of Lyme borreliosis

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Objectives
Acrodermatitis chrnica atrophicans (ACA) is a chronic inflammation of the skin, usually of limbs, leading to sclerosis and atrophy of the skin,caused b the spirochete Borrelia burgdorferi. The objective of the study was to analyze the epidemiological data, clinical picture, diagnostic methods results of patients with ACA.
Methods
9 patients (5 women, 4 men; mean age: 58.7±14.3 yo, 4 (44%) inhabitants of cities) with ACA were included to the study. All patients had serology for Borrelia spp. (ELISA and Western blot) and histopathological examination of skin lesion performed. 7 patients had PCR in skin biopsy performed. Epidemiological data, clinical picture, diagnostic methods results were analyzed.
Results
8 (88%) of all patients remembered a tick bite. 4 (44%) patients were treated because of EM. In 5 (56%) cases the disease was work-related. The duration of symptoms was from 2 months to 2 years. In 7 (77%) patients skin lesions were localized within the lower limbs. 2 patients had co-existing skin lesions in abdomen (Pictures). The mean diameter of skin lesion was 13.5 ±50 centimeters. 1 (11%) patients suffered from headache, 6 (66%) from muscle and joints pain. Specific B. burgdorferi DNA was detected in 44% of the skin biopsy specimens. IgM anti- B. burgdorferi -specific antibodies were present in serum of 33% of patients (mean titer: 9.1±17.1 BBU/ml) and IgG antibodies in 100% of patients (mean titer: 72.2±26 BBU/ml). All positive results in ELISA test were confirmed by Western blot. In 7 cases diagnosis was confirmed by histopathological examination, in 2 cases microscopic picture showed chronic inflammatory status, not described as ACA clearly. Response to 28 days III generation cephalosporin therapy was various. In 2 cases the changes disappeared completely, in others faded, but skin thinning and discolouration of bluish pink remained.

Conclusions
Late stages of Lyme borreliosis still is seen in clinical practice in Europe. Diagnosis should be based on anamnesis (tick bite), serological tests and histopathological examination results. Effects of antibiotic treatment varies.

Lipid peroxidation in Lyme neuroborreliosis

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Objectives: Increase in number of Lyme disease (LD) is observed. In Europe, 85.000 new cases, and in North America 15-20.000 are noted yearly. Not fully clarified LD pathogenesis and difficulties in diagnostics show necessity of searching indicators, which may let predict the course of the disease. Discovery of biomarker of LD should help in diagnostics. The goal was to exam if neuroborreliosis (NB) effects on neuronal phospholipids peroxidation. We aimed to measure concentration of PUFA's cyclization and fragmentation products, antioxidants protecting PUFA's against peroxidation ? glutathione peroxidase and vitamin E in NB patients.
Methods: 22 patients with NB were included to the study. GSH-Px ? EC.1.11.1.6 activity was assessed spectrophotometrically. Phospholipid arachidonic acid (AA) and docosahexaenoic acid (DHA) were determined by gas chromatography. Lipid peroxidation was estimated by measuring of reactive aldehydes 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal (4-HHE), malondialdehyde (MDA), acrolein, crotonaldehyde, 4-oxononenal (4-ONE), F2-isoprostanes (8-isoPGF2?) and A4/J4-neuroprostanes (NPs). Commercial assay kits were used to determine PLA2 and PAF-AH activities.
Results: Decrease in the antioxidant abilities against lipid peroxidation in CSF and plasma was observed. The activity of GSH-Px and vitamin E concentration in both body fluids decreased. Concentration of 8-isoPGF2? increased in the CSF, plasma and urine. The total concentration of 8-isoPGF2was higher in CSF and plasma. Concentration of plasma free 8-isoprostanes was twice enhanced and of neuroprostanes increased 20 times in CSF and 16 times in plasma. MDA, 4-HNE, 4-HHE and 4-ONE CSF concentration was 1.5-4.0 times higher. The plasma 4-HNE and 4-ONE concentration was over 7 and 3 times higher in NB. Concentration of plasma 4-HNE, and 4-HNE-His-protein adducts increased in plasma. The urine MDA, 4-HNE, 4-HHE and 4-ONE concentration increased by 4, 3, 2, 5 times. The CSF fatty acid concentration decreased by about 18% and 10% for DHA and AA respectively. Concentration of plasma AA and DHA was lower by 29% and 28% respectively. Phospholipase A2 and PAF acetylhydrolase activities were lower in plasma and in CSF.
Conclusions: Lipid peroxidation plays role in the pathogenesis of NB. Measurement of its products concentration or enzymes activity may help in diagnostic process of NB.

Surveillance of human leishmaniasis in Italy: towards the development of an epidemiological model for autochthonous human cases

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Zoonotic visceral (VL) and cutaneous leishmaniasis (CL) caused by Leishmania infantum are endemic in Italy, however the risk of exotic Leishmania introduction is emerging. An epidemiological approach for autochthonous human leishmaniasis surveillance is reported. A human database was implemented by ISS-Italy EDENext partner and applied during 2011-2014. Harmonization of diagnostic methods was achieved following WHO guidelines for case definition. Attention was addressed to the geographical origin of infections. Cases, georeferenced and identified by clinical form, were classified as autochthonous, imported, of unknown origin, and exported. In 2011, ISS diagnosed or collected information from 35 human cases (27 VL; 8 CL, 1 multiple - VL with tegumentary involvement) of which 28 were primary infections; 26 were autochthonous, 2 imported, 6 of unknown origin, and 1 exported to Netherlands. In 2012, 57 patients were included (41 VL; 13 CL, 1 mucocutaneous (MCL) and 2 multiple) with 46 primary infections; 46 were autochthonous, 8 imported, 2 of unknown origin and 1 exported to Germany. In 2013, we recorded 42 patients (33 VL; 7 CL, 2 multiple) including 37 primary infections; 41 were autochthonous and 1 imported. Through November 2014, 33 patients (15 VL, 17 CL and 1 MCL) were recorded of which 32 were primary infections; only 1 case was imported. Five Leishmania species were identified: L.infantum, the only species detected in autochthonous cases, and L.tropica, L.major, L.donovani complex and L.panamensis, isolated from imported cases. Data confirmed that leishmaniasis is endemic in all Regions of Italy. Active VL foci were found in Emilia Romagna, Campania, Latium, Liguria, Lombardy and Tuscany Regions while CL foci appeared scattered all over the country. Of note: i) Campania, the most VL endemic Region in years 1990s-2000s showed a sharp drop of cases; ii) an outbreak of 16VL cases occurred in Bologna province from November 2012; iii) the few HIV- coinfected cases were almost represented by relapses of old infections. Thanks to a better CL surveillance, the number of cases recorded in 2014 were similar to those of hospitalized VL cases.
This study was carried out in the frame of FP7-UE EDENext collaborative project, Contract Number: 261504.

State of the art of modelling approaches for assessing vector control strategies to contain human West Nile fever in Europe

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Introduction: West Nile virus is an arbovirus maintained in an enzootic bird-mosquito cycle and transmitted to equids and humans. Since 2010, the number of European countries reporting human cases of West Nile fever (WNF) has increased. As no vaccine or specific treatment is available, prevention and control of infections rely on early detection of active transmission and mosquito abatement. To optimize resources allocation and vector control effectiveness, a comprehensive overview of modelling approaches developed for WNF has been undertaken. Its results will be used to design and develop a model to assess vector control strategies.
Methods: Three people, a librarian plus two experts, performed the systematic review following the PRISMA Statement. Literature search was performed from their earliest dates to October 22nd, 2012 via 11 peer-reviewed and grey literature sources (MEDLINE, CISMEF, BDSP, CAIRN, SUDOC, Science direct, Web of Knowledge, arXiv, BioOne, OpenGrey, HAL). Retrieved studies were reviewed using predefined inclusion criteria for modelling and for suitability to address vector control.
Results: Of the 1518 records, 1206 were unique publications of which 38 (26 dynamic and 12 statistical models) were selected for appraisal (see Table 1). Eleven studies (7 dynamic and 4 statistical models) were found relevant since fulfilling both criteria for modelling quality and for suitability to address vector control. The 7 dynamic models include 5 ordinary differential equations (ODE), 1 partial differential equations and 1 multi-agents models. The 4 statistical models include 1 logistic regression, 1 GIS-based model and 2 risk analysis approaches.
Conclusions: Modelling approaches for WNF can be classed into dynamic (68%) and statistical (32%) models. Dynamic models mostly involve ODE and statistical models mainly use environmental factors and GIS-based approach for constructing risk maps. These two classes of models appeared quite complementary for modelling transmission dynamics of WNF.

Network "Rodent-borne pathogens" in Germany: An Overview

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Rodents and other small mammals play an important role for the transmission of zoonotic pathogens to both humans and domestic animals. In addition, rodents habour a large number of rodent-specific pathogens with no or unknown zoonotic potential. The network «Rodent-borne pathogens» aims to study the influence of changes in rodent populations on the prevalence and molecular evolution of various pathogens. For this purpose a monitoring of small mammals was performed at selected sites in Germany during 2010-2014.
Our investigations in Germany demonstrated the presence of different hantaviruses with strong host-specificity and very rare spillover infections of non-reservoir animals. Leptospires of different genomospecies and Rickettsia spp. were detected Germany wide, but without an obvious reservoir-specificity. In contrast to a high orthopox virus seroprevalence, only in very rare cases viral DNA was detected in voles. In addition, novel herpes-, papilloma-, hepe- and paramyxoviruses were discovered in different rodent species. Recently, a novel hepatitis C virus-like agent was detected in bank voles which may allow the future development of a novel hepatitis C animal model.
Future investigations will have to prove potential interactions of the pathogens detected. A pilot study in rodents from Austria indicated single infections with eight of eleven investigated viruses, bacteria and parasites, but also simultaneous infections with two or three pathogens.

West Nile virus positive blood donation and subsequent entomological investigation, Austria, 2014

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Background: West Nile virus (WNV) is maintained in a mosquito-bird transmission cycle; humans and horses are considered dead-end hosts. Most human infections are asymptomatic, however approximately 20% of cases develop a febrile illness with flu-like symptoms (West Nile fever, WNF) and less than 1% West Nile neuroinvasive disease (WNND), the latter associated with a mortality rate of about 10%. The vast majority of patients acquire WNV infection through the bite of an infected mosquito. Other possible routes of transmission include blood transfusion and solid organ transplantation. In the eastern part of Austria, WNV has been detected in various bird species, predominantly goshawks, and mosquitoes since 2008. Three WNV human infections were diagnosed retrospectively in 2009 and 2010, respectively.
Objective: In this study we report an acute WNV infection in a Viennese blood donor and the results of subsequent entomological investigations.
Methods: As a blood donation from August 2014 tested positive for WNV RNA, mosquito sampling was initiated in Vienna focusing on the residential area of the patient. A total of 45 mosquito pools were investigated by WNV PCR. The WNV positive human blood sample and the mosquito pools were analysed in detail by several PCRs and sequencing, and compared phylogenetically.
Results: The blood donor was a 44-year-old Viennese female. Shortly after her blood donation she developed myalgia, and later a generalized maculopapular rash. Of the mosquitoes investigated, two pools proved positive for WNV RNA, both consisting of immature Culex pipiens individuals collected in 500m distance to the patient's home. Complete sequences of the Austrian human- and mosquito-derived lineage 2 WNVs were generated. They were more closely related to Czech WNV strains isolated in 2013 than to each other. Both newly determined Austrian WNV strains clustered within the Central/Southern European lineage 2 WNV group together with the goshawk-derived Austrian strains.
Conclusion: The detection of WNV in a blood donation originating from an area with rather low WNV prevalence in humans is surprising and emphasizes the importance of screening of blood donations by nucleic acid testing (NAT) even in areas of low WNV prevalence, along with an active mosquito surveillance program.

The distribution of Ljungan-virus reactive antibodies in Finnish human population is incompatible with rodent-borne zoonotic transmission

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Ljungan virus (LV, genus Parechovirus, family Picornaviridae) is a small non-enveloped ssRNA virus) that is considered to be a rodent-borne virus. Thus far it has been isolated from the bank vole (Myodes glareolus), and two other species of subfamily Arvicolinae.LV RNA has been detected also in the field vole (Microtus agrestis) and several species of mice (Apodemus and Mus), laboratory rats and Eurasian red squirrels.. At the moment, there is little uncontroversial evidence for transmission to humans, yet there have been speculations of an association to human disease. In this study, we aimed to characterize the demographic and geographic variation in the prevalence of LV-reactive antibodies in the Finnish human population and possible LV exposure. We selected for the study altogether 1378 serum samples from archived sample panels of Helsinki University Hospital, Finland, collected in 2006?2014, representing 17 of the 20 Finnish hospital districts. The samples included 1155 patients with suspected acute Puumala virus (PUUV) infection and tested for PUUV IgG and IgM antibodies, 135 children and adolescents with suspected acute central nervous system infection, and 88 healthy, pregnant women (N=88). Children, adolescents and women were underrepresented in PUUV panel, and therefore the dataset was supplemented with additional sera.
In total, we found 36% of the 1378 sera to be positive in LV-IFA. The likelihood of LV-IFA positivity increased from 33% in infants to 60% at the age of 14 years, declining thereafter gradually to 22% by age. Males were significantly more often seropositive than females ($p = 0.007$). The presence of LV-reactive antibodies was neither associated with being suspected nor having contracted NE. These findings markedly differ from human serological patterns of rodent-borne pathogens such as PUUV, and were more compatible to those of human parechoviruses, close relatives of LV.

Wild bird surveillance of West Nile virus in an endemic area, 2011 - 2013

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West Nile virus (WNV) is maintained in a cycle between Culex mosquitoes and wild birds in nature. Susceptibility of wild birds to WNV infection varies with age and among different bird species. To better understand the complex mechanism of WNV maintenance and transmission in an endemic area, a serosurvey and RT-qPCR screening were carried out. Migratory and resident wild birds of 26 different species were sampled in three consecutive years, 2011, 2012, and 2013. Sampling was carried out from October 2011 to January 2012 (winter collection 1), from May to July 2012 (summer collection 1), from September 2012 to February 2013 (winter collection 2), and from March to September 2013 (summer collection 2) near Tulcea, in the Romanian Danube Delta. Altogether, 1205 serum samples were screened for WNV antibodies with INGEZIM West Nile COMPAC ELISA. ELISA positive and questionable sera were subjected to a plaque-reduction neutralization microtest 90 (PRNMT90) with Vero cells and the Egyptian topotype Eg-101 WNV strain. Cross reactions with related flaviviruses TBEV, USUV, and BAGV were excluded. Oral and cloacal swabs taken from 1193 different birds, and 179 pooled organ samples including lung, kidney, spleen, liver, and brain were tested for presence of WNV nucleic acid by RT-qPCR. One hundred ninety-seven out of 1205 sera samples reacted positive in the ELISA but only 87 sera were verified positive by PRNMT90. Antibodies specific to WNV were detected in birds of the families Passeridae (42/620), Corvidae (30/177), Sylvidae (6/59), Ardeidae (3/3), Oriolidae (3/3), Upupidae (2/2), and Lanidae (1/34). No WNV nucleic acid was detected in any of the wild birds sampled. Our serological and virological data were not indicative of any acute/persistent infections or WNV shedding in the investigated wild birds. However, considering the great biodiversity and large amounts of wild birds in this endemic area our sample size was restricted. Moreover, only a limited number of birds may be involved in the WNV transmission cycle. On the basis of our results, we assume that there was limited WNV circulation in wild birds in Tulcea from October 2011 to September 2013.

Chikungunya outbreak, Montpellier, France, October 2014

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On October 1st 2014, a probable autochthonous case of chikungunya was identified in Montpellier, southern France, a town colonized by Aedes albopictus since 2010. A few days later, the University Hospital notified to the regional health authorities four suspected cases among a family living in Montpellier. None of them had traveled abroad recently. These cases were then confirmed by the National Reference Centre for Arboviruses.
The analysis of the chikungunya and dengue cases surveillance database allowed the identification of the index case, imported from Cameroon and living in the same area.
Active case finding (door to door and from health professionals) enabled the detection of other cases. Altogether, 12 autochthonous chikungunya cases were identified. All lived in or had visited the same area of Montpellier, a square of 250 m each side enclosing small buildings and individual houses.
Entomological investigations conducted in the neighbourhood of the cases showed high densities of Aedes albopictus larvae and adults and numerous gardens providing mosquito breeding and resting sites. Mosquito-control treatments and elimination of mosquito breeding sites were performed in the outbreak area.
In this episode, the conditions for autochthonous transmission of chikungunya virus were gathered: a densely populated neighborhood with a non-immune population, high densities of Aedes albopictus and the introduction of an adapted viral strain. However, the size of this outbreak remained small. Several factors can explain the interruption of the transmission: the effectiveness of the vector control measures, meteorological conditions and the diapause of Aedes albopictus. The implementation of the national dengue and chikungunya preparedness and contingency plan facilitated a prompt and coordinated multidisciplinary response.
In 2014, the chikungunya and dengue virus surveillance system in mainland France has been challenged by numerous imported cases due to the chikungunya epidemic in the Caribbean Islands. Nevertheless, this autochthonous outbreak was caused by a chikungunya strain imported from Africa and belonging to the ECSA genotype. This confirms the high susceptibility of French Aedes albopictus to such CHIKV strain and reminds us that chikungunya is an emerging disease in Europe.

Poster 5.19.

NOT PRESENTED

Status of Insecticide Susceptibility and Natural Leishmania Infection in Wild-Caught Sand Flies Collected From Coastal Areas of Izmir Province, Turkey

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In Turkey, vector control programs are mainly based on indoor residual spraying with pyrethroids against mosquitoes and no special control program is available for sand flies. Most insecticide susceptibility tests were done for mosquitoes but not for sand flies. We therefore aimed to determine the insecticide susceptibility against commonly used insecticides, on wild-caught sand fly populations collected in coastal areas of Izmir Province of Turkey.
Sand flies were collected from several towns of Izmir province and tested to determine insecticide susceptibility against 7 different synthetic pyrethroids (Permethrin, Deltamethrin, Etofenprox, Lambdacyhalothrin, Cyfluthrin, Lambda cyhalothrin, Alpha cypermethrin). WHO approved Tube test was performed and 3 different dosages (0.005%; 0.01%; 0.025%) were used in bioassays to determine effective dosage of tested insecticides. Evaluations were made using EPA Probit V1.5 and KDT50/KDT100 values were determined. Head and genitalia parts of sand flies were dissected for identification and rest of the body parts were pooled according to the species for PCR analysis. Conventional ITS1 PCR was performed to detect Leishmania spp. DNA in pooled samples.
In the lowest dosage (0.005%), by the end of 24 hour period, the most and less effective insecticides were etofenprox and permethrin with 66% and 43% of death rate, respectively. Death rates for the control tubes were noted as 4% that makes bioassays within the confidential limits. One pool containing 10 bodies of P. similis was found positive for Leishmania spp. A total of 6 Phlebotomus and 1 Sergentomyia species were found in the study area. The species and abundance of Phlebotomus were; P. tobbi (32.19%), P. neglectus (32.12%), P. papatasi (17.56%), P. alexandri (11.70%), P. similis (0.97%), P. mascittii (0.48%).
These results clearly pointed out more attention are needed by the authorities involved in control programs for sand fly-borne diseases. It is also needed to create devices and guidelines (by WHO or expert committee) for applying insecticide susceptibility tests using sand flies because of the tubes prepared for mosquitoes are not actually fit for sand flies.
This study was carried out in the frame of FP7-UE EDENext collaborative project, Contract Number: 261504 and TUBITAK Project No 112T270

Rift Valley fever in Mayotte in the Indian Ocean: from surveillance to genome detection

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Rift valley fever (RVF) is a zoonotic disease which circulates in many African countries as well as in the Arabian Peninsula. Epizootics are characterized by large sweeping abortion storms and significant mortality in adult livestock (primarily sheep, goats, and cattle), with newborn animal mortality approaching 100%. In most human cases, the disease is characterized by a self-limiting febrile illness progressing to more serious complications in only 1 to 2% of infected individuals (hepatitis, encephalitis, blindness or hemorrhagic syndrome. Further to the confirmation of a first human case of RVF in 2007 in Comoros, isolation of the virus was successfully achieved on suspected human cases. These viruses are genetically closely linked to the 2006-2007 Kenyan isolates. Serological surveys for antibodies to Rift Valley fever (RVF) virus were carried out in ruminants on Mayotte following the confirmation of a first human case of RVF in Comoros in 2007. The results suggested low level circulation of RVF virus on Mayotte as early as 2004, with neither human nor animal population experiencing outbreaks of the disease.

Swedish city rats ? a potential health threat?

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Background:
The number of rats rapidly increases in our cities and are well-known carriers of various microorganisms pathogenic to humans like plague (Yersinia pestis), leptospira and Seoul hantavirus (SEOV). SEOV causes hemorrhagic fever with renal syndrome (HFRS) expressed mainly by fever, fatigue and kidney problems. SEOV is found mainly in rats in China and Southeast Asia, but has recently also been found in European wild rats in France, Belgium, England and the Netherlands. Very little is at present known about any potential pathogens carried by Swedish city rats. In this study we are investigating potentially dangerous microorganisms, like Seoul hantavirus, carried by wild brown rats (Rattus norvegicus) in Sweden.

Methods:
Together with actors in the pest control industry, and own trapping efforts, we have collected hundreds of wild-caught rats as well as droppings from rats, mainly from different Swedish cities. Rats have been dissected and organs such as spleen, kidneys and brain are being analysed. The methods for analyses of previously known microorganisms in the collected rat tissues are predominantly ELISAs, immunofluorescence assays (IFA), neutralization tests (NT), RT-PCR, Q-PCR, genome sequencing, and cell culture isolation. We are searching for various pathogenic and zoonotic viruses, bacteria and protozoa.

Results:
We will present the first results of our analyses.

Conclusions:
The project will clarify whether wild rats in Sweden can be a threat to human health, regarding a selection of pathogens.

Assessment of the efficacy of insecticide control strategies in Rome (Italy) using novel monitoring and statistical approaches

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Urban mosquitoes in temperate regions are target of control activities aimed to reduce their nuisance and the risk of arbovirus transmission. Common practices to reduce this burden often imply larvicide treatments of street catch basins ? i.e. the main non-removable urban breeding site ? and/or insecticide spraying. The planning of these interventions, as well as the evaluation of their effectiveness, rarely benefit of adequate monitoring of the mosquito abundance and dynamics.
We present the results of the assessment of mosquito control activities (i.e. bi-monthly treatments of catch basins with Insect Growth Regulators and Low-Volume insecticide spraying) carried out in Rome in 2012 and 2013 in the main campus of Sapienza University and in the area of the University Hospital, respectively. Monitoring of mosquito abundance and population dynamics in these areas and in control un-treated areas was carried out by adhesive traps (instead than by more widely used ovitraps) in order to directly target the adult populations. To investigate how observed spatio-temporal variations in mosquito (Aedes albopictus and Culex pipiens males and females) counts was affected by several explanatory variables (e.g. mosquito collection methods, type and number of treatments) advanced statistical models were carried out by implementing both Generalized Linear Mixed Models and Change Point Analysis approaches.
The analysis of 2012-treatments showed: i) inhibition of adult emergence in most monitored treated catch basins, although these were repeatedly visited by adult females; ii) significant reduction of adult abundance in the treated area than in the control one after the adulticide spraying carried out at the peak of the mosquito reproductive season; iii) spatial heterogeneities in the effect of the treatments. The analysis of 2013-treatments showed a significant decrease in correlation in mean female counts between the treated and untreated sites during the peak of Ae. albopictus population expansion, even though the two sites experienced similar climatic changes.
Overall the results support the approach used (i.e. mosquito monitoring by adhesive traps associated to advanced statistical analyses) in assessing the efficacy of control measures and in providing indications on the effectiveness of common mosquito control strategies carried out against mosquito in European urban areas.