

### A user-friendly, Windows based risk mapping program

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A risk map shows the modelled distribution of a particular disease or vector over a region, and is usually created by applying an algorithm to a set of training data (of known disease presence, absence or abundance) associated with a set of predictor variables often derived from climatological databases, or from earth-orbiting satellites. Risk maps identify areas of possible disease risk across the entire area covered by the training set, and often also regions well beyond the training set limits. The EDEN LRRS HIT team has developed a set of non-linear discriminant analytical algorithms for risk mapping and these have been applied to a number of datasets from EDEN's specialised disease Sub-Projects and using inter alia the LRRS team's temporal Fourier processed MODIS data as predictors. Currently there is no commercial or free soft-ware for non-linear discriminant analysis of this sort and so the LRRS team had to write its own (originally in MS QBasic). Various refinements to the basic modelling algorithm have been added during the life-time of EDEN, including bootstrap sampling of the training set data and the use of new selection criteria for, and new ways of, identifying important discriminatory predictors. The team has also developed approaches for using administrative level data of disease presence/absence to produce pixel level risk map predictions. In order to share more widely the expertise developed within EDEN, the LRRS modelling approach has now been encoded in MS VBasic Express (i.e. free PC software from Microsoft) to produce a user-friendly GUI suite that carries out in a single pass all of the operations that used to involve at least seven separate programs. To the extended capability mentioned above we have also added a Bayesian option for discriminant analysis and further options allowing the use of alternative algorithms for risk map production (e.g. random forest models). The GUI application begins with a standard form, with a Windows 'look and feel', in which the user selects the training set data (which may be in raster or text file format) and the set of predictor variables to be used (prominent among which are usually the temporal Fourier processed MODIS data). The user is then guided through a sequence of pop-up windows to select options determining the scaling of the data; the removal of outliers; the number of clusters into which to divide the data; the variable selection method to be used; whether or not bootstrap samples should be taken, and the results averaged; and so on. All output maps are saved as IDRISI raster files for input into GIS software. Model statistics and a log of all the options selected by the user during the current model run are saved as text files. Results are averaged and summarised into a further text file when bootstrap samples are taken. Both map and text output are also displayed on-screen as each model is created. We have developed this software so that users can create their own risk maps from their own data. Every attempt is made to guide the user through the modelling process (using help files) and to explain in biological terms the meaning of the model results. We strongly believe that statistical risk mapping is only as good as the biological conclusions that can be drawn from it. We aim to make this new software a tool to help biologists understand their vectors and diseases better.

### Bayesian Spatial Discriminant Analysis for Risk Mapping

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The standard discriminant analytical risk mapping approach of EDEN's LRRS HIT group, applied to many of EDEN's datasets, is based on calculating the Mahalanobis Distances (a co-variance adjusted measure of separation) of each point in the training set from the centroids of the multi-variate clusters defined by the entire set of training data of presence/absence or abundance observations (Hair et al. 1998). A Bayes' theorem then determines the posterior probability of the point belonging to each of the distributions in turn (Marden 2000). Posterior probabilities define the output prediction of 'risk' when they are summed across all absence and all presence probabilities (Rogers 2000).

In common with most other risk mapping algorithms in use at the present time the above method does not take into account either the geo-spatial arrangement of the training set data nor the uncertainty of the values of the real world parameters introduced by taking any training set sub-sample to define the relevant multi-variate distributions used in the discrimination algorithm (Fraley and Raftery 2002).

In this talk we propose a new algorithm that combines Discriminant Analysis, Bayesian statistics and Geostatistics (Bayesian Spatial Discriminant Analysis) for improved disease risk mapping. In this new approach the means and the covariance matrix for each cluster are considered random variables and are corrected for spatial (and/or temporal) autocorrelation. This involves the use of priors (Gelman et al. 2004) for each of the parameters in the components of the geo-spatial model (kernel trend, variogram, distribution of the mean values, variance-covariance distribution and probabilities in the discriminant function). The Bayesian approach estimates the probability that the location belongs to the cluster  $j$  with local mean,  $\mu$ , and variance-covariance matrix  $\Sigma$  given the available data (Anderson et al. 2000).

The new method offers several advantages over the older one, such as the ability to combine soft information (interval or qualitative data), laws (e.g. immunity increases with age) and observed values (hard data); to work with different distributions for each of the parameters in the model; to assess the uncertainty of the model and its predictions. The disadvantages are the heavy computational requirements of even relatively modestly sized training sets; the difficulties in the prior selection; and the more complex interpretation of the results.

The new method is illustrated with an application to one of EDEN's disease datasets. This example shows the potential of the new method, and also some of its limitations. The future perspectives of Bayesian Spatial Discriminant Analysis are finally discussed.

## Understanding the abiotic and biotic drivers of vector-borne disease emergence

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Vector abundance and seasonal dynamics are important determinants of variation in the risk of vector-borne infections. Seasonal and spatial patterns in vectors are influenced by both abiotic (climate, land use) and biotic (hosts, conspecifics) processes whose impacts vary between species. These differential impacts are best understood using model frameworks in which ecological responses are compared across candidate vector species in a region and linked to their ecological characteristics such as life-history and resource use.

Orbiviral pathogens of livestock (the causal agents of bluetongue and African Horse Sickness) display typical features of vector-borne disease systems that complicate our understanding of patterns in transmission. Population sizes of their *Culicoides* biting midge vectors can vary seasonally and over short distances (< 1km) by several orders of magnitude in relation to environmental factors. Within a single region, transmission can involve multiple ruminant hosts and multiple vector species. The latter often include complexes of cryptic *Culicoides* species, making critical, species-specific, data on habitat use, host or pathogen interactions and demography unobtainable without molecular taxonomy.

Here, comparative analyses are applied to understand patterns in *Culicoides* vectors for both a temperate and a tropical orbivirus system. Firstly, local scale abundance patterns of constituent species of the palearctic *C. obsoletus* and *C. pulicaris* groups across Scotland are related to landscape, host and remote-sensed climate factors within a hierarchical GLM model framework. Abundance patterns were better explained by models combining climate with host or landscape factors than by climate models alone. Key predictors and model accuracy varied between species with poorest performance for generalist species such as *C. pulicaris s.s.* In the second case study, the mortality rates of two key vectors from the *C. imicola* complex – *C. bolitinos* and *C. imicola s.s.* - are estimated for sites across South Africa and related to seasonal, remotely-sensed, temperature and rainfall changes using a Bayesian Hierarchical Non-linear Mixed Model. Density dependent factors play a

substantial role in regulating populations of both species, probably because high reproductive output of females and scarcity of breeding sites in dry conditions leads to larval competition for resources. Conditions of high temperature and moisture levels favour survival of both species but to different extents. Remotely sensed models of mortality outperformed those based on meteorological data and could be extrapolated to identify zones and seasons of high *C. imicola* s.s and *C. bolitinos* mortality. We illustrate how similar vector model outputs have been integrated into  $R_0$  model frameworks to evaluate how species-specific sensitivities to environmental conditions translate into changing risk of orbivirus establishment.

## **Ecosystem complexity, biodiversity dynamics and infectious diseases. An introduction to community epidemiology**

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Infectious disease represents a critical problem for human well-being today. These diseases are caused by pathogen agents which are mainly originating from wildlife, and their understanding cannot be done without considering the whole ecosystem in which they are involved. Here, we show how the different components of parasite ecosystem can influence disease agent transmission. To do that, we have apply the concepts issued from community ecology and to the field of epidemiology. We analyse how the structure of species communities, the ecological relationships between species and the links that connect them to each other and their life history traits can impact on parasite transmission within the environment. After developing a generic model for vector-borne diseases in wildlife living in an heterogeneous environment, we show how the spatial distribution of host reservoir and vector species can affect the general characteristics of pathogen transmission. At last, we discuss on a better consideration of ecological literacies in epidemiology, and their importance in public human health. We conclude on the necessity to conduct a more global approach in epidemiology to study the transmission of these pathogens in wildlife, and we outline new avenues of research.

## **R0; models and risk maps**

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An important aim of the EDEN project has been the development of tools to study the effect of different (climate) scenarios on the risk of vector-borne diseases. The basic reproduction number (or  $R_0$ ) is such a tool; it is a measure for the risk that a disease can establish after introduction. Deriving a correct expression for  $R_0$  for each disease system and calculating  $R_0$  values can be done by means of the next-generation method.

The EDEN Modelling Horizontal Integration team has focussed on exploring the usefulness of  $R_0$  and the next-generation method in the context of predicting the risk of establishing of emerging vector-borne diseases in a changing European environment. We here present a number of case studies:

In the West Nile virus case study, we demonstrated that an additional transmission route — which was shown experimentally, but for which the relevance in the field is unknown — can easily be included in the calculation of  $R_0$ . The contribution of a transmission route to  $R_0$  can then be assessed by performing a simple sensitivity analysis. Our results suggest that transmission between crows via close contact could have a considerable impact on establishment of WNV, especially at places or at moments with a low density of ornithophilic mosquitoes or a high density of crows (e.g., in large communal roosts), but proof of bird-to-bird transmission in the field is needed before stronger conclusions can be drawn.

The second case study, on tick-borne infections, shows that the next-generation method can also be applied to very complex disease systems. In chapter 3, this is demonstrated by deriving an expression for the basic reproduction number for two tick-borne diseases: Lyme borreliosis and tick-borne encephalitis (TBE). Both are

transmitted by the bite of infected ticks of the species *Ixodes ricinus*, a common European tick species. In the case of TBE and Lyme borreliosis, the next-generation matrices have the same structure and differ only in the values taken by the elements of the matrix, which means that direct comparison is possible.

In the third case study, we show how an  $R_0$  map can be constructed, using canine leishmaniasis in a region in the South-west of France as an example. All the horizontal integration teams in EDEN were involved in this project, where field data from the Leishmaniasis team were used as input for a prediction model. This prediction model is unique in that it uses predictor variables derived from high resolution satellite data (land cover, landscape fragmentation, etc) as well as low resolution satellite data (climatic variables and patterns of seasonality). The resulting sand fly abundance predictions are then used as input in a mechanistic temperature dependent model to calculate the  $R_0$  values to produce an  $R_0$  map.

## Session 2 - Tick-borne diseases (Tick-borne pathogen distribution and prevalence)

### Detection of tick-borne pathogens in Estonia

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Tick-borne infections are widely distributed in Europe. The most frequent tick-borne diseases (TBD) are Lyme borreliosis and tick-borne encephalitis, which are caused by spirochetes of *Borrelia burgdorferi* sensu lato complex and tick-borne encephalitis virus (TBEV), respectively. Also there is an increasing frequency of human granulocytic anaplasmosis and human babesiosis cases reported in Europe. These tick-borne diseases are caused by the bacterium *Anaplasma phagocytophilum* and protozoa of *Babesia* species, respectively. There are two tick species of the *Ixodes* genus circulating in Estonia, *I. ricinus* which is prevalent throughout the entire territory, and *I. persulcatus* found in eastern Estonia.

The aim of study was to detect tick-borne pathogens circulating in Estonian tick populations during 2006-2008.

Ticks were collected during their activity period (April- October) in seven different regions of Estonia by standard flagging. For the detection of TBEV, *Anaplasma* and *Babesia*, ticks were pooled according to collecting sites, species, development stage and sex. For the presence of *Borrelia* species ticks were investigated individually. Detection of TBEV and *A. phagocytophilum* was carried out using real-time PCR. The presence of *Borrelia* and *Babesia* species was determined with Reverse Line Blot hybridization (RLB).

In 2006-2008, a total of 10503 ticks (3089 larvae, 5324 nymphs and 2090 adults) were collected, of which 3205 (798 adults, 1343 nymphs of *I. ricinus* and 725 adults/339 nymphs of *I. persulcatus*) were analysed.

A total of 2283 ticks (456 pools) were analysed for the presence of TBEV, 46 of which were positive. The majority of TBEV infected tick pools (33/46) were collected in two closely situated localities in the south-east of Estonia (Laeva, Järvselja). The presence of *Babesia* spp was detected in 36 of 461 pools; *B. microti* and *B. divergens* were found in 18 and 2 tick pools, respectively, while in 15 tick pools *Babesia* species could not be identified by RLB. Twenty one out of 36 positive tick pools were collected in the coastal region of south-western Estonia (Puhtu, Are, Kilingi-Nõmme), and 8/36 of *Babesia* positive tick pools were collected in south-eastern part (Laeva, Järvselja). *A. phagocytophilum* was found in 23 tick pools out of 3112 analyzed, with the majority of positive pools (18/23) collected in South-West Estonia (Puhtu, Are, Kilingi-Nõmme).

*B. burgdorferi* s.l. was detected in 30 of 758 individually analyzed ticks. Out of the positive ticks, 11 were infected with a single species (4 ticks with *B. garinii* and 7 with *B. afzelii*), 4 ticks had double infections (3 with *B. garinii/B. afzelii* and 1 with *B. afzelii/B. lusitaniae*), while 5 ticks had triple infections (*B. garinii/B. afzelii/B. lusitaniae*). In one third (10/30) of *Borrelia*-positive ticks, the infecting species could not be

determined.

A majority of the ticks carrying pathogens were collected in southern part of Estonia. *Borrelia*, *Babesia* and *Anaplasma* were detected in South-Western Estonia while TBEV and *Babesia* were more prevalent in the South-East of Estonia.

## **Lyme disease and human granulocytic anaplasmosis in Latvia, epidemiological situation and molecular analysis in ticks**

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Ticks of the Ixodidae family are vectors of various human and animal pathogens, including *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*. Epidemiological significance has been observed for only two *Ixodes* tick species in Latvia: *Ixodes ricinus* L., spread on the whole territory of the country, less in its eastern part, where *I. persulcatus* P. Sch. is prevailing.

From the beginning of 1990s a significant increase in Lyme disease cases was observed with the highest number of cases in 2003 (714 or 30,62 cases per 100 000 inhabitants). An almost similar high level was noticed in 2004, whereas in 2005 and subsequent years there was variable incidence of the disease (488-619 cases). Epidemiological data were analysed using incidence rates for the time period 2002-2008. Differences in morbidity by gender were found; morbidity increased with the age. The seasonality was analysed according to the registration month of cases and most of cases were notified during the tick activity season. During the observation period, mostly local skin forms of Lyme borreliosis were registered, the incidence of this form was significantly higher than the incidence of neuroborreliosis, and there were differences in distribution of the different forms by regions.

The first case of human granulocytic anaplasmosis was diagnosed serologically in 2001 and up to 2007 altogether there had been 16 cases registered; an additional 25 ehrlichiosis cases appeared in 2008. Monitoring and collection of *I. ricinus* and *I. persulcatus* developmental stages was performed in 2006-2008 during the tick activity season, once a month at 8 sites in Riga, Liepaja, Saldus, Tukums, Madona, Kraslava and Jelgava regions.

Reverse Line Blot Hybridization method was used for detection of *Borrelia* species, and Real Time PCR was used for detection of *Anaplasma phagocytophilum* in ticks collected from the field. *Borrelia burgdorferi* s.l. has been found so far in 24,5% of ticks, contained several *Borrelia* species: 10 ticks (of 49) contained *B. afzelii*, three - *B. lusitaniae* and one - *B. garinii*. Three ticks contained mixed *Borrelia* species.

Molecular analysis of *Ixodes* tick pools pointed to higher prevalence of *Anaplasma phagocytophilum* in adult ticks than nymphs (4.4% and 1.6% of pools, respectively). Positive results for *A. phagocytophilum* were obtained for ticks collected from sites from western and central parts of Latvia, however not from eastern parts.

The unequal incidence distribution of incidence rates as well as clinical forms by regions could present evidence of true differences in rates due to unequal distribution of pathogens, could be explained by different strategy in diagnostics, treatment, and notification of Lyme disease and confirms the need of further investigation.

## **Spatio-temporal distribution of *Ixodes ricinus* ticks and prevalence of tick-borne pathogens in Slovakia**

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Based on previous epidemiological surveillances of tick-borne diseases in Slovakia, six tick collection sites were selected (Western Slovakia: Malacky, Devín, Medné, Topolcianky, Eastern Slovakia: Zádiel, Rozhanovce) in order to study seasonal dynamics of tick activity and prevalence of tick-borne pathogens. Questing ticks were collected by standardised methods (flagging, 100 m<sup>2</sup>) in monthly intervals in 2006-2008. Molecular diagnostic methods were applied to detect pathogens in questing *Ixodes ricinus* ticks: qRT-PCR for tick-borne encephalitis virus (TBEV) and *Anaplasma phagocytophilum*, touch-down PCR followed by reverse line blot (RLB) for species detection of the *Borrelia burgdorferi* s.l. complex and *Babesia* spp. Pathogen prevalence was detected either in individual ticks or pooled samples, where the maximum likelihood estimation (MLE) of infection rate was calculated.

Numbers of collected ticks and prevalence of tick-borne pathogens varied considerably among sites and during the seasons. *Ixodes ricinus* dominated (95%; range 72-100%, depending on site and year). *Haemaphysalis concinna* and *H. inermis* were present in W Slovakia and *Dermacentor marginatus* and *H. inermis* in E Slovakia. Temporal changes in tick density patterns were observed between the collection sites. In 2006, the highest average *I. ricinus* density was in Malacky (252 ind./100 m<sup>2</sup>) and the lowest in Zádiel (3.7 ind./100 m<sup>2</sup>), whereas in 2008 the tick density in Malacky decreased to 23.20 ind./100 m<sup>2</sup> and the highest density was registered in Topolcianky (64.12 ind./100 m<sup>2</sup>). Peaks of *I. ricinus* seasonal activity were detected between April and June.

Presence of TBEV was confirmed in the known focus at Topolcianky. MLE for TBEV infection rate in adult ticks was 4.46% in 2006. Based on sequencing and BLAST analysis, the detected TBEV was assigned to the HYPR strain. Molecular diagnostics revealed the presence of several genospecies of the *B. burgdorferi* s.l. complex, *A. phagocytophilum* and *Babesia* spp. in both adult and nymphal *I. ricinus* at all study sites, as well as spatial and temporal differences in the distribution and prevalence of the pathogens. Overall *A. phagocytophilum* and *Babesia* spp. infection rates reached 3.2% and 2.7%, respectively. Both pathogens were most prevalent at Malacky (MLE: *A. phagocytophilum* 10.1% - adult ticks, 3.8% - nymphs; *Babesia* spp. 5.8% - adults, 3.5% - nymphs). 42.8% and 4.7% of *Babesia*-positive samples were *B. microti* and *B. divergens*, respectively, whereas 47.6% of the positive samples have not been detected to species level. These samples will be further analysed by sequencing. Presence of *B. microti* (50%) and *B. divergens* (10%) was also confirmed at Topolcianky, the other positive samples have been identified only as *Babesia* spp. Overall *B. burgdorferi* s.l. prevalence was 25.9% and 31.7% in W and E Slovakia, respectively. Spatial heterogeneity in representation of individual *Borrelia* genospecies was obvious. In W Slovakia *B. garinii* and co-infections of *B. afzelii* and *B. burgdorferi* s.s. prevailed, whereas *B. lusitaniae* and *B. garinii* were dominant in E Slovakia.

The results confirmed that humans in selected sites of Slovakia are exposed to high risk of infection with tick-borne pathogens, mainly borreliae, however, risk of infection with other pathogens must also be considered.

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## Genetic characterization of TBE virus obtained from *Ixodes ricinus* and *Dermacentor reticulatus* ticks

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Tick-borne encephalitis virus (TBEV) is an arthropod-borne virus, which is transmitted to vertebrates by chronically infected ticks. In Europe *Ixodes ricinus* tick species are regarded as vectors for TBEV. TBEV

causes encephalitis (TBE) or meningitis in humans, occasionally with serious sequelae, and has a significant impact on public health in endemic areas. In Poland, sudden increasing of TBE cases has been reported in 1993 and up to date, the TBE morbidity stays at the high level. Almost half of Polish TBE human cases are registered in north eastern part of Poland (Bialystok region). In this endemic area two tick species, *I. ricinus* and *Dermacentor reticulatus*, co-exist. The importance of *D. reticulatus* ticks for TBEV maintenance in endemic areas is unclear. Several TBEV strains have been isolated from ticks *I. ricinus*, patients and small mammals in Poland in the past, but genetic characterizations of virus have never been performed.

Aims of the study were: to detect TBEV in questing tick in Bialystok region; to characterize genetically TBEV strains circulating in Bialystok endemic region.

Material and methods: Ticks (nymphs and adults of *I. ricinus* and adults of *D. reticulatus*) were collected by flagging from vegetation throughout the whole season (April to October) in 2007. Collected ticks were washed with 70% ethanol and stored at -80°C. Ticks were pooled according to species, stage, sex, and collection place. A total, 270 tick pools of *I. ricinus* (20 nymphs and of 10 adults in pool) as well as 120 tick pools of *D. reticulatus* (5 adult ticks/pool) were prepared. Presence of TBEV was detected by real-time PCR. The minimum infectious rate (MIF) of TBEV in tick pools was calculated with the assumption that a positive pool contains only one infected tick. For genetic analysis positive samples were amplified in the E gene of TBEV by nested PCR with subsequent sequencing. Phylogenetic analysis of partial E gene was performed using the PHYLIP program .

Results: In total 3600 *I. ricinus* (1800 adults and 1800 nymphs) and 600 *D. reticulatus* adult ticks were analyzed by real-time PCR. Six tick pools of *I. ricinus* were found positive, 3 were detected in adults and 3 in nymphs pools. Surprisingly, the presence of TBEV was detected in two *D. reticulatus* pools. The TBEV MIF in questing ticks was calculated as 0.16% for *I. ricinus* and 0.33% for *D. reticulatus*. Nucleotide sequences of partial E gene (1168 nucleotides) were recovered for 6 (4 from *I. ricinus* and 2 from *D. reticulatus*) out of the 8 positive tick pools. Genetic analysis of these samples revealed that all of them belong to European TBEV subtype (Eu-TBEV). The nucleotide sequence homology within subtype was 99.2% for *I. ricinus* and 98.2% for *D. reticulatus* TBEV strains. On the phylogenetic tree, TBEV strains of *D. reticulatus* were monophyletic and formed a well supported lineage, while strains from *I. ricinus* shared common ancestors with strains from Latvia and Estonia.

Conclusions: Two species of ticks were infected with TBEV. Two tick species may exchange TBEV strains in the same foci.

## **The prevalence of Crimean-Congo haemorrhagic fever virus in the host seeking ticks in an endemic area of Turkey**

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The recent 6 years epidemic of CCHF in Turkey has induced special attention on the importance of the *Hyalomma marginatum* tick, which is the main vector of the virus in Balkans, Crimea, Southern Russia and Turkey. Human-tick contact level and the prevalence of infected ticks are among the main indicators of the epidemiological status of the disease. In this study we aimed to understand the prevalence of the CCHFv in host-seeking ticks and to obtain information about the human-tick contact in an endemic area of Turkey.

Ticks were collected from 6 villages of Cekerek and Kadisehri districts of Yozgat province in July 2008. Crop fields, where human activities were concentrated, were selected with the aim of collecting active host-seeking *Hyalomma* ticks. Collection was made by using human volunteers, where one collector spending 15 minutes sitting/standing by the bushy border of the field in order to attract hiding ticks. Sampling was done between 10 and 12 in the morning and 14 and 16 hours in the evening for total of 4 hours per day. Approaching or already crawling ticks were collected in to vials, kept alive in a cool place at approx. 85%RH and later were transferred to the laboratory. Ticks were identified at species level (4) and were further crushed and stored in liquid

nitrogen until RNA extraction. RNA extraction and cDNA synthesis were done by using commercial kits. Semi-nested PCR protocol amplifying a specific part of CCHFv S genome was conducted. Positive samples were subjected to sequencing and 593bp region of obtained S segment parts was blasted against known sequences and a phylogenetic tree was constructed. Some samples were selected for viral load determination by qRT-PCR (4) according their low, medium and high visual band intensities on the agarose-gels

A total of 280 host-seeking ticks were collected during 15 days of study period. Of these, 276 (98.57%) were *H. marginatum*. Also 2 individuals of each *Dermacentor marginatus* and *Rhipicephalus turanicus* were found crawling on collectors, probably acquired from vegetation. Sex distribution was as 158 female and 122 male ticks. All ticks were individually subjected to semi-nested PCR and 46 (16.43%) were positive for CCHFv RNA presence. Although female ticks (28, 60.87%) had higher infection rate compared to male ticks (18, 39.13%) the difference was not significant. Prevalence of infected ticks among 6 localities varied between 6% and 30%. Five positive samples with comparatively low, medium and high visual band intensity on the gels were tested for viral load determination by qRT-PCR. Viral load ranged between  $4.88 \times 10^1$  and  $8.64 \times 10^4$ .

Although tick-bite rate was high, only 152 persons from these study sites attended the local hospitals for tick removal during study period. All removed ticks were *H. marginatum*. In the same time 5 persons at these villages, regardless of hospital tick removal, developed clinical signs and CCHF was confirmed in all by laboratory testing. It seems that clinical manifestations appear at a low level compared to the high infection rate among ticks. Thus some factors, such as the timely removal of ticks, viral load in ticks or individual immune responses, may be important in limiting viral transmission.

Tick presence showed a scattered distribution and the abundance of ticks varied among different sites, but our data could not identify any significant sign for hot-spots.

### Session 3 – From endemic Mosquito-borne diseases in Africa to emerging Mosquito-borne diseases in Europe (Endemic cycles in Africa)

#### Rift Valley fever surveillance systems in Senegal, West Africa

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RVF is a viral disease of veterinary and public health importance. Periodic severe animal epizootics are accompanied with human epidemics in Africa (Egypt 1997, 1993, Mauritania 1987, 1993, 1998, Eastern Africa 1997-98) with recent extensions in the Arabian Peninsula (Saudi Arabia and Yemen in 2000). In Senegal, following the first RVF outbreak in 1987 in the Senegal River Delta, a surveillance program of this disease in domestic ruminants was conducted since 20 years (from 1988 to 2008). The main objectives were to establish an early detection of the disease based on sentinel herds sero-monitoring and proper and rapid disease reporting through the country.

A network of sentinel herds (small ruminants) located in potential high risk areas for RVF epizootics were visited during the raining season (June to November) and subject to clinical examination like also several cattle herds, randomly selected during the Pan African Rinderpest Campaign, which were sampled. Collected sera were analysed by Neutralization Test and Elisa for IgM and IgG antibodies in order to reveal recent and/or past viral infection. Virus isolation from organs and tissues was performed in Vero cells culture and by inoculating suckling mouse.

Recent transversal entomological studies were conducted in the Senegal River Delta to identify potential RVFV mosquito vectors that occur in the region. These entomological results were correlated to serosurvey data to determine the high risk periods of RVFV transmission.

The serosurveys conducted in sheep and goats and entomological data showed the following:

In the Senegal River Basin, the RVF antibody prevalence that reached a peak of 70% after the 1987 epizootic, dropped to 30% in 1988 and, then decreased continuously until 1993. This decrease of RVF prevalence



corresponded to period of low rainfall

RVF virus activity re-emerged as epizootics in 1994, 1999, 2002 and, 2003 amongst herds in the Senegal River basin and bordering areas such as the Ferlo plateau.

In the Ferlo, an enzootic cycle of RVF virus was shown involving mostly *A vexans* and *A ochraceus* mosquito species as its vectors during the raining season.

In the Delta, *Culex tritaeniorhynchus*, *Cx poicilipes*, *Cx neavei* and *Mansonia uniformis*, 4 species candidates vectors for RVFV were identified. The critical period of transmission would be situated between September-November, corresponding to the second half of the rainy season, according to the results on the dynamics of abundance of these mosquitoes.

Communication and training materials (10 periodic bulletins, 400 booklets, 200 videos and 2000 posters) were produced and distributed to raise local awareness with regard to RVF burden and cost on livestock and human health.

A computerized regional database was developed with more than 20,000 data set (serosurveys, suspected cases and, outbreaks notifications) collected in Senegal and neighbouring countries (Mali, Mauritania) from 1988 to 2008.

Although data obtained from satellite imagery were used in order to assist in predicting and preventing future RVF epizootics and epidemics, the immune status of animal herds, herd movements and, local conditions favouring the mosquito breeding might play a more significant role in the inter-annual variability of outbreaks than environmental factors (i.e.: rainfall, land cover).

Our RVF surveillance is done by the clinical and serological survey system according to local settings, specially herd owners agreement, cost and effectiveness. Moreover, the selected diagnostic tools like ELISA assay allowed an efficient IgG and IgM detection, and IgM detection is a helpful indicator of recent infections. Virus circulation increases the risk of epizootic, and subsequently a risk of epidemic due to vector activity. The etiological diagnosis is to be associated with sustained awareness of RVF in order to prevent major RVF emergences.

## **Culex neavei (Diptera, Culicidae), a potential West Nile vector bridging birds and horses in Ross Bethio, Senegal**

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West Nile virus (WNV) is a widespread pathogen transmitted by mosquitoes. Its epidemiological cycle of involves birds. Humans and horses are accidental, dead end hosts. Migratory birds are believed to play important roles in its dissemination through the Palaearctic migration, as well as local migration between wintering sites. However studies on a smaller scale of local transmission dynamics may help to better understand the epidemiological cycles. In this work, we have studied the potential mosquito vectors involved in the transmission using a general trapping method (CDC traps) and then narrowing it to more host specific methods (horse-baited trap, henhouse sentinel chicken baited-trap, and pigeon-baited trap). Previous seroprevalence studies had indicated that horses and domestic birds are frequently exposed to WNV in the Senegal River delta. Our abundance and feeding-pattern results have indicated that *Cx tritaeniorhynchus* and *Cx neavei* may play a key role in the WNV transmission dynamics. Moreover, *Cx neavei* is both an ornitophilic, and a mammophilic species, thus giving to this mosquito species a high potential to be a bridge WNV vector between mammals and birds.

## **Changes in mosquito fauna of Tunisia**

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Urbanization and agriculture practices (dams, irrigation systems) generally imply ecosystem disturbances including breeding sites and mosquito population. This has prompted us to undertake an entomological study to monitor and evaluate the new situation of mosquito fauna. Our aim was to study the diversity of mosquito fauna in all regions of the country : collecting (dipping) and identifying larvae from potential breeding sites. We compared the previous and present geographic distributions and abundance of mosquito in Tunisia. In addition, we have investigated the ability of some strains of Tunisian *Culex pipiens* to transmit Rift Valley Fever virus (RVFV).

The identification of the collected field larvae showed the presence of 9 species of *Anopheles* (among 12 listed in the literature) including *An. labranchiae*, *An. sergenti* and *An. multicolor* which has been incriminated in Plasmodium transmission. Breeding sites of *An. labranchiae* have disappeared from several regions where malaria was endemic. Nine species of *Culex* (among 10 cited in the literature) were identified in our collection. *Cx. pipiens* is the most frequent and abundant species found. Several field collected *Cx. pipiens* strains were susceptible to disseminate RVFV with an infection rate of 14.7%. However, *Cx. perexiguus*, rarely collected in the past, seemed to be more common. Six *Aedes* species were encountered in our collection. Among them, *A. caspius* and *A. detritus* which occupied halophilic sites, were the most abundant and frequent species in coastal regions. Several generations of these two species emerged per year due to erratic rainfalls and mild winters. Our survey made it possible to find the majority of previously described species mosquitocited in Tunisia. The drop in rainfall and the intensive use of water for irrigation could be a source of spatial and temporal variations of breeding sites and scarcity or even disappearance of some mosquito species. Pollution of water (urbanization) has encouraged *Culex pipiens*, which has become a threat by its density and ability to transmit certain pathogens including West Nile virus, responsible for two outbreaks in Tunisia with 17 deaths (1997 and 2003). This species is active almost all year round because of mild winters; this increased high resistance, with respect to most organophosphate and pyrethroid insecticides.

## **West Nile virus circulation among a sentinel horse population in the Senegal River Delta**

Nicolas D. DIOUF

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From August 2007 through July 2008, blood samples were collected from 570 horses living in the Senegal River Delta from 3 different zones: Ross Bethio, Richard-Toll and St.-Louis. After that, sera were analyzed by indirect ELISA method. Out of 570 horses, only 38 horses were negative from West Nile antibodies: 21 horses from St.-Louis, 11 from Ross-Bethio and 6 from Richard-Toll. These negative animals constitute sentinel horses and were followed-up in a longitudinal survey by nearly two months intervals sera collections during 11 months.

During this period, WN antibodies were found in 19 horses after ELISA test that means, in 50% of sentinel animals. We tested them again by PRNT method and had confirmation for 8 of them, a global incidence of 21% [8; 34] was found for neutralizing antibodies: 14.28% in St-Louis, 27.27% in Ross Bethio and 33.33% in Richard-Toll. These results show that horses were infected between October and January and confirm a West Nile virus circulation. 12h30-12h45 West Nile disease in Morocco wild birds (Ouafaa Fassi Fihri, Institut Agro-Vétérinaire Hassan II, Morocco)

## **West Nile disease in Morocco wild birds**

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Within the framework of the Eden project we have studied the circulation of West Nile virus in wild birds in Morocco.

During the years 2008-2009, we have sampled blood of 365 wild birds from 31 species at 5 localities in the

dwelling neighbourhoods (farms, villages, small towns) and near wetlands (rivers, ponds, snares). We have focused our work in declared disease zones during the 1996 and 2003 outbreaks. The field work took place in summer, winter and spring in low altitude and mountain, in the north and the south, near the sea and inside the continent.

Serum samples were tested by the ELISA-Ab competition technique. Overall WNV antibody prevalence was 8%. Positives were reported for 7 species and all the visited stations revealed the presence of the virus circulation with a rate of prevalence rather close except that to the Ifrane station. Antibodies prevalence was specially high for blackbirds (*Turdus merula*), a non-migratory species, with prevalences ranging from 16,7% to 37,5% in the different localities.

Antibodies against Usutu virus were found in some birds in the Sidi Kacem station (Gharb).

These results suggest the silent circulation of West Nile virus in Morocco.. New studies are needed to determine the propagation conditions of the virus, confirm its circulation in on all wetlands and determine the conditions favouring outbreaks among humans and horses.

## Session 4 - Leishmaniasis (Mediterranean environments of leishmaniasis)

### Can we predict the distribution of canine leishmaniasis in West Europe using historical seroprevalence data and environmental variables?

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A systematic search was conducted of published and unpublished surveys of the frequency of canine leishmaniasis in Portugal, Spain, France and Italy. Seroprevalence data were used and a multivariate logistic regression model was built to find the main predictors of the prevalence of canine leishmaniasis (CanL) in the study region, from amongst the following factors: altitude, latitude, long-term averages of temperature, rainfall, relative humidity, enhanced vegetation index and middle infra-red. The analysis was adjusted for clustering by survey location. Three CanL prevalence endemicity classes were considered: low (<5%), medium (5-20%), and high risk (>20%). The predictive performance of the model was assessed using a 10-fold cross-validation approach, by determining the capacity of the model to (1) predict point-values of CanL prevalence, and (2) predict the correct endemicity class using the area-under-curve (AUC) of a receiver-operating-characteristic (ROC) curve. The statistical model was then applied to estimate and map the CanL prevalence across the whole study region.

2,059 potentially relevant surveys were identified. Amongst these, 947 surveys were included in the analysis, representing 504,369 dogs tested for *Leishmania* infections from 1971 to 2006. Most of the surveys included in the analysis were conducted in Italy (40%), followed by Spain (22%), Portugal (20%) and France (18%). The overall prevalence was 23.2% (range: 0-100%). The median prevalence was 10%, being highest in Italy (18%), followed by France (8%), Portugal (7%) and Spain (6%). The environmental factors associated with CanL prevalence in the final multivariate model were: altitude, night-time land surface temperature and enhanced vegetation index. The model did not perform well for predicting point-values of CanL prevalence ( $r=0.341$ ), but it performed reasonably well for correctly predicting the low and high risk endemicity classes (ROC AUC=0.71 and 0.72, respectively).

This work was funded by EU grant GOCE-2003-010284 EDEN ([www.eden-fp6project.net](http://www.eden-fp6project.net)).

## Phlebotomine sandfly vectors of *Leishmania* in the Algarve Region, Portugal

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The Algarve Region (AR) situated in southern Portugal was considered a leishmaniasis endemic focus in the 1980s. We report a survey carried out to update the distribution, abundance and vectorial roles of phlebotomine sandflies in the AR. From May to November 2006 and from March to November 2007, sandflies were captured by CDC miniature light traps. Kinetoplastid DNA-PCR and nuclear ribosomal ITS1-PCR were used to screen female sandflies for *Leishmania* infections.

A total of 2137 sand flies, 474 in 2006 and 1663 in 2007, were collected and identified morphologically. Specimens from the five phlebotomine species previously known to exist in Portugal (*Phlebotomus perniciosus*, *P. ariasi*, *P. papatasi*, *P. sergenti* and *Sergentomyia minuta*) were collected. The highest phlebotomine density was observed in July, when all species were collected. The predominant species was *P. perniciosus* and it was found infected with *L. infantum*. A simultaneous survey of canine leishmaniasis (CanL) revealed dogs infected with *L. infantum*, showing that the AR remains an active focus of CanL.

Climate warming would enhance the number of days favourable for *Leishmania* transmission, due to higher densities and activities of sandflies during a longer period each year, which could increase the incidence of leishmaniasis not only in dogs but also in humans. In addition, our results reinforce the earlier conclusion that *P. perniciosus* is the principal vector of leishmaniasis in the AR, due not only to the fact that it was the only species found infected with *L. infantum* but also to its high abundance and distribution. On the other hand, *P. sergenti* and *P. papatasi*, vectors of *L. tropica* and *L. major* respectively, were also identified. Although neither parasite is currently being transmitted to humans in Portugal, *L. major/L. infantum* hybrids have already been identified in autochthonous human leishmaniasis cases. There is a real risk of introduction of new *Leishmania* species from travellers or immigrants from North Africa and the Indian subcontinent, because their vectors exist in the country, emphasizing the need for on-going surveillance of *Leishmania* reservoir hosts and vectors. This work was supported by the EU/FEDER project POCI/CVT/56357/2004 from Fundação para a Ciência e Tecnologia (FCT), Portugal. C. Maia holds a FCT fellowship (SFRH/BPD/44082/2008).

## Visceral leishmaniasis in Tunisia: spatial distribution and association with climatic factors

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The study concerned 18 governorates of northern and central Tunisia where Mediterranean visceral leishmaniasis (MVL) is known to be endemic. Data were collected by directly consulting medical records from all paediatric departments centralising cases from the study area. All MVL cases under 5 years were recorded for the period 1996-2006. Mean incidence rates were calculated for each of the 215 districts in the study area. Averages of annual rainfall and extreme values of low-winter and high-summer temperatures were used to characterize the climate of each district according to its continentality index and bioclimatic zone. GIS and local indicator of spatial association (LISA) were used to summarize the spatial properties of MVL distribution. Poisson spatial regression was performed to study the relationship between MVL incidence rates and climatic parameters.

Eight hundred and fifteen patients under 5 years of age were geographically located at a district level. Annual averages of 74 MVL cases and mean incidence rate of 10.75 MVL cases/100,000 children under 5 years were recorded for the whole study area. The yearly number of MVL cases from 1996 to 2006 shows an oscillatory pattern with peaks in 1998-2000 and 2005-06. Positive correlation was found between the number of MVL cases of the year (y) and the annual rainfall value of 2 years earlier (y-2) ( $r=0.87$ ). Mean annual incidence rate by district varied from 0 to 96.7 MVL cases/100,000 inhabitants under 5 years. Spatial autocorrelation tests showed a clustered distribution of the disease with the presence of one hot spot region comprising 35 inland districts.

Districts that composed the hot spot i) were mostly localised in the semi-arid bioclimatic zones with cool or warm winters (32 out of 35, 91%), ii) had mean annual rainfall of 495 mm (SD: 77 mm), iii) had a semi-continental climate ( $I_c > 30^\circ\text{C}$ ). MVL incidence rate was positively correlated with mean yearly rainfall and continentality index. An entomological survey undertaken in 20 sites belonging to the hot spot region showed that *Phlebotomus perniciosus* is locally the most abundant vector of the subgenus *Larroussius*.

This work was supported by the Ministry of Higher Education and Research, Tunisia, and carried out within the framework of the research laboratory "Parasitoses Emergentes" LR 05SP03.

## Spatial and environmental models for human and canine leishmaniasis in the Aydin Mountains in western Turkey

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In Turkey, most zoonotic visceral leishmaniasis (ZVL, caused by *Leishmania infantum*) is reported from the Aegean and southern Mediterranean regions, whereas anthroponotic cutaneous leishmaniasis (ACL, caused by *Leishmania tropica*) predominates in the southeast of the country. Interestingly, however, both clinical forms of the human disease and canine leishmaniasis (CanL, with the domestic dog as the reservoir host of *L. infantum*) occur in the Aydin Mountains and its surroundings, near the Aegean coast of western Turkey. The aim of the present study was to investigate and contrast any environmental factors associated with the spatial distribution of each of the three types of leishmaniasis in our study area (48x88 km) in the Aydin Mountains.

The locations of human cases were obtained from the health authorities in Aydin city. For CanL, an active survey was carried out to estimate the spatial variation in seroprevalence. Uniform sampling was attempted based on a grid of 66 cells, each of 16 km square, with at least one settlement being chosen in each square for collecting blood samples from dogs. The overall seroprevalence of CanL was found to be 9.0% for 4-19 samples from each of 25 villages.

Separate statistical models were built for CanL and ACL using multivariate logistic regression and negative binomial regression, respectively. The distributions of the two transmission cycles overlapped little at the local level. The association between the distribution of each disease and environmental variables will be discussed in the oral presentation.

The GIS approach taken was found to be useful for evaluating sustainable interventions to control both forms of leishmaniasis by the local health authorities.

This work was funded by EU grant GOCE-2003-010284 EDEN ([www.eden-fp6project.net](http://www.eden-fp6project.net)).

## Leishmaniasis and Toscana virus infections in sandfly populations in two regions in the south of France

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Leishmaniasis and Toscana virus infections are two sandfly borne diseases that are considered as emerging or re-emerging in Europe. While the range of canine leishmaniasis covers almost all of the south of France, Toscana virus (TOSV) seems to be restricted to an area ranging from the Italian border to the city of Marseille. The reasons for this limited distribution of Toscana virus are unknown, as phlebotomine populations are not considered to be significantly different eastern or western of Marseille. We conducted an entomological, parasitological and virological survey in all of the south of France, in previously identified leishmaniasis foci. Phlebotomine sandflies were caught from June to September 2009 with CDC light-traps. After morphological identification, all phlebotomine females were tested for the presence of *Leishmania* (PCR) and both males and females were tested by RT-PCR for phleboviruses (TOSV, SFNV - Sandfly fever Naples virus, SFSV - Sandfly fever Sicilian virus).

More than 4000 sandflies were trapped. We found four of the five species principally described in south-France: *Phlebotomus perniciosus*, *P. ariasi*, *P. mascittii* and *Sergentomya minuta*. In areas without reported TOSV human cases, *P. ariasi* was the main species and only a few specimens of *P. perniciosus* were caught. In areas where human TOSV infections had already been reported, we found that *P. perniciosus* and *S. minuta* were the main species trapped. *Leishmania infantum* infections had been found in all studied areas. In one region close to the city of Marseille where three Toscana infections (human cases) had been observed, we could detect TOSV by RT-PCR in one female and one male of *P. perniciosus* and isolate the 2 viruses in VERO cells. Virus identification in male and female sandflies in the same area suggests a high level of transmission. All these strains belong to the Spanish lineage of Toscana. We could not find any evidence of SFNV or SFSV circulation in analysed sandflies. The infected *P. perniciosus* have been trapped in the same geographical zone that appears to be a large focus of Toscana virus transmission including different biotopes and human land use: forested and deforested hills with or without agricultural activities, peri-urban residential area and urban area. As mentioned in other European countries of the Mediterranean basin, *P. perniciosus* seems to be responsible for TOSV transmission and is encountered in the same densities in biotopes with and without anthropic modifications. The growing urbanisation of the countryside (with the arrival of urban TOSV-naïve populations) suggests that TOSV infection will probably be an extensive emerging disease in future years.

## Session 5 – Malaria

### Risk maps for the re-emergence of Malaria in Portugal

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Malaria is considered by the WHO an eradicated disease in Portugal, since 1973 (Antunes et al., 1987). Three decades after the last reported autoctonous case, the receptivity of the country for the re-emergence of the disease was estimated by entomological surveys and the use of spatial information analysis based on Geographic Information Systems (GIS). A three-years study on a selected region (Comporta) characterised the vectorial capacity of *Anopheles atroparvus* Van Thiel, 1927, the former malaria vector in Portugal; experimental infections of local mosquito populations assessed the atroparvus vector competence for tropical

strains of *Plasmodium falciparum* Welch, 1897 (Sousa, 2008) and a predictive model of *atroparvus* habitat suitability was designed to estimate mosquito abundance throughout Portugal (Capinha et al., 2009). These three elements were applied into the development of a risk model for malaria transmission in mainland Portugal. Risk maps were generated using a  $R_0$  index based on the variables of receptivity and infectivity of *A. atroparvus* and vulnerability of mainland Portugal for malaria transmission. Results showed that the highest risk areas are located in northeast and central-south Portugal (Alentejo), and in the hidrographic basins of the Tagus and Sado, especially at the river mouths. This spatial pattern is very similar to the former malaria distribution in the country but the current risk for the occurrence of a malaria outbreak in Portugal is low.

## **Receptivity to malaria in a previously endemic area in Spain: A Risk assessment approach for Malaria re-emergence**

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The study has been conducted on the Ebro Delta, an area of the Mediterranean coast with risk for Malaria (re)-emergence in Spain. The aim of this study was to know the vector's spatial and temporal distribution using field data and remote sensing techniques and to determine the Malaria receptivity.

In the initial study phase comprising the entomological survey, *An. atroparvus* was identified as the only *Anopheles* species present. Results showed it to be a zoophilic, endophilic and endophagic mosquito in that area. A map, including the main biotopes where *An. atroparvus* was collected, was constructed on an Eurasian Corine Land Cover image. Due to the scarce larval captures in the Ebro Delta, *An. atroparvus* capture data and environmental data from La Camargue (France) were used to test for associations between the presence of *Anopheles* larvae in breeding sites and land cover variables. A logistic-regression model was developed and applied to the Ebro Delta Land Cover map. Using ArcGIS, the *An. atroparvus* Larval Index map for the Ebro Delta was obtained. Field survey data in the Delta were used to validate this index. Assuming that adult abundance depends on the presence of the breeding sites in the surroundings, the Larval Index map was used to derive an Adult Index map. Due to the huge extension of the rice fields, the Adult Index map showed a great area with a high value. However, this map did not appropriately fit, because the biological characteristics of *An. atroparvus*, the entomological data, our field experience, and mainly the distribution of the farms in the surroundings, all indicated a very low probability for adult specimens to survive in an uninhabited area. Consequently, a detailed spatial location and distribution of the animal farms would increase the resolution and accuracy of that map.

In order to assess Malaria receptivity, space-independent parameters as trophogonic cycle, sporogonic cycle of *Plasmodium* species, vector biting rate, survival rate and anthropophily were calculated. Using Adult Index map, UV-Light traps capture and human landing captures an estimation of the human biting rate for each pixel and each month was obtained. Finally, the mean value of the malaria receptivity was assessed from June to October for each pixel of the Delta map. July and August and *P. vivax* showed the highest value of receptivity. A follow-up of the entomological surveys is key to validate and possibly also improve this preliminary model and mapping for the Ebro Delta area. According to the entomological results, there is a potential risk for Malaria (re)-emergence in the Ebro Delta, as *An. atroparvus* shows a density and spatial and temporal distribution similar to those it presented during the past Malaria endemic period. The spatial and temporal distribution of Malaria receptivity has been determined in the Ebro Delta region. The current risk for malaria re-emergence will be forecasted regarding other factors such as susceptibility of local *An. atroparvus* populations to different *Plasmodium* species and the number of Malaria imported-cases.

## **The use of multi-agent simulations in spatial epidemiology: advantages, challenges a example application**

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The transmission of vector-borne and zoonotic diseases is intrinsically a spatial process as it results from interactions between pathogens, vectors, and hosts (including humans), each of them interacting with the environment. The combination of factors explaining disease transmission and the complexity of such systems led to the development of an innovative, spatially-explicit modelling method: multi-agent simulation (MAS). MAS can be used to integrate the movements and behaviours of the different agents that could influence disease transmission - people, vectors, hosts and the landscape - in a spatially-explicit environment. It allows simulating the spatial and temporal variations in contact rate between vectors and hosts, and the number of new infections that could emerge from these contacts. MAS can be used as virtual laboratories to test the impact of changes in potential drivers of disease emergence including biological processes, geographical settings and the social context.

We will illustrate this with a MAS developed in the frame of the EDEN project to assess the risk of malaria re-emergence in a non-endemic area where *Anopheles* (Culicidae) live, in Southern France (Linard et al., 2009a). The MALCAM model showed that people-vector contacts not only depend on the spatial distribution of people and potential vectors, but also on their temporal distribution, their behaviours and interactions (Linard et al., 2009b). Simulations showed that the risk of malaria re-emergence is low in Southern France, and could only occur under a combination of unfavourable conditions. These conditions would include the introduction of a large population of infectious people or mosquitoes, combined with high levels of people-vector contacts resulting from significant changes in land use, tourism activities, agricultural policies, biological evolution of mosquitoes, and climate (Linard et al., 2009b). Results from scenario analyses can help local public health authorities in formulating policies.

The development of a MAS necessitates a considerable amount of data and a good preliminary knowledge of the disease system. The simulation of local interactions between agents, based on simple individual rules, and their integration in a MAS allow observing macro-scale dynamics at the level of the system. MAS is a powerful tool to represent the spatio-temporal dynamics of vector-host contacts and to test various hypotheses related to vector-borne disease dynamics. This is principally due to its ability to represent spatial heterogeneity and the behaviour of multiple, autonomous agents, and to the multidisciplinary approach of the system. MAS is able to resolve many modelling challenges, but also raises a number of other challenges including the verification, calibration and validation of the model, the operational modelling, and the way the model can be communicated and shared. Advantages and challenges associated with the development of MAS in spatial epidemiology will be discussed.

## **Characterization of immunogenic proteins of *P. falciparum* -infected salivary glands of *An. gambiae*: Toward a biomarker of infecting bite?**

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Emergence and/or re-emergence of vector-borne diseases in the developed and in the developing world, constitutes a major public health problem. Among them, malaria remains the main cause in the developing countries. Malaria causative agent, i.e. plasmodium parasite, is transmitted to human during the blood meal of the anophele mosquito. During blood feeding, the vector injects parasite and also saliva into the vertebrate host skin. This saliva contains bioactive components which may play a role in pathogen transmission and in host-vector relationships by inducing an immune response in the vertebrate host. Our team has previously developed a serological biomarker to assess the human exposure to mosquito bite. This tool is based on the evaluation of human antibody response against mosquito salivary proteins. The goal of this study is to investigate whether a salivary antigen could be specific of the infecting bite and then a biomarker of the risk of disease. To assess this question, we have performed an immuno-proteomic study to compare antibody response against salivary



extracts infected or not by *P. falciparum*.

Experimental infections of *An. gambiae* by *P. falciparum* were carried out and salivary glands were dissected 14 days post-infection. The infection status of each salivary gland was confirmed by PCR. Then two-dimensional western-blot were realized with different pools of infected vs non infected sera. These pools were constituted with sera from Senegalese 1-2 y.o. children leaving in deeply exposed village to anopheles and presenting or not a high parasitemia.

The results of 2D-blot showed that immunogenic proteins around 70kDa are detected in both infected and non infected vector. Mass spectrometry analyses identified these proteins as the 5' nucleotidase and Apyrase, proteins which inhibiting platelet aggregation during blood meal. Furthermore one immunogenic protein from infected salivary glands extracts was detected only with sera from infected children by *P. falciparum*. Mass spectrometry analysis on this protein is underway. These first results indicated that human immune system could discriminate between an infective bite and a non infective bite. This work opens the way to design epidemiological tools to define areas with heavy risk of malaria in case of (new) epidemic, but also have strong implications for the vector control monitoring.

## The *Anopheles gambiae* gSG6 salivary protein: a serological marker of exposure to African malaria vectors

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With the final aim to develop novel additional tools for the evaluation of exposure to malaria vectors we started an analysis of the human humoral response to recombinant salivary proteins of the major African vector *Anopheles gambiae*. We started focusing our efforts on gSG6 (*gambiae* Salivary Gland protein 6), a small protein specifically expressed in adult female glands where it plays some important role in blood feeding (Lanfrancotti A et al., 2002; Arcà B et al., 2005; Lombardo F et al., 2009). This protein seemed especially suitable because it is relatively abundant in the *An. gambiae* saliva, is restricted to anopheline species and earlier studies indicated its immunogenicity to humans (Orlandi-Pradines E et al., 2007; Poinignon A et al., 2008).

We optimized conditions for expression and purification of a recombinant version of gSG6 and we used it to measure by ELISA the anti-gSG6 IgG response in human sera collected during three consecutive years in a rural malaria hyperendemic area of Burkina Faso. The IgG response in the exposed population varied according to malaria transmission and was short-lived, as indicated by a drop in the IgG levels during the dry low transmission season. The response started very early, with a maximum in one-two years old children, and then decreased according to age; a similar pattern has been previously observed with whole mosquito saliva (Peng Z et al., 2004) and interpreted as possible desensitization to salivary antigens. Interestingly, the response was significantly different in Mossi and Fulani, two sympatric ethnic groups already shown to differentially respond to several *Plasmodium falciparum* antigens (Modiano D et al., 1996; Torcia MG et al., 2008).

*An. gambiae*, *Anopheles arabiensis* and *Anopheles funestus* are the three main African malaria vectors and they all carry in their saliva the SG6 protein. Since the *An. arabiensis* (aSG6) and the *An. funestus* (fSG6) homologues share 98% and 80% identities with gSG6, it is likely (although verification is needed) that the antibody response to gSG6 may be used to evaluate exposure to all three main African malaria vectors. In conclusion, our study provides strong support to the idea that anopheline-specific salivary antigens may represent a solid serological marker of exposure to malaria vectors and indicates that a reliable evaluation should also take into consideration the age and, if pertinent, the ethnic group of the populations analyzed. Moreover, comparative analysis of mosquito sialomes also highlights the existence of a relatively large group

of culicine-specific salivary proteins suggesting their possible exploitation as indicators of exposure to *Aedes* and *Culex* vectors.

## Session 6 – Tick-borne diseases (Ecological impacts on epidemiology)

### Infestation with ticks and prevalence of *Anaplasma phagocytophilum* in roe deer in Germany

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Roe deer (*Capreolus capreolus*) is a very common mammal in the European landscape, where locally it may reach fairly high densities. This species actually moves between various habitat types and thus moves ticks and the pathogens they harbour between these habitats. The aims of this study were therefore to identify and quantify the ticks found on deer, and screen this host for *Anaplasma phagocytophilum*.

Skin samples were collected from 184 culled deer from Bavaria, Baden-Württemberg and Thuringia between May and mid-January (hunting season) from 2007 to 2009. In 91 cases, it was possible to collect the entire pelt for quantitative tick sampling. The pelt was then spread inside a white plastic tray. This first tray was then placed in the middle of a second white tray filled with water. Ticks crawling away from the pelt eventually fell into the water where they were easily detected and collected. A total of 3941 ticks were thus collected from 91 deer. All ticks were identified morphologically as *Ixodes ricinus*. Tick frequency distribution was aggregated (variance > mean: 5265.6 > 43.8). 74.7% of the deer examined were infested with ticks, hosting between 1 and 504 ticks per deer. Adult ticks were more frequent (72.5%) than nymphs (65.9%) and larvae (56.0%). Most interestingly, all stages were actually feeding on deer. Engorged ticks represented 36%, 30% and 68% for larvae, nymphs and female ticks, respectively. Infestation levels peaked in May, matching the peak of tick questing activity. Tick-free deer were observed during winter months only. Although there is a relation between tick questing activity and tick infestation, the proportion of observed variability explained by any model remains low: 3% for larvae, 12% for nymphs, and 40% for adult ticks.

Estimates of deer abundance were obtained by the distance transect method. No relation could be found between estimates of deer density and infestation with ticks.

Intact DNA could be extracted from 147 deer samples. These were screened for *Anaplasma phagocytophilum* by real-time PCR. The prevalence of *A. phagocytophilum* ranged from 85.5% in Thuringia to 90.7% in Bavaria. No morulae were detected on blood smears from 22 *Anaplasma* PCR-positive deer.

The frequent co-occurrence of all tick stages feeding on *Anaplasma*-positive skin, demonstrates the high potential of the transmission of this pathogen via co-feeding ticks on deer. Prevalence estimates of *Anaplasma* infection in questing ticks imply that most ticks become infected through their nymphal blood meal. This very strongly suggests that circling between immature and adult ticks occurs through co-feeding. The main amplification host must therefore simultaneously harbour larvae, nymphs and adult ticks. These conditions are met by deer.

### Empirical assessment of deer density threshold for the risk of Tick-borne encephalitis under natural conditions

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Tick-borne encephalitis (TBE) risk areas are characterized by an extremely heterogeneous distribution allowing them to be identified and mapped as TBE foci. Although many studies have been devoted to understanding TBE spillover, i.e. why it emerges as a human disease from silent sylvatic cycles, comparatively little research has been devoted to estimating the quantitative contribution of the key hosts involved in TBE transmission (especially rodents and deer) with the aim of identifying the threshold conditions for TBE persistence. While the environmental and climatic conditions permitting TBE emergence have been evaluated with regard to the vector (e.g. seasonal synchrony of tick stages), the tick cycle itself depends on host abundance and distribution. A first analysis based on infection seroprevalance indicated that TBE risk depends on both the abundance of mice and game, particularly deer. In addition, recent studies have shown that TBE risk can be predicted at a regional scale by models including rodent habitat and local deer abundance, while empirical and theoretical studies have investigated the relation between deer abundance, tick intensity and TBE persistence. In this study, in Italy and Slovakia we set up an empirical comparison between sites with contrasting TBE prevalence, including control sites with no TBE. In all sites, we geo-referenced an 80 ha quadrat, where deer density was assessed using a pellet group count-plot sampling technique. In the same area, we collected host-seeking ticks by dragging a 1 m<sup>2</sup> white blanket along a 100 m transect, stopping every 2.5m to count and collect ticks. In addition, two parallel lines of 50 traps each were used to live-trap rodents. For each individual rodent, we determined species, sex and class age and assessed tick infestation by recording the different life stages of feeding ticks. Both tick dragging and rodent trapping were performed in 4 sessions from April to October 2006. The incidence of TBE in humans was derived from official medical statistics. TBE incidence was associated with the number of co-feeding ticks on rodents, and deer density ("deer" and "deer<sup>2</sup>") We hypothesised that the association between TBE cases and deer (also previously observed at a large scale) was mediated by the relation between rodent tick infestation and deer abundance. This was confirmed by our results: the number of feeding ticks on rodents was best explained by deer density ("deer" and "deer<sup>2</sup>"), and region. Starting from a threshold density, deer were negatively associated with rodent tick infestation, since available ticks were diluted on these larger hosts (feeding ticks on rodents/total ticks =  $-0.07 \cdot \text{deer density} - 1.63$ ,  $p < 0.05$ ). The threshold density value of deer may correspond to the spatial scale at which deer and rodents can ecologically interact.

In memory of Prof. Milan Labuda

## **The role of co-feeding transmission in the ecology of *Borrelia afzelii*: a glance at the intra-specific level.**

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Lyme borreliosis is a disease caused by spirochetes of the *Borrelia burgdorferi* complex. In Europe, *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* are the genospecies most frequently reported in humans. These spirochetes are maintained in nature by a transmission cycle involving *I. ricinus* ticks and some of their hosts. Various *Borrelia* genospecies can circulate between ticks and hosts in one area. However, the situation is even more complicated since diversity also exists at the intra-specific level. Studies showed that ospC variation within a local population of a single *Borrelia* species is almost as great as the variation of a similar sized sample of the entire population. The genetic diversity at the ospC locus allowed a classification into groups of alleles. Here, we analysed the ospC diversity in *B. afzelii*, a species associated with small rodents, over 3 years, in two areas in Switzerland.

The diversity of ospC groups among 485 *B. afzelii* isolates from questing ticks, ticks infesting small rodents and xenodiagnostic ticks was investigated by PCR and cold single strand conformational polymorphism.

Questing tick density was higher in Neuchâtel than in Staatswald, whereas rodent density was higher in Staatswald. The proportions of rodents infested by ticks were similar in each site (59.4% vs 61%) but the mean number of ticks per host as well as the proportion of rodents infested by larvae and nymphs simultaneously were higher in Staatswald ( $P=0.00014$ ). The distribution of *B. afzelii* ospC groups in questing ticks was different from the distribution in ticks feeding on rodents in Neuchâtel (Fischer test,  $p=0.0015$ ) but not in Staatswald. Alternative hosts, probably squirrels, contribute to the diversity of groups in questing ticks in

Neuchâtel.

In both sites, rodents, infested by larvae only transmitted a lower mean number of ospC groups to larvae feeding on them than rodents infested by larvae and nymphs (Neuchâtel: 1.13 vs 2.6, Wilcoxon test,  $p=0.04$ , and Staatswald: 1 vs 1.75, Wilcoxon test,  $p=0.003$ ). Co-feeding transmission appears to play a role in maintaining the diversity of *B. afzelii* at the intra-specific level.

## **Role of sand lizards in the ecology of Lyme and other tick-borne diseases in the Netherlands**

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**Background:** Changes in the environment influence the distribution of various vertebrates. This changes compositions and abundance of tick host communities and has the potential to affect the incidence of tick-borne pathogens. Lizards are considered zooprophylactic for almost all *Borrelia burgdorferi* species, probably because of the presence of borreliacidal factors in their blood. Whether lizards also act as dilution hosts for *B. burgdorferi* and how they influence other tick-borne pathogens in Western Europe is not clear. We studied the influence of sand lizards (*Lacerta agilis*) on the distribution of *Borrelia*, *Rickettsia*, *Anaplasma* and *Babesia* species in Dutch heather areas in comparison to woodlands.

**Results:** Ticks were collected from sand lizards, their habitat (heather) and from an adjacent wood area. DNA of tick-borne pathogens was detected by PCR followed by reverse line blotting. Tick densities were measured at all four locations by blanket dragging. Nymphs and adult ticks collected from lizards had a very low (1.4%) prevalence of *Borrelia burgdorferi* spp., compared to questing ticks in heather (24%) or woodland (19%). *B. lusitaniae*, the only *Borrelia* species that is suspected of being transmitted by lizards, was not detected during this study. The prevalence of *Rickettsia helvetica* was higher in ticks from lizards (19%) than those from heather (15%) or woodland (10%). For *Anaplasma* species, a lower prevalence was observed in ticks collected from lizards (5.4%) than in questing ticks (12% and 14% for heather and woodland, respectively). Tick densities were lower in the heather areas than in woodlands at all for sites.

**Conclusions:** Despite their low reservoir competence, the presence of sand lizards had insignificant impact on the *B. burgdorferi* s.l. infection rate of ticks. In contrast, sand lizards might act as proliferation hosts for *R. helvetica*. The public health risk from these two pathogens is lower in heather than in woodland, due to the low tick densities in heather. We are currently undertaking efforts to evaluate the genetic variety of *Rickettsia* and *Borrelia* species in the Netherlands which will enable us to understand the ecology of these pathogens better and to detect changes in species distribution during coming years. A shift to warmer climate could lead to an increased abundance of lizards and therefore could influence the prevalence of *Borrelia*, *Rickettsia* and *Anaplasma* in future.

## **Strong genetic differentiation between Eurasian and North-African *Ixodes ricinus* ticks**

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*Ixodes ricinus*, the most common tick in Europe, is a vector of human (e.g. Lyme disease) and animal diseases (e.g. Babesiosis). To develop effective control methods against these diseases, a better understanding of tick-pathogen interactions is essential, notably the genetic variability of vector.

We collected individual ticks to be representative of three geographical scales, namely the local scale, the regional scale and the distribution area scale. We combined different types of molecular markers including 2 mitochondrial genes and 4 nuclear genes (with housekeeping genes and genes potentially involved in pathogens

transmission).

We observed low levels of nucleotide variability when taking the 3 spatial scales as a whole (2.63% of the 6963 pb were variable sites) contrasted with high levels of haplotype diversity ( $H_d = 1$ ). Similar levels of nucleotide diversity were observed between the local and regional scale. When considering only the Eurasian ticks, the distribution area scale showed higher, but not significant, level of genetic variability than the local and regional scales. Adding the North-African populations increased the nucleotide variability level of the distribution area scale two-fold (without North-Africa:  $\pi = 0.00449$ , including North-Africa  $\pi = 0.0074$ ).

Phylogenetic trees were performed on each gene separately. We observed similar tree topologies between the different genes: an absence of a genetic and geographic structure between the Eurasian ticks contrasted with a strong genetic differentiation of the North-African ones that constituted a divergent clade.

Several hypothesis may explain the homogeneity and lack of deep structure in Eurasian ticks: (i) important gene flow due to the passive dispersal of ticks mainly linked to transportation by host animals during infestation, notably by highly mobile mammals or birds; (ii) recent range expansion northward from southern peninsular refugia of *I. ricinus* populations in Eurasia, as evidenced from the high haplotype diversity contrasted by low nucleotide diversity, and also the lesser mismatch of the distribution of the Eurasian ticks to the demographic expansion model (raggedness Harpending index,  $p=0.74$ ) than to the constant population size model (raggedness Harpending index,  $p=0.43$ ). The strong genetic divergence of the North-African populations could be explained by (i) the important effects of the genetic drift on these populations that are geographically isolated and of small size, and (ii) the selection pressures that may occur due to different ecological conditions (e.g. seasonal activity and hosts differences have been recorded for North-African ticks).

Our results suggest that the North-African *I. ricinus* ticks may be evolving towards reproductive isolation from the Eurasian ticks, as a consequence of ecological and genetical differences. The impacts of our results on the epidemiology of the vector borne diseases will be discussed.

## Session 7 – From endemic Mosquito-borne diseases in Africa to emerging Mosquito-borne diseases in Europe (Modeling West Nile and Rift Valley Fever)

### Rift Valley Fever (RVF) in Barkedji (Senegal), Zones Potentially Occupied by Mosquitoes (ZPOMs), dynamics, mapping and RVF risks

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This communication presents an analysis of the interaction between the various variables associated with Rift Valley Fever (RVF) such as the mosquito vector biology, available hosts and rainfall distribution. To that end, the varying Zones Potentially Occupied by Mosquitoes (ZPOM), rainfall events, ponds dynamics, and the associated exposure of hosts to the RVF virus by *Aedes vexans*, were analyzed in detail in the Barkedji area of the Ferlo (Senegal), during the 2003 rainy season. Ponds remotely-sampled from images were obtained by the processing of high-spatial resolution SPOT-5 images. Additional data on ponds dynamics and rainfall events (obtained from the Tropical Rainfall Measuring Mission) were combined with *in-situ* entomological measurements and hydrological information, and the localization of vulnerable ruminant hosts (obtained from Spot 5 and QuickBird satellites). Hydrological modelling (filling-up and flushing-out of the ponds) was derived from TerraSAR-X data and field measurements.

Since “*Aedes vexans* productive events” are dependent on the timing of rainfall for their embryogenesis (six

days without rain are necessary to trigger hatching), the dynamical spatio-temporal distribution of *Aedes vexans* density was based on the daily rainfall amount and associated ponds dynamics. Detailed ZPOM mapping was obtained on a daily basis and combined with aggressiveness temporal profiles. Risks zones, i.e. zones where hazards and vulnerability are combined, are expressed by the percentages of parks where animals are potentially exposed to mosquito bites. This new conceptual approach, using precise remote-sensing techniques, simply relies upon rainfall distribution also evaluated from space. It is meant to contribute to the implementation of operational Early Warning Systems for RVF based on environmental risks linked to climatic and environmental changing conditions: natural and anthropogenic. In addition, future developments of the system will be tackled in the framework of the 'AdaptFVR' project of the research program 'Management and Impacts of Climate Change' or 'Gestion et Impacts du Changement Climatique (GICC)' in French, managed by the Ministry of Ecology, Energy, Sustainable Development and Sea (Ministère de l'Ecologie, de l'Energie, du Développement Durable et de la Mer, MEEDDM, in French).

## **A diffusion model to predict spatial and temporal population dynamics of Rift Valley fever vectors in Northern Senegal**

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We have developed an explicit spatial and temporal model to predict the population dynamics and dispersal of the two main mosquito vector species (*Aedes vexans* and *Culex poicilipes*) involved in Rift Valley fever virus (RVFV) transmission in Senegal (western Africa). Covering an area of 11x10 km around the village of Barkedji, and located in the Ferlo valley (Northern Senegal), the study area is characterized by a complex and dense network of water bodies and ponds that are filled by rainfall during the rainy season (from July to mid-October). These water bodies are known to be the principal mosquito breeding sites in the area.

A spatial diffusion model (Raffy and Tran, 2005) is applied in combination with a vector population dynamic model (Soti et al., 2009), and which takes into account the hydrological conditions of the system (Soti et al., submitted). The vector population dynamic model has been more specifically derived from Porphyre et al. (2005). Daily mosquito abundance for each pond was fed into the spatial diffusion model to simulate the spread of vector populations around their breeding sites, taking into account the spatial distribution of night camps of the sensitive hosts (essentially small ruminants such as sheep) and the influence of the landscape variables (vegetation type and density) extracted from a Quickbird satellite image. We have validated the simulation results using apparent mosquito-abundance data collected during the 2002 and 2003 rainy seasons, using sheep-baited traps located at different distances from the ponds (Chevalier et al., 2004). This original approach will allow predicting how mosquitoes move and disperse spatially and temporally, such information being useful for orienting health protection measures towards areas of higher risk for RVF.

## **From high-spatial resolution remote sensing to modeling the dynamics of habitats of mosquito vectors of Rift Valley Fever vs rainfall in Barkedji area, Senegal**

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High activity of the Rift Valley Fever (RVF) virus is partly related to a tremendous increase in the number of associated mosquito vectors and of the widespread and proliferation of their reproduction habitats, which follows periods of heavy rainfall in suitable environments [1]. The rationale being that rainfall creates ecologically humid environments that insure the proliferation of breeding sites and the development of RVF vectors. In the context of Barkedji, we are dealing with mosquitoes of genera *Aedes* and *Culex* which are found to play complementary roles of vector-reservoir and vector-amplifier, respectively, in the RVF transmission [1]. To forecast the dynamic of mosquito populations at the scale of a single pond, we have developed a theoretical framework, based on the physical mechanism of the breeding habitat formation and on bio-ecology of mosquitoes, that combines two dynamical models [2]: first model describes the time evolution of a rain fed pond, considered as the main mosquito breeding habitat, and the output of this latter model is next used in a second model for the calculation of the population dynamics of mosquito vectors.

Our aim in this study is to extend the previous approach developed in Ref.[2] to the entire area of Barkedji for forecasting the mosquito populations and, thus, determining areas and periods at risk of RVF activity. In other words, the main issue of this work is rationalize where and when does one find mosquito vectors of RVF in the pond system of Barkedji area. To tackle this problem, we proceeded in 4 steps as follows: 1 – System of Ponds: following the method in Ref.[3], a high-spatial resolution SPOT5 satellite image of Barkedji area (42km x 42km centered on the Barkedji village) was used to construct a map (coordinates, shape, surface and perimeter) of ~ 1400 ponds in Barkedji.

2 – Field data: a sample of few well studied ponds in Barkedji was used to characterize and estimate both parameters for the pond discharge dynamics and the potential of each pond to producing mosquitoes. 3 – Rainfall and a Runoff: a disaggregation model was employed to generate daily rainfall at the pond scale and a hydrological model was developed to generate water inputs in each pond. 4 – Habitat and Population Dynamics: the extended version of the approach in Ref.[2] was run for each pond of the system.

As a result, we stochastically generated a dynamical map providing the probability in space and time of finding *Aedes* and *Culex* mosquitoes versus rainfall in the Barkedji area. This modeling was next used in the analysis of the impacts of climate variability and changes on the mosquito dynamics.

## **O, deer! Modeling the role of wildlife in potential spread and persistence of Rift Valley Fever Virus in The Netherlands and Belgium**

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Rift Valley Fever (RVF) is a vector-borne viral infectious disease of various mammal species including man. Until a decade ago RVF remained confined to Africa, but outbreaks on the Arabian peninsula gave rise to the fear of expansion to Europe. As shown by the recent establishment of bluetongue virus in North-West Europe, the possibility of a rapid invasion and persistence of a new vector-borne disease is realistic.

Rift Valley Fever virus (RVFV) is transmitted between mammal hosts by mosquito vectors (*Aedes spp.* and *Culex spp.*) and mechanically by the stable fly (*Stomoxys calcitrans*). Some univoltine *Aedes spp.* are known to transmit the virus to their eggs. Thus, the time scale of these various transmission routes is different (hours, days, years). One or more potential vectors are present in The Netherlands and Belgium.

RVFV can infect a large range of African wildlife including rodents, livestock and human. The infection is thought to persist during inter-epidemic periods by infected *Aedes* eggs, and / or in a sylvatic cycle, depending on the area and its climate. In the Netherlands and Belgium wildlife populations are roaming the nature reserves and rural areas. These include feral cattle, such as Heck-cattle, and several species of deer: Roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*). Although unknown for deer species, it is likely that they are susceptible to RVFV. This leads to our research question:

Can RVFV spread and persist in the Netherlands and Belgium after an accidental introduction of the virus, and what is the role of different hosts such as farm animals, and wildlife?

We developed a model for the transmission of the infection and described this in a system of ODE's. From this we derived an expression for the basic reproduction ratio,  $R_0$ , for RVFV in the low countries.  $R_0$  is subsequently quantified for various areas differing in biotope. The population density of vectors and hosts differs between these biotopes. The method allows to aggregate population density estimates based on different biotopes for one epidemiological reasonable spatial scale (i.e. the flight range of vectors). We show the sensitivity of predicted risk areas including or excluding the risk added by deer and rodents. We determine the final size by numerical solving the ODE's of the model for different seasons in the year of virus introduction. We conclude that for a proper estimate of RVFV risk in temperate zones, such as the Netherlands and Belgium, it is of importance to know the susceptibility of local wildlife, which means in the case of North Western Europe, the deer species.

## **Impact of vector and host distribution on the processes of introduction, amplification and emergence of a multi-host infectious disease**

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West Nile Fever (WNV) is an emerging vector-borne disease affecting several continents: Africa, Europe, Asia and more recently the Americas. Due to the great numbers of hosts and vectors species potentially involved in the transmission processes, the epidemiology of such multi-host infectious disease is complex.

In the current study, we propose an ecological and landscape approach to assess the transmission processes likely to explain the patterns of West Nile Virus (WNV) transmission in the Camargue region (southern France). We postulated that the epidemic processes of introduction, amplification and emergence of WNV are related to the existence of specific associations of hosts and vectors species in time and space.

Our analysis included three primary components: i) we first mapped seasonal distribution of mosquito and bird populations using expert-based knowledge and geographic information systems (GIS); ii) we used GIS modelling functionalities to simulate relative risk maps corresponding to different hypotheses of introduction, amplification and emergence of WNV - the hypotheses tested were chosen according to the literature; iii) we compared model outputs of each scenario (combining the different steps of introduction, amplification and emergence) with results of seroprevalence measured in horses and birds populations over the study area.

Our results suggest that some of the simulated scenarios do explain the observed spatial patterns of WNV transmission in the study area, whereas others hypotheses may be rejected in the Camargue context. These results are discussed in terms of surveillance and in comparison with other European and African wetland areas with past WNV outbreaks.

## **Session 8 – Leishmaniasis (Evidence for the emergence of leishmaniasis in Europe)**

### **The emergence of zoonotic visceral leishmaniasis in northern Italy: a compendium of 2005-2009 studies**

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**Background and studies** - Zoonotic visceral leishmaniasis (VL) emergence and spread in 5 northern continental regions of Italy were confirmed through a dedicated national project in 2003-2005 (M. Maroli et al., 2008. TMIH 13: 256-64). Since 2005, monitoring and surveillance has continued through the EU-FP6 EDEN-LEI subproject in selected sub-Alpine and sub-Apennine territories, together with a parallel study undertaken in central-western Italy in 2007 to compare latitudinal and altitudinal parameters associated with the distribution of *Leishmania* vectors. In 2008, a risk assessment investigation was carried out in the northern-most province of the eastern Alps, Bolzano-South Tyrol (G. Morosetti et al., 2009. Geospat Health 24:115-27), and in 2009 the expansion of natural foci into the Padana valley and neighbouring areas was actively monitored in 10 selected provinces of 3 regions.

**Methods** - All surveyed sites were geo-referenced, and standard human and canine questionnaires were submitted as appropriate. Surveys on phlebotomine sandflies were performed by standard sticky traps, and those on canine leishmaniasis (CanL) by standard IFAT serology combined with clinical, parasitological and molecular methods. Both passive and active detection of human cases was carried out.

**Results and conclusions** - The most competent *L. infantum* vector, *P. perniciosus*, was collected in both sub-Alpine and sub-Apennine areas of northern Italy, associated with *P. neglectus* in the former (206-570m altitude) and *P. perfiliewi* in the latter (52-440m). The large Padana valley was found apparently free from sandfly colonization, probably acting as a natural barrier. In central-western Italy, *P. perniciosus* and *P. perfiliewi* were confirmed to be the only vector species, the former being widespread in the area and recorded up to 772m, the latter being prevalent in the range 300-599m but apparently absent above 610m. Also, the absence of *P. neglectus* in this territory suggests that sub-Alpine populations of this species originated from eastern Europe rather than from populations endemic in southern Italy.

CanL investigations confirmed the northwards spread of VL, with an increase of mean seropositive rates in pre-Apennine sites (from 2% to 4%), which were significantly higher than in pre-Alpine sites (an average of 1.8%). Besides the continuous detection of imported CanL, autochthonous cases were newly identified in 6 provinces of 3 regions (Piedmont, Lombardy and Emilia-Romagna), but not in the Padana plains nor, despite the presence of *P. perniciosus*, in the northern-most eastern Alps. In 2005-2009, autochthonous human cases of VL, and also cutaneous leishmaniasis, were recorded in the newly endemic regions; several *L. infantum* strains were typed from dogs and humans.

This study was carried out within the framework of the EU FP6 Integrated Project GOCE-2003-010284 EDEN "Emerging Diseases in changing European environment" ([www.eden-fp6project.net](http://www.eden-fp6project.net)), subproject Leishmaniasis (EDEN-LEI).

## **Is leishmaniosis wide-spread in Spain? First data on canine leishmaniosis in the province of Lerida (NE Spain)**

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Globally, environmental and social changes have resulted in the emergence or re-emergence of leishmaniasis and other vector-borne diseases in some parts of the world. In Spain, where the first case of canine leishmaniasis was reported in 1913, knowledge of the disease is heterogeneous, with very few data from the north of the country, including Lerida province (Catalonia, NE of Spain). The disease is also common in dogs in the south of France, including the border with northern Spain.

For this reason, an epidemiological survey of canine leishmaniasis was carried out in the Lerida region of the Pallars Sobirà (1,355.22 km<sup>2</sup>, 5.1 inhabitants/km<sup>2</sup>), located in the Pyrenees and pre-Pyrenees areas and bordering France in the north and Andorra in the east. The climate is Mediterranean at altitudes up to 1,500 m above sea level (a.s.l.).

Since vaccination against rabies is currently not mandatory in Catalonia, unlike other parts of Spain, collecting blood samples from dogs for epidemiological studies is very difficult. It was thus necessary to actively search for dog owners with the help of a veterinarian of the area. A total of 145 blood samples were obtained by cephalic vein puncture of 145 dogs with the agreement of the owners. The dogs belonged to 23 owners from 16

localities situated between 500 and 1500 m a.s.l. The majority were hunting dogs living in kennels in periurban or rural areas.

The diagnostic technique used was an in-house ELISA with whole promastigotes as antigen and protein A as conjugate and using a single concentration of the sera (1/400). The reaction results were quantified as units (U) related to a positive serum used as calibrator and arbitrarily set at 100 U. The cut-off was established at 20U. Forty-two of the samples were considered positive, with a seroprevalence of 29%. The minimum, maximum and mean values obtained were 0, 311 and 36, respectively. The distribution of the titres was as follows: 0-15 (59%), 16-19 (12%), 20-30 (8%), 31-50 (7%), 51-100 (3%), >100 (11%). The positive dogs were found from 500 m to 1,500 m a.s.l. and in 12 of the 16 (75%) localities studied. Twenty-two of the seropositive dogs were asymptomatic. The results show the presence of canine leishmaniasis in an area where it was not considered endemic until now and with a high seroprevalence. The high seroprevalence detected might be explained by the fact that the analyzed dogs lived in periurban or rural kennels, which could favour conditions for the presence of sandfly vectors and, as a result, for disease transmission in the area.

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## Surveillance of leishmaniasis in Madrid Region (Spain)

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Leishmaniasis is an endemic zoonosis in the autonomous region of Madrid, Spain, transmitted by the bite of female phlebotomine sandflies (genus *Phlebotomus*), with the domestic dog acting as the main reservoir.

The Environmental Health Service of the Autonomous Government of Madrid launched in 1991 a Surveillance System of this disease following the recommendations set up by the World Health Organization (WHO Expert Committee, 1990). Leishmaniasis is also included as a Disease of Compulsory Declaration by the regional sanitary institutions.

This surveillance includes the study of stray dogs captured in the Madrid Region and kept in Animal Protection Centres. These are tested by indirect immunofluorescence (IIF) on blood samples in order to investigate the prevalence of *Leishmania* infection in the canine population. Recently this survey has been extended to other possible vectors like foxes and stray cats.

Since 2008, a systematic monitoring of sandfly populations has been launched through the capture of the phlebotomines in risk areas, following the experience of a previous study of the vector carried out in 1991 and 1992. In the year 2008, sand flies were collected in nine different Animal Protection Centres. In each site, sticky traps were used to capture sandflies in appropriate habitats from June to October. In two locations, CDC light traps were also used in order to check their efficiency relative to sticky traps. Collected specimens were preserved dry at 4°C and afterwards slide-mounted in Hoyer medium for identification of species and sex using published morphological keys and descriptions.

More than 1,000 sandflies were recovered. Altogether five species were identified, consisting of *Phlebotomus perniciosus*, *P. ariasi*, *P. papatasi*, *P. sergenti* and *Sergentomyia minuta*. *Phlebotomus perniciosus* was the predominant species (89.1%), followed by *P. ariasi*. The first species is considered the principal vector of *L. infantum* in the Madrid Area. The phenology of this species showed one major peak in September. Densities reached more than 60 sandflies per m<sup>2</sup> in one location.

Comprehensive systems of surveillance of sandfly populations and the prevalence of *Leishmania* in dogs (and other possible reservoirs) will improve our understanding of the epidemiology of this zoonotic disease and provide tools to design public health interventions for prevention and control.

In addition to leishmaniasis, sand fly fever caused by Toscana virus is receiving growing attention in recent years. *Phlebotomus perniciosus*, the predominant phlebotomine in Madrid, has been reported to be the competent vector in Spain.

## Validation of a novel approach to estimating prevalence of canine leishmaniasis [in

## Cyprus]

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Canine leishmaniasis is a serious veterinary problem throughout the Mediterranean Basin and evidence indicates an expansion in the range of the disease and its vectors in some regions in the last 30 years. Surveillance and monitoring of the disease in dogs is very important since the number of infected dogs in an area is the main factor determining the risk for human infection. An accurate, inexpensive and rapid method to carry out an epidemiological survey of the dog population is therefore very important. The EDEN-Leishmaniasis sub-project developed an epidemiological tool, a veterinary questionnaire, for this purpose. The aim of the current work was to evaluate the questionnaire by comparing the data collected from the veterinarians practising in Cyprus with an active epidemiological survey conducted at the same time. The results of the serological survey were consistent with the information given by the veterinarians concerning: disease prevalence in different areas; geographical spread of the disease on the island; best diagnostic practices used (clinical symptoms, laboratory tests); and, assessment of the preventive measures employed, which proved inadequate. Thus, the EDEN-Leishmaniasis veterinary questionnaire proved a valuable tool for estimating the prevalence of canine leishmaniasis in Cyprus and assessing local monitoring practices.

This study was carried out within the framework of the EU FP6 Integrated Project GOCE-2003-010284 EDEN “Emerging Diseases in changing European eNvironment” ([www.eden-fp6project.net](http://www.eden-fp6project.net)), subproject Leishmaniasis (EDEN-LEI).

## Surveys for the presence and spatial distributions of canine leishmaniasis and its sandfly (Diptera: Psychodidae) in Hungary

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Hungary is regarded as free of leishmaniasis because, prior to the EDEN FP6 project, only imported cases had been recorded, consisting of a few dozen humans and one domestic dog. However, southern Hungary has a Mediterranean climate, and so EDEN decided to include the country in its surveys of the northern limits of canine leishmaniasis (CanL) and human visceral and cutaneous leishmaniasis, which are all caused by *Leishmania infantum* in many rural areas of southern Europe. There had been no surveys of sandflies in Hungary, although there was a report of collections in 1931-2 of the vector *Phlebotomus perfiliewi*, which was originally identified using the current synonym *P. macedonicus*. The numbers of travelling and imported dogs have increased in the last decade, and this raised concerns about the introduction of CanL.

Sera samples were collected in 22 localities of 6 counties from 725 dogs that had never travelled to endemic countries, as well as from 185 red foxes and 13 golden jackals shot countrywide. These canids are all proven or potential reservoir hosts of *L. infantum*. All the samples were tested by the indirect fluorescent antibody test, but all except two dogs were sero-negative using the OIE cut-off of 1:80 serum dilution. The first autochthonous cases of CanL were confirmed in the two dogs by the presence of classic symptoms (skin abnormalities, lymphadenomegaly, splenomegaly and ocular disorders) and by cytological, serological and molecular diagnoses. Both dogs were living in a kennel of 20 animals located in central Hungary, and they were born in the same place. Neither had been abroad or received a blood transfusion. All the other dogs in the kennel were seronegative. The mode of transmission is still unclear, because no sandfly vectors were caught at

the site.

From 2006 to 2009, phlebotomine sandflies were sampled in the summer months at 68 peridomestic and sylvatic sites in 47 localities of 8 counties. They were trapped with castor-oil impregnated sticky-paper traps, CDC miniature light traps (John W. Hock Co. USA) or dry-ice baited Mosquito Magnet® X traps (American Biophysics Corp.). Small numbers of two vectors of *L. infantum* were trapped. *Phlebotomus neglectus* was found in three villages near to Croatia and one in north Hungary, latitude 47° N, a northern record. *Phlebotomus perfiliewi* was trapped at two sites in a southeastern county, close to the collection sites of 1931-2. A possible vector of CanL, *Phlebotomus mascittii*, was trapped in three counties, and a specimen of *P. papatasi* was trapped in the southeast.

We are unable to conclude that CanL has recently spread northwards into Hungary from the endemic Mediterranean region, because of the absence of previous surveys. However, our results are a base-line for future surveys, and they are also being used to help produce risk maps of the vectors based on environmental variables. There is a need for on-going monitoring of CanL in Hungary, and the risk maps will help inform where and when interventions might be used to try to limit the northward spread of the canine and human diseases.

This work was funded by EU grant GOCE-2003-010284 EDEN ([www.eden-fp6project.net](http://www.eden-fp6project.net)).

## Session 9 – Malaria

### **Anopheles darlingi (Diptera: culicidae) is not the only malaria vector in French Guiana**

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Since the beginning of the last decade, malaria has persisted under a stable pattern in French Guiana, with approximately 4000 cases reported each year. One fourth of the 200 000 inhabitants is living in endemic areas. *Plasmodium falciparum* and *P. vivax* are equally responsible for malaria cases whereas only 2.5% of cases are due to *P. malariae*. *Anopheles darlingi* is largely distributed all over the territory and is considered as primary vector because of its high densities, its anthrophilic behavior and its natural infections rates observed in infested areas. In French Guiana, anopheline fauna is represented by over 20 species. Some of them are known as local or secondary malaria vectors in the Amazonian region but whose involvement in malaria transmission has not yet been proven in French Guiana.

The medical entomology unit at the "Institut Pasteur de la Guyane" is leading research programs on mosquito bionomics and dynamics related to disease transmission such as dengue, other arboviruses and malaria in both anthropogenic and natural environments. From 2006 to 2009, all anopheline mosquitoes collected through these programs were morphologically identified and subjected to an enzyme-linked immunosorbent assay (ELISA) in order to detect the presence of *P. falciparum*, *P. vivax* and *P. malariae* circumsporozoite protein (CSP).

Apart from *An. darlingi*, five specimens belonging to three different species were found positive for *P. falciparum*. In November 2006, animal bait and human landing collections performed in a secondary forest near St-Georges de l'&rsquo;Oyapock yielded, respectively, one positive specimen of *Anopheles nuneztovari* and two of *Anopheles intermedius*. In February 2008, an additional *An. intermedius* was diagnosed as positive during human landing catches in a cultivated clearing near the village of Cacao. Finally, in May 2008, one positive specimen of *Anopheles oswaldoi* was collected on human in an abandoned Amazonian village in the region of Camopi.

The role of *An. intermedius*, *An. nuneztovari* and *An. oswaldoi* in malaria transmission has already been suggested in the Amazonian region but it is the first time that these species are found to be naturally infected by *P. falciparum* in French Guiana.

These results reinforce the hypothesis of a malaria transmission pattern due to local or secondary vectors in

French Guiana. Their role in maintaining malaria endemic needs to be further investigated.

## Evolution of the risk of Malaria re-emergence in Romania

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Romania experienced about 300,000 malaria cases yearly, and "malaria stratification" established in 1938 on the basis of vector species distribution in correlation with the ecological factors led to the adequate elaboration and application of malaria eradication programme. The so called "anophelism without malaria" period after the eradication in 1962 is characterized by the permanent risk of malaria re-emergence. The risk is generated by the simultaneous presence of very abundant anopheline populations in the former endemic areas including the previous vector species with capacity (experimentally documented) to transmit the imported Plasmodium strains and the increase of the number of imported malaria cases including the possibility of appearance of the Plasmodium sp. new reservoir. The evaluation of the evolution of the risk level of malaria re-emergence to anticipate, prevent and control the malaria re-introduction was performed. Analysis of both the current and historical data regarding the presence, dynamics and vectorial capacity of the anopheline vector populations and the presence of the imported Plasmodium strains in correlation with the evolution of the environmental, social and economical conditions in the areas with human populations at risk was performed. The main elements increasing or decreasing the risk of malaria re-introduction have been recorded. Four periods (including the present one) have been detected after the eradication in Romania with different risk level of malaria re-emergence. The risk level resulted from the balance of parallel action of the elements increasing or decreasing the risk at a moment in different areas. The present risk has been defined and the risk areas mapped. The permanent surveillance of the factors influencing malaria re-emergence risk is needed to prevent and control the re-appearance of this disease.

## Effects of environmental changes on malaria vectors in Algeria

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Thanks to the malaria eradication programme launched in 1968 for ten years, the incidence of autochthonous malaria cases in Algeria had significantly decreased from 70,000 cases in 1950 to only thirty cases in 1978. However, with the opening of the Trans-Saharan road the number of imported malaria cases, mostly from sub-Saharan Africa, had increased, sometimes reaching seven hundred cases per year.

Nowadays, autochthonous malaria occurs in residual foci located in the oases of Tamenrasset, Ouargla Adrar, Illizi, Ghardaia and Béchar. The disease is caused mainly by Plasmodium vivax. Anopheles labranchiae is the main potential vector in the northern areas, while An. sergenti and An. multicolor are considered as potential vectors in central and southern parts of the country. Recently, during a P. falciparum outbreak in Tinzaouatine (extreme south of Algeria), An. gambiae was observed for the first time in this area and incriminated as vector.

Since the remarkable work of Etienne and Edmond Sergent and colleagues on malaria and their vectors in the last century, very few studies on the biology of malaria vectors have been conducted in Algeria, particularly in the East of the country and in the Sahara, where malaria constitutes a real threat due to the influence of human practices and environmental changes.

Here, we present data on the effect of environmental changes on An. labranchiae and An. multicolor biology and

population dynamics and on malaria risk in two Algerian areas: El Kala, a wetland area in the North East of the country, and Ouargla, an oases in the south of the country where the increase in ground water constitutes a major environmental factor in relation to An multicolor larval breeding sites. Finally, climatic changes and human population migration are incriminated as putative factors in the establishment of An gambiae, and malaria, in southern Algeria.

## **Impact of human activities on the dynamics of insecticide resistance in Anopheles gambiae: Case of Benin (West Africa)**

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Vector control for malaria prevention remains mainly based on the use of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS) in Sub-Saharan Africa. The effectiveness of these tools is threatened by insecticide resistance of An. gambiae s.s., one of the main African vectors of human Plasmodium. Historically, resistance against DDT in natural populations of African malaria vector since the 60's has been selected by insecticide use in public health. Nowadays, it is also clear that most cases of resistance against others classes of insecticides in An gambiae s.s. are the result of massive use of these products against crop pests in vegetable and cotton growing areas in the whole West African region.

In addition, some of the pesticides used in agriculture are used against other pest insects (cockroaches, fleas, termites ...), increasing insecticide pressure on Anopheles mosquitoes and representing also a risk to the health of humans exposed to these products. This phenomenon remains poorly studied.

To develop food resources, extension of cultivated areas and the need of modern agricultural practices with high yield have to be implemented in sub-Saharan region of Africa. This could further increase the amount of pesticides and selection pressure for resistance in mosquitoes knowing that much more insecticides are used for agriculture purpose than public health. In this situation, what can we do to ensure the efficacy of current or news resistance management strategies and therefore prevent the failure of vector control tools used? Impact of human activities on the dynamics of insecticide resistance in *Anopheles gambiae*:

### **Why is there no anopheline in the Seychelles?**

Vincent ROBERT (IRD Montpellier),

Simon JULIENNE (Ministry of Health, Seychelles),

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The anophelines are distributed worldwide, over all continents. The only few exceptions include Antarctica, islands such as New Caledonia, some Pacific islands such as Polynesia, as well as the Seychelles.

The Seychelles consist of several archipelagos of about a hundred islands in total, located in the Indian Ocean and linked to the African continent. The islands forming the centre of the archipelago are granitic and have a strong pluviometry and a climate a priori suitable to anophelines. Those granitic islands have never been totally submerged, at least since the break-up of the Gondwana. Other islands &mdash; such as Aldabra, the biggest raised atoll of the world &mdash; are coralline and extremely flat.

The anophelines being absent of the Seychelles islands is a curiosity of paramount importance for public health and tourism economy, that both benefit by a malaria-free environment. One should put this absence into perspective since anophelines of undetermined species, which did not succeed in maintaining their presence, proliferated and triggered outbreaks with Plasmodium vivax in 1908 within Aldabra, and with P. falciparum in 1930 within Aldabra and Assumption.

With a field survey carried out in December 2008, we confirmed the absence of anophelines in the Seychelles. The hypotheses aiming to explain this absence are necessarily speculative and distinguish between with ancient

causes (at a million-year scale) and recent causes (at a century scale). In the granitic and Amirantes archipelagos the climate is favourable, but the environmental is unfavourable due to the low availability of stagnant waters useable as larvae habitat, itself consequence of the steepness of the terrain. In the southern islands (Aldabra and Providence-Farquhar groups), which are close to Madagascar (less than 300 km from Farquhar), the presence of a long dry season of c.9 months and the total absence of permanent natural freshwater reservoirs prevent anophelines to breed and become established. Native terrestrial mammals are absent with the notable exception of bats, which does not facilitate the blood-feeding accessibility and might explain why endemic anopheline species do not exist in the granitic islands &mdash; especially in mountain streams &mdash; when many of them can be found on the eastern slopes of Madagascar Island. However, many speciations took place in several genera of non anopheline mosquitoes, in the Seychelles. Recently, the low occurrence of successful accidental introductions of anophelines from Madagascar Island or the African continent is likely to be due, on the one hand, to limited maritime and aerial exchanges, and on the other hand, to an efficient health system of border controls that has certainly reduced the success probability of those introductions.

## Session 10 – Tick-borne diseases (Environmental and human factors in risk of TBDs)

### Mapping the risk of tick-borne encephalitis in Sweden by use of low-resolution remote-sensing

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Tick-borne encephalitis (TBE) is caused by TBE virus TBEV, genus *Flavivirus*, family *Flaviviridae*, and transmitted through bites of *Ixodes ricinus* ticks. Across Europe TBE incidence has doubled since the 1980s, with the major increase occurring in the early 1990s. In Sweden, however, two step-increases in 1984 and 2000 caused an overall 4.5-fold rise, with foci appearing in new regions. In 2008 224 cases were diagnosed, a new all time high in Sweden.

Remotely sensed environmental conditions at 1,704 geo-referenced localities of human TBE in Sweden (1986-2007), derived from the MODIS sensor on the NASA Terra satellite, were temporal Fourier processed to extract environmental signatures of seasonality, using middle infra-red, day- and night-time Land Surface Temperatures, and the Normalised Difference and Enhanced Vegetation Indices data. The TBE cases and satellite data were used within a bootstrap, non-linear discriminant analytical framework to produce risk maps of TBE in Sweden.

Overall, kappa values were highly significant, ranging between 0.990 ( $\pm$  0.0024) for the best ten models and 0.943 ( $\pm$  0.0185) for the worst ten models of the 100 bootstrap samples. The equivalent figures for sensitivity were 99.6% ( $\pm$  0.52) and 98.9 ( $\pm$  0.85) and for specificity were 98.5 ( $\pm$  0.53) and 97.7 ( $\pm$  0.82) respectively. Daytime Land Surface Temperature (LST) Minimum of the sample sites was the best discriminator of TBE presence (selected in 100/100 models), followed by the Digital Elevation Model (94/100) and Daytime LST Variance (65/100).

Observations from screening of serum samples from sentinel species support obtained predictions. TBE antibody positive samples were found in areas where previously the risk of TBE was unrecognised, thus highlighting the risk in new foci.

These results suggest a limiting and regulating influence of LST Min and Variance on tick activity and the degree of the critical co-feeding between nymphs and larvae, respectively, thereby modifying TBEV transmission. The effect of elevation is most likely due to local climate where *I. ricinus* reaches its northern boundary in Sweden. These results will help raise public awareness on TBE risk and give recommendations on

TBE vaccination regionally.

## **Landscape and vector-borne and zoonotic diseases: a complex interaction illustrated with tick-borne encephalitis in Latvia**

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Vector-borne and zoonotic diseases have strong ties with the environment in relation to the habitat and survival of vectors and hosts, and pathogen development. Among environmental influences on the distribution of vector-borne and zoonotic diseases, a number of factors can be identified at the landscape level. Such factors, however, do not simply describe suitable habitats for vectors and hosts. Indeed, landscapes result from the combination of a wide diversity of physical, biological and human elements that are potentially relevant to the distribution of vector-borne and zoonotic diseases, especially if the degree of human exposure is considered. Several major characteristics of landscapes can be considered: whether their environment is suitable for vector and host populations (depending on land cover); whether the local human population is likely to enter the vector or host habitats on a regular base (depending on land use); and whether the spatial distributions of these two aspects are likely to intersect, through access rules (as a function of land ownership). We will illustrate this concept based on a collaborative study of tick-borne encephalitis (TBE) in rural municipalities of Latvia, carried out in the framework of the EDEN project. The study used official records of TBE in Latvia and data extracted from high-resolution satellite imagery, agriculture, forestry and population censuses. The data were analysed using binomial negative regression with a spatial component. The results indicated that complex combinations of land cover, land use and land ownership all contribute to explaining and predicting the spatial distribution of TBE cases in rural areas of Latvia. This case study also highlighted the role of forest management features, such as felling and clear-cuttings, for the first time. The concept of landscape is here given new depth by considering its physical structure, its use by human populations, and its accessibility as modulated by ownership. It also points to the potential role played by landscape management in modifying disease risk for humans.

## **Nature's calendar: Spatial and temporal variation in *Ixodes ricinus*, associated *Borrelia burgdorferi sensu lato* genospecies and tick bites in the Netherlands**

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Lyme disease incidence in the Netherlands has increased three-fold over the past decade. Here, we describe a study where internet-based self-registration of tick bites provides spatial and temporal information on the prevalence of tick bites in the Netherlands. Furthermore, population densities of the Lyme disease vector *Ixodes ricinus* and their associated *Borrelia burgdorferi* s.l. infections were monitored at 24 sites from July 2006 to December 2007. Ticks were sampled monthly by blanket dragging by a volunteer network, which sent all samples to a central collection centre. Nymphal ticks were investigated for the presence of *Borrelia* genospecies. *I. ricinus* was found present in all selected locations, but with large significant spatial and temporal variations in density. In the web-based survey over 2006 and 2007, 3437 tick bites were registered. Tick bites were recorded at all months, but peaked between April and August. Most tick bites (39%) were picked up in the forest, but a large proportion (35%) was picked up in gardens. Most tick bites occurred during the activities walking (32%) and gardening (24%) in the age groups of 46-65 (37%) followed by age category



of 26-45 (24%). In the tick monitoring study, some locations showed *I. ricinus* activity throughout the year, including winter activity, which was accompanied by a continued reporting of tick bites through our web survey. In contrast to typical bimodal activity, *I. ricinus* phenology showed a wide unimodal distribution in 2007, with largely synchronized activity of nymphs and larvae. Mean monthly *Borrelia* infections in nymphs varied from zero to 29%, and differed significantly between locations. Five species of *Borrelia burgdorferi* s.l. were found, with *B. afzelii* being dominant at most sites. Significant differences in the diversity of *Borrelia* species were found between locations. Considering the different habitats, higher *Borrelia* infection percentages were found at locations with lower tree cover. Moss cover and litter layer were positively related to nymphal and adult densities.

This study shows that *Borrelia* infected ticks in The Netherlands are widespread, ranging from dune areas to inland deciduous and coniferous forests, many of which are heavily used for recreational activities such as hiking, running and biking. Surprisingly, gardens also seem to be a suitable setting for tick bite incidence. The study also shows that tick densities and *Borrelia* infections relate differently to specific vegetation characteristics. These results can be applied to improve prevention of Lyme borreliosis through risk mapping and more efficient public information.

## **Animal disease risk in the countryside: providing appropriate information, avoiding alarm**

Mariella Marzano, Darren Moseley and Chris Quine,

Forest Research, Edinburgh, UK

Results will be presented from a UK-based interdisciplinary project on 'Assessing and communicating animal disease risks for countryside users'. The project, involving Forest Research UK and the Universities of Oxford and Surrey, explores the potential impact of zoonotic diseases on the development of recreation in rural areas within an overarching framework of risk communication. The research focuses initially on Lyme disease (or Lyme borreliosis), an infection caused by the tick-borne bacterium *Borrelia burgdorferi*. Project results will be relevant to other zoonoses, but Lyme disease is an ideal case study because it is relatively well understood and appears to be increasing in the UK. This paper examines how and why organisations communicate risk of zoonotic disease to their staff and various publics through an in-depth assessment of the issues and constraints faced by organisations in information provision.

We collected information on Lyme disease provided by 44 organisations that are involved in health and recreational policy, land management or recreation and access to the countryside. The wide range of information sources on ticks and Lyme disease were then analysed to assess current understanding of the disease and preferred methods for communicating to different audiences. A content analysis of warning information was undertaken through coding each component of the 'messages'. The message components were divided into: descriptive information (of ticks and Lyme disease); imagery used (ticks, rashes, removal devices); precautionary information (prevention, removal advice, infection periods, symptoms, medical referral); and risk (risk periods, risk areas, and fear factors). Principle component analysis was used to identify the major factors in relation to organisational type, message format, and intended audience. In addition, 17 semi-structured interviews were conducted with relevant health and safety, management, education or recreational representatives of organisations who are providers of precautionary information and might be affected by an increase in Lyme disease.

Analysis of the material suggests marked differences in approach between organisations, in particular the use of illustrations and text, and between information provided for staff and that provided for publics. We found a co-occurrence of fear factors in relation to levels of precautionary information, with a step change once a detailed level is reached, raising the question 'in trying to provide adequate information, are organisations potentially alarming their audience?'. Our interviews identified a difference between organisations in where information is held, how it is provided (and in what format) and to whom. Results from the analysis will be integrated with research conducting by other project partners on risk perception and behavioural responses of individuals. The overall aim is to develop practical advice for policy-makers, countryside managers and users on approaches to balancing the promotion of greater awareness and preventative behaviour without increasing public perceptions of risk.

## Human-tick interaction: developing an agent-based model of risk of tick bites during recreation

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Recreation in the countryside can lead to exposure to environmental hazards including the tick *Ixodes ricinus*, a vector of the bacteria that cause Lyme borreliosis (Lyme disease). The probability of countryside users coming into contact with ticks must be calculated as a combined function of tick population biology and human behaviour; seasonal and spatial variation in the use of an area by both ticks and humans will lead to differential levels of risk. In this study, the risk of tick contact is assessed in three contrasting recreational environments in the UK: Richmond Park, the New Forest and Exmoor National Parks. Variation in both the timing and magnitude of peaks in tick abundance across a full range of habitats is recorded by fine-scale field surveys, and the mechanisms behind such variation demonstrated by mathematical modelling. Analysis of fine-scale spatial use by human visitors will combine behavioural observation and GPS tracking data with a risk awareness survey of ticks, Lyme disease and preventative behaviour to develop an agent-based recreational activity model of potential human-tick interaction.

The research is funded by DEFRA's Rural Economy and Land Use programme, with partners at the University of Surrey (Prof. David Uzzell, Dr Julie Barnett, Dr Afrodita Marcu) and Forest Research (Dr Chris Quine, Dr Mariella Marzano, Dr Darren Moseley, Liz O'Brien).

### Session 11 - Rodent-borne diseases (Hanta epidemiology)

## Hantavirus infections in Europe: from virus carriers to major health problem

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In Europe, hantavirus disease or Hemorrhagic Fever with Renal Syndrome (HFRS) is an endemic zoonosis that affects tens of thousands of individuals. The causative agents are viruses of the genus Hantavirus, family Bunyaviridae, rodents and insectivores act as carriers. In all European countries there is a seroprevalence for hantaviruses in the general population but not all countries also report cases for various reasons. Here we give an overview of the hantavirus situation in Europe.

A survey was conducted on behalf of the European Network for diagnostics of Imported Viral Diseases (ENIVD), a European Centre for Disease Prevention and Control (ECDC) network. ENIVD members were invited to complete a questionnaire. Data from this questionnaire were statistically analysed.

Analysis of the ENIVD data provided insight in the various national regulations, the geographical boundaries of co-circulation of Puumala (PUUV), Saaremaa (SAAV) and Dobrava (DOBV) virus in Europe, incidence rates, clinical presentation and epidemiological patterns in different European Union countries. In several European countries HFRS is not yet a notifiable infection.

The understanding and recognition of hantavirus infections have greatly improved in Europe over the past few decades. During the past decade both the amplitude and magnitude of hantavirus outbreaks have been increasing. This could partly be due to increased awareness, better diagnostics and intensified research into reservoir species, but human invasion of previously undisturbed habitats (by building houses, factories, etc) and changing climatic conditions (warmer winters, increased frequency of mast events) certainly contribute to the problem.

## Why is Nephropathia epidemica so common in Finland?

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Nephropathia epidemica (NE) is the most common hantaviral disease in Europe, and is caused by Puumala hantavirus (PUUV). Both the virus and its host, the bank vole *Myodes glareolus* are found in most of Europe, but the disease incidence is highest in Northern Europe: ever since PUUV was discovered in Finland in 1979, more than 70% of NE cases in the European Union have been recorded in Finland. Although the incidence of NE has increased in many European countries, the disease is still most common in Finland, with a new high of 3 216 cases in 2008.

One suggested reason for the high NE incidence in Northern Europe is the high-amplitude vole density fluctuations that only occur in the boreal zone. Mathematical models on PUUV transmission suggest that the rapid increase in the number of susceptible hosts is critical for the emergence of NE epidemics. In our 7-year monthly mark-recapture study of bank voles we found that natural populations indeed follow the transmission dynamics predicted by the mathematical models: the abundance of recently infected hosts increases right after the peak of total host density, coinciding with periods of high NE incidence.

Another possible reason for the epidemiological pattern is the differences in landscape: in temperate Europe, forests are patchy, but in the boreal zone continuous. However, intensified forestry has led to fragmentation of mature forests, so that only 1/5 of Finnish forests are in climax phase, which is the preferred habitat of bank voles, and 1/5 is younger than 20 years. We investigated whether the fragmentation of climax forests restrains the spread of PUUV hantavirus. In a local scale study, we captured bank voles in four forest succession phases during the vole cycle peak year in Northern Finland. In our country-wide study, we studied bank voles on 30 sites across the country. Our results show that fragmentation of old forests has no impact on the spread of PUUV. Bank voles were most abundant in the oldest forests, but PUUV infected bank voles were equally abundant over all succession phases. PUUV infected bank voles were accordingly found in most of the habitats throughout the whole country.

Our results indicate that human NE risk is equally high from recent clear-cuts to climax-phase forests, and forests habitats altogether cover over 80% of Finnish land cover. Our findings further explain why NE is so common in Northern Europe: besides yielding a high viral load in the environment via reaching high population growth rates in peak years, boreal bank vole populations can spread without barriers, leading to continuous existence of PUUV over large areas.

## **Spatio-temporal variation in bank vole hantavirus infection and relation to nephropathia epidemica in humans during an epizootic**

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Understanding transmission dynamics of emerging zoonotic pathogens in their reservoir host populations and the relationship with accidental human infection occurrence is a key aim in current epidemiological research. Puumala hantavirus (PUUV), the causal agent of Nephropathia epidemica (NE) in humans, is one of the many hantaviruses (Family Bunyaviridae; genus Hantavirus) included in the list of emerging pathogens as, over the last five years, the number of NE cases is on the rise throughout continental Europe (Heyman et al. 2009).

Bank voles (*Myodes glareolus*), the PUUV reservoir hosts, were monitored in nine independent study sites during the NE outbreak of 2005 in Belgium. Within a defined high risk cluster for NE, study sites were selected based on preferred bank vole habitat and local occurrence of Nephropathia epidemica in humans. Rodent trappings were carried out in spring, summer and autumn. Bank vole sera were tested for presence of IgG against PUUV. The seasonal pattern of PUUV infection in bank voles was compared to NE occurrence in the study area and using logistic regressions, the spatial relationship between seasonal vole population structure and PUUV infection occurrence was analyzed. A logistic mixed model was built to investigate the temporal variation in individual characteristics and their relative importance to PUUV infection in bank voles.

We found that in our study region, occurrence of NE in humans coincided with the local number and the spatial occurrence of seropositive bank voles. Our analyses show that an increased bank vole number and a high proportion of sexually active males are important features of patches with high PUUV occurrence or a high

intrinsic transmission rate. However, under peak conditions of spatial infection occurrence, local population features did no longer play a major role in determining local PUUV infection risk.

Relative risk calculations for individual vole characteristics related to PUUV infection in the reservoir host show that reproductive activity dominates infection risk. Even the known gender effect is only found in reproductively active voles, where reproductively active males have the highest infection risk. However, our results also revealed a clear seasonal variation in the importance of reproductive activity linked to PUUV infection. In contrast to the main effect in most seasons during the epizootic, no difference in risk ratio was found between reproductively active and non-active voles in the spring period. Combined with increased infection risk for the non-active group at that time, these results indicate a shift in the transmission process due to changes in bank vole behaviour, physiology or climate features. Hence, our results suggest that mathematical models should take into account seasonal shifts in transmission mechanisms between the breeding season and the non-breeding winter period. When these results are combined with the seasonal changes in population structure during the epizootic period, we identify bank vole reproductive activity and length of the breeding season in close combination with bank vole abundance as the main drivers of PUUV epizootics in temporal models for west-central European regions. A clear overview of external factors that shape vole reproduction patterns is needed.

## Predicting high risk of human hantavirus infections in Sweden

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In Sweden, hemorrhagic fever with renal syndrome (HFRS, nephropathia epidemica) is caused by Puumala virus (Bunyaviridae, Hantavirus) shed by bank voles (*Myodes glareolus*). During July 2006 to June 2007, a new record of HFRS was reached in Sweden with 1,394 cases concurrent with high bank vole numbers.

Also observed during this period, was a remarkably abrupt increase of HFRS cases in midwinter followed upon heavy snow melt and loss of the protective snow cover to voles, hypothesized to affect their behavior to enter human dwellings more than usual and increase the vole-human encounter rates.

Data on bank vole abundance within the HFRS-endemic region have been available since fall 1971 through biannual monitoring. Thus, before the HFRS season July 2007 to June 2008, bank vole numbers in autumn 2007 were predicted and subsequently verified by trapping to be at the highest level since the early 1970s.

Consequently, the predicted risk of acquiring HFRS during autumn/winter 2007/2008 was also high. The predicted autumn trapping indexes, and subsequent risk of HFRS, were based on the observed spring trapping index 2007 and the estimated bank vole population growth rate over summer. Based on these a priori predicted indices (low/medium/high) of bank voles, the number of HFRS cases during July 2007 to June 2008 was estimated to reach 1,152 (low), 1,451 (medium), and 1,741 (high), respectively. Despite a high degree of public awareness and clinical preparedness, the total number of reported HFRS cases in Sweden during this period reached 1,483.

## Network “rodent-borne pathogens” in germany: molecular epidemiology of hantavirus and leptospira infections in rodent hosts

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Rodents are important reservoirs for a large number of zoonotic pathogens with direct- or vector-mediated transmission to humans (e.g. hantaviruses, *Leptospira* spp., Tick-borne encephalitis virus, *Borrelia* spp.). The knowledge of the geographical distribution and genetic diversity of rodent-borne pathogens and of the reasons for clusters of human infections in Germany is limited. Therefore the network « Rodent-borne pathogens » was initiated for a synergistic collaboration on rodents and rodent-associated pathogens including viruses, bacteria and parasites. Embedded in the network, a total of about 7,400 wild and commensal rodents and other small mammals were collected in 15 federal states of Germany since 2001. In bank voles (*Myodes glareolus*) trapped in regions in southern and western Germany with previous human hantavirus cases a high prevalence (up to 60%) of Puumala virus (PUUV) was observed. Initial longitudinal studies in Cologne and at different trapping sites in a rural region close to the city of Osnabrück demonstrated a continuing PUUV prevalence in the bank vole populations. The striped field mouse (*Apodemus agrarius*) was identified as reservoir host of Dobrava-Belgrade virus in northeastern Germany. Interestingly, spillover infections of this virus lineage to the yellow-necked mouse (*A. flavicollis*) were observed. Tula virus (TULV) was demonstrated to be present in two *Microtus* species (*Microtus arvalis*, *M. agrestis*) in Brandenburg and Lower Saxony suggesting a broad host range of this virus. *Leptospira*-PCR analysis of 332 rodent and small mammal kidney samples resulted in the detection of 67 positive samples originating from seven federal states and six rodent species (average prevalence 24% with a range of 3.2% and 64.7%). In conclusion, our investigations revealed an unexpected broad geographical distribution of different hantavirus and *Leptospira* species in rodents in Germany. The initiated studies will set the basis for understanding the molecular evolution as well as changes in the distribution and prevalence of these and other rodent- and small mammal-borne pathogens. This may become very important for setting up a risk assessment for human infections especially with the changes in the epidemiology of these pathogens expected in the light of climate changes in the future.

## Session 12 – West Nile in Europe

### Identification of West Nile virus vectors in France

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In 1962, a large cluster of equine encephalitis occurred in association with human cases in the Camargue (southern France). West Nile virus (WNV) was isolated in 1964 from *Culex modestus* females and from the blood of two entomologists who were collecting them. It was then considered the main WNV vector, even if the involvement of *Culex pipiens* was not excluded. After decades, when WNV seemed to persist, but failed to produce human or equine cases, France faced new WNV episodes. Large equine outbreaks were reported in the Camargue in 2000 and 2004, with sporadic human and/or equine cases in the Mediterranean littoral in 2003 and 2006. These episodes in dry areas inappropriate for *Cx. modestus* development suggest other vectors.

A research program was thus undertaken to study WNV vectors. Bird, human and horse baited trap collections were conducted to identify the potential vectors. Mosquito exposure to virus under natural conditions was assessed by testing field-collected individuals for the presence of WNV. Experimental infections allowed us to evaluate the susceptibility of potential vectors to replicate and transmit WNV. These studies showed that *Cx. modestus* is an extremely efficient laboratory WNV vector. Thus, this species, as a bird-feeder, could be involved in very efficient rural WNV cycles between wetland birds in breeding or resting sites, such as reed

marshes, where both mosquito and bird densities are high. As this species is able to feed aggressively on mammals, it could be responsible for sporadic human and equine cases in these sites. *Culex pipiens* was a moderately efficient laboratory WNV vector. This species is found throughout the Mediterranean littoral, where it is the main bird-feeder. It could be considered the sole enzootic WNV vector in dry areas and the secondary enzootic WNV vector in wetlands. *Culex pipiens* is also the most abundant species collected engorged on horses in dry areas, and it could thus be considered the main WNV epidemic vector in dry areas responsible for human and equine cases. According to host preference and current data on vector competence, the role of the most abundant *Aedes* species may remain anecdotal.

Even if WNV is present and transmitted in the Mediterranean littoral since several decades, large WNV epizootics were recorded only in the 1960s and the 2000s. Since World War II, the Camargue has faced important anthropogenic ecosystem modifications, especially in rice cultivation, leading to fluctuations in the abundance of *Cx. modestus*, the natural WNV vector in this region, as supported by historical data. From World War II to 1971, *Cx. modestus* abundance increased as rice cultivation expanded in the region in a political context of supporting agriculture, and then fell likely due to decreased cultivation and increased pesticide usage to control an introduced rice pest. Abundance rose again in the 2000s with the advent of more targeted pest management strategies mainly due to European regulations. We will discuss the potential consequences on WNV transmission of these modifications due to an inter-twined influence of political context, environmental constraints, technical improvements and social factors.

## Mosquito surveillance for West Nile virus in central Europe

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Six viral isolates were obtained from 23,243 female mosquitoes (examined in 513 pools) belonging to 16 species and collected along the lower reaches of the Dyje River in South Moravia (Czech Republic, central Europe) during 2006-2008: five isolates of Orthobunyavirus *Tahy* (TAHV, California group, family Bunyaviridae: 3 isolations from *Aedes vexans*, 1 from *Ae. sticticus*, 1 from *Culex modestus*), and one isolation of Flavivirus West Nile (WNV, Japanese encephalitis group, family Flaviviridae) &minus; strain Rabensburg (proposed lineage 3 of WNV) from *Aedes rossicus*. All viral isolates were recovered from mosquitoes collected in 2006 (15,882 mosquitoes examined) while no virus was isolated from mosquitoes trapped in 2007 and 2008, when 1,555 and 5,806 mosquitoes were examined, respectively. The population density of local mosquitoes was very low in 2007 and 2008 due to warm and dry summer including a considerably low water table, compared to environmental conditions favorable for mosquito development in 2006. The virus isolation procedure was based on intracerebral inoculation of newborn mice; the isolates were identified by neutralization with mouse immune sera, and confirmed by reverse transcriptase polymerase chain reaction (the WNV isolate was also sequenced). In parallel, more than one-third of the samples (183 pools consisting of 8470 individual mosquitoes) were also examined by inoculating Vero cell cultures in Leighton tubes. However, the latter method detected only 3 of the 6 virus isolates (including WNV&minus;Rabensburg). *Aedes rossicus* is a new potential vector for WNV&minus;Rabensburg. This species feeds mostly on mammals including man; this raises the question whether this virus lineage is adapted to an alternative mosquito&minus;mammal cycle in the South-Moravian natural focus.

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## West Nile circulation in Doñana, Spain (2003-2009)

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The prevalence of West Nile virus neutralizing antibodies in wild birds in Doñana (southern Spain) has been

monitored from 2003 to 2009. Seroneutralisation test revealed high interspecific differences in the prevalence of antibodies. Larger and migratory species presented higher prevalences of WNV neutralizing antibodies. We focussed a detailed capture-recapture study on the Common coots (*Fulica atra*) due to the high prevalence of antibodies against WNV and their preference for mosquito rich habitats. Birds were captured through the year, individually marked and bled at each capture. Prevalence of antibodies widely changed across the study. Rapid seroconversion of some individuals confirmed local circulation of West Nile virus. The highest prevalences were found in 2003 and 2008 and the lowest in 2006. Environmental factors related to changes in West Nile virus circulation rates remain under study.

## West nile virus circulation in Romania – the past and the present

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The circulation of West Nile virus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals including humans as tangential hosts. The WNV circulation was documented in Romania beginning with the &lsquo;50s by serological investigations on healthy humans and domestic animals and the confirmation of this virus as etiological agent of some sporadic human neurological disorders with various clinical aspects. An outbreak of more than 800 human cases of WNV neurological infections (mainly meningo-encephalitis) appeared in South Romania in 1996. This was the European signal of the increase of WNV circulation especially because of the global environmental changes including climatic ones. The WNV neurological infections continued to appear yearly after the outbreak in Romania on more extended areas. The multidisciplinary investigation using entomological, immunological, virology and molecular biology techniques have been performed in 2001 &ndash; 2009 on large territories in Romania on the main elements of the transmission cycles of WNV involving mosquito vectors, domestic and wild birds and horses in natural and anthropic ecosystems. The significant high values of the seroprevalence of specific IgG antibodies against this virus in its vertebrate hosts over the large territories and their variations in correlation with the environmental factors have been put in evidence. The virus was detected by immuno-chromatographic test included in RAMP system (Rapid Analyte Measurement Platform) in females of *Culex pipiens*, *Coquillettidia richiardii*, *Ochlerotatus caspius* and *Anopheles maculipennis* s.l. species. Males of *Cx. pipiens* and also over wintering females of this species have been positive for the virus. Besides of isolation from *Culex pipiens* in Bucharest during the epidemic in 1996 the WNV have been isolated from this species by inoculation on suckling mice in 2002 in Bucharest and in 2007 and 2009 in a Bucharest suburb. The isolated viral strains are molecularly characterized by using a multi-level protocol including RT-PCR, nested PCR and sequencing and also the Lab-on-a-chip technology for checking the quality and quantity of total viral RNA. It has been demonstrated the intensive and permanent circulation of WNV on extended territories in the country and the permanent risk of its transmission to humans. The risk areas have been mapped. The permanent surveillance of WNV endemic circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health.

## West Nile virus circulation in Emilia-Romagna (Italy) in 2009: the integrated surveillance system

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During the late summer 2008 a large epidemic of West Nile Virus (WNV) occurred in North-Eastern Italy involving an area of more than 7,000 Km<sup>2</sup>, in 3 Regions including Emilia-Romagna where it caused 23 cases in

horses and 3 human cases of neuroinvasive form of West Nile Disease.

Aiming at an early detection of the reactivation of virus circulation in 2009 an integrated surveillance system was planned and activated with the collaboration of medical and veterinary practitioners, entomologists and epidemiologists.

Human surveillance: The activity was performed on the whole regional territory, from 15th June to 31st October, period of vector activity in Emilia-Romagna. First of all the human surveillance includes WNV and/or antibody investigation on blood and cerebro-spinal fluid for all suspected cases with viral encephalitis or meningoencephalitis observed on the regional territory. The second activity is an active surveillance for all exposed persons who live or work in an area of documented viral circulation, like workers of farms where emerging infected horses have been identified.

Horse surveillance: In Italy all suspected signs of WND in horses has to be compulsory notified to the Official Veterinary Services.

Furthermore, in some Provinces of Emilia-Romagna region some seronegative unvaccinated equine sentinels were selected in 2009 spring. They were serologically tested twice after the selection, at the beginning of August and September.

Any suspect case is confirmed by virus neutralisation test, IgM ELISA or PCR.

Wild birds surveillance: Monitoring has been carried out in all the Provinces along Po river, in the plain area of Emilia-Romagna. Every 1,600 km<sup>2</sup>, a monthly sample of about 40 wild birds was collected. Samples of organs each bird were pooled and examined by PCR.

Entomological surveillance: Surveillance system was based on the collection of mosquitoes in fixed stations and in the sites where birds, humans or horses signalled WNV circulation. Mosquito collections were conducted using 92 CO<sub>2</sub> baited traps positioned in fixed stations. 52 stations were running from long time, while 40 ones were newly planned stations to cover the surveillance area using a grid of 100 km<sup>2</sup>. Moreover mosquito collections were promptly performed in sites following the detection of positive horses and human cases by CO<sub>2</sub> and gravid traps. The surveillance system was activated in the period 15th April - 10th October, collected mosquitoes were pooled (max 200) by species, date and site of collection and examined by PCR. In addition overwintered mosquito females were collected in early spring by manual aspirator inspecting rural buildings in the area where WNV activity was observed during 2008.

Results (update 2009, November 23): Out of 74 possible human cases, 9 have been confirmed (3 deaths). 26 WND cases were confirmed out of 45 suspected horses. Seroconversion in sentinel horses was detected in three Provinces (Ferrara, Modena and Reggio Emilia). 44 wild birds (out of 1,239 tested by PCR) resulted positive to WNV. About 190,000 mosquitoes were collected, pooled and PCR tested (1,789 pools of &le; 200 individuals/pool). Twenty-seven pools, all consisted of Culex pipiens, resulted WNV positive.

## Session 13 – Malaria

### **Multigenic and phylogenetic analyses of anopheles species from Spain, inferred from ribosomal and mitochondrial dna markers**

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Renewed interest in the distribution of Anopheles mosquitoes and their ability to transmit malaria has arisen in recent years due to the potential effects of global warming on insects and insect-borne pathogens in Europe. Spain is the country having suffered the largest people immigration phenomenon in recent years, including immigrants from malaria endemic areas, mainly Latin America but also Africa. Because of its close proximity to Africa, Spain has played an important bridge role in the traffic of sub-Saharan migrant workers. In that way, malaria transmission risk by anophelines, both indigenous or migrating from neighbouring Mediterranean countries which could become infected from gametocyte-bearing migrants, has increased substantially. Thus, the characterization of present potential vector populations is of high importance in southern countries of Europe where the impact of global warming is predicted to be highest.

The current trend in molecular population genetics is to use increasing numbers of genes in the analysis. Here,



we describe a genetic analysis of DNA polymorphism data from multiple genes and spacers of the ribosomal and mitochondrial DNA of *Anopheles* species populations collected in several areas of Spain which were endemic for malaria in the past, including species from other European countries for comparison.

The complete sequence of several ribosomal markers (18S rRNA gene, ITS-2 and D2 and D3 expansion domains of 28 rRNA gene) and mitochondrial markers (mtDNA *cox1* and *cox2*) were obtained for first time from different *Anopheles* species and populations from Spain (Murcia, Huelva, Tarragona, Cadiz, Cuenca, Salamanca, Gran Canarias), Georgia and Italy: I) *An. algeriensis*; II) *An. atroparvus*; III) *An. sergentii*; IV) *An. cinereus hispaniola*; V) *An. plumbeus*; VI) *An. maculipennis* s.s.; and VII) *An. labranchiae*.

Combined multigenic sequence analysis allows us to describe haplotypes and haplogroups for *Anopheles* species and populations studied. Unique-event polymorphism (UEP) and short tandem repeat sequences (STRs) were analysed for specific combined haplotype adscription. DNA sequence analysis showed that ribosomal 18S, ITS-2, D2 and D3 markers were different in each *Anopheles* species. Polymorphic sites detected were not indicative of intraspecific or geographical differences using those markers. ITS-2 and D2 were the most variable of these ribosomal markers. The *cox1* and *cox2* markers of mtDNA allow us to define different haplotypes for each *Anopheles* species as well as for different populations of given species. In combined studies on insects, it is well known that nuclear genes perform better than mitochondrial genes. Within the large insect literature about this better performance, there are even impressive situations in which nuclear genes outperformed mitochondrial genes in most measures of phylogenetic utility.

Results on genetic distances and phylogenetic analyses fully support the supraspecific arrangements included in the most recent classification of *Anopheles*. When comparing all genes and spacers used, the ribosomal markers show to be the best candidates for the reconstruction of anopheline phylogenetics and to help systematic and taxonomy towards an evolutionary classification of this genus of high medical interest. At a low level of divergence, as for studies of closely related taxa (cryptic species and population genetic studies), useful information is furnished by the silent substitutions of mtDNA codifying genes. The use of a standardized composite haplotype code nomenclature for both nuclear rDNA and mtDNA markers is strongly encouraged to avoid difficulties in comparative studies.

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## Population structure of the European malaria vector *Anopheles atroparvus*

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Populations of the mosquito species *Anopheles atroparvus* were among the main malaria vectors of Europe, particularly in southern West-Central rural regions of the continent. In the advent of malaria resurgence in Europe, the rate of spread of introduced *Plasmodium* parasites is likely to be influenced by patterns of population structure of the vector. We have therefore used microsatellite and morphometric analysis to unveil levels of differentiation among European populations of *A. atroparvus*.

We have genotyped 10 microsatellites and conducted discriminant multigroup analysis based on wing measurements in 20 mosquito samples collected from 5 countries (Portugal, Spain, France, Italy and Romania). Results from both microsatellites and morphometric analysis suggest a shallow population differentiation and weak correlation with distance of *A. atroparvus* populations spanning up to ca. 3,200 km. Estimates of effective population size based on temporal samples varied between 2,500-20,000 in Portugal, being highest in the south. These were ca. 10 fold greater than the one obtained for the sample of north Italy (ca. 500). Mutation-drift equilibrium tests provided a weak signal of population expansion for some of the sites.

The shallow levels of population substructure can be explained by considerable gene flow among populations

or by relatively recent demographic expansions that are likely to be associated with past anti-vector measures implemented by the former European malaria eradication programmes.

## Risk assessment of Malaria reintroduction in Italy

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### Background

Due to genetic, historical, social and epidemiological reasons, *Anopheles labranchiae* is the only species that may represent a potential risk of malaria re-emergence in the countries facing the Western side of the Mediterranean Basin. In the light of the predicted global warming and of the possible spread of exotic pathogens and vectors outside their natural areas studies on the possibility that malaria could be reintroduced in Italy, in at &ldquo;risk areas&rdquo;, were implemented. We focused our investigations in Maremma plain, a historical malaria hyper-endemic area until 1950&rsquo;s. This study was mainly aimed to evaluate the &ldquo;malariogenic potential&rdquo; of the study area, by the assessment of the 3 parameters that determined it, receptivity, infectivity, vulnerability. The theoretical vectorial capacity of *An. labranchiae* was also assessed. Results of the study were compared with the existing literature (Romi et al., 1997; 2001).

### Methods

The area selected was a small portion of the Maremma, a coastal plain that extend along the Tyrrhenian side of the country, where the re-colonization of the territory by *An. labranchiae* and its seasonal abundance were favorite by human activities (intensive rice cultivation, introduced in the late 70&rsquo;s). Entomological data were obtained by longitudinal and sporadic surveys carried out in selected sites in 2005-2009 (Di Luca et al., 2009). Historical data on the distribution and bionomics of anopheline species in Maremma, past data on malaria incidence and present data on imported cases (gametocyte carriers) were also acquired.

### Results

Our findings showed a very high receptivity of the study area because of the relevant abundance of the potential vector *An. labranchiae*, and a known infectability of the species with *Plasmodium vivax* (Baldari et al., 1998), opposite to an apparent poor competence to transmit afro-tropical *P. falciparum* strains (NF54 lab-strain). The study area also shows a very low vulnerability due to the scarce presence of gametocyte carriers potentially circulating during the favourable transmission season. The theoretical values of vectorial capacity were assessed by site and by period.

### Major conclusion

In the study area, as in other areas of the country where *An. labranchiae* still persist in relevant densities, the return to a situation of endemic malaria should be considered not possible (also due to the improved cultural and socio-economics conditions). Nevertheless the occurrence of isolated cases appears improbable but not impossible as already occurred in this area in 1997 (Baldari et al., 1998). In fact it is possible that illegal immigrants, coming from endemic malaria countries as seasonal workers, and afraid to attend public health facilities, may introduce malaria gametocytes in rural &ldquo;at risk&rdquo; areas. It should be also considered that in the summer tourists, largely occurring in the area, could be exposed to mosquito bites more than resident people. Moreover, the theoretical values of vectorial capacity obtained, (i.e. the measure of the disease spread from a single malaria case), appears not reliable, suggesting a critical revision of the parameters commonly used for assessing this epidemiological factor in a developed malaria-free countries.

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## Factors affecting the size of a mosquito population in a favourable environment

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Mosquitoes are vectors of major human and animal pathogens (agents of the malaria, dengue and West Nile

viruses&hellip;). Transmitted diseases, which are often largely distributed across the world, have dramatic health, socioeconomic and political consequences. Model vector abundance and understand factors of influence may improve our knowledge of the pathogen transmission by vectors. Our objective is to identify factors affecting the size of a mosquito population located in a favourable environment (providing accessible hosts, rest shelters and breeding sites) by a modelling approach. We develop a mechanistic model of the temporal dynamics of mosquito abundance over several years and according to environmental conditions. The model represents each stage of the life cycle: egg, larvae, pupa, and adult (emerging, nulliparous and parous). The parameters and the transition functions between stages are adapted to species of the genus *Anopheles* present in the Camargue (France). This zone is a favourable environment wetland where independent field data were available on two successive years to validate our model. Only nulliparous enter into diapause during winter and survive from one year to the next. On the one hand, development parameters (larvae, pupae, engorged adults which become gravid) depend on temperature. On the other hand, mortality rates (larvae, pupae, adults) depend on temperature. Temperatures are chosen to be representative of the Camargue. The sensitivity of the model to parameter variations is assessed. Our model is consistent with field data. Parameters influencing abundance concern adult stages (basal or additional (related to host-seeking behaviour) mortality, development rate of emerging adults, engorgement rate, oviposition success and medium number of eggs laid by nulliparous) and the emergence period (pupa carrying capacity, success of emergence, pupa mortality). These parameters must be precisely assessed to enhance the precision of our model prediction and because of their influence on the mosquito abundance. Nevertheless, estimation of most of these parameters and particularly their dependence to climatic factors are weakly documented or still lacking for most of the main vector species. Entomological research should then be focused on the field and lab estimations of these parameters. Moreover, in a more constrained environment, mosquitoes may have to move to accomplish their life cycle, resulting in a spatially structured population. Factors affecting mosquito abundance in such an environment may be different and should also be identify. A spatio-temporal model driven by climate is therefore needed.

## Larval malaria vector control: a closer look into the mosquito nursery

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In combination with existing efforts to combat malaria, interventions aimed at mosquito larvae can contribute significantly to sustainable malaria control. We will start by presenting insights into the fundamental, ecological relationships within the aquatic habitat of African malaria vectors (competition, impact of climate etc.). Then, we will present our latest findings regarding the development of new tools for controlling the aquatic stages of malaria vectors. These include the application of monomolecular surface films to large water bodies as well as the opportunities to use entomopathogenic fungi in the aquatic environment. We will present results from field trials in western Kenya on how environmental management techniques can be included in the farming system and how these can significantly reduce aquatic vector populations. Finally, we will discuss how changing environmental conditions could affect the aquatic ecology of malaria vectors and how this, on its turn, may alter the choice and/or efficacy of intervention tools.

## Session 14 - Tick-borne diseases (Biological features in the emergence of TBDs)

### Infection by *Borrelia burgdorferi* sensu lato influences *Ixodes ricinus* survival under challenging climate conditions

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As global warming is growing into a worldwide concern, it becomes necessary to explore what impacts this phenomenon may have on primary European tick vector *Ixodes ricinus* and the major disease it transmits, i.e. Lyme borreliosis. In the present study, *I. ricinus* ticks sampled in the Neuchâtel region in May 2009 were

analyzed for fat content. Others were rehydrated over water in the dark at 4°C for 4 weeks before being tested in harsh climatic conditions using saturated salt solutions generating various relative humidities (RH) (13%, 32%, 51.5%, 61%, and 89%) at two different temperatures (12.5 and 25°C) in order to determine whether *Borrelia burgdorferi* s. l. detected by Real-Time PCR (RT PCR) and Reverse Line Blot (RLB) influences tick survival after 48-hour climatic stress.

Fat contents varied significantly between nymphs and adults (Mann-Whitney test; nymph-male:  $P=0$ , nymph-female:  $P=0.004$ ) but not between males and females (Mann-Whitney test;  $P=0.114$ ). There were significant differences in survival rates among life stages: that of females was highest (77.6%), followed by that of males (51.6%) and that of nymphs (43.2%). The climatic factor that most determined survival was saturation deficit (SD), a measure of the drying power of the atmosphere depending on both temperature and RH. As SD increased, tick survival rate decreased in all three stages.

Among the 1500 tested ticks, 34.8% ( $n=522$ ) were infected by *B. burgdorferi* s.l. Adult infection rate (39.6%) was significantly higher than that of nymph (25.5%). Infection load in RT PCR ranged from 1 to  $1.23 \times 10^7$  spirochetes per tick: infected females contained very high numbers of spirochetes more frequently than infected males and nymphs. Genospecies identification by RLB was possible in 519/522 ticks. Single (84%), double (15%) and triple infections (1%) were detected. *B. afzelii* (38.8%), *B. garinii* (33.9%), *B. valaisiana* (18%), *B. burgdorferi* ss (8.5%), and relapsing fever-like spirochetes (0.7%) were recorded. Mixed infections mainly consisted of *B. garinii* and *B. valaisiana* (46.5%), *B. afzelii* and *B. burgdorferi* s.s. (23.3%), and *B. afzelii* and relapsing fever-like spirochetes (11.6%) infections. *B. garinii* was present significantly less in nymphal than in adult ticks, whereas *B. afzelii* displayed the opposite trend.

Nymph survival rate was significantly enhanced by *Borrelia* infection, whatever the infection load, while that of adults, more markedly females, behaved similarly up to some threshold point (about 160,000 spirochetes), where an increasing number of spirochetes reversed to a negative effect. Moreover, *B. afzelii*-infected ticks survived better than other ticks (infected or not). The present results clearly suggest that *B. burgdorferi* s.l. infection and more strikingly *B. afzelii* infection confer *I. ricinus* ticks, nymphs even more than adults, survival advantages under climatic stress conditions.

## Ticks, birds and tick-borne diseases: the terrestrial cycle

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The importance of birds as reservoir hosts for tick-borne diseases has been recognised for many decades. Due to the difficulties inherent in studying birds in natural and laboratory conditions, the knowledge and appreciation of their role as reservoirs have lagged behind that of hosts that are easier to study, e.g. rodents. We are currently studying the basic ecological processes involved in the interactions between Ixodid ticks and terrestrial songbirds. Biological and epidemiological parameters were obtained from experiments and long-term observational data (12 years) of the sheep tick (*Ixodes ricinus* L.) and the tree-hole tick (*I. arboricola* Schulze and Schlottke) parasitizing one of Europe's commonest songbirds, the great tit (*Parus major* L.). The great tit has been used as a model organism in many ecological studies, and is recognised as a reservoir host for *Borrelia burgdorferi* s.l. Immature developmental stages of *I. ricinus*, the most important vector of tick-borne zoonotic diseases in Europe, are frequently found on songbirds. *I. arboricola* is a wide-spread nidicolous tick, adapted to a lifestyle inside tree-holes where it infests roosting and breeding birds, e.g. the great tit. In addition, this tick is considered as a competent carrier of *B. burgdorferi* s.l. and tick-borne encephalitis virus, though its ability to transmit those pathogens and therefore maintain it in birds has not been determined yet. We will report on the main factors that explain spatio-temporal infestation risks, defined by ecological risk models making use of detailed vegetation maps in conjunction with micro-climatological variables and birds' life-history parameters. Furthermore, we will present experimentally obtained data on the virulence of ticks, birds' resistance mechanisms, as well as the host-mediated transmission mechanisms. Future research will fully establish the importance of resident passerines as reservoirs of tick-borne diseases in particular habitats. Additionally, in hole-breeding birds the possibility of closed enzootic cycles between birds and *I. arboricola*, with *I. ricinus* as a bridge vector, will receive further attention.

## Ticks and tick-borne pathogens transmitted by birds in Norway and Lithuania

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Birds could play an important role for the long-range migration of *Ixodes ricinus* from Eastern and Central Europe to Scandinavian countries, affecting the tick population dynamics and structure, and transporting infecting ticks. It is also possible that migratory birds are dispersing infected *I. ricinus* in new areas in Norway, where they could raise a public health risk.

The aim of this study was to investigate the prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* pathogens in ticks collected from migratory birds and to define specific species of birds and ticks in the diseases spreading.

A total of 876 *I. ricinus* ticks were collected from 230 migrating birds during 2005-2008 in Lithuania (Ventes Ragas) and in Norway (Jomfruland and Lista). The majority of ticks 82 % (716 from 876) were nymphs. The bird species most infested by ticks in Lithuania were *Luscinia luscinia* (2.4 ticks per bird), and in Norway, Jomfruland - *Turdus merula* (11.12) and *Hippolais icterina* (9.5), and Lista - *Sturnus vulgaris* (19) bird species.

*B. burgdorferi* s. l. and *A. phagocytophilum* in ticks was determined by using PCR, Real Time-PCR, and sequencing. The infection rates of *B. burgdorferi* s. l. in ticks collected from birds in Norway were 13 % (43 ticks from 340) and 5 % (13 from 262) in Jomfruland and Lista, respectively. *B. burgdorferi* s. l. pathogens were detected in ticks collected from *Carduelis cannabina*, *Carpodacus erythrinus*, *Fringilla coelebs*, *Oenanthe oenanthe*, *S. vulgaris*, *T. merula*, *Turdus philomelos* and *Turdus pilaris* bird species. *B. afzelii*, *B. burgdorferi* s. s., *B. garinii* and *B. valaisiana* genospecies were identified in infected ticks. *B. garinii* was the most dominant genospecies: 77 % (33 ticks from 43) in Jomfruland and 67 % (8 from 12) in Lista. Co-infection of different *Borrelia* genospecies was detected in 4 % ticks (2 from 43) collected from birds in Jomfruland.

*A. phagocytophilum* infection rate in ticks collected from birds in Jomfruland was 11 % (42 from 371), in Lista 2 % (6 from 297). This pathogen was detected in ticks collected from *Erithacus rubecula*, *F. coelebs*, *Sylvia borin*, *S. vulgaris*, *T. merula* and *T. philomelos* bird species. *A. phagocytophilum* infection was not detected in ticks collected from birds in Lithuania, however, other rickettsial infection was detected in 32 % (37 from 116) of ticks.

## Is the introduced Siberian chipmunk (*Tamias sibiricus*) a reservoir host for *Borrelia burgdorferi* sensu lato in suburban forests?

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In Europe, Lyme borreliosis is caused by the Spirochaetae *Borrelia burgdorferi* s. l. complex and is transmitted by the tick *Ixodes ricinus*. The main reservoir hosts are identified among small rodents and birds. We have previously suggested that the introduced Siberian chipmunk (*Tamias sibiricus*) is an important reservoir host for Lyme borreliosis. A reservoir host is able to maintain and to directly or indirectly, i.e. through a vector, transmit the infection.

We conducted a longitudinal field study between 2006 and 2008 in a suburban forest near Paris (Sénart, France) to 1) demonstrate and quantify the reservoir competence of chipmunks for *B. burgdorferi* s.l. and 2) analyze the diversity of the *Borrelia* species hosted by the chipmunk compared to a native reservoir host, the bank vole (*Myodes [Chlethrionomys] glareolus*).

To demonstrate that chipmunks are reservoir, blood remnants in infested questing nymphs were screened for chipmunks DNA. To partly quantify the reservoir competence of chipmunks, we measured the rate of infected engorged larvae collected on infected chipmunks. In addition, we characterised the temporal variation of infection in chipmunks using capture-mark-recapture. Juveniles were monitored to determine when and how long they hosted *Borrelia*. *Borrelia* species were identified using molecular analyzes and culture from ear biopsies of rodents.

Results of analyses of nymphal blood meals and engorged larvae will be available in early 2010. Juvenile chipmunks were not infected in the first days after their emergence from the nest, but acquired the infection during the tick season. Once contaminated, most chipmunks remain infected even during the hibernation period. 156 of the 352 chipmunks analysed (44.3%), were infected by *B. burgdorferi* sl. Among these, 45%, 44%, 5% and 6% carried *B. burgdorferi* s. s., *B. afzelii*, *B. garinii* and a combination of two of these species, respectively. By contrast, only 90 out of the 623 bank voles investigated (14.4%), were infected and *B. afzelii* was identified in all. Thus, we showed that the *Borrelia* infection rate was significantly higher in the introduced chipmunks than in native bank voles, and was associated with a higher diversity of the *Borrelia* species. An increased immune tolerance towards *Borrelia* may explain such pattern of infestation in Siberian chipmunk. The effects of the introduction of the exotic species *T. sibiricus* on the acarologic risk and human Lyme disease burden in the Sénart area are currently being investigated.

## Ticks and tick-borne disease in South Bohemia (Czech Republic)

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In the epidemiology of infectious diseases and particularly vector-borne diseases, complex models for disease risk prediction are increasingly employed. A frequent output of the field studies of vector-borne diseases is the prevalence rate of pathogens in their vectors. The biggest limitation of such data is that they can hardly be generalized on a larger geographic area or time period. Therefore, complex models for prediction of disease risk independent on prevalence data are developed. In the Czech Republic as well as in many other European countries an increase in the incidence of tick-borne diseases (TBD) is recorded. Regarding TBD, the Region of South Bohemia is one of the areas with highest risk in the Czech Republic.

The work, currently realized in the South-Bohemian Region, is part of a cross-border co-operation project 'Ticks and tick-borne diseases under the conditions of South Bohemia and Bavaria', with the following aims: 1) mapping the occurrence of *Ixodes ricinus* ticks; 2) mapping causative agents of tick-borne encephalitis (TBE) and Lyme borreliosis (two most important TBD in Europe); 3) identification of the key factors that influence the distribution of the risk on the basis of the data acquired by mapping; and 4) development of model for TBD risk prediction on a regional scale.

On the basis of analysis within a geographic information system (GIS) regarding various criteria influencing the distribution of ticks and clinical cases of TBD (altitude, vegetation cover, occurrence of TBE disease cases, tourism attractiveness etc.), 30 locations were selected for the collection of ticks and estimation of tick activity. Variability in the habitat types suitable was emphasized in the selection procedure.

The field work was conducted in 2008 in three time periods according to the seasonality of the tick populations (May, June, September). Ticks (*I. ricinus*) were found at all 30 localities. The localities differed in the total abundance of ticks and in the seasonal pattern of tick activity. A total of 20 057 individuals was collected. The mean host-seeking activity of ticks (number of ticks collected per hour) reached 101 ticks/hour (range 28-177) in May, 58 ticks/hour (13-139) in June and 64 ticks/hour (5-221) in September.

In the continuation of the project, the ticks will be tested for the presence of tick-borne encephalitis virus and *Borrelia burgdorferi* s. l. spirochets (*B. burgdorferi* genospecies will be identified). The TBD risk will be

evaluated for individual localities. By the means of statistical analysis the factors influencing the TBD risk distribution will be identified and on their basis a model for TBD risk prediction for the Region of South Bohemia will be developed. The same work will be conducted in the region of Bavaria and the results will be compared.

The project is partially financed from the European Regional Development Fund of the European Union.

## Session 15 - Rodent-borne diseases (Ecology of Puumala hantavirus)

### Degree of synchrony in dynamics of bank vole populations and Puumala hantavirus persistence: a study through stochastic modelling

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Our previous modelling work (Sauvage et al. 2007) suggested the main impact of the bank vole demography coupled to the virus shedding dynamics by infected rodents to explain the occurrence and the geographic distribution of Nephropathia epidemica (NE) epidemic events. NE is a human disease caused by the Puumala hantavirus whose reservoir species is the bank vole, *Myodes glareolus*. Schematically, bank vole populations exhibit large multi-annual fluctuations of density in northern areas of Europe where the largest NE epidemics and the highest incidences take place. In addition, the virus shedding by infected voles is much stronger during the first two month of infection than during the later chronic phase.

The large and rapid increase of the density in bank vole populations appeared necessary to obtain the high number of acute-infected voles required to provoke the peak of viral concentration in the environment that leads to the human contamination. On the other hand, periods of locally very low densities in bank vole populations associated to low detectability of the virus circulation ask for a better understanding of rodent population synchrony to allow spread of the virus and improve its persistence at a larger scale.

We are currently developping stochastic models to explore the consequences of the degree of bank vole population demographic synchrony on the Puumala hantavirus probability of persistence.

### The role of maternal antibodies in the transmission dynamics of Puumala hantavirus in vole populations

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Females that are, or have been, infected may transfer maternal antibodies (MatAbs) to their offspring, protecting them transiently against the infections the mother has encountered. Despite maternal protection being a well-know phenomenon, its role in the transmission dynamics of infectious diseases in wildlife has been largely neglected. Here we investigate the role of Puumala hantavirus (PUUV)-specific MatAbs in PUUV dynamics, using seven years' data from a cyclic bank vole population in an endemic area in Finland. For the first time, we partition data on seropositivity from a natural population into separate dynamic patterns for MatAbs and infection. Likelihood that the young of the year carried PUUV-specific MatAbs during the breeding season correlated positively with infection prevalence in over-wintered voles in the preceding spring. The probability of being infected by PUUV itself varied between seasons, being highest in spring and lowest in

late summer, but was also, notably, negatively related to the MatAb prevalence in summer, as well as to the infection prevalence earlier in the breeding season. Our results suggest that high infection prevalence in the early breeding season leads to a high proportion of transiently immune young individuals, which causes delays in transmission. This suggests, in turn, that MatAb protection has the potential to affect infection dynamics in natural populations.

## **Does the Puumala virus impact the survival of its host, the Bank Vole *Myodes glareolus*? Insights from a capture - mark - recapture analysis**

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From the point of view of humans, hantaviruses are the etiological agents of zoonotic diseases called haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in Americas. Mortality in humans ranges from <0.5% in Nephropathia epidemica to 5% to 10% for other HFRS and 45% for the more severe HPS. From the point of view of hantaviruses, humans act as evolutionary dead ends and each hantavirus is associated predominantly with a reservoir species, typically a rodent. In this paper, we will focus on the Puumala virus, responsible for a mild form of HFRS, called Nephropathia epidemica and its reservoir species, the bank vole, *Myodes glareolus*. A few studies have already reported that outbreaks of hantavirus disease occur following outbreaks of reservoir populations (LeDuc 1987). Thus, if we are to understand the transmission of hantaviruses to humans, we have to understand the population dynamics of the reservoir species.

For small mammals, survival is generally considered as a demographic parameter of crucial importance to understand the population dynamics. In the context of a species reservoir of hantavirus, it is thus particularly relevant to investigate whether or not the virus decreases survival of its host. The literature provides actually mixed results. Some authors have reported no effect of hantavirus on the survival of its hosts in the wild (e.g. Verhagen et al. 1986). Others have reported a decrease in survival at least for some groups of infected individuals, i.e. juveniles, or seasons in the year, i.e. winter (Douglass et al. 2001, Kallio et al. 2007).

However, as far as we know, previous work has used methods that fail to take into account the imperfect catchability of individuals in the wild (e.g. enumeration methods) and that thus yield biased estimates of survival unless very restrictive assumptions are fulfilled. We have used instead a capture mark recapture multi-state model that includes probabilities of recapture correcting for the imperfect catchability of voles and four states crossing sexual activity and infection status of voles. Further, this model allows us to estimate survival for each state and also to work out the probability of maturation from sexually inactive to sexually active individuals as well as the probability for becoming infected.

Data were collected from July 2004 to October 2006, with an outbreak in 2005, at nine sites in broad-leaved forest patches in Belgium. Trapping was conducted on a 10 X 10 grid of 100 traps during three consecutive nights per site at each spring, summer and autumn (1072 individuals captured). Individuals were classified as sexually active or inactive depending on several reproductive criteria. Although the sparseness of data impedes to draw definite conclusions, the model does suggest that the survival of infected individuals is substantially reduced for sexually inactive individuals.

## **Risks of Hantavirus occurrence and emergence: insights from rodent immunogenetics at regional and European geographic scales**

Nathalie Charbonnel<sup>1</sup>, Maxime Galan<sup>1</sup>, Emmanuel Guivier<sup>1</sup>, Julie Deter<sup>1</sup>, Pierre-Jean Malé<sup>2</sup>, Marie Pages<sup>1</sup>,



Over the past few years, the molecular variation of immune genes in reservoir populations has provided new perspectives for the understanding of disease epidemiology. In this context, we demonstrate that hantavirus occurrence and emergence may be better predicted from a comprehensive assessment of the spatial distribution of immune molecular variation involved in hantavirus control. Moreover we show that immunogenetic keys can help to determine a priori the possibility for rodent species to carry hantaviruses potentially pathogenic for humans.

In this talk, I will first summarize our immunogenetic studies on Puumala virus (PUUV) and its rodent reservoir, the bank vole *Myodes glareolus*, at European and regional geographical scales.

We focused on four Mhc (major histocompatibility complex) genes. We found local positive and negative correlations between some allelic forms of the two Mhc class II genes (Dqa and Drb) and PUUV infection in *M. glareolus* populations. The molecular variation at these two genes was very high, as 19 Dqa exon 2 and 71 Drb exon 2 alleles were detected over the whole European distribution area of the bank vole. Strong signatures of positive selection were detected within the antigen binding sites. Neither Dqa nor Drb genes displayed clear geographical patterns contrary to what was observed for neutral genes. These results clearly support that the two Mhc class II genes experience balancing selection that creates and maintains their polymorphism, and that consequently erases the footprints of *M. glareolus* phylogeographic history. Challenging our initial prediction, Dqa and Drb molecular variation may thus have only a low role in predicting the geographic distribution of PUUV distribution in Europe.

A better assessment of the risks associated with PUUV emergence was provided by analysing TNF-alpha expression and polymorphism. We compared TNF-alpha expression of bank voles trapped in geographic areas of medium-high endemicity and of low endemicity at the European scale. Geographic areas of high PUUV endemicity exhibited significantly lower levels of TNF-alpha expression. Mounting immune inflammatory response has important physiological costs. It is therefore possible that unfavourable environmental conditions select for both low inflammatory responses and tolerance to PUUV, whereas more favourable ones would select for higher inflammatory responses and consequently resistance to PUUV. The analysis of TNF-alpha promoter polymorphism in European bank vole populations confirm that these differences in TNF- expression were driven by genetic components. An extensive study of TNF-alpha promoter genetic variation at the European scale could provide an interesting view of the risk of PUUV emergence in bank vole populations.

In the second part of this talk, I will investigate the role of integrin genetic variation in the capacity for rodent species to carry pathogenic hantaviruses. Interestingly, alphaIIa-beta3 integrins mediate the cellular entry of HFRS- and HCPS-causing hantaviruses whereas non-pathogenic or low pathogenic hantaviruses (e.g. PHV or TULV) use alpha5-beta1 integrins. We sequenced a part of the beta3 chain integrin for 15 rodent species. Although the genetic variation globally reflected the neutral phylogeny of rodent species, we found one variable site that was under positive selection in rodent species that do not carry any pathogenic hantaviruses and that was non variable in rodent species that do carry pathogenic hantaviruses. This site of the beta3 integrin could thus help determining a priori the possibility for rodent species to be reservoirs of pathogenic hantaviruses. We will confirm this hypothesis by testing whether amino-acid changes at this site could mediate conformational changes that would prevent the fixation of pathogenic hantaviruses.

## Immunogenetics in wild population reservoir: a key for landscape epidemiology

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In the context of (re)-emergent zoonoses, studying immunogenetics in wild animal reservoir populations appears as one important challenge for evolutionary biologists. This approach seems now indispensable to understand the geographic distribution of zoonotic agents and to determine the risk of emerging infectious diseases in humans.

Our research focuses on the mild form of hemorrhagic fever with renal syndrome (HFRS), nephropathia epidemica (NE), in Europe. This infectious disease is caused by the Puumala hantavirus (PUUV) which is carried by the bank vole *Myodes glareolus*. The landscape influences the dispersion and geographic distribution of host and its associated parasite community. The evolutionary forces as gene flow, genetic drift and parasite pressure, determines the potential for local adaptation in host/parasite system. Our objective is to estimate the importance of these factors as a key to understand the geographic distribution of Puumala at the landscape scale.

In Ardennes, French endemic area, we analysed the neutral genetic pattern of bank vole populations at the landscape scale and we determined the diversity of parasite community in the different habitats. We focused also on TNF-alpha gene, a pro-inflammatory cytokine known to be involved also in human susceptibility to Puumala. We studied the geographic variability of TNF-alpha expression in bank vole populations, with regard to landscape and Puumala seroprevalence.

Our main result indicates a strong effect of habitat fragmentation on gene flow and a high variability of parasite community between the different landscape components. We observed also a significantly higher level of TNF-alpha expression in edge habitats (low Puumala seroprevalence) compared to large forests (high seroprevalence). TNF-alpha is under-expressed in populations where seroprevalence levels are higher.

We concluded that the landscape influences the genetic structure of bank vole populations and the associated parasite community. Therefore, the difference of TNF-alpha expression observed between *M. glareolus* populations could be a genetically selected component which characterizes the population reservoir. The immunogenetics characteristics of bank vole population seemed to be an important component in evaluation of NE emergence risk.

## Session 16 – West Nile in Europe

### West Nile transmission in resident birds in Italy

Rossella Lelli, Paolo Calistri, Rossana Bruno, Federica Monaco, Giovanni Savini

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Migratory birds are considered one of the main sources for West Nile virus (WNV) introduction into European countries. Following the WNV epidemic in the late summer of 1998 in a marshy area of Tuscany (Padule di Fucecchio), an extensive ornithological surveillance programme was carried out in the infected areas from 2006 to 2008. Several species of migratory and resident birds were trapped, sampled and tested serologically and virologically. The results of this surveillance programme gave a useful indication of potential sources of WNV re-introduction and spread into Italy. The area under study was also investigated and classified in ecological areas through a satellite image processing. In August 2008 the WNV infection re-emerged in Italy in the area surrounding the Po river delta, involving three regions: Lombardy, Emilia Romagna and Veneto. Several surveillance activities were immediately put in place, including the extensive monitoring of wild birds found dead or trapped in the framework of other surveillance programs. These activities were also prolonged in the 2009, when the virus circulation re-occurred at the border of the area already infected in 2008. A comparison between the results of the EDEN project activities, obtained from 2006 to 2008 in the Padule di Fucecchio area with those observed during 2008-2009 epidemics was made. The possible epidemiological role of the different species of migratory and resident birds is discussed, in relation to the different ecological patterns identified in the area and their potential ability to introduce, spread and support the endemisation of WNV infection.

### The Danube Delta: birds, mosquitoes and West Nile virus at the crossroads (Mihai Marinov, Danube Delta National Institute, Romania)

Mihai Marinov Jr, Botond J. Kiss, Florian L Priotesa, Elena Falcuta, Vasile Alexe, Gabriela Nicolescu, , Aurora Alexe, Paul Reiter

The Danube Delta—the largest wetland area in Europe—is situated on a major flyway of birds migrating to and from Africa and presents an ideal site for study of the zoonotic transmission of West Nile virus. Our studies of the mosquito, avian, and equine fauna in the area (Danube Delta Biosphere Reserve and the Dobrogea Tableland) confirm active transmission and, in tandem with studies in other deltaic regions of the EDEN project, support the hypothesis that reed-beds are an important focus of transmission.

## **The spatial modelling of biodiversity hotspots for endemic mosquito species in temperate Europe, implications for field sample design and spatial MBD risk analysis.**

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Ongoing large scale economic and environmental changes create suitable conditions for the (re)emergence in Europe of vectorborne diseases in general and mosquito borne diseases (MBD) in particular. Vector biodiversity hotspots may play an important role in the introduction, establishment and spread of such diseases. In temperate Europe little is known of the distribution patterns of mosquito species.

In this paper, based on experience gained in the Benelux, we propose a cost-efficient methodology for the area-wide spatial modeling of mosquito biodiversity. From 2006 to 2009 a series of cross-sectional surveys were conducted in the framework of the MODIRISK project to inventory mosquito species in Belgium using CO<sub>2</sub>-baited traps deployed over a total of 978 trapping sites. Trap sites were selected using a stratified sampling approach discriminating between urban, rural, natural and import-risk habitats. The sampling strategy was specifically designed to prepare for spatial distribution modeling at a one kilometer resolution. At a second stage this spatial data base was further completed with recent and new records from The Netherlands and model results were validated.

A total of 22 mosquito species were identified including two invasive species. Of the 20 endemic species, and based on the developed spatial models, a group of four common species could be identified as being representative of mosquito biodiversity: *Culex pipiens*, *Aedes cinereus/ geminus*, *Coquillettidia richiardii* and *Aedes vexans*. Of the six recorded rare endemic species, all occurred within the identified biodiversity hotspots and 70% of the positive catches were recorded in the high ranking biodiversity zone which represents only 10% of the land surface. Overall 53% of the positive catches for all mosquito species occurred in the &lsquo;broadersquo; biodiversity zone covering 23% of the land surface. These results are discussed regarding their implications both for (a) planning area-wide cost-efficient sampling campaigns applicable to temperate Europe and (b) assessing the risk of introduction of MBD such as West Nile.

## **A metapopulation model to simulate West Nile virus circulation in southern Europe and the Mediterranean basin**

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In Europe, virological and epidemiological data collected in wild birds and horses suggest that a recurrent circulation of West Nile Virus (WNV) could exist in some areas. Whether this circulation is permanent due to overwintering mechanisms or not remains unknown. The current conception of WNV epidemiology combines an enzootic WNV circulation in tropical Africa with seasonal introductions of the virus in Europe by migratory birds. The objectives of this work were to (i) model this conception of WNV global circulation; (ii) evaluate whether the model could reproduce data and patterns observed in Europe and Africa in vectors, horses, and birds.

The model is a deterministic discrete time meta-population model with a daily time step. The epidemiological system is represented by a set of host populations (passerines) that share during their annual life cycle a set of locations where vector populations (*Culex* mosquitoes) live. Incidental hosts (sentinel chickens and horses) also are living in each location and are exposed to infectious bites. Three locations were considered: a wet African area, a dry African area, and a European Mediterranean area. Three resident bird populations live in these areas, as do three vector populations. Two migratory bird populations link the three areas: long distance migrants (wet African area-European area) and short distance migrants (wet African area-dry African area). Population dynamics of both vectors and hosts as well as Infection dynamic parameters were fixed according to literature data. Age-specific bite relative risk and site-specific vector-host ratios were estimated using published seroprevalence data collected in wild birds of a wet African area (the Senegal river Delta, Senegal), a dry African area (Ferlo, Senegal), and in a southern European area (Seville area, Spain). Two age classes were considered for the variations of the bite relative risk: nestlings, chosen as the reference class, and flying individuals (juveniles and adults). A systematic univariate sensitivity analysis was conducted to study the effects of parameter variations on the estimated values of the juvenile and adult bite relative risk, and of site-specific vector-host ratios.

The model was validated using independent, published studies: seroprevalence in migratory and resident wild birds, minimal infection rates in vectors, as well as seroprevalence and incidence in horses. According to this model, overwintering mechanisms are not needed to reproduce the observed pattern. However, the existence of such mechanisms cannot be ruled out. This model will be used to test the efficiency of several surveillance systems (sentinel horses and chickens, mosquito trapping) as well as climatic scenarios.

## **Epidemic West Nile virus: Experiences in Indiana and Texas**

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West Nile virus was first detected in the United States in 1999, and since has rapidly spread throughout the Americas. Field epidemiologic studies were conducted in Indiana (2002) and Texas (2002-2004) to describe WNV spread, to identify environmental risk factors, and to develop forecasting models. In most cases, initial and subsequent epidemics developed during late summer and early autumn, and ended by November of each year, with a classic epidemic curve pattern of occurrence. Spatio-temporal clustering of disease was apparent. Environmental factors that increased disease risk included hydrological features (lakes, rivers, swamps, canals and river basins), land cover (tree, mosaic, shrub, herbaceous, cultivated and artificial), elevation, and climate (rainfall and temperature).

Remotely sensed data – the normalized difference vegetation index – was found to be useful to identify locations at increased risk of WNV disease. Forecasting epidemics was found to be regional-specific: in some regions, a temporal window of 4–5 months was found. In these situations, forecasting periods of increased risk of disease is feasible. Meteorological data was not found to be as useful for forecasting disease occurrence. In addition, in some situations disease in horses may predict the occurrence of disease in humans within the same local area.

Findings suggest that WNV disease epidemics can be predicted, based on landscape- and climate-associated features, with moderate accuracy. These epidemiological relationships potentially allow for the creation of forecasting systems as an early-warning of disease occurrence and to enhance disease surveillance. However, to date, such applications have either not been developed or do not appear to have made a substantial impact. Local variation in WNV occurrence appears to be a barrier to the development of such systems. This is a trade-

off between spatial resolution and accuracy, and broad-scale, generic application.

## Session 17 – Leishmaniasis (Predicting the emergence of leishmaniasis in Europe and its territories)

### Impact of human activities on diversity of phlebotomine sandflies (Diptera: Psychodidae) in the Amazonian forest, region of Cacao, French Guiana

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With about 300 new cases notified each year, including some multiple and severe lesions, cutaneous leishmaniasis remains a public health problem in French Guiana. *Leishmania guyanensis* is the predominant species, responsible for more than 95% of the local cases even if other *Leishmania* species, such as *L. braziliensis* and *L. amazonensis*, also infect humans. In the past, sandflies have been studied throughout French Guiana and about 80 species are listed today. *Lutzomyia umbratilis* seems to be the primary vector of *L. guyanensis*. Also, *Lu. flaviscutellata* has been found infected by both *L. amazonensis* and *L. guyanensis*. However, vectors of *L. braziliensis* have not yet been identified in French Guiana. During the last decades, forested environments have been modified in many places due to human pressure arising from the development of urbanized and cultivated areas, the construction of roads and tracks and the expansion of legal and illegal gold mining activities. In the context of such ecological changes, we examined the distribution of sandfly species in a panel of different locations, in order to update entomological data and estimate the epidemiological risk factors linked to potential distribution changes of suspected vector species.

The study was carried out in the surroundings of the village of Cacao, located in the Amazonian forest, 1.5 hours drive from the capital Cayenne. In 2007 and 2008, sandfly collections were conducted with CDC light traps in three locations characterized as anthropogenic (cultivated areas, essentially orchards), semi-anthropogenic (abandoned cultivated areas overgrown by secondary forest) and natural (primary forest). Two successive nights of trapping were organized both during the dry (October) and the rainy (April) seasons for each of the three locations. Seven light traps were set up exactly in the same places in each location for each night during the whole study.

A total of 168 traps-nights produced a total of 13,099 sandflies (7,149 females, 5,950 males). All 3,250 males and females collected in October 2007 were dissected and mounted for morphological identification. The number of collected sandflies was greater during the dry season (60.7%). Overall, 56.9% were collected in the primary forest compared with only 20.4% in the cultivated areas. Yet, species diversity in the natural environment was curiously very poor. In October 2007, species of the subgenus *Trichophoromyia* clearly predominated in the primary forest. The situation was very different in the cultivated areas, where the proportion of species from the subgenus *Nyssomyia* was more important, with *Lu. umbratilis* and *Lu. flaviscutellata* being the abundant species. We discuss the capacity of these anthropophilic sandflies to adapt to man-made environments and how this might change the risk of leishmaniasis transmission in French Guiana.

### Modelling sandfly vector distribution in the Madrid region of central Spain using a geographic information system

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Cross-sectional surveys of sandflies were performed in 2006 and 2007 in central Spain (mainly in the Madrid province) where canine leishmaniosis (CanL) is endemic. One hundred and twenty three sites were sampled and 9557 sandflies captured. The working hypothesis was that environmental and meteorological factors affect the density and distribution of both vectors (*Phlebotomus perniciosus* and *P. ariasi*) and that spatial and temporal associations between these variables might help predict the risks of CanL prevalence.

Sampling sites were linked to environmental and meteorological parameters using the geographic information system ArcGis v.9.2 software (ESRI, Redlands, CA, USA). Two different statistical approaches were taken to estimate the number of sandflies per square metre of trap. Both models assumed an over-dispersed Poisson distribution of the data (negative binomial regression). The first model-building strategy included the following land cover categories: cultivated areas, forest & shrubland, sparse vegetation, urban areas and water bodies. The second model included percentages of land cover categories in a 500m buffer. Models with best Bayesian information criterion (BIC) were chosen. Bootstrapped samples (1000) were used to assess the predictive performance of the model determining point-values of apparent density per square metre of trap (AD).

Variables included in model 1: autumn, winter, spring and summer rainfall; summer minimum temperature; autumn, winter and spring mean temperature; distance to urban settlement; altitude; aspect; land cover; distance to livestock & equine, fowl and hen farming.

#### Final Model 1

AD	IRR	95% LL	95% UL	P-value
Urban_hm	1.053	1.020	1.088	0.0010
Altitude_hm	0.833	0.766	0.906	0.0000
Livestock 'equine	0.659	0.492	0.883	0.0050

Variables included in model 2: autumn, winter, spring and summer rainfall; summer minimum temperature; autumn, winter and spring mean temperature; distance to urban settlement; altitude; aspect; percentages of land cover categories; distance to livestock & equine, fowl and hen farming.

#### Final Model 2

AD	IRR	95% LL	95% UL	P-value
Spring rainfall _mm	0.710	0.530	0.949	0.0210
Autumn mean t. °C	1.296	1.147	1.464	0.0000
Urban_hm	1.056	1.023	1.090	0.0010
Forest & shrubland	0.470	0.228	0.969	0.0410
Livestock & equine	0.670	0.501	0.895	0.0070

In conclusion, model 1 did not perform too bad for predicting point-values of AD [ $r^2=0.37$ ; 95%CI (19-71)], but model 2 performed better [ $r^2=0.45$ ; 95%CI (23-73)]. The predictive performance might be better if AD classes were used. Although models could be improved, environmental and meteorological parameters were found to be decisive in modelling sandfly vector distribution.

The Spanish Meteorological Agency (AEMet) provided meteorological data. Ministry of the Environment and Rural and Marine Affairs supplied livestock farming registries inside the study area. This work was partly funded by EU grant GOCE-2003-010284 EDEN ([www.eden-fp6project.net](http://www.eden-fp6project.net)).

## Uncertainties in predicting future habitat suitability of European sandflies in the face of climate change

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*Phlebotomus* species, which are native to Southern Europe, are important vectors for sandfly-borne diseases (e.g. leishmaniosis). Several species are recorded, but the density of records is rather low in many regions. These cold-blooded insects cannot regulate their body temperature themselves. Activity phase and survival are strongly connected with the local environmental conditions.

As a consequence of climatic changes, the northward spread of these vectors is expected. This is supported by recent sandfly catches and autochthonous cases of leishmaniasis in humans and their domestic animals in Central Europe, e.g. in Germany. Nevertheless, until now most infections of leishmaniasis at higher latitudes are reported from overseas travellers or dogs imported from the Mediterranean Region, the preferred holiday destination of Europeans.

In this investigation we highlight the restrictions and options for the projection of habitat shift for various *Phlebotomus* species. Bioclimate envelopes are modelled for species which prefer maritime climatic conditions without strong changes in temperature - *P. ariasi*, *P. perniciosus*, *P. mascittii* (assumed but unproven vector competence) - as well as for species with a south-eastern focus of distribution (*P. neglectus*, *P. perfiliewi*, *P. tobbi*).

Uncertainties in projections of the influence of climatic variables are fundamentally dependent on the realization of emission scenarios for greenhouse gases. Then, scale-dependent differences in mechanistic projections between global or regional climate models have to be considered. In addition to thermal factors, the precipitation regime is a source of uncertainty as it is highly variable already by nature. Here, the future projections differ strongly as well in time as in space. Furthermore, it is difficult to estimate the magnitude and frequency of increasing extreme climatic events, which may be decisive for the establishment and disappearance of vectors, respectively.

Anyhow, even if future suitable climatic habitats for *Phlebotomus* species are detected - more or less accurate, depending on model quality - they should not be taken to provide certain forecasts. Whether sandflies can follow their spatially and temporally moving potential climatic or environmental niche is regulated by many other factors, including dispersal ability and barriers. The Alps still can be seen as a natural barrier, which prevents a direct and short-track spread from northern Italy to Central Europe, even if climatic conditions on the northern side are suitable or will become suitable. Furthermore, uncertainties are related to the limited knowledge of sandfly ecology and, beyond that, on the role of potential evolutionary effects that can not be ignored in such short-lived species.

Despite all uncertainties about future tendencies in a rapidly changing environment, one fact is quite clear. Phlebotomine sandflies will occur close to humans and their domestic animals, which provide the preferred blood-meal for the females.

## Ecological risk mapping of canine leishmaniasis in France

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Canine leishmaniasis is a zoonotic disease due to *Leishmania infantum*, a trypanosomatid protozoan transmitted by phlebotomine sandflies acting as vectors. Leishmaniasis is endemic in southern France but its range as well as the influence of environmental and climatic variation on transmission remains poorly understood.

From a retrospective database, including all the studies reporting prevalence or incidence of canine leishmaniasis in France between 1965 and 2007, we performed a spatial analysis in order to: i) map the reported cases in France and ii) produce an environment-based map of the areas at risk for leishmaniasis.

We performed a Principal Component Analysis (PCA) followed by a Hierarchical Ascendant Classification (HAC) to assess if the locations of canine leishmaniasis had to be considered as different groups, delimited according to environmental variables related to climate, vegetation, and human and dog densities. For each group, the potential distribution of canine leishmaniasis in France was mapped using a species niche modelling approach (Maxent model).

Results reveal the existence of two groups of leishmaniasis cases. The first group is located in the Cévennes region, characterized by relatively cold temperatures, intense rainfall and forest cover. The second group is located in the Mediterranean littoral zone, characterized by warmer temperatures, lower precipitation and less forest cover. These two groups may correspond to the ecology of the two species of phlebotomine sandfly vectors in France, *Phlebotomus ariasi* and *P. perniciosus*, respectively. The ecological niche modelling of these two groups of leishmaniasis foci permitted us to produce the first risk map of canine leishmaniasis in France. The results show how an ecological approach can contribute to improving our understanding of the spatial

distribution of canine leishmaniasis in France.

## **R0 maps for canine leishmaniasis**

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An important aim of the EDEN project has been the development of tools to study the effect of different (climate) scenarios on the risk of vector-borne diseases. An R0 map is such a tool: it is a map that indicates the value of R0 for different areas. R0 (or basic reproduction number) is a measure of the risk that a disease can establish after introduction. Here, we show how an R0 map for a vector-borne disease can be constructed, using canine leishmaniasis as an example. In this integrative study, biological field data, high- and low-resolution satellite imagery-based prediction models and mathematical modelling had to be combined. Hence all the horizontal integration teams in EDEN were involved.

The study region is an area of southern France where intensive sandfly trapping was carried out by members of the Leishmaniasis team in the summer of 2005. Trapping results for the two vector species present in this area, *Phlebotomus ariasi* and *P. perniciosus*, were used to predict the abundance of these species for the non-sampled places. This was done using a combination of two different approaches for spatial analysis that to our knowledge had never been combined before: negative binomial regression based on high-resolution remotely sensed data, and non-linear discriminant analysis using low-resolution remotely sensed data. These two approaches have been combined into one integrated model to predict the spatial variance in the sandfly abundance. Combining high and low resolution satellite data is essential, since the presence of a vector may very well depend on variables measured at low resolution (climatic variables such as temperature, vegetation indices and patterns of seasonality) as well as on high resolution variables such as land cover and landscape fragmentation. The vector abundance predictions resulting from the combined model - together with dog density data and literature-derived values for sandfly and parasite related parameters - are then used as input for the R0 formula. In this formula, the temperature dependency of several sandfly and parasite related parameters was taken into account. The resulting R0 map provides an estimate for R0 for every pixel in the raster for which the vector abundance had been estimated by the integrated statistical model. The maps constructed here are a first step towards an integrated method that combines all relevant biological, epidemiological and environmental data, as well as statistical and mathematical modelling techniques, in a Geographical Information System.

**Session 18 – From endemic Mosquito-borne diseases in Africa to emerging Mosquito-borne diseases in Europe (Assessing and managing the risk)**



## **Geographical multifactorial analysis of risk of dengue transmission in Guadeloupe according to urban contexts**

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**Background:** During these recent years, dengue epidemics have progressed in the America region with an increase of rate of hemorrhagic dengue incidence. In this study, we were interested in Guadeloupe (French West Indies) over the 2000-2008 period. Epidemiological data (suspect and confirmed cases) were correlated with environmental factors (urbanization, entomology and meteorology) in order to explain the morbidity of dengue in the Department.

**Methods:** The use of a Geographical Information System (ArcGis) has made it possible to describe the incidences of suspect and confirmed cases at level of communes and sections of the Department but also to define the demographic and urbanization contexts (natural, agricultural, urban). Entomology has made the object on the ground of capture of *Aedes aegypti* mosquito, the sole vector in Guadeloupe in four areas representative of main environmental contexts (urban, peri-urban, residential, rural). On the other hand, temporal and spatio-temporal analyses on suspect cases with SatScan software were carried out in order to detect clusters. Finally, a preliminary retrospective analysis of meteorological data (pluviometry and temperature) was carried out.

**Results:** Calculations of incidence have showed an aggravation of epidemiological situation in the Department during the study period with marked epidemics (2001, 2005 and 2007). The appearance of suspect cases detected by the sentinel doctors show the same trend. Spatio-temporal clusters detected throughout the area are heterogenous with the most probably cluster in the region of the county town of Guadeloupe (Basse-Terre) and surrounding areas, at the end of 2007 during epidemic period. According to the contexts of capture, entomological studies have also revealed a heterogeneousness of period of activity and aggressiveness of vector mosquito; the residential and peri-urban being areas the most exposed at the risk of transmission. At level of the different urban contexts, the evolution between 1996 and 2004 shows extension of these two contexts at risk.

**Conclusion:** The demonstration of progressive extension of areas at risk in Guadeloupe makes it essential to improve of surveillance systems and to develop models in order to improve the prediction and therefore the management of future foci of dengue in the region.

## **Risk Assessment of the emergence of arboviruses diseases transmitted by mosquitoes (Diptera: Culicidae). A case study in the Autonomous Province of Trento: the RISKTIGER project**

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The invasive vector *Aedes albopictus* has spread rapidly around the world implying risks for arboviruses transmission as the recent Chikungunya outbreak in Italy proved. In addition, West Nile virus has recently caused several outbreaks in Northern Italy. The Risktiger Project is being develop from 2007 to 2010 to evaluate and prevent the emergence of arboviruses cycles in the Autonomous Province of Trento, Northern Italy.

This project implements a multidisciplinary approach including evaluation of optimal areas with remote sensing, ovitraps survey to evaluate current distribution, BG-trap intensive survey for population characterization, molecular research of arbovirus with RT-PCR, and integration of all data to prevent

arboviruses emergence in this area.

Satellite data analysis based on reconstructed MODIS land surface temperature maps combined with ovitraps surveys contribute as a methodology to the evaluation of current and optimal distribution areas; intensive BG-trap survey contributes as an useful tool for seasonal dynamics studies and for obtaining samples for arbovirus screening. Furthermore, generic RT-PCR contributes for the identification of a wide range of arboviruses.

Results are presented including: seasonal dynamics and influence of climatic variables in host-seeking females, detection of other mosquito species and blood-fed females, optimal and current distribution of *Ae. albopictus* in Northern Italy, competition with autochthonous species, screening for flavivirus in *Ae. albopictus* and *Culex pipiens*, characterization of two new sequences of flavivirus and influence of climatic and ecological variables in distribution, seasonality, activity period of adults and overwintering of *Ae. albopictus* in this area. All these data will be exposed with concluding remarks about the application of several methodologies and implications of this research in the prevention and study of mosquito-borne diseases in Europe.

## **The Chikungunya Disease in Réunion Island: Chemical control versus Biological control.**

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In 2005 and 2006, Réunion Island faced two epidemics of Chikungunya: the epidemic in 2006 was particularly dramatic. Chikungunya is a vector-borne disease, usually localized in Asia and East-Africa. In 2005, it was the first time that a developed country, like Réunion Island, was affected by this virus. In July 2007, a small outbreak raised in Italy, indicating that the South of Europe is potentially threatened. The principal vector of the Chikungunya virus in Réunion Island is *Aedes albopictus*.

In recent works, we have proposed and studied a mathematical model, called hereafter the L-SEIR model, to explain the outbreak of 2005 and possible links with the explosive epidemic of 2006 [1].

Recently, we also have focused our study in the comparison of different mosquito control tools, like adulticide (Deltamethrin), larvicide (Bti), mechanical control, and combinations of them, in order to know if it would have been possible to contain or to stop the epidemic of 2006 [2]. In particular, using very recent biological results, we have proved that an appropriate combination as well as an early use of the control tools would have been appropriate to contain the explosive epidemic or to significantly reduce the risk of an epidemic. Unfortunately, this chemical control is effective as long as the mosquitoes are sensitive to Deltamethrin, the only authorized adulticide.

The previous control tools are always used in Réunion Island when a Chikungunya case is confirmed. But, Réunion Island is well known as a hot spot of endemicity and massive spraying can, sometimes, be very detrimental to the environment and thus must be limited. It is also necessary to study and to check the feasibility of other type of controls. For instance, the Sterile Insect Technique (SIT) could be an interesting tool for vector-control in Réunion Island.

The aim of this talk is to present some mathematical results and numerical simulations to assess the efficacy of the vector-control tools already used in Réunion Island. In particular, our simulations take into account the protocol used by the DRASS Agency, with different start dates and periodicities for the treatments. In addition to a suitable combination of vector-control tools, we showed that mechanical control, that is very cheap and sustainable, can be very efficient to lower the risk of an epidemic.

Finally, we present a Mathematical model for the SIT coupled with our L-SEIR epidemiological model [3]. After some theoretical results, we show several simulations and compare these results with the ones obtained for the chemical control. We discuss the results and the advantages/disadvantages of the SIT in comparison with the chemical vector control.

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## **Potential transmission of Rift Valley Fever in Europe: Preliminary review of candidate**

## mosquito vectors

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Rift Valley Fever (RVF) is an acute arthropod-borne virus infection with a wide range of vertebrate disease hosts affecting notably ruminants and humans. Rift Valley fever virus (RVFV) can be transmitted to ruminants and humans by a range of arthropods, although mosquitoes of the genera *Aedes* and *Culex* are considered to be the most common vectors of the disease. Human infections may also result from direct contact with the tissues and body fluids of infected ruminants during slaughter or the abortion of infected animals. In addition to the impact on veterinary and public health, restrictions on the trade of domestic animals and animal products from infected areas can lead to severe economic consequences. RVF is endemic in many Sub Saharan African countries and has recently spread to Arabic Peninsula (Saudi Arabia, Yemen) and Comoros archipelago (Mayotte Island). RVF risks to spread into Northern Africa and Southern Europe. In the following review, we predicted mosquito species that could transmit RVF should the virus enter the European Union by listing African RVF vectors also present in Europe that exhibited vector competence in the laboratory and checked them against ecological parameters characteristic of Palaearctic populations. Our list includes: the predominantly ornithophilic *Culex pipiens* Linné and its anthropophilic biotype *molestus* Forskal; salt marsh-adapted *Ochlerotatus caspius* Pallas and *Aedes vexans* Meigen from different types of temporary rain pool. Further studies are needed to better appreciate the vector capacity of potential European mosquito vectors - notably for those not present in Africa and thus not yet studied on this report - so that rises of emergence of RVF in the old continent can be better estimated. The prediction of suspect RVF vector species should facilitate risk assessment, prevention and control programs.

## Session 19 – Rodent-borne diseases (Other than rodent hantas)

### Masting, rodent dynamics and tick-borne encephalitis risk

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Tick-borne encephalitis (TBE) is a zoonotic infection of increasing concern for public health in Europe [1]. Although a safe and efficient vaccine is already available, disease control and cost-effective actions would be greatly improved by the availability of early warning predictors of the inter-annual variation in infection risk in areas where TBE is already known to occur in endemic foci [1-2]. The fundamental ecology of TBE in natural foci is now better known [2-4]: TBEV circulates within a tick-rodent cycle with an intensity that is modulated by a combination of biotic and abiotic factors, such as climatic conditions, availability of rodents (competent hosts) and density of wild deer (reservoir hosts). Rodent density has been shown to fluctuate over time as a result of forest tree seed availability (including masting events) and these fluctuations are known to be associated with infection risk for rodent-borne infections such as Hantavirus [5-6]; temporal variation in rodent density affects also tick abundance [7]. Up to now, long-term collection of TBE epidemiological data across Europe indicates temporal fluctuations in incidence that has been attributed to climatic and socio-economical factors [8]; however, this variation is not entirely explained by these changes in human behaviour. Therefore, our hypothesis is that masting could be an early warning predictor of infection risk, not only for rodent-borne pathogens, but also for tick-borne infections such as TBE, which include rodents as reservoir hosts.

We examined the temporal relationships between masting events in beech forest, rodent density, density of feeding ticks on rodents and TBE incidence in humans using long-term monitoring data obtained in Trentino (Northern Italy) during the period 1999-2008. A significant correlation between annual rodent abundance and

masting indices recorded in the previous year was found (GLM with Negative binomial error: Chi-squared<sub>1,34</sub>=42.44, P<0.001). In addition, overall density of feeding larvae was higher where and when rodent density was higher (GLM with Negative binomial error: Chi-squared<sub>1,47</sub>=47.84, P<0.001), while for feeding nymphs no such effect was observed (GLM with Negative binomial error: Chi-squared<sub>1,47</sub>=1.56, P=0.21). The best explanatory variable for feeding nymph density in any given year was the larval density of the previous year (GLM with Negative binomial error: Chi-squared<sub>1,38</sub>=5.69, P<0.017). Finally, a significant relationship was observed between the overall feeding nymph density and TBE human incidence recorded in Trentino in the same year (Linear model: F<sub>1,47</sub>= 5.93 P=0.019)

TBE risk increases in the year following rodent population peaks, which are in turn related to masting events occurring in the previous year. The increase in TBE risk at year at t+2 after the masting event, t, is linked primarily to a higher risk of encountering infected nymphs at t+2 which fed as larvae on the large number of rodent hosts present in the previous year (t+1). It is probable that during rodent population peaks, the potential circulation of TBE virus is favoured also because the total number of larvae co-feeding on rodent with infected nymphs also increases, but this and other hypothesis need to be tested experimentally.

## **A mathematical epidemiological model of Bartonella infections in the invasive Rattus rattus**

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We study the dynamics of Bartonella in Murid rodents living commensally with humans with the aim of possibly facilitating effective prevention and treatment strategies of rodent-borne Bartonellosis. We develop a mathematical model in the form of a set of ordinary differential equations representing a dynamical system involving the host (*Rattus rattus*) and two ectoparasite vectors, by using a compartmental approach. The relationships between the contributing factors of Bartonella infections in a rodent population are studied within the setting of this model. The parameters of the model relate to the transfer rates to and from compartments. Largely these values have been estimated from the available studies. However, where such data are not available, numerical simulations were used to derive such estimates. Under the (unrealistic) assumption of constant population the dynamical system has a single endemic equilibrium. If a seasonal change of the populations of the vectors is assumed the system has an endemic limit cycle. A sensitivity study with respect to the parameters, which is carried out via numerical simulations, quantifies the system's response to perturbations (e.g. due to measuring errors or natural fluctuation). This, on the one hand reflects the significance of the assumptions made in the model and on the other hand may direct future research to accurately establish the values of key parameters in this system. Naturally, computer simulations are also an ideal first testing ground for any measures of control that may be considered. Therefore, apart from giving a better understanding of the relationships and their significance for the sylvatic cycle of Bartonella, the model may impact on future research direction and the design of control measures.

## **Presence of Mopeia Virus, an African arenavirus, related to individual host characteristics and biotope**

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Mopeia virus (MOPV) is an East-African arenavirus closely related to the West-African Lassa virus (LASV). Unlike MOPV which is probably not pathogenic to humans, LASV can cause haemorrhagic Lassa fever, a severe emerging disease.(1) Because these two viruses have the same rodent species *Mastomys natalensis* as reservoir host besides being phylogenetically closely related, MOPV offers a unique opportunity to safely study transmission dynamics and ecology, allowing to extrapolate findings to LASV and thus contribute to more efficient disease control.

Our study investigates the occurrence of MOPV in three contrasting habitats, testing the hypothesis that virus presence will differ according to host characteristics and population dynamics. In particular, we expected virus prevalence to be lower in habitats less suitable to the host.

Three strongly differing habitats were chosen for trapping: woodland (dense stands of young acacia trees, dry sandy soil), vegetable garden (fertile grounds, irrigated year-round for growing fruits and vegetables) and fallow land (used for maize crops a few months to about 2 years before time of trapping). Of each habitat 5 sites were sampled using two-night capture-mark-removal trapping. A total of 515 *M. natalensis* individuals were captured and host body characteristics were measured. Seroprevalence of anti-MOPV IgG antibodies (Ab) as well as viral RNA were determined using resp. immunofluorescence assays (IFA) and RT-PCR targeting the L segment.

We found that although the habitats differed strongly in abiotic environment as well as in average host densities (4/ha, 56/ha and 37/ha for resp. woodland, garden and fallow land), there was no significant difference in seroprevalence.

Density, body mass index (BMI), age, weight, gender, habitat and sexual maturity were tested using separate generalised linear models, of which the model fit was compared using the DIC value(2). Of these factors, BMI, age, weight and sexual maturity were significantly negatively correlated with the seropresence of viral RNA, with BMI being the best predictor of infection probability.

As young animals generally have a lower BMI(3), this effect of BMI is partly due to an age factor, but also due to a negative relationship between body condition and infection risk.

41 individuals were RNA+/Ab-, 52 were RNA-/Ab+, and 6 were RNA+/Ab+. Interpreting these data using results from experimental infection results from previous studies on other arenaviruses(4,5), this would mean that 6 individuals have been infected between 7 and 21 days before trapping, while 41 are recent infections not more than 7 days old. As this is quite unlikely, this means that the known epidemiology of the arenaviruses described in abovementioned studies is not readily applicable to MOPV, and that infection experiments on MOPV are necessary to properly interpret our results. The same studies have also shown that when infection occurs at a young age, individuals are more likely to become lifelong chronic virus shedders, while this is not so for older individuals.

Relating these findings to the significant effect of age and BMI on virus presence, this shows that both the transmission modes and the timing of infection are important for determining transmission dynamics, and that the relative importance of transmission modes may change according to natural cycles in population composition.

## **An emerging paradigm shift in Hantavirus evolution from the identification of newfound soricid and talpid reservoir hosts**

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Rodents (Order Rodentia) serve as reservoirs of myriad pathogenic microbes, including disease-causing hantaviruses in Eurasia and the Americas. By contrast, shrews (Order Soricomorpha, Family Soricidae) and moles (Family Talpidae) have been largely unstudied in the ecology of zoonotic diseases. Recent analysis of the full genome of Thottapalayam virus, a hantavirus isolated from the Asian house shrew (*Suncus murinus*), indicating a separate phylogenetic clade consistent with an early evolutionary divergence, suggests that other soricomorphs harbor hantaviruses. In launching an aggressive search for hantaviruses in shrews and moles, we relied heavily on the decades-old historical accounts of serological and antigenic evidence of hantavirus infection in non-rodent hosts, and we systematically accessed archival frozen tissues, principally the meticulously curated collection housed at the Museum of Southwestern Biology.

Lung, liver, kidney and/or heart tissues from 784 shrews and 113 moles, captured in Europe, Asia, Africa and North America between 1980 and 2009, were analyzed for hantavirus RNA by RT-PCR, using oligonucleotide primers designed from conserved regions of hantavirus genomes. Hantaviral sequences, aligned and compared using ClustalW, were analyzed phylogenetically using maximum-likelihood and Bayesian methods

(PAUP\*4.0b10, RAxML BlackBox and Mr.Bayes 3.1.2), under the best-fit GTR+I+G model of evolution (JModelTest v0.1). Host-parasite phylogenetic comparisons were analyzed using TreeMap 2.0b.

Genetically distinct hantaviruses were detected in the Chinese mole shrew (*Anourosorex squamipes*), northern short-tailed shrew (*Blarina brevicauda*), Eurasian common shrew (*Sorex araneus*), Laxmann's shrew (*Sorex caecutiens*), masked shrew (*Sorex cinereus*), dusky shrew (*Sorex monticolus*), flat-skulled shrew (*Sorex roboratus*), Ussuri white-toothed shrew (*Crocidura lasiura*), Asian lesser white-toothed shrew (*Crocidura shantungensis*), Japanese shrew mole (*Urotrichus talpoides*), American shrew mole (*Neurotrichus gibbsii*), European common mole (*Talpa europaea*) and eastern mole (*Scalopus aquaticus*). Extensive host sharing was found for Seewis virus (in *Sorex araneus*, *Sorex tundrensis* and *Sorex daphaenodon*) and Jemez Springs virus (in *Sorex monticolus*, *Sorex bairdi*, *Sorex palustris*, *Sorex trowbridgii* and *Sorex vagrans*) throughout their vast geographic ranges. Phylogenetic analyses showed that hantaviruses harbored by soricomorphs segregated into three lineages: one, paraphyletic with rodent-borne hantaviruses, included soricine shrew- and shrew mole-borne hantaviruses; the second and third, evolutionarily distant from all other hantaviruses, included crocidurine shrew- and mole-associated hantaviruses, respectively. Co-phylogeny mapping showed congruent topologies in the phylogenetic relationships between hantaviruses and their soricomorph hosts, with occasional exceptions suggestive of host switching.

The discovery of genetically divergent hantaviruses in shrews and moles suggests that ancestral soricomorphs, rather than rodents, may have served as the early or original mammalian hosts of primordial hantaviruses. Also, the close associations between distinct hantavirus clades and specific subfamilies of rodents, shrews and moles are likely the results of episodic host-virus co-divergence and local host-specific adaptation through deep evolutionary time.

## Isolation and characterization of novel insectivore-borne hantaviruses from Finland

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Hantaviruses (family Bunyaviridae) are important human pathogens that cause hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome. Rodents have been regarded as principal reservoir hosts of hantaviruses, but recently many novel hantaviruses have been recovered from insectivores (order Soricomorpha). For many of these newcomers, only sequence data are so far available, and attempts to isolate these viruses have earlier often failed. This has hampered the analysis of their infectivity and pathogenicity of these viruses to humans and other vertebrates.

In this study, we have searched for novel insectivore-borne hantaviruses in Finland. We first screened 44 lung tissue samples of 5 different insectivore species by nested-RT-PCR that was targeted to the highly conserved domain of the RNA-dependent RNA-polymerase. Of these samples, 43% were hantavirus-positive, and novel hantavirus sequences were recovered from the pygmy shrew (*Sorex minutus*) and water shrew (*Neomys fodiens*). We also found Seewis virus (SWSV) from the common shrew (*Sorex araneus*), captured at 4 different locations in Finland. Furthermore, we recovered hantavirus sequences from the masked shrew (*Sorex caecutiens*) that has been reported to carry Artybash virus in Siberia, Russia. We designated the new viruses Asikkala virus (ASIV), carried by the pygmy shrew, and Laihia virus (LAIV), carried by the water shrew. Both of these new viruses as well as SWSV, were isolated in Vero E6 cells. Sequence analysis revealed that ASIV is closely related to SWSV, while LAIV is more distantly related to other soricine-borne hantaviruses. This reflects well the host taxonomy.

In conclusion, we have isolated and characterized two novel hantaviruses, and we have also isolated SWSV, of which sequence data had been reported earlier. More isolations from other European insectivore species are in progress. The cell culture isolates will allow for serological screening of human samples, enabling us to determine whether these viruses are pathogenic for humans.

## **West Nile viruses responsible for the 2008 and 2009 Italian outbreaks**

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Following the 1998 Tuscany outbreak of West Nile virus (WNV), no further WNV clinical cases were observed in Italy either in horses or in humans until late summer 2008. Clinical signs characterised by posterior weakness, ataxia, and loss of equilibrium, were firstly observed on the 20th of August 2008 in a 2 year racehorse of a stable located in the Ferrara Province, close to Po river. The WNV infection was confirmed both serologically and virologically. An emergency veterinary surveillance program aiming to define the geographical extension of the infection and the animal populations involved was immediately put in place.

In 2009 new cases of West Nile Disease appeared in the Reggio Emilia province. Clinical signs of the disease confirmed by laboratory tests were firstly observed on the 24th of July.

The immediate implementation of the measures and the activities included in the National surveillance plan allowed to identify other outbreaks in the Emilia Romagna, Veneto, Lombardy, Tuscany, Lazio and Friuli-Venezia Giulia regions and to isolate the WNV strains responsible of the outbreaks. The genomic sequence analyses performed on the Italian WN viruses isolated in 2008 and 2009 showed a high homology not only within them but also with the WNV strains responsible for the recent Western Mediterranean outbreaks. To our knowledge this is the first time that in Western Europe two large epidemics occurred in the same areas in two consecutive years. The high identity between 2008 and 2009 WNV strains (>99%), and the earlier period and the area involved in this re-occurrence strongly support the overwintering hypothesis and the endemisation to local host populations

## **A Multiplex-PCR to identify Vertebrate hosts from mosquito bloodmeals**

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Identification of the bloodmeal taken by a haematophagous insect provides information on host preferences under natural conditions and is essential to develop efficient control policies. The feeding preference of mosquitoes on humans is an important element in their vectorial capacity and rule in transmission of vector-borne diseases, and the knowledge of other mosquito hosts reveals the relative importance of reservoirs in zoonotic or enzootic infections. Blood meals of hematophagous arthropods have traditionally been identified by serologic techniques such as the precipitin test, latex agglutination, and the enzyme-linked immunosorbent assay (ELISA), even if these methods are not suitable for distinguishing between closely related species and can generate cross-reactions. On the contrary, DNA analysis greatly increases the speed and cost-effectiveness and the bloodmeal identifications by DNA study provide a more specific tool and allow a multiple approach: host identification, vector species confirmation, pathogenic infections, and population genetics.

However several DNA-diagnostic tests based on PCR (RFLP or HDA) have resulted cumbersome when analysing large numbers of samples. The development of specific Multiplex-PCR has allowed to directly and rapidly overcoming these drawbacks but to date it can identify only a limited number of vertebrate host sources.

In this paper is presented a novel Multiplex-PCR that permits to identify a wider range of hosts (19 different species) of engorged mosquitoes caught during field collections. It was chosen as molecular marker the Cytochrome b, a well-characterized protein from complex III of the mitochondrial oxidative phosphorylation system. The choice was addressed by the high copy number of gene and by the sufficient genetic variation at the primary sequence level among vertebrate taxa.

This diagnostic test was designed to be applied to any environment where humans and domestic animals are present as potential blood hosts. For this purpose it was developed a vertebrate multiplexed primer set specific for bird and for 12 mammals: one forward universal primer and thirteen animal-specific reverse primers manually selected from a multiple alignment of cytochrome b sequences obtained from GenBank. Primer sequences were checked for melting temperature compatibility and self-complementarity with Primer3 software. From whole blood from domestic cattle and poultry was extracted the total DNA and tested for the assay. Expected products were designed to differ in size by at least fifteen bases for unmistakable identification

on agarose gels.

Mosquito specimens from lab-colonies after feeding on membrane apparatus were processed and used as positive controls; unfed specimens were also tested simultaneously for nonspecific amplification by the vertebrate primer set.

Species-specific cytochrome b fragments of the predicted size were obtained using template DNA from whole blood, mosquitoes fed on blood of known animal origin, and blood-fed field females. Moreover the PCR products from birds were subsequently analysed with the restriction enzyme HphI allowing to distinguish 7 bird species, commonly occurred in a farm.

This diagnostic assay identifying the blood sources from 12 mammals and 7 birds will be extremely useful for entomologically based studies concerning blood-feeding behaviour and vectorial capacity of hematophagous arthropods for the vector-borne diseases.

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## **West Nile in the Mediterranean: The significance of the newly arising NS3-249Pro genotype**

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West Nile virus (WNV) is the paradigm of an emerging pathogen. In the last ten years it has expanded its geographic range dramatically, now being considered the most widespread arbovirus in the world [1]. In parallel, significant changes have been observed in its epidemiology, virulence and range of species affected. While once WNV was considered a pathogen of less importance, causing a mild disease in humans, during sporadic, small, self-limiting outbreaks resolving spontaneously, now this notion is changing as WNV is causing large, persistent epidemics, with thousands of human and veterinary cases, and relevant morbidity and mortality. Even in Europe, where this virus was disregarded for decades, significant changes have occurred, leading to increasing outbreak number and persistency, human affection, and virulence for wild birds. Whether this new situation is a consequence of environmental changes (for instance, climate change, global commerce, or other factors) or is rather caused by newly arising viral genotypes, is still in question.

Recently, a particular WNV genotype, NS3249-Pro, has been found associated with an increase in pathogenicity for wild birds [2]. This genotype has arisen independently in different locations: Egypt (1951), Eastern Europe (Romania 1996; Russia, 1999), Israel (1998, connected to the North American epidemic since 1999) and Southern Europe [1, 3]. It has been proposed that this genotype is associated with more virulent and persistent WNV outbreaks [2]. We have studied this issue in the Western Mediterranean region, where since 1996 WNV outbreaks occur sporadically, and nucleotide sequence analysis indicate that all isolates obtained to date belong to a single monophyletic group of common origin, likely representing a single introduction in the area [4]. In 2007 we isolated WNV from golden eagles in Spain [5]. Genomic analysis showed that these isolates were the first identified in the Western Mediterranean bearing NS3249Pro, instead NS3249Thr [4]. In 2008, the worst WNV epidemic in the Western Mediterranean ever observed initiated in the Po river region of Italy, with hundreds of horses affected and 28 human cases observed to date. Genome analysis of WNV isolates from this outbreak showed that they also bear the NS3249Pro genotype [3]. We have performed nucleotide sequence comparisons among Spanish and Italian isolates, demonstrating that this genotype has arisen independently in both locations. Moreover, through experimental inoculations in animal models (both avian and mammal) we have demonstrated that bearing the NS3249Pro genotype does not necessarily mean an increase in virulence. This result remarks the difficulties to secure general concepts for this virus, and that, at least in terms of pathogenicity, what appears to be a rule with a particular strain or cluster of viruses, might turn up wrong for others.

The current WNV recrudescence in Europe and in other parts of the world demands an explanation. We are beginning to glimpse the first data on the virulence determinants for this virus. More investigation is still



needed to elucidate which part of the current WNV recrudescence is attributable to new virus genotypes arising, and which part to environmental changes.

## **Serologic evidence of West Nile virus infection in patients with exanthema in Hungary, 2008**

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Earlier studies showed that West Nile virus (WNV) has been present in Hungary for 30 years. However, it was only in 2003 when it caused animal illness and death. A goose farm became infected and in connection with this outbreak the first human cases were also recognised and serologically confirmed by the National Center for Epidemiology. Since then an average of 6 acute human WNV infections were confirmed every year, the majority of which occurred in the Eastern part of Hungary. The virus has shown higher activity with eighteen cases of meningoencephalitis in Hungary in 2008. Most of the cases occurred in the mentioned region but the virus has also appeared in TBE endemic territories of the country (such as counties Győr-Moson-Sopron and Vas), which may increase the potential of cross reaction (and antibody-dependent enhancement phenomenon) in anti-TBE positive persons infected with West Nile virus.

Samples sent to WHO National Reference Laboratory for Viral Exanthematous Diseases of the Virology Division, from patients with exanthema and fever have been examined for West Nile virus antibodies in a retrospective study. While infection with common viruses causing rash (such as measles, rubella, parvovirus B19) could be excluded as the source of the symptoms, in seven (15%) of forty-five patients the etiology of WNV has been confirmed. Two of 7 patients live in the western (TBE endemic) part of the country. Some of the patients had animal contact (horse-riding, working on chicken farm) or an outdoor hobby (fishing).

The laboratory diagnosis was based on a serological method (indirect fluorescent assay — IFA) for the detection of IgG and IgM antibodies in the patients' sera on in-house prepared slides.

## **Risk of introduction and establishment of African Horse Sickness in the Netherlands**

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African Horse Sickness (AHS) is a vector-borne viral disease of equids that is transmitted by *Culicoides* spp. (*C. imicola* and *C. bolitinos*). Mortality in horses can reach 95%. AHS virus is an orbivirus belonging to the family Reoviridae and closely related to bluetongue virus and epizootic haemorrhagic disease virus. The emergence of bluetongue virus serotype 8 in North-western Europe in 2006 has demonstrated that other *Culicoides* spp. than *C. imicola* can be competent vectors for these orbiviruses. This has resulted in increased awareness of the risk of AHS in the Netherlands. However, not much information and data are available to evaluate this risk. Risk modelling is a useful tool to provide insight into the major variables influencing the risk of introduction and establishment of diseases and can help in optimizing prevention and control strategies.

To analyze the probability of introduction and establishment of AHSV in the Netherlands by import of equids, an import risk analysis has been conducted. Countries worldwide were grouped into three risk categories: (a) high risk, i.e., those countries in which the virus is circulating, (b) low risk, i.e., those countries that have experienced outbreaks of AHS in the past or where *C. imicola* is present, and (c) very low risk, i.e., all other

countries. Imports of equids were grouped according to species (horses; donkeys, mules and hinnies; zebras) and purpose (for life; for slaughter; temporary imports for participation in international events). The probability of establishment was calculated taking into account both seasonal (temperature) and regional (host and vector density) effects. A pathway diagram was constructed to outline all steps required for successful release of AHS virus in the Netherlands and subsequent establishment. This pathway diagram was the basis for a risk model in Excel and @Risk that is under development to quantify the contribution of risk regions and groups of equids to the overall risk of AHS virus introduction into the Netherlands. More insight into the relative risk of these risk regions and groups of equids will help decision-makers in prioritizing preventive measures.

Reported imports of horses from high risk areas into the Netherlands have been restricted to South-Africa in the last decade and are low in numbers. No other equids have been imported from high risk areas. Imports of equids from low risk areas outnumber those from high risk areas and include small numbers of donkeys, mules and hinnies. First model calculations indicate that establishment of AHS in the Netherlands is only possible when average daily (24h) temperatures are greater than 10°C, i.e., in the months May up till October. The probability of establishment is highest in August. The vector-host ratio in the model is given by the abundance of vectors and the possible dilution effect of other ruminants. Both are uncertain factors and have large impact on absolute model output. Further sensitivity analysis will be performed to indicate those parameters that are major sources of uncertainty in the risk model and those with the best options for prevention.